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Cultivo Integral de dos especies de escómbridos: Atún rojo del Atlántico (*Thunnus thynnus*, L. 1758) y Bonito Atlántico (*Sarda sarda*, Bloch 1793)

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MEMORIA

Cultivo Integral de dos especies de escómbridos: Atún rojo del Atlántico (*Thunnus thynnus*, L. 1758) y Bonito Atlántico (*Sarda sarda*, Bloch 1793)

Presentada para optar al Título de Doctor por

Aurelio Ortega García

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A Nieves, por ser mi compañera y estar siempre ahí.

A Lucia y María, que han iluminado mi vida. Ojalá puedan aunar como yo hobby y trabajo.

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INDICE

Glosario

AB: Atlantic bonito, bonito Atlántico
ABFT: Atlantic bluefin tuna, atún rojo del Atlántico
C: Cholesterol, colesterol
Dph: days post hatching, días después de la eclosión
DHA: Docosahexanoic acid, ácido docosahexanoico
EFA: Essential fatty acid, ácidos grasos esenciales
EPA: Eicosapentanoic acid, ácido eicosapentanoico
FA: Fatty acids, ácidos grasos
FAME: Fatty acids methyl ester, metil ésteres de ácidos grasos
HPTLC: High perfomance thin layer cromatography,
Cromatografía de capa delgada de alto rendimiento.
LA: Linoleic acid, ácido linoleico
LC-PUFA: long chain, polyunsaturated fatty acid, ácidos grasos
poliinsaturados de cadena larga
MUFA: monounsaturated fatty acids, ácidos grasos monoinsaturados
PBFT: Pacific bluefin tuna, atún rojo del pacífico
PC: phosphatidylcholine, fosfatidil colina.
PE: phosphatidylethanolamine, fosfatidil etanolamina
PI: phosphatidylinositol, fosfatidil inositol
Ppt: parts per thousand, partes por mil
PS: phosphatidylserine, fosfatidil serina
PUFA: polyunsaturated fatty acids, ácidos grasos poliinsaturados
SAT: saturated fatty acids, ácidos grasos saturados
SD: standard deviation, desviación standard
SE: Sterol ester, ésteres de esterol

RESUMEN

Los escómbridos comprenden una familia de peces pelágicos con un gran potencial para la acuicultura debido a su rápido crecimiento y alto valor comercial. Con la excepción del Atún rojo del pacífico (*Thunnus orientalis*), especie en la que se ha completado el ciclo vital, la acuicultura integral de las especies pertenecientes a la familia de los escómbridos o es inexistente o se basa en la captura de especímenes adultos con capacidad de madurar sexualmente y reproducirse en condiciones controladas de confinamiento.

A pesar de que algunos de los conocimientos obtenidos con el atún rojo del pacífico puedan ser extrapolables a otras especies, se hace necesario realizar una investigación rigurosa sobre las mismas que permita controlar la reproducción y cerrar el ciclo en cautividad, aumentar los conocimientos y mejorar los resultados en cultivo larvario y avanzar en los conocimientos de requerimientos nutricionales y formulaciones de piensos específicos, así como mejorar la tecnología de engorde.

Esta tesis pretende establecer las bases de la reproducción y cultivo larvario de dos nuevas especies de escómbridos: el atún rojo del Mediterráneo y el bonito Atlántico.

Los objetivos a alcanzar pueden resumirse en tres:

a) Desarrollar una tecnología de cultivo que permita capturar adultos, establecer las condiciones adecuadas para su mantenimiento y maduración en cautividad, recolectar huevos fertilizados de buena calidad y finalmente llegar a cerrar su ciclo en cautividad.

b) Describir el desarrollo embrionario del Bonito Atlántico y el Atún rojo del Atlántico y evaluar el efecto de la temperatura sobre el mismo.

c) Determinar la composición lipídica de huevos y larvas y estudiar su composición lipídica (con especial referencia a PUFAs) a lo largo del desarrollo, ensayando diversos alimentos y cuantificando los resultados obtenidos

La tecnología de captura de peces está suficientemente descrita en el caso de atunes rojos mantenidos en jaulas en el mar, por lo que se ha puesto el foco en la captura y manipulación de escómbridos de menor tamaño como es el caso del Bonito. Se establece la metodología de captura para peces de 1-2 kg y de su adaptación a la cautividad en condiciones controladas, logrando la maduración y puestas espontáneas no sólo del Bonito Atlántico sino también del Atún rojo del Atlántico. Ambas especies se han reproducido durante cuatro años, exponiéndose los resultados obtenidos en términos de cantidad de huevos obtenidos, delimitación de la época de puesta, patrón de las mismas y caracterización de los huevos fecundados obtenidos: diámetro, número de

gotas de grasa y tasas de eclosión. Cuando ha sido posible se ha determinado asimismo la fecundidad relativa de las hembras, y se ha conseguido cerrar el ciclo del bonito en cautividad. En el caso del atún rojo estamos próximos a lograrlo, ya que en la actualidad existen en Murcia atunes rojos de más de 20 kg fruto de la colaboración público privada (Instituto Español de Oceanografía, grupo Fuentes y Fortuna Mare) en la que se enmarcan gran parte de las investigaciones aquí relatadas. Se ha descrito el desarrollo embrionario de los huevos obtenidos y se ha estudiado el efecto de la temperatura sobre los mismos, estableciendo la temperatura más propicia para su cultivo.

Asimismo se ha establecido la composición lipídica de los huevos fecundados y su evolución a lo largo del desarrollo embrionario y la etapa de alimentación endógena de las larvas.

Con relación al cultivo larvario, se han comparado los resultados obtenidos en atún rojo con dos presas diferentes, no solo en cuanto a crecimiento y supervivencia, sino también en cuanto a composición lipídica, con especial referencia a los PUFAs y dentro de estos al DHA y a la relación DHA/EPA, considerada esencial para el éxito del cultivo. Asimismo se ha estudiado el comienzo de la fase piscívora de ambas especies, determinando el momento más adecuado para su inicio.

Por último se incluye el proyecto biológico realizado para la construcción de una instalación en tierra capaz de albergar reproductores de atún rojo. Este proyecto es pionero en Europa y alberga las instalaciones más grandes que existen en la actualidad a nivel mundial. El mantenimiento de reproductores en tierra es fundamental para el adecuado desarrollo de una acuicultura integral de atún rojo, ya que permitirá independizarse totalmente de las condiciones meteorológicas y, mediante control del foto y termoperiodo, alargar la época de reproducción de dichas especies. Además, el control de la reproducción del Bonito Atlántico, puede suponer con el tiempo una ventaja añadida para la reproducción del atún rojo: con un menor tamaño corporal y tiempo generacional más corto, los reproductores de bonito podrían usarse como sustituto de reproductores de atún rojo Atlántico para la producción de gametos funcionales viables mediante el implante de células germinales primordiales de atún rojo en el tejido reproductor del bonito.

El trabajo que se presenta en esta tesis pretende ayudar al desarrollo de la acuicultura de estas dos especies de escómbridos, sentando algunas bases para afianzar la reproducción y el cultivo larvario de los mismos. Aún queda camino por recorrer, pero la acuicultura integral comercial del bonito, y especialmente del atún rojo, serán una realidad en muy poco tiempo.

OBJETIVOS

1. Describir técnicas que permitan capturar, manipular y adaptar a la cautividad escómbridos de pequeño tamaño y conseguir un sotck de reproductores de Bonito Atlántico

2. Desarrollar una tecnología de cultivo que permita reproducir el Bonito Atlántico en cautividad. Describir los patrones las puestas obtenidas en tanques de cultivo.

3. Establecer las bases para el cultivo larvario y engorde de Bonito Atlántico para conseguir cerrar el ciclo de la misma en cautividad

4. Establecer las condiciones adecuadas de mantenimiento de reproductores de Atún rojo del Atlántico en jaulas flotantes para conseguir la maduración y puesta espontánea de los mismos.

5. Determinar la biometría de las puestas de Atún rojo del Atlántico

6. Testar el uso colectores para recoger el huevo obtenido en las jaulas ubicadas en el mar

7. Determinar la composición lipídica de huevos de Atún rojo del Atlántico y de Bonito Atlántico

8. Estudiar la evolución de los lípidos, con especial referencia a los ácidos grasos poliinsaturados durante la fase de alimentación endógena de las larvas de Atún rojo Atlántico y Bonito Atlántico.

9. Testar los resultados de diferentes esquemas de alimentación con presas vivas sobre el cultivo larvario del Atún rojo, tanto a nivel de parámetro biométricos (supervivencia y crecimiento) como a nivel de composición lipídica.

10. Examinar las diferencias encontradas entre larvas de escómbridos alimentadas con zooplancton y con larvas de peces, determinando el momento óptimo de transición a la piscivoría

11. Describir el desarrollo embrionario del Bonito Atlántico y el Atún rojo del Atlántico

12. Determinar el efecto de la temperatura de incubación sobre la velocidad del desarrollo y la tasa de eclosión de huevos fertilizados de las dos especies.

13. Diseñar una instalación en tierra capaz de albergar atunes rojos adultos para controlar su ciclo reproductor.

INTRODUCCION

Los escómbridos son una familia de peces con gran interés comercial y objeto de importantes pesquerías a lo largo de todo el mundo. Incluye 15 géneros y unas 50 especies. La familia incluye dos subfamilias, estando casi todos los géneros incluidos en la subfamilia Scombridae. Dentro de ella hay cuatro tribus: dos más primitivas, SCOMBRINI, que incluye a las caballas, SCOMBEROMORINI, y dos más evolucionadas, SARDINI y THUNINI, que incluyen respectivamente a los bonitos y los atunes.

La tribu Sardini incluye 4 géneros, el más importante de los cuales es el género *Sarda* (bonitos) que incluye a su vez 4 especies: *Sarda sarda, Sarda orientalis, Sarda chiliensis* y *Sarda australis*

La tribu Thunini incluye 5 géneros: *Allothunnus, Auxis* (melva)*, Euthynnus* (Bacoreta)*, Katsuwonus (*listado) y *Thunnus* (atunes). Entre estos podemos citar al rabil (*Thunnus albacares)*, el patudo (*Thunnus obesus),* el bonito del Norte (*Thunnus alalunga)* y las tres especies de atún rojo. Dentro de éste nombre genérico se incluyen tres especies diferentes:

- Atún rojo del Atlántico, en adelante ABFT por sus siglas en inglés*, Thunnus thynnus*
- Atún rojo del Pacífico, en adelante PBFT por sus siglas en inglés*, Thunnus orientalis*
- Atún rojo del Sur, en adelante SBFT por sus siglas en inglés*, Thunnus maccoyii*

En general los escómbridos son peces con el cuerpo alargado y fusiforme y con un hocico puntiagudo. La boca es bastante grande y presentan dientes en las mandíbulas. Los ojos pueden presentar una papila adiposa. Tienen dos aletas dorsales separadas entre sí. La primera dorsal tiene de 9 a 27 radios, suele ser corta y comenzar bastante atrás de la cabeza y se puede replegar totalmente en un surco. Poseen dos filas de 5 a 12 pínulas o falsas aletas que se extienden por detrás de la segunda dorsal y de la aleta anal. Las aletas pectorales son altas y las pélvicas son pequeñas y se sitúan casi debajo de las pectorales. La aleta caudal es robusta y ahorquillada y sus radios cubren totalmente la placa hipural. Presentan dos quillas pequeñas a ambos lados del pedúnculo caudal (algunas especies presentan una tercera quilla intermedia más grande).

La línea lateral es simple. De 31 a 66 vértebras. Generalmente el cuerpo cubierto de pequeñas escamas, aunque algunas especies pueden presentar unas escamas más grandes y gruesas en el coselete (región que se corresponde con la parte posterior de la cabeza y las aletas pectorales). Algunas especies, como la bacoreta, solo presentan escamas en la línea lateral y en el coselete.

La coloración dorsal es verdosa o azulada y la ventral es plateada, pudiendo presentar en los flancos manchas negras que pueden formar bandas.

Peces epipelágicos, marinos o de aguas salobres, algunas especies son costeras pero otras viven lejos de la costa. Se distribuyen por los mares tropicales y subtropicales de todo el mundo. Presentan un comportamiento muy activo, algunos son muy veloces y realizan grandes migraciones, pudiendo formar grandes cardúmenes.

A pesar de que unas pocas especies (principalmente el género *Scomber*) son planctófagos, la mayoría de las especies son predadores, alimentándose de peces, crustáceos y cefalópodos.

Presentan sexos separados con escaso o nulo dimorfismo sexual. Las hembras de muchas especies son de mayor tamaño que los machos. La puesta de la mayoría de las especies se realiza en aguas tropicales o subtropicales, frecuentemente en aguas costeras. Sus larvas son pelágicas y se caracterizan por poseer una cabeza grande y unas mandíbulas muy desarrolladas y provistas de dientes. La mayoría presentan espinas en la zona del preopérculo que desaparecen con el desarrollo.

Casi todas las especies son apreciadas comercialmente. Su carne es sabrosa y puede consumirse fresca, en salazón o en conserva. Se pescan con muy diversos artes: almadrabas, currícán, palangre, cerco, arrastre, artes de enmalle, etc.

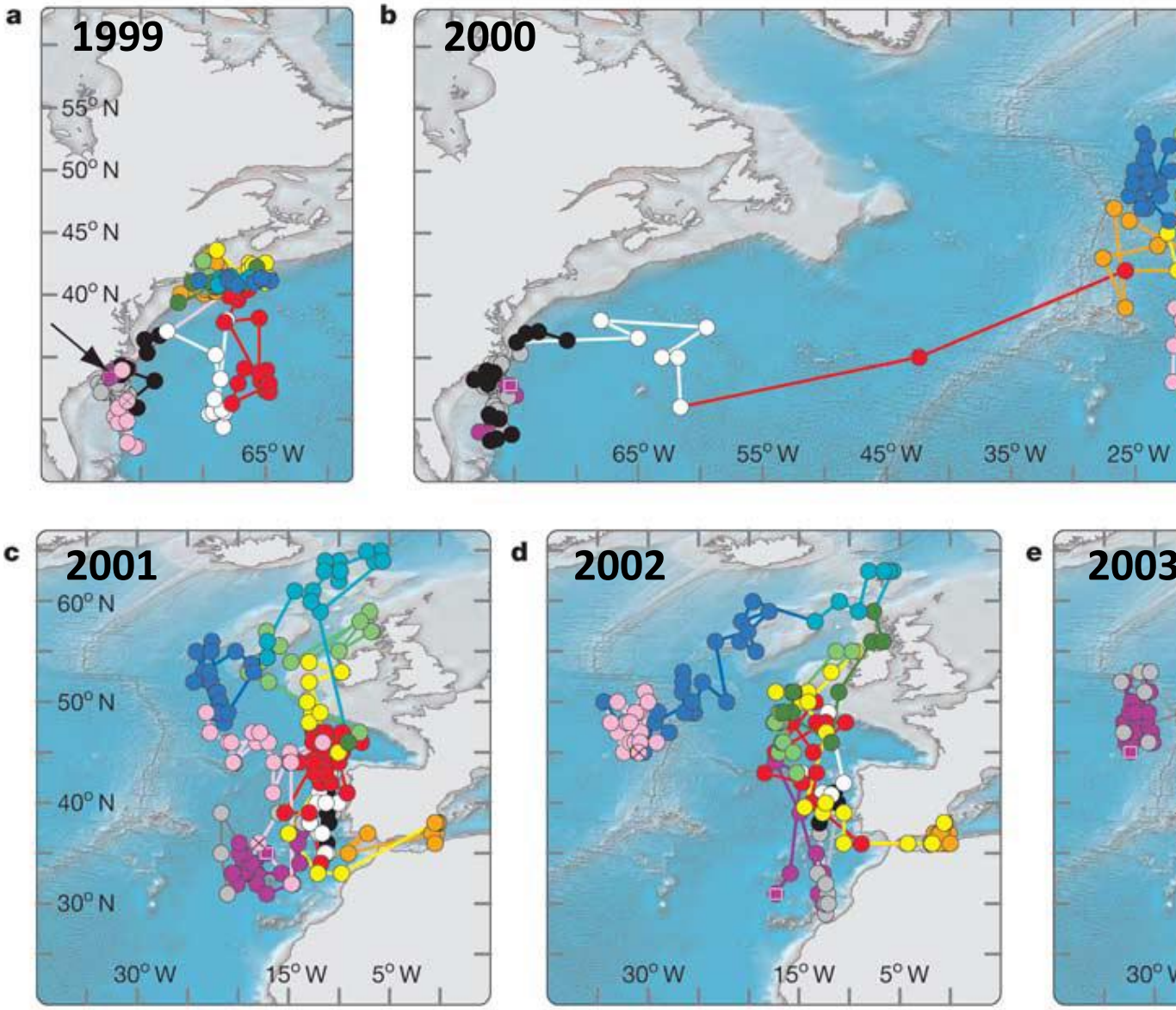
Algunas de las especies más evolucionadas tienen un sistema vascular especializado para el intercambio térmico denominado “rete mirabili”.

ATUN ROJO (*Thunnus Thynnus)*

El ABFT, *Thunnus thynnus* (L., 1758) es un teleósteo perteneciente a la familia Scombridae (Collette *et al.,* 2002). Tiene un cuerpo fusiforme y robusto, perfectamente adaptado para la natación (Figura 1). Posee vejiga natatoria en el estado adulto (Smith-Vaniz, 1986; Collette, 1986). Es una especie de gran tamaño, que puede alcanzar 3 metros de longitud y 600 kg de peso (Cort, 2007). El mayor peso registrado corresponde a un atún de 679 kg desembarcado en Nueva Escocia (Canadá) en 1979 (Collette and Nauen 1983). Mencionar que las otras especies de atún rojo parecen crecer menos, y así el máximo registro de un *Thunnus orientalis* es de 555 kg (Foreman and Ishizuka 1990) y de 260 kg para *Thunnus macoyii* (Nakamura 1990). Se han citado ABFT de más de 15 años (Cort, 2007), y un atún marcado con una edad estimada de 2 años fue recuperado al cabo de 18 años, lo que indicaría que el atún rojo puede alcanzar, al menos, una edad de 20 años (Abascal, 2004).

Figura 1: Atún rojo del Atlántico

Se trata de un gran nadador, alcanzando una velocidad media de 5,9 km/h y una máxima de entre 13 y 31 km/h (Lutcavage *et al.,* 2000) pudiendo cruzar el Océano Atlántico en menos de 60 días. Es capaz de desplazarse miles de kilómetros y está considerada como especie altamente migratoria según el Anexo I de la convención de 1982 sobre la Ley del Mar (FAO, 1994). Es capaz de realizar a lo largo de su vida varios viajes entre las costas orientales del Atlántico Norte y Mediterráneo y las occidentales, y así lo ponen de manifiesto numerosas publicaciones (Block *et al.*, 2005). La figura 2 pone de manifiesto los movimientos realizados por un atún rojo marcado y recapturado posteriormente.



Una de las principales características de esta especie, que comparte con algunos de sus congéneres y con ciertos escualos (Dickson y Graham, 2004), es su capacidad para elevar y mantener su temperatura corporal por encima de la temperatura ambiente, en ocasiones más de 20 ºC. Esto lo consigue mediante un sistema de contracorriente vascular denominado *rete mirabile*. Este sistema actúa como un intercambiador de calor que transfiere el calor producido en el metabolismo y en la contracción muscular, de la sangre venosa a la arterial, volviendo a los tejidos en los que su acumulación produce el aumento de la temperatura. Este hecho le permite mantener un metabolismo elevado y constante, parecido al de los homeotermos, así como habitar zonas del océano con muy amplio rango de temperaturas (Graham y Dickson, 2004; Dickson y Graham, 2004).

Con se ha mencionado anteriormente, con el nombre genérico de atún rojo se conocen tres especies: ABTF, *Thunnus thynnus,* PBFT, *Thunnus orientalis* y SBFT, *Thunnus maccoyii*. Hasta hace poco tiempo, el atún rojo del Atlántico y el atún rojo del Pacífico eran considerados como subespecies de la misma especie (*Thunnus thynnus thynnus* y *Thunnus thynnus orientalis*).

El ABFT se encuentran en aguas que van desde Terranova hasta Brasil, en el lado occidental del Océano Atlántico, y desde Cabo Blanco (20 ºN) hasta Noruega y en todo el Mediterráneo y el Mar Negro, en el lado oriental (Cort, 2007). Sin embargo su presencia en aguas del Norte de Europa es casi nula desde principios de los años 60 (Mac Kenzie y Myers, 2007).

Como se comentó anteriormente es una especie altamente migratoria. Se reconocen dos *stocks* (separados por el meridiano 45 ºW) determinados por sus áreas de puesta (Block *et al.,* 2005): el occidental, con el área de puesta en el Golfo de Méjico, y el oriental, que se reproduce en el Mar Mediterráneo. Sin embargo, la proporción de mezcla entre ambos es mayor de lo que se pensaba hace unos años. Esta mezcla se produce mayoritariamente en el Atlántico Central en las zonas de alimentación (Block *et al.,* 2005).

Es un pez pelágico, marino oceánico que se distribuye en un rango de profundidades desde 0 hasta 1.000 m (Abascal, 2004). Aunque se trata de una especie de aguas abiertas, estacionalmente puede acercarse a la costa, tolerando una amplia gama de temperaturas (Collette y Nauen, 1983). Los esquemas migratorios estacionales dependen de la edad y el tamaño de los peces, estando relacionados fundamentalmente con la búsqueda del alimento y la reproducción. El caso de migraciones de peces adultos en dirección a las zonas de puesta en el Mediterráneo y su posterior regreso al océano para alimentarse intensamente, se conoce desde hace miles de años (Cort, 2007). Existe constancia de su pesca desde tiempo de los romanos, y registros de pesca de las almadrabas desde al menos el siglo XVI, aunque ya en el siglo XIII se habla de ellas. Parece ser que constituyó un importante alimento en

época de los romanos, siendo varias las factorías para su salazón que se datan en aquella época, una de las cuales se encuentra en Mazarrón (Murcia).

En cuanto a su morfología, presentan el cuerpo fusiforme y robusto, perfectamente adaptado para la natación. Color azul oscuro en el dorso, con las zonas inferiores del vientre de color blanco plateado con líneas transversales incoloras alternándose con hileras de puntos. La primera aleta dorsal es de color amarillento o azulado; la segunda rojiza o marronácea; la aleta anal y las pínnulas son de color amarillo oscuro, ribeteadas de blanco; las carenas caudales son negras en los adultos y transparentes en los juveniles. Radios duros dorsales (total): 12-14; Radios blandos dorsales (total): 13-15; Ausencia de radios duros anales; Radios blandos anales: 13–16; Vértebras: 39. Segunda aleta dorsal más alta que la primera; las aletas pectorales son muy cortas, menos del 80 % de la longitud de la cabeza. Presencia de vejiga natatoria (Smith-Vaniz, 1986; Collette, 1986).

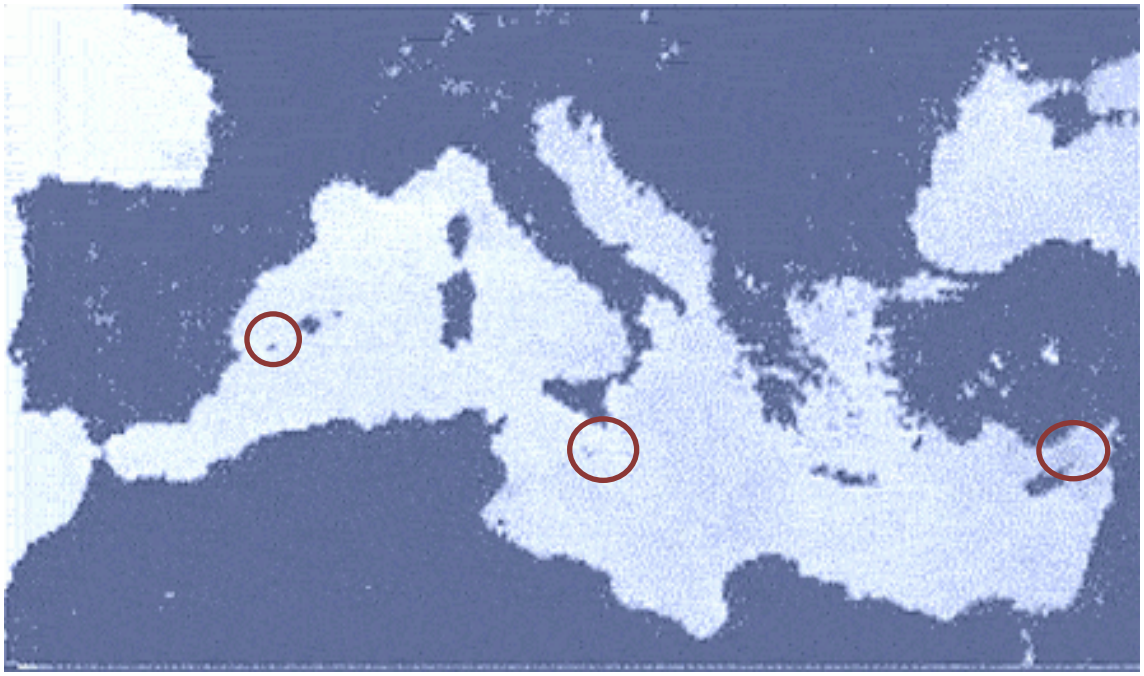
Los atunes se alimentan en zonas costeras de latitudes elevadas a fin de aumentar la grasa corporal que permita hacer frente al gran gasto energético que supone la migración y la reproducción (Chase, 2002). Persiguen a los bancos de pequeños pelágicos: lanzones, boquerones, arenques, caballas, jureles y calamares (Sinopoli *et al.,* 2004), aunque su alimentación no está limitada sólo a ellos, ya que en zonas de aguas someras el 20 % de su dieta está constituida por especies demersales, como cangrejos (*Munida* sp.). La presencia en ocasiones de esponjas en su estómago indica que también se alimenta de especies bentónicas (Chase, 2002). Puede ser considerado, por tanto, como un predador oportunista capaz de explotar una gran variedad de recursos. La composición del alimento varía considerablemente según la zona y a lo largo de la vida del pez. En este caso, dependiendo del tamaño de los atunes ocupan nichos ecológicos diferentes (Stergiou y Karpouzi, 2002; Sara y Sara, 2007). Recientes estudios con juveniles demuestran una gran plasticidad ecológica a la hora de alimentarse. Peces, cefalópodos o, como se ha comentado, incluso crustáceos pueden constituir la base de su dieta según la zona, época del año y especies predominantes en la zona (Varela *et al.*, 2010)

Como en otros escómbridos, el desarrollo del ovario es de tipo asíncrono, en el que pueden encontrarse simultáneamente ovocitos en todas las fases del desarrollo (Tyler y Sumpter, 1996). Así, el atún rojo puede considerarse como un pez de puestas múltiples, que puede ovular varias partidas de ovocitos en una estación reproductora.

La reproducción tiene lugar a finales de la primavera, principios de verano, fundamentalmente en dos zonas: el Mediterráneo y el Golfo de Méjico (Lutcavage *et al.,* 1999), aunque de forma esporádica en otras como en Bahamas y Atlántico noroccidental (Rooker *et al.,* 2007). Los esquemas reproductivos de las poblaciones oriental y occidental del Atlántico son

marcadamente distintos, y mientras que la población oriental alcanza la madurez entre los 3 y 5 años de vida (Corriero *et al.,* 2005), la población occidental no lo hace hasta mucho más tarde, entre los 6 y los 8 años (Baglin, 1982).

En el Mediterráneo, y aunque puede ocurrir en otros lugares, se conocen principalmente tres zonas de puesta; al sur de Italia (alrededor de Sicilia), Baleares y el Mar Levantino (Turquía) (Rooker *et al.,* 2007). La estación de puesta dura entre 30 y 45 días en cada una de las tres zonas, y parecen estar ligeramente desfasadas en el tiempo (Corriero 2003; Karakulak *et al.* 2004; Heinisch *et al.* 2008). Mientras que en las zonas más al Este comienzan hacia mediados de mayo, en las zonas más occidentales (Islas Baleares) comienzan a mediados de junio. No obstante algunos estudios recientes (Gordoa & Carreras, 2014,) ponen de manifiesto que las puestas también pueden comenzar en las aguas de las Islas Baleares a finales de mayo.

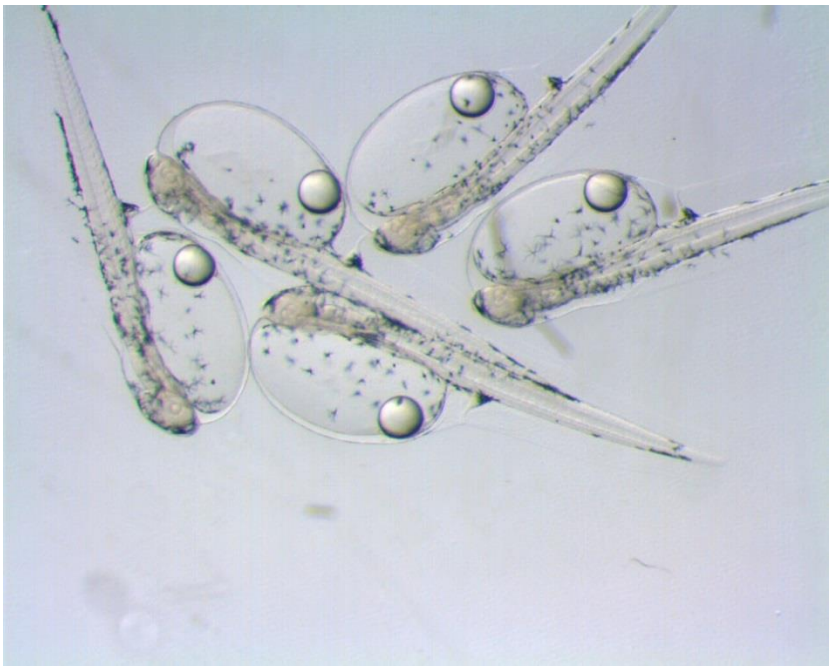


La edad y talla de la primera madurez en el ABFT varía según los autores. En el Mediterráneo, para Corriero *et al.* (2005) la longitud furcal a la que el 50% de los atunes están maduros es de 103.6 cm (3 años de edad), y el 100% está maduro a una talla de 135 cm (4-5 años de edad). Sin embargo Diaz 2011 y Schirripa 2011, estiman la edad correspondiente al 50% de madurez en 4 años.). En cualquier caso, son edades más tempranas que las estimadas para la madurez en el stock del Atlántico Oeste.

El intervalo entre dos puestas consecutivas, según un estudio realizado por Medina *et al.* (2007) con capturas del cerco, es de 1.2 dias, y la puesta pueden prolongarse durante un mes o mes y medio según los autores (Medina *et al.*, 2007, Corriero *et al.*, 2005, Gordoa & Carreras 2014).

Un último dato a tener en cuenta es que se cree que los individuos sexualmente maduros no ponen todos los años (Secor 2007), ya que se han visto individuos sexualmente maduros fuera de las zonas de puesta (Lutcavage *et al.*, 1999) y se han detectado individuos de gran tamaño reproductivamente inmaduros en las zonas y épocas de puesta (Zupa *et al.* 2009)

Para realizar la puesta de huevos, los atunes emigran formando grandes bancos que eligen las áreas más apropiadas en función de numerosas variables ecológicas y ambientales (Cort, 2007). Las hembras de entre 270 y 300 kg de peso pueden producir del orden de 10 millones de huevos por sesión de puesta. A 24 °C, el desarrollo embrionario dura unas 32 horas y los estadíos larvarios unos 20 días. El diámetro de los huevos está en torno a 1 mm y la longitud de la larva al eclosionar sobre los 3 mm. Los huevos de atún poseen por lo general una única gota de grasa.



La larva recién nacida mide unos 3 mm y presenta un saco vitelino bastante voluminoso, que consume a gran velocidad. A los 2-3 días comienza la alimentación exógena, que se basa en pequeños animales planktonicos como nauplius de copépodos. El crecimiento es rápido, y un par de semanas después ya ha finalizado la flexión de la notocorda y la aleta caudal está bien formada, estando preparado para comenzar su etapa piscívora.

A partir de este momento, su crecimiento se acelera y ayudado por la mayor capacidad de natación comienza a abandonar los campos de puesta y a dirigirse hacia aguas más someras cerca de la costa buscando alimento.

Cuando son jóvenes muestran un fuerte comportamiento gregario. Los cardúmenes de juveniles se orientan mediante el sentido de la vista, aunque también se observan de noche. Por tanto, otros sentidos y particularmente la línea lateral parecen intervenir en este comportamiento.

Estos juveniles pueden quedarse en el Mediterráneo durante uno o varios años o bien cruzar al Atlántico donde suelen permanecer por las costas de Marruecos y tras unos meses migrar, generalmente en dirección Norte en paralelo a las costas portuguesas y hacia el Golfo de Vizcaya (Mather *et al.*, 1995).

El área de distribución de los adultos es muy amplio, tanto en el Mediterráneo como en el Atlántico Norte, pudiendo cruzar dicho Océano hasta aguas de las costas de Norteamérica.

La pesca comercial del atún rojo se ha realizado tradicionalmente en el Atlántico oriental y en el mar Mediterráneo con el arte de la Almadraba. Hacia los años 70-80 comenzó la pesca del cerco, que es la modalidad pesquera que mayores capturas obtiene en la actualidad, entre el 70 y el 86 % de la captura total reportada (Ottolenghi, 2008). Otras modalidades de pesca son el cebo vivo, caña y palangre.

Las almadrabas españolas se encuentran en la provincia de Cádiz, y realizan su actividad pesquera aprovechando la migración reproductiva de esta especie al entrar en el Mediterráneo (abril-mayo). En cuanto a la flota de cerco, su actividad se centra en un periodo muy concreto, coincidente con la agregación de miles de reproductores cerca de la superficie en las zonas de puesta del Mediterráneo (mayo-junio). La captura mediante cerco supone el único método utilizable para capturar los ejemplares manteniéndolos vivos, condición esta indispensable para su puesta en cautividad de la que hablaré más adelante.

Dado el importante aumento del esfuerzo pesquero que se produce sobre todo a partir de los años 80, y que pone al atún rojo en una situación de sobrepesca, la Comisión para la Conservación del Atún Atlántico (ICCAT) decidió establecer medidas correctoras, la principal de las cuales es el establecimiento de Capturas Máximas Permitidas, también llamadas cuotas. Así mismo, se incrementa el tamaño mínimo de captura, pasando este de 6,5 kg a los 30 kg actuales y se limita el periodo de captura para la flota de cerco a unos pocos días entre finales de mayo y mediados de junio. La cuota para todo el atún del Atlántico Oriental y Mediterráneo fue 22 000 t en 2009 y 13 500 en 2010. Esta situación se mantuvo durante unos años y ya a partir de 2014-2015 ha

comenzado a aumentar debido a los efectos positivos de las medidas adoptadas. Así este año 2015, la cuota fue de 16 100 Tm y para el próximo 2017 será de 23 100 Tm.

La parte correspondiente a España, que era de 4.117 t en 2009 y de 2.526 t en 2010 se ha empezado también a recuperar, rozando las 3.000 en este año 2015. Estas capturas se repartieron en torno al 89 % para la flota de cerco del Mediterráneo, un 27 % para las Almadrabas, un 22 % para la flota del cebo vivo del Cantábrico, un 14 % para las flotas de palangre y línea de mano y un 6 % para la flota de cañas y líneas de mano del Estrecho. La flota de cerco que se dedica al atún rojo en España está formada por sólo 6 barcos, todos con base en el puerto tarraconense de L'Ametlla de Mar.

La producción se destina en su mayor parte al mercado japonés, en donde alcanza precios más elevados que en los mercados españoles y europeos (Mylonas *et al.,* 2010), aunque cada vez se está prestando más atención a los mercados europeos (fundamentalmente del Norte de Europa) y a Norteamérica.

Engrase de atún rojo

Básicamente, el cultivo del Atún Rojo del Atlántico es un semicultivo consistente en engrasar durante un periodo de tres-siete meses los atunes pescados con el arte del cerco. Durante su migración y reproducción, los atunes pierden una gran cantidad de grasa, lo que influye en su calidad como producto en el mercado. Por ello, durante los meses que permanecen en las jaulas, lo que se pretende es aumentar su grasa corporal alimentándolos con especies de bajo valor comercial y alto contenido graso, como la caballa, sardina, alacha, etc. Esta actividad cada vez se prolonga más en el tiempo, de tal modo que se puedan mantener abastecidos los mercados de producto fresco durante todo el año.

La actividad comenzó en Canadá a finales de la década de los 60 del pasado siglo, mientras que la primera granja de engrase en el Mediterráneo se estableció en Ceuta en 1979. (Miyake *et al.*, 2003), con los atunes obtenidos en una Almadraba. El atún se mantenía hasta Navidad, cuando se vendía como producto de gran calidad en Japón.

Pero no fue hasta la década de los 90 cuando comenzó el cultivo como se realiza en la actualidad, pescando peces con el arte de cerco y trasladándolos a las zonas de engrase. Miyake *et al.* (2003) y Katavic *et al.* (2003a) reportan que esta actividad comenzó en 1996 en España (concretamente en Murcia) y en Croacia, incrementándose con rapidez y alcanzando las 7700 Tm

exportadas a Japón en el año 2001. A partir del año 2000 comenzó también la actividad en otros países: Malta, Italia, Tunez y Turquia, y posteriormente Chipre y Libia.

Los actividad consiste en la pesca del atún con artes de cerco en la época de reproducción, cuando se reúnen en ciertas áreas del Mediterráneo, caracterizadas fundamentalmente por sus condiciones oceanográficas (temperatura, salinidad, etc.), formando grandes cardúmenes en donde se realiza la reproducción. Y es en estas zonas, o cuando están en camino hacia ellas, cuando son capturados por los artes de cerco.

Una vez cercado el cardumen, éste es transferido a una jaula flotante de transporte, circular o hexagonal, y de dimensiones que oscilan entre los 22 m de lado en hexagonales y los 50 m de diámetro en las circulares. Estas jaulas son remolcadas hasta los lugares donde se realizará su cultivo. El transporte se realiza a baja velocidad (1-1.5 nudos), y la mortalidad desde las zonas de pesca a las zonas de engrase es baja, alrededor del 2% (Ottolenghi, 2008).

Al llegar a las zonas de engrase, el atún es trasferido a grandes jaulas, usualmente redondeadas de 50 - 90 m de diámetro, o rectangulares de 120 x 50 m., con profundidades que oscilan entre los 20 - 30 m. La densidad de cultivo es baja, normalmente inferior a 5 Kg/m3 (Belmonte y De la Gándara, 2008).

La alimentación se suele realizar una vez al día a saciedad. Esta operación suele ser supervisada por buceadores para ver el comportamiento de los atunes y detenerla cuando los atunes dejan de alimentarse. Generalmente se suministra el alimento a través de un tubo que se conecta desde el barco y se dirige al centro de la jaula y junto con el agua bombeada se mezcla el alimento para que éste se desplace a través del interior de dicho tubo y llegue al centro de la jaula.

La alimentación es a base de sardina (*Sardina spp*.), alacha (*Sardinella spp*), jurel *(Trachurus trachurus),* caballa (*Scomber scombrus),* estornino (*Scomber japonicus),* arenque (*Clupea harengus*) y algunas especies de cefalópodos. Huntington (2008) estimó que en el año 2004, la alimentación suministrada en todas las granjas del mediterráneo fue de 225 000 Tm de alimento.

Sin conocer con certeza el número y peso de los atunes al comienzo del engrase, es muy difícil estimar índices de conversión y crecimiento, aunque varios autores los han conseguido estimar. Los índices de conversión del alimento son elevados, alrededor de 15-20:1 para los peces pequeños, de menos de 30-40 kg (Farwell, 2003; Katavic *et al.*, 2003b) y de hasta 40:1 (De la Gándara and Ortega, 2013) para individuos de mas de 100-150 kg. Tras 6-8 meses de engorde, los atunes pueden alcanzar un incremento de peso del

orden de un 40-50% in los peces pequeños y un 10-30% in los mas grandes (NORITA, 2003).

La mortalidad es baja, y se ha ido reduciendo cada año hasta llegar a ser casi insignificante, excepto en el caso de accidentes o catástrofes naturales que han llegado a ocasionar la pérdida del total del stock (Mylonas *et al.*, 2010).

El sacrificio suele hacerse en el mar, bien mediante una escopeta de cartuchos con una bala hueca (para que al hacer impacto se deforme y no atraviese el cuerpo del animal evitando dañar a otros que se encuentren próximos), bien mediante la lupara, que consiste en un cartucho que explota por contacto con la cabeza del pez, y puede aplicarse mediante un rifle o mediante un arpón. Estos métodos tienen la ventaja de que el sacrificio se produce de forma individualizada e instantánea. Hay que tener muy en cuenta que el objetivo de todos los métodos de sacrificio es el de producir la muerte del pez lo más rápido posible, evitando la formación de lactato en el músculo, que confiere un sabor metálico a la carne cuando se consume cruda. Este hecho, llamado síndrome de la carne quemada (en japonés *yake)* hace que el precio de venta disminuya e incluso que no sea aceptado por el mercado japonés.

Hacia la acuicultura integral del atún rojo del Atlántico

Debido a las restricciones a la pesca acordadas por ICCAT y mencionadas anteriormente, esta actividad se ha visto considerablemente reducida. En España llegaron a coexistir hasta 14 empresas operando, pero en la actualidad solo cuatro mantienen su actividad.

Este hecho, junto con el aumento del conocimiento generado con el mantenimiento de los peces en cautividad y por las investigaciones desarrolladas en Japón, pusieron el foco en conseguir dirigir esta actividad, o al menos una parte de ella, hacia la acuicultura de ciclo cerrado, que permita independizar la actividad de las capturas del medio. La Comisión Europea ha financiado hasta 4 proyectos encaminados a conseguirlo entre 2002 y 2014: DOTT, REPRODOTT, SELFDOTT y TRANSDOTT, todos acabados en DOTT por la siglas en inglés de Domesticación del atún rojo (Domestication of *Thunnus thynnus*), y en la actualidad hay centros de investigación y empresas privadas trabajando en la reproducción, cultivo larvario y engorde de alevines en el mar en España, Croacia, Malta y Turquía, además de algún otro intento en Italia y Grecia.

La reproducción de ABFT en jaulas se lleva desarrollando desde hace algunos años en varias instalaciones en Europa, y debido a las mejoras en el cultivo larvario, el cierre del ciclo podrá conseguirse a corto plazo. Las primeras puestas en cautividad fueron obtenidas en Italia en 2008 (De Metrio *et al.*, 2010) y en España en 2009 (Anon, 2010; De la Gándara *et al.*, 2011).

La reproducción en cautividad del PFBT se realizó por primera vez en la Universidad de Kinki en 1979, pero en los 80 dejaron de obtenerse puestas, que no se reanudaron hasta que a partir del año 1994 comenzaron ya a obtenerse con cierta regularidad (Masuma *et al.*, 2011). En 2002 consiguieron cerrar el ciclo en cautividad (Sawada *et al.*, 2005). El PBFT ha sido también capaz de reproducirse en instalaciones en tierra. Concretamente se ha reproducido en el acuario de Tokio (Mimori *et al.*, 2008) y en el año 2014 en el Seikai National Fisheries Research Institute en Nagasaki. En el año 2010 también se consiguió reproducir en tanques el SBFT

Son numerosas las publicaciones que tratan sobre cultivo larvario de PBFT (Miyashita, 2002; Seoka *et al.*, 2007; Kato, 2008; Biswass, 2010; Masuma *et al.*, 2011; Kurata *et al.*, 2013), tanto aspectos de cultivo como aspectos fisiológicos y nutricionales. Pero en cuanto al ABFT las aportaciones son mucho más escasas (Caggiano *et al.*, 2011; Ortega *et al.*, 2011; Anon, 2010; Anon 2012; Yúfera *et al.* 2014). No obstante, una empresa española y otra turca se encuentran en la actualidad engordando juveniles de ABFT nacidos en cautividad. Y la primera de ellas, con la que el Instituto Español de Oceanografía mantiene una estrecha relación, posee ejemplares de 30-40 kg que cumplirán los cuatro años de edad en la próxima estación de puesta de

2016, con lo que el cierre del ciclo vital del ABFT en cautividad puede ser cosa de meses.

No obstante habrá que perseverar en las investigaciones para alcanzar un mayor conocimiento de la fisiología y requerimientos nutricionales de esta especie, adecuando su cultivo a estos hallazgos. Y se deberá hacer un mayor esfuerzo en conseguir una tecnología de engorde en jaulas que aumente las tasas de supervivencia y en encontrar formulaciones que mejoren las tasas de conversión y optimicen el crecimiento, disminuyendo además la dependencia de materias primas de origen marino para su alimentación.

BONITO ATLANTICO (*Sarda sarda)*

El Bonito Atlántico*, Sarda sarda (Bloch 1793)* (AB en adelante)*,* es también un teleósteo perteneciente a la familia Scombridae, y dentro de estos a la tribu de los *Sardini.* Es objeto de pesquerías, principalmente estacionales, en nuestras costas, bien conocido en el Mediterráneo y apreciado por el mercado. Es una especie que presenta un crecimiento rápido, alcanzando 1 kg de peso antes de los seis meses de vida (Santamaria *et al.*, 2005) y capaz de reproducirse al final de su primer año de vida (Rey *et al.* 1984).

Su cuerpo es ligeramente alargado y estrecho. La boca es grande y las mandíbulas son alargadas pudiendo alcanzar el borde posterior del ojo. No tiene dientes en la lengua y presenta párpados adiposos poco desarrollados.

Las dos aletas dorsales se repliegan al tiempo, y la primera dorsal es bastante alargada, pudiendo tener entre 20 y 23 radios espinosos. La línea lateral es ondulada y conspicua. Presenta dos quillas pequeñas y otra central más desarrollada y dos huesos intermusculares unidos a cada lado de la parte posterior del cráneo.

El cuerpo está cubierto de pequeñas escamas excepto en la zona del coselete, donde son grandes. El dorso y la parte superior de los flancos es de color azulado-verdoso, con 5-11 bandas oblicuas de color oscuro. En los individuos jóvenes se pueden apreciar hasta 12-16 bandas oscuras verticales.

No tiene vejiga natatoria y el bazo es grande y prominente en visión ventral. El intestino se dirige recto desde el estómago hasta el ano, aunque tiene dos lazos adicionales.

Puede llegar a medir 80 cm de largo y pesar más de 5-6 kg, aunque las capturas más comúnes son de individuos de 1-3 kg., que corresponden a peces de hasta tres años de vida.

Especie pelágica, suele vivir cerca de la superficie en zonas costeras. Puede realizar importantes migraciones y a menudo forma numerosos cardúmenes Como los demás escómbridos es un ventilador pasivo, lo que implica que tiene que nadar continuamente para que el agua pase a través de las branquias.

Se distribuye a ambos lados de la zona tropical y subtropical del Océano Atlántico, pudiendo llegar hasta las Islas Británicas. Frecuente en el Mediterráneo.

Especie dioica, el ovario madura asincrónicamente y la puesta se produce hacia finales de la primavera en el Mediterráneo y en el verano en el Atlántico Norte (Rey *et al.*, 1984, Macias *et al.,* 2005 a). Cada hembra puede realizar varias puestas en cada estación reproductora, con fecundidades absolutas estimadas que oscilan entre 304 000 y 1 150 000 oocitos (Macias *et al.,* 2005b). Las puestas se realizan al atardecer o en las primeras horas de la noche.

Los huevos de bonito son grandes, sobre 1.3 mm, y presentan varias gotas de grasa. La larva se desarrolla muy rápidamente y los juveniles de poco más de 3 cm de longitud ya muestran las típicas bandas verticales, que se mantienen hasta que alcanzan los 25-30 cm.

Se pesca durante todo el año con numerosos artes: arrastre, cerco, artes de enmalle, palangre y almadrabas. Su carne es muy sabrosa y apreciada y se consume fresco, en salazón o en conserva.

Predador activo con una dieta que está dominada fundamentalmente por otros peces adultos, aunque circunstancialmente se puede alimentar de otras presas, fundamentalmente cefalópodos. En el Mediterráneo, las presas más comúnmente ingeridas son los clupeiformes. El primer escalón lo ocupa la alacha, *Sardinella aurita* seguido de la sardina*, S. pilchardus* y del boquerón *E. encrasicolus* (Campo *et al.*, 2006*)*. Algunos autores como Demir (1963) se refieren al BA como un predador insaciable, capaz de adaptarse a las especies más abundantes. Es capaz de tragar presas de gran tamaño relativo, y así bonitos de 40 cm de longitud pueden llegar a ingerir peces de unos 20 cm. Tanto los adultos como los juveniles presentan canibalismo, Zusser (1954)

Es frecuente que los bonitos capturados durante el día presenten presas frescas o parcialmente digeridas, lo que indica que es un pez que se alimenta de día, con una actividad alimentaria mucho más vigorosa a primeras horas de la mañana y al atardecer, como ocurre con la mayoría de los escómbridos.

La práctica inexistencia de crustáceos, moluscos y peces demersales en el estómago de bonitos, que como se ha dicho se alimenta mayoritariamente clupeiformes, evidencia que preda en la provincia nerítica de los océanos, no alcanzando casi nunca el fondo

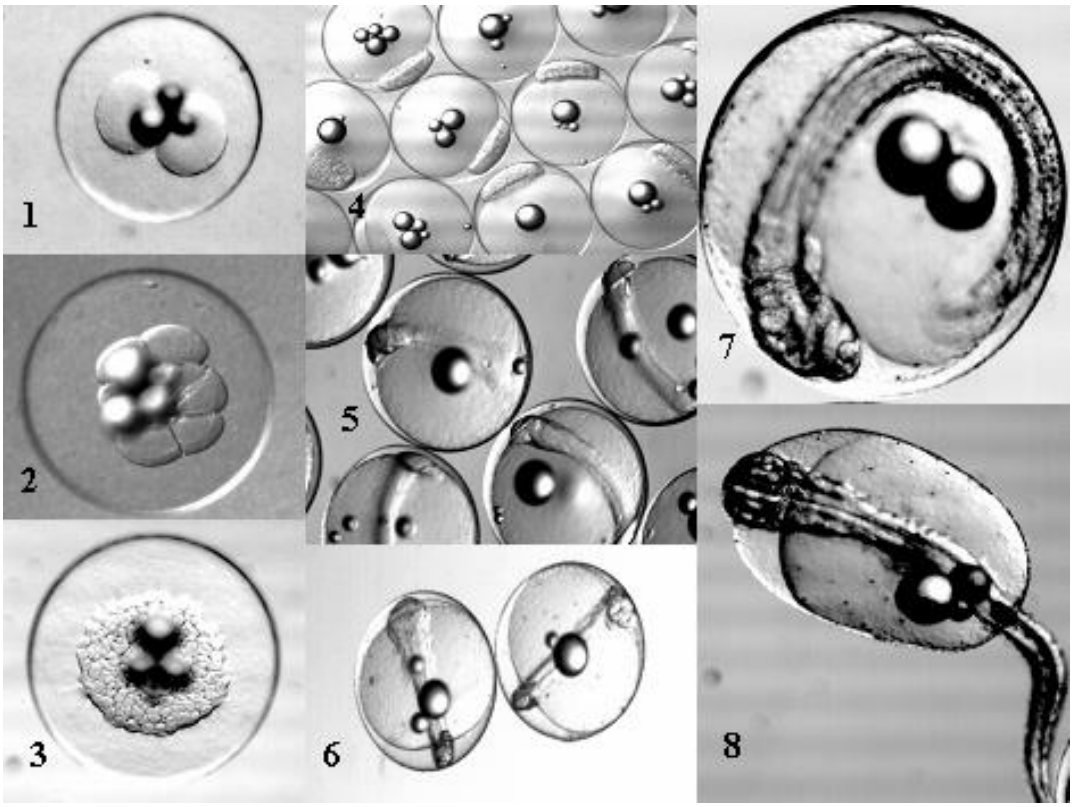
A pesar de que los datos de su crecimiento no son unánimes y dependen de la época, en general se acepta que presenta un rápido crecimiento alcanzando a finales de verano los 25-30 cm. Y alrededor de los 38–41 cm al final de su primer año de vida, los 53–57 cm en el segundo y los 60–64 cm en el tercero (Numann, 1955). Según Rey *et al.* (1984), crece a una media que puede llegar a ser de 3 to 4 mm por día durante los tres primeros meses de vida. Y según Santamaría *et al.* (2005) es capaz de alcanzar 1 kg al cabo de 4-6 meses de vida.

El BA es una especie con una vida media relativamente corta, y son muy escasos los ejemplares que se capturan de la clase de edad 3^{+} en adelante.

A pesar de que se han conseguido puestas en acuarios de otras especies de bonito como el bonito listado, *Sarda orientalis*, (Shimizu *et al.*, 1998, Kaji *et al.*, 2003) y el bonito Pacífico, *Sarda chiliensis*, (McFarlane *et al.*, 2000), no se han

reportado puestas en cautividad de AB hasta hace poco (Ortega & de la Gándara, 2007a).

Su desarrollo embrionario y larvario ha sido descrito por Ortega & de la Gándara (2007b). A 22ºC las primeras divisiones comienzan aproximadamente 90 minutos tras la fecundación, y la eclosión comienza a las 48 horas.



En cuanto a su desarrollo larvario, se pueden diferenciar seis estadíos:

ESTADIO 1: Las larvas recién nacidas flotan pasivamente en la superficie del agua, y tienen varios melanóforos distribuidos a lo largo de todo el cuerpo. Miden sobre 4 mm y el saco vitelino es muy voluminoso.

ESTADIO 2: Corresponde con la reabsorción total del saco vitelino. La boca está abierta y hay una ligera protusión de la mandíbula inferior. Se vuelve mas activa, se pueden observar las aletas pectorales y comienzan a verse melanóforos en la parte superior de la cabeza. El ojo está pigmentado y abren la boca para comenzar su alimentación exógena. Esto sucede el día 2 de vida.

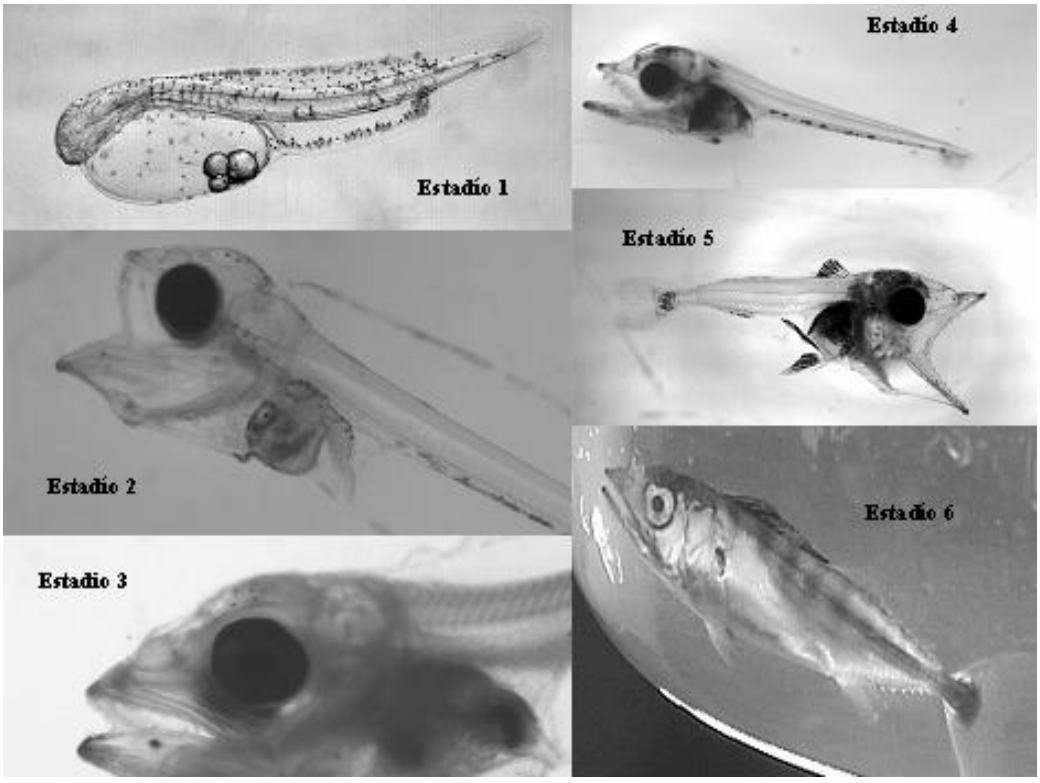
ESTADIO 3: Ya se pueden observar los dientes de ambas mandíbulas. Hay un mayor número de melanóforos en la cabeza y en el hocico. La larva ya caza activamente. Tiene 5-6 días de vida.

ESTADIO 4: Hay una diferenciación de la cabeza, aumenta el diámetro del ojo y ya pueden observarse las aletas ventrales. Aparece la musculatura ventral y dorsal del tronco. Sus hábitos alimentarios comienzan a cambiar y aparece el canibalismo. La larva es muy activa, tiene unos 8-10 días de vida, y su crecimiento se acelera considerablemente. Empieza a alimentarse de larvas de peces.

ESTADIO 5: Se corresponde con una metamorfosis bastante pronunciada: se diferencian las aletas dorsal y caudal, se flexiona la notocorda. Los peces aumentan significativamente su ingesta y su crecimiento. Su alimentación es básicamente piscívora. Las larvas tienen 12-14 días de vida

ESTADIO 6: Se caracteriza por una segunda metamorfosis que comienza sobre el día 17-20 de vida y en la cual ya adquiere las características del adulto. Se desarrollan todas las aletas, incluyendo las pinnulas, y comienza a platear los opérculos y toda la zona abdominal. Los radios de la aleta caudal se tiñen de negro. La larva mide sobre 2-2,5 cm.

A partir de un mes de vida, el juvenil ya ha finalizado totalmente la metamorfosis y tiene ya el aspecto del adulto. Mide sobre 5-6 cm y pesa entre 1 y 2 gramos.



La secuencia alimentaria es rotífero (entre los días 2 y 9), metanauplios de Artemia enriquecidos (entre los días 5 y 12) y larvas de peces recién eclosionadas (a partir del día 8-9). El destete puede comenzarse a partir del día 20 o incluso algo antes.

Al igual que sucede con el atún rojo, se hace necesario a) controlar la reproducción y cerrar el ciclo de la especie, b) aumentar los conocimientos y mejorar los resultados en cultivo larvario, c) avanzar en los conocimientos de requerimientos nutricionales y formulaciones de piensos específicos y d) mejorar la tecnología de engorde.

El trabajo que se presentará en esta tesis pretende ayudar al desarrollo de la acuicultura de estas dos especies de escómbridos, sentando algunas bases para afianzar la reproducción y el cultivo larvario de los mismos. Aún queda camino por recorrer, pero la acuicultura integral comercial del bonito, y especialmente del atún rojo, serán una realidad en muy poco tiempo.

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CAPITULO 1:

Reproducción

Reproduction

1.1. Capture and adaptation to captivity of Atlantic bonito (*Sarda sarda,* Bloch 1758) broodstock

Resumen

Captura y adaptación a la cautividad de reproductores de bonito atlántico (*Sarda sarda*).

Este estudio muestra los primeros resultados de captura, trasporte y mantenimiento en tanques de reproductores de bonito Atlántico, *Sarda sarda* (Bloch, 1793) bajo condiciones controladas. Los peces fueron capturados en una Almadraba para pequeños túnidos existente en La Azohía (costa SE de España) y trasladados), en tanques de transporte acondicionados, hasta las instalaciones del IEO en Mazarrón (Murcia), donde fueron estabulados en tanques cilíndricos de 20 m^3. Se describen los métodos de pesca y transporte empleado, así como las condiciones en las cuales se mantuvieron los peces.. Un mes después de la captura, la supervivencia registrada fue del 57.6%, y los peces estaban perfectamente adaptados a la cautividad.

Abstract

This study deals with the first results on capture, transport and maintenance in tanks, of AB (*Sarda sarda,* Bloch, 1793) broodstock, under controlled conditions. Fishes were caught in an Almadraba trap for small tunids off La Azohía (SE coast of Spain), and then transferred, by conditioned transported tanks, to IEO facilities in Mazarrón (Murcia), where they were placed in two cylindrical tanks of 20 m^3 of capacity. Fishing and transport methods employed, as well as the conditions in which fishes were maintained during their first days in captivity are described and discussed. Survival one month after capture was 57.6%, and remaining bonitos were fully adapted to captivity.

Introduction

AB, *Sarda sarda* (Bloch, 1793), is a coastal migratory fish belonging to the Scombridae family. It is a well known species in the Mediterranean and well appreciate by consumers. It is a fast growing species, able to reach 1 kgr total weight in 4-5 months (Santamaria *et al.*, 2005) and attain reproduction by the end of this fisrt year of life (Rey *et al.* 1984; Macias *et al.* 2005). Bonitos are pelagic fishes, ram ventilators and very sensitive to handle, and although some close species like stripped bonito, S*arda orientalis*, (Kaji *et al.* 2003) and Pacific bonito, *Sarda chiliensis*, (McFarlane *et al.* 2000) have been cultured no one to date has carried out the experience of rearing and breeding BA in captivity.

Scombrids are pelagic fishes which swim great distances by open and coastal sea during their migrations. Their handling is difficult by several reasons: their skin is very sensitive, they have not scales or these are quite small, they stress easily and they need to swim constantly to avoid sinking as well as maintaining fresh oxygenated water currents through their gills for respiration. They are ram ventilators which means that they are unable to move their operculum to pump water through their gills when stationary (Farwell 2000).

Either for a sustainable aquaculture as well as for exhibition in aquaria centers or to keep the fishes alive for research purposes, you must be able to capture and transport them alive and in good health conditions to housing tanks. This have been achieved and described for many marine fish species, most of them non-pelagic, non ram ventilators and small size species (Rimmer and Franklin, 1997, James *et al.*, 1988, Harmon, 2009). However, experiences with Scombrids are quite scarce, most of the time for aquariology, in part due to the difficulties that these species show for transport and maintenance in captivity.

Bluefin tunas are moved from the fishing areas to culture cages, and the survival rate is higher when small fish (less than 1 kg total weight) are captured one by one by rod and line fishing. Hook is carefully released and fish are placed in cylindrical tanks to be transported up to tanks or cages (De la Gandara *et al.*, 2009). Similar methodology is currently used to capture of live Scombrids (Bourke *et al.* 1987; Sepulveda and Dickson 2000; Wexler *et al.* 2003).

But this capture and movement is only possible with small fish when schooling. Scombrids need to swim continuously and anaesthetics are not effective and should not be used. Stress during transport could be very high, and waste metabolites (CO_2, ammonia, lactate, cortisol, etc…) are accumulated in the captured fish blood at high rates (Korsmeyer and Dewar 2001) and it could lead to immediate death of the fish. Efficient removal of wastes and replenishment of the taken oxygen requires high flow rates of water and often, dissolving supplemental oxygen.

Since 2005, several campaigns to capture live ABs have been developed at Murcia Oceanographic Center, (MOC) COMU, in order to house fishes of this species in their facilities for research purposes. First attempts were developed in collaboration with L'Oceanografic aquarium from Valencia, during winter 2005 and spring and summer 2006 with various results. Acquired knowledge was used in the following attempts carried out during spring 2007, to capture and transport fishes to COMU facilities to be adapted to captivity in order to establish an AB broodstok.

Material and Methods

In spring 2007, 99 ABs were captured in La Almadraba trap in La Azohia, Cartagena, SE Spain. This fishing net gear consists of a long piece of netting which is placed perpendicular to the coast. Migratory fish swim parallel to the coast line, and when they find the netting sheet change their direction and start to swim along the net until they are trapped in an enclosure of about 1500 m^2 named "matador". When ABs are inside matador, fishermen start to pull at the net (Figure 1), making the enclosure progressively smaller. When this is about 30-40 m^2, ABs are captured one by one from auxiliary boats with a special hand rubber net. ABs are then placed in a cylindrical tank (130 cm diameter and 75 cm in depth, Figure 2) provided with a double bottom system containing an oxygen diffusor and a pump to recirculate the water inside the tank. In this way, is avoided that any device inside the tank might disturb the behaviour of the fishes and affect their swimming.

Once captured, the fish must be handled as gently as possible, ensuring minimal contact with the skin. Scombrids lack the protection of large scales, and have a tendency to shed their scales when captured, which makes them more vulnerable to skin infections.

Figure 1: Fisherman dragging at the net

The tank containing the ABs was fast moved to port where fish were transferred to a similar tank placed in a van and then transported to COMU. When arriving, ABs were extracted with rubber hand nets (Figure 3) and housed in three 20 m^3 cylindrical rearing tanks. Total transport time oscillated between one and one and a half hours.

Figure 2: Transport tank

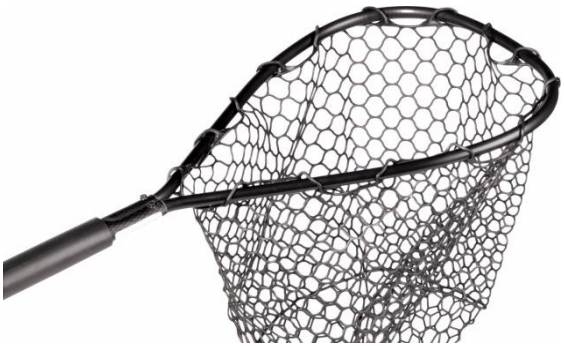

Figure 3: Hand rubber net

Previous experiences with ABs showed preferences for cylindrical and dark color painted tanks, so tanks with these characteristics were used. Fish were disinfected by submersion in a solution of formaldehyde in water (150 ppm, for 1 h) and peroxide of hydrogen solution (200 ppm) in alternate days. These treatments were administrated during the first week. Bait fish was supplied to ABs as food from the third day in captivity onwards. Bait species used were round sardinella (*Sardinella aurita)* and anchovy *(Engraulis encrasicholus)*, offered to satiety. A yellowtail (*Seriola dumerilii*), well adapted to captivity, was

introduced in the tank with the ABs to get easy adaptation of fish to confinement and learning to feed on an inert diet ration.

ABs were captured in three different periods: April 16 to 18th, April 19 to 20th and May 3rd. Fish coming from every period were placed in the same type of tanks, so that three 20 m^3 cylindrical black tanks were used. The experience lasted 45 days for the different tanks. Water temperature during capture ranged between 15.5 and 17.2ºC, and during the following weeks, by the end of experiment, increased to 21ºC. A photoperiod of 13 hours artificial light was used and the flow of fresh sea water through the system was about 5000 l/h, salinity was 38 ppt and oxygen was maintained above 90% saturation.

During the first two weeks, to initiate fishes to eat, food was offered several times per day, seven days per week. From the third week onwards, when ABs started to eat normally, food was supplied ad satiety once per day early morning, five days per week. Non eaten food was siphoned off every day to maintain water quality in the tanks.

Results

In the whole experiment, total mortality rate in the first two weeks after capture was 42.4%. In the following month, mortality rate was 0%, and ABs fed normally showing a normal behavior, so it can be considered that ABs were perfectly adapted to controlled conditions confinement. A total of 8 AB trap capture operations and transport to land based facilities were accomplished, in order to allocate fishes in the tanks and study performance results. Three different tanks were used so that three different capture and transport events could be investigated:

- 3 first captures were carried out between April 16th and 18th. In these trials temperature was 15.5ºC and different age classes were fished: ABs with an average of 1050 gr of total weight and supposed to belong to 0^+ class, in addition to ABs belonging to the 2-3^+ class with a total weight average of 3200 gr. Class estimations of age by weight and size were made according to correlations age/total weight reported for this species by Rey *et al.*, (1984), Santamaria *et al.*, (1998) and Valeiras *et al.*, (2008). Density during transport ranged between 9 and 20 kg/m^3, and mortality rate during first 15 days in COMU was 75%, with two groups clearly separated: large fishes had a 93% mortality rate while small ones had a mortality rate of only about 40%.

- 3 following captures were performed between April 19 and 20th. In these trials, temperature was 15.7ºC and most of the ABs belonged to the 0^+ age class. Fish showed an average of 1150 gr of total weight, and density during transport events ranged between 17-21 kg/m^3. Mortality rate registered in the following two weeks was 20%.

- Last two capture events were carried out on 3rd April. Water temperature was 17.1ºC and the transport density was higher, ranging between 21 y 30 kg/m^3. Average total weight was 1280 gr and mortality rate was about 45%.

Bonitos started to feed ten days after fishing and conditioning conditions .A week later, ingestion rate showed to be between 5 and 8% of the biomass weight per day. This high food intake rate was maintained during the following month, reaching levels close to 10% of the total weigh biomass every day. Bonitos accepted well both offered bait fish species: anchovy and round sardinella.

Discussion

AB is a species difficult to handle, and mortality rates are high unless some special cares are taken into consideration. Previous trials in 2006 pointed out the importance of operating in an adequate way. Fish must be handle with special netting, using rubber nets and avoiding conventional nylon ones. Handling should also be done with plastic tarpaulin in order to avoid causing injuries or loss of scales and fish should not ever be touched with naked hands. A defective handling may lead to high mortalities.

We have also determined the importance of temperature in previous trials; an attempt conducted on June 2006, with temperatures ranging between 23 and 24ºC failed, with 100% mortality rate after three days of capture. However, the first fishing and transport events completed successfully were performed in March 2006, when temperatures were below 15ºC. In trials performed in these experiment, temperature had been quite stable (15.5 to 17ºC) and no detrimental effects were detected.

From these trials, the importance of size of the fish and density (biomass per cubic meter) during transport can be inferred. First captures were comprised of bonitos of different sizes and class ages but mainly composed of large fishes (more than 3 kg). These fish are more difficult to handle, being difficult to capture and be introduced in hand nets. They are more vigorous and stronger fishes and tend to hit the net trying to avoid being captured as well as once

captured suffer collisions against tank walls. These fishes show uncomfortable in small transport tanks and often collide among themselves. Even when the number of large ABs in the transport tank was low (5-7 fish per tank) stress and injuries caused the death of most of ABs in the following days. Practically, 100% of the surviving fish in this batch were smaller fish, which also had a greater mortality than the one observed in the second capture batch, which could have been caused by collisions with bigger fish.

Transport density also played an important role in trial performance results. This was probably the factor that caused the greater mortality rate observed in the last capture/transport batch. During transport, collisions among ABs was considerable high; when a fish is disoriented and start to swim in opposite direction to the rest of the school, probability of collisions against other fishes increase with the density of fish in the tank (personal remark).

As it has been pointed out in the introduction section, transport of young sombrids like Atlantic Bluefin and Yellowfin tunas have been carried out in tanks where fish can swim freely (De la Gandara *et al.*, 2009; Wexler *et al.*, 2003). In the last year, a new transport method consisting in proper conditioned tubular tanks for small tunids has also been used as an alternative to the above traditional described method (McPherson, 2004; Bar *et al.*, 2015). This method consists in introducing live tunas (head down against a water flow) inside tubes and forcing the water to pass through these tubes and fish gills at the same time. Tunas cannot swim with this method but there is a constant oxygen enriched water flow pumped passing through their gills. Bar *et al.*, (2015) transported different scombrid species with a small size (under 1 kg) but obtained mortality was higher than 80%. Besides, the longer time of transport and high temperatures could have also contributed to these poor results.

Some improvement suggested by Bar et al (2015) to get a better fish survival, dealed with maintaining low stress levels during transport. This can be achieved either by improving water quality parameters or by slowing down the fish metabolism. Some possible methods to improve water quality parameters are described by Correia *et al.*, 2011, to transport different species. Others have suggested the use of immersion anaesthetic sedatives or cooling the tank water by 5–10°C as a measure to slow down metabolic rates of the transported fish (Berka, 1986; Rimmer and Franklin, 1997; Harmon, 2009). These practices are mainly applicable to closed transport tanks, which recirculates and reuse the treated water, Yazawa *et al.* (2015) showed that stress and activity levels can be reduced in mackerel tunas if fishes are kept in dark conditions during handling and transport.

Once transferred to the holding facilities, fishes face major challenges to recover from capture, handling and transport, stress and injuries, such as skin abrasion and head collisions in the transport tank walls. In addition, fishes need

to adapt to tank conditions and begin to feed on the supplied food. To assist recovery, fishes are usually treated with antibiotics to prevent bacterial infections (oxytetracycline, sodium nifurstyrenate solution, etc…), bath submersions in a formalin solution (200 ppm, for 1 h) to remove external parasites (Wexler *et al.* 2003), or in peroxide of hydrogen solution (200 ppm, for 1 h). We have only treated ABs with prophylactic solutions of formalin and hydrogen peroxide, which have an antiparasitic activity but at the same time disinfect the fish skin. This treatment is important to prevent from opportunistic pathogen which can colonize skin injuries. When temperatures are low, using these prophylactic solutions can be adequate with no need of antibiotic treatment.

Once overcome handling stress and instigating feeding during the first few days in the tank, which seem to be the most crucial factor for fish adaptation to confinement, survival rates increased significantly.

As concluding remarks, as it has been shown in these trials, capture and transport of small ABs to inland facilities with high survival rates it is possible. Meanwhile,temperatures should be kept below 17.5ºC, transport to inland facilities take less than two hours and transport density not exceeding 20 kg/mf AB biomass.

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1.2. Reproduction performance of Atlantic bonito (*Sarda sarda*, Bloch 1973) in captivity

RESUMEN

Rendimiento de la reproducción del Bonito Atlántico (*Sarda sarda*, Bloch 1793) en cautividad.

Los escómbridos componen una familia de peces pelágicos con un gran potencial para acuicultura debido a su rápido crecimiento y alto valor comercial. La mayoría de los esfuerzos de investigación se han dedicado a cultivar especies del género Thunnus, principalmente el atún rojo. Sin embargo, otros miembros de la familia deberían ser tenidos en cuenta. Este artículo trata sobre la reproducción en condiciones controladas en cautividad del Bonito Atlántico, una especie que presenta un rápido crecimiento en sus primeros meses de vida y que es capaz de madurar sexualmente antes del año de vida (clase de edad 0^{+}). El bonito es una especie gonocórica, con un desarrollo asincrónico de la gónada y capaz de adaptarse a vivir en tanques en tierra y reproducirse después de unos meses en cautividad. Además, si las condiciones son adecuadas, los bonitos son capaces de extender su estación de puesta durante varios meses, presentando al menos 15 puestas en todo el periodo.

ABSTRACT

Scombridae comprise a family of pelagic fish species with a great potential for aquaculture due to fast growth rates and high commercial values. The majority of research efforts have been dedicated to breed and culture species belonging to the *Thunnus* genus, mainly to bluefin tuna. However, another members of the family should also be considered as meritorious candidates. This article deals with the reproduction under controlled conditions in captivity of AB, a species with a fast growth rate during the first months of life and the capacity to attain sexual maturation in less than one year (0^{+} class). Bonito is a gonochoric fish species with an asynchronous development of the gonads and capable to be acclimatized to live in inland facilities and reproduce after some months in captivity. Moreover, if environmental conditions are adequate enough, bonitos can extend their spawning season during several months with at least fifteen spawning events.

INTRODUCCION:

AB (AB), *Sarda sarda (Bloch, 1793)*, could be a new candidate to aquaculture for several reasons: it has a fast growth rate, reaching 1 kg total weight in a few months of life (Santamaria *et al.*. 2005), and attains sexual maturation and reproduction by the end of the first year of life (Rey *et al.*, 1984; Macias *et al.*, 2005a), in addition to be a valuable and well known species. In contrast, at the same time, is a delicate and sensitive species to handling, easy to stress and difficult or nearly impossible to sedate. However, it is noteworthy to point out that although some close related species like Striped bonito, *Sarda orientalis*, (Shimizu *et al.*, 1998, Kaji *et al.*, 2003) and Pacific bonito, *Sarda chiliensis*, (McFarlane *et al.*, 2000) have been successfully cultured, nobody has tried to date to breed and grow (under controlled conditions) AB for aquaculture purposes.

With regard to reproduction, AB represents a gonochoric species, with asynchronous gamete development, which mean that several spawning events can be attained during the spawning season.

IEO started a AB culture program back in 2005 and first spawning in captivity was reported in 2006 by Ortega *et al.* (2007a). This was the first reproduction in captivity, under controlled conditions of *S. sarda*, but the quality and quantity of spawns reflected variable and sometimes badly. Through the following seasons, results were improved, and predictability and reliability of the spawning events were enhanced. In this article, it is described the reproductive performance and behaviour in captivity of two selected groups of *S. sarda* broodstock during the period between 2009 and 2013.

MATERIAL AND METHODS:

ABs were fished in Mazarrón Bay with a small tunids fishing trap called Almadraba according to the technique described by Ortega *et al.* (2007b). Bonitos got inside the trap where they stayed alive until the net was lifted. Just then, fish were caught with a rubber hand net and placed in a 500 l cylindrical tank supplied with oxygen and open water flow system to create a water stream inside the tank. This led the fish to swim against the current, avoiding and diminishing the collision among individuals and against the tank walls. ABs were transported to the nearest port ans then transferred into another similar tank placed in a van and brought to Murcia Oceanographic Centre (COMU) facilities in Mazarrón (Murcia, SE Spain). During this last transport, no water exchange

was made, but the current was kept by a recirculation system. Total transport time was about 1 hour. Once in COMU, fish were placed in a 20 or 55 m^3 black cylindrical tanks (4m or 6m diameter and 1.8 or 2m depth) with a small transparent lateral window to allow watching the bonito's behaviour without stressing them.

During all the experiment the tanks were provided with continuous open flow system of filtered sea water, which was regulated in order to maintain dissolved oxygen concentration over 90% saturation level. Water temperature was daily registered and it ranged between 12ºC (winter) and 28ºC (summer). Salinity was 38 ± 0.5 ppt and natural photoperiod (it ranged between 9:30 and 15:30 hours day length). At the beginning of May, when spawning season was approaching, the water flow was changed to force surface water outlet. A 500µ net was placed, as egg collector, in the outlet and it was checked daily for floating spawned eggs.

Spawning events occurred at nightfall, and eggs were collected the following early morning every day. After cleaning and separating buoyant from non-buoyant, eggs were counted and buoyancy rate calculated. When just fertilized, this rate it can be considered as equivalent to fertilization rate. A small sample of floating eggs was extracted to measure total diameter, number of oil globules and general appearance for quality assessment. Another sample was taken out to calculate hatching rates: 24 eggs were incubated during 48 hours in a 24 wells (2 ml capacity each) plate, and one egg was placed in each well. As it was reported by Ortega A. *et al.*, (2008b), the length of embryonic development period depends on the temperature, and at 20-23ºC it usually ranged between 48 and 60 hours, which means that eggs placed in the plates hatched about two days after recollection.

Broodstock A:

A total of 18 bonitos with an average weight of about 1.8 kg (1.6-2.0 kg) were fished in La Almadraba trap in La Ahohía in early May 2008. According to data growth reported for this species by Rodríguez-Roda (1981), Rey *et al.* (1984), Santamaria *et al.* (1998) and Valeiras *et al.* (2008) in the Mediterranean Sea, it is suggested that they should belong to the 1^+ class, so when they were fished they were practically two years old (approx. 23-24 months old).

After fishing, bonitos were placed in a 20 m^3 cylindrical tank (G4 tank). One fish died because of the injuries caused during fishing and transport.

After three weeks not eating and ignoring food, the bonitos started to feed on fresh European anchovy (*Engraulis encrasicolus*), reaching a high feeding rate

(about 8% of their total weight as a ration) in only one week. From this moment onwards, the feed was changed to defrosted fish, firstly anchovy and afterwards a mixture of anchovy, European pilchard (*Sardina pilchardus*), Round sardinella (*Sardinella aurita*) and mackerel (*Scomber scombrus*), supplied to satiation. The fish were feed once per day, five days per week, and the ration varied between 2 and 8% of fish biomass depending mainly on the temperature, size of the bonitos and other behavioural and environmental factors.

From the beginning of 2009, and in order to improve the feeding quality, mussels and squids were added every Monday instead of fish. Using cephalopods as food vehicle to supply fat-soluble vitamins and pigments, which are known to improve spawning performance and the quality of spawned eggs in different species, as reported by Watanabe and Vasallo-Agius in 2003.

These fishes were kept in this tank during 2 years, with natural photoperiod and thermoperiod (maximum 27.9ºC in early September 2008 and minimum 12.1ºC in the middle of January 2009). They started to spawn in captivity in May 2009, when they were 3 years old, and they went on spawning in 2010.

Broodstock B:

A total of 46 ABs were fished in April 2010 in La Almadraba trap in La Azohía in the same way described above. Average total weight was 910 g, and accordingly, these fishes belonged to the 0^+ yearly class and about 10-11 months old (Rodríguez-Roda, 1981; Rey *et al.*, 1984; Santamaria *et al.*, 1998; Valeiras *et al.*, 2008). This lot was place in Z4 tank (Figure 1), an outdoor 55 m^3 black cylindrical tank (6 m diameter and 2.2 m in depth) covered with a shadowless net to prevent algal growth in the tank. In the first week, three prophylactic baths with 150 ppm formaline (40% formaldehyde in water solution) during one hour, were applied to avoid infections with the injuries caused by handling. However, a total of three bonitos died during this period. Ten days after capture, ABs started to feed, firstly anchovy but after some days, food source was changed to round sardinella.

Figure 1: Tanks used for AB broodstock: Z (left) and G (right).

Temperature and oxygen levels were daily registered and no artificial light was used but for cleaning and maintenance of the tanks.

Bonitos were kept in this tank until October 2011 when they were transferred to A tank (an indoor 80 m^3 rectangular tank). They were kept in this tank until the end of the spawning season in 2012 (30^{th} July) when they were moved to Z3, an indoor 55 m^3 tank. From the moment that bonitos were placed in indoor tanks, artificial light was used to provide natural photoperiod.

During all the trial, the main species used to feed bonitos were mackerel (*Scomber scombrus)* and round sardinella (*Sardinella aurita*), but also and occasionally, European pilchard (*Sardina pilchardus*) and herring (*Clupea harengus*) were supplied. The feeding schedule was to apparent satiation (food was offered to fish until rejection) five days per week. We also fed the broodstock on squids once per week during the maturation and spawning seasons.

RESULTS

The size of the ration fed oscillated between 0.5% (January 2010) and 10% (June 2009) of total biomass weight, according to temperature and size of the fish. Thermoperiod was natural and ranged between 12.5-13.0ºC in February (minimum temperature recorded was 12.3 in February 2013) and 27-28ºC in August (maximum was 27.8ºC in August 2010).

Broodstock A:

During the first year in captivity, a total of six fish died, three of them during a prophylactic treatment with hydrogen peroxide (150 ppm, one hour duration), and two because jumped out of the tank.

At the beginning of May 2009, when temperature increased from 15 to 18ºC, fish started to show a typical courtship behaviour: several males, most of them with a vertical bars pattern in the skin, swam quickly persecuting females. This behaviour was patent all the day round, but mainly during the afternoon and evening.

Spawning started on May 13th, and last spawning was collected on July 10th. Broodstock spawned during a total of 53 days, and only skipped spawning 6 days during the whole period. Bonitos spawned every day (except one) during the first 47 days, but during the last days of reproduction, spawning pattern became more erratic, with five days without collecting eggs.

Fish spawned at dusk, usually between 19 and 22 h, and the eggs were collected early morning. The number of total eggs collected daily during the spawning season ranged between 26,000 and 572,000, with a daily average of 127,350 (Figure 2). A total of 6.75 million eggs were collected in total, and the mean fertilization rate was 48.5 ± 17.9 % (Figure 3). Total number of bonitos in the tank during the spawning season comprised 11 fishes: 6 females and 5 males. One male died because a collision in the middle of June. Average total weight of bonitos was about 2.1 kg (average for females 2.3 kg). This means a total fecundity of 1.125 million of eggs $fish^{-1}$ and a relative fecundity of 490 eggs g^{-1} of female during the whole reproductive season.

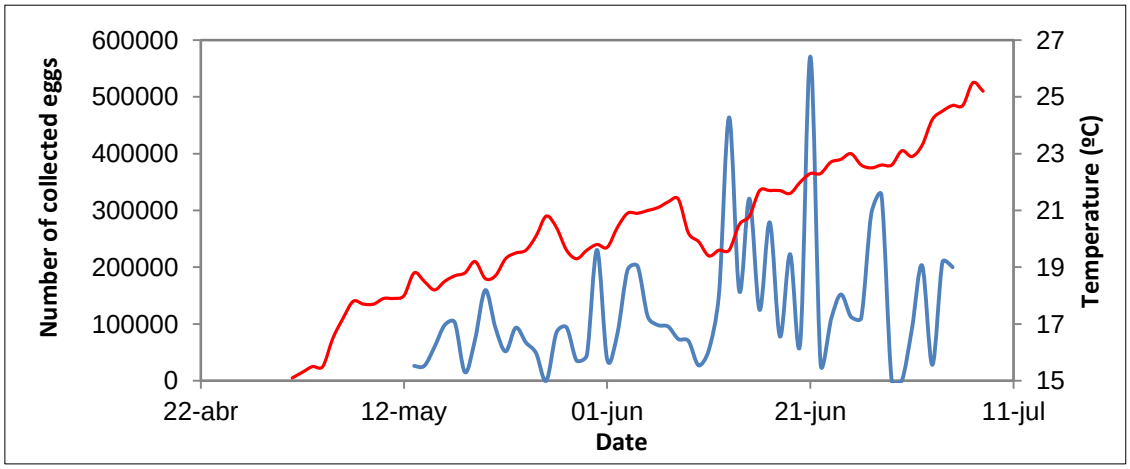

Figure 2: Total Eggs collected from broodstock A during 2009 spawning season.

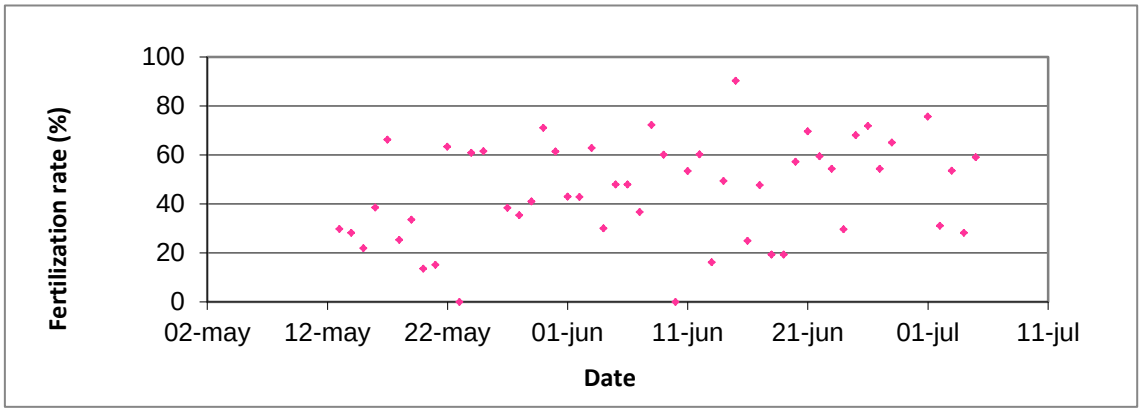

Figure 3: Fertilization rates in broodstock A during 2009 spawning season.

Average egg diameter was 1,300.3 ± 34.4 µm, and except from an upturn at the end of the spawning season, tended to diminish through that period. The average number of oil globules per egg was 5.6 ± 0.98 and it did not show significant changes during the whole spawning season. Average hatching rate of the fertilized eggs was 66.3 ± 15.3 %.

In July 2009, broodstock was composed by 10 bonitos. During all the year round, a total of 7 fish died. In spite of some treatments with formaline and some products to enhance their immunity response (glucans) mortality went ahead. At the beginning of May, only a total of 3 fishes (2 females and one male) started the reproductive season. In this moment, bonitos were four years old.

Bonitos spawned during a 66 days period (from 28th April to 2nd July 2010) and a total of 970,000 eggs were collected, as it is shown in figure 4. These eggs were obtained in 29 days of the total spawning period, with an average of 33,450 eggs per spawning event. Maximum daily egg production was 70,000, and mean fertilization rate was 75.2 ± 20.6 % (Fig. 5). Mean weight of the females was 2.45 kg, so this means an average of about 485,000 eggs per female and an annual relative fecundity of 198 g^{-1}of female. Average hatching rate was found to be 78.8 ± 16.9 %.

Average egg diameter was 1,326.0 ± 33.2 µm, and the tendency was to decrease through spawning season. Number of oil globules per egg was 5.5 ± 0.8 and fairly constant during the period.

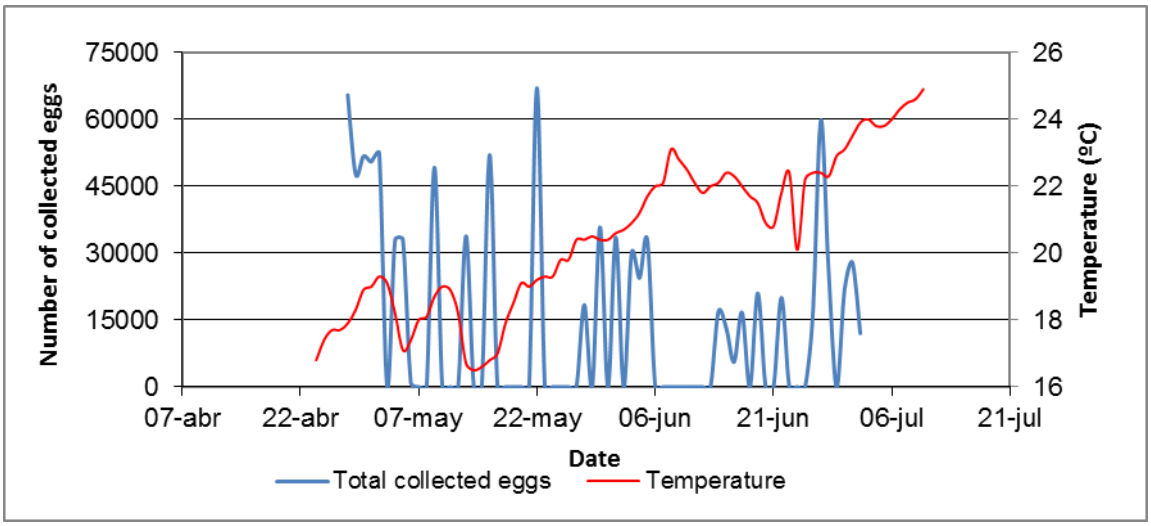

Figure 4: Daily egg production of Broodstock A during 2010 spawning season.

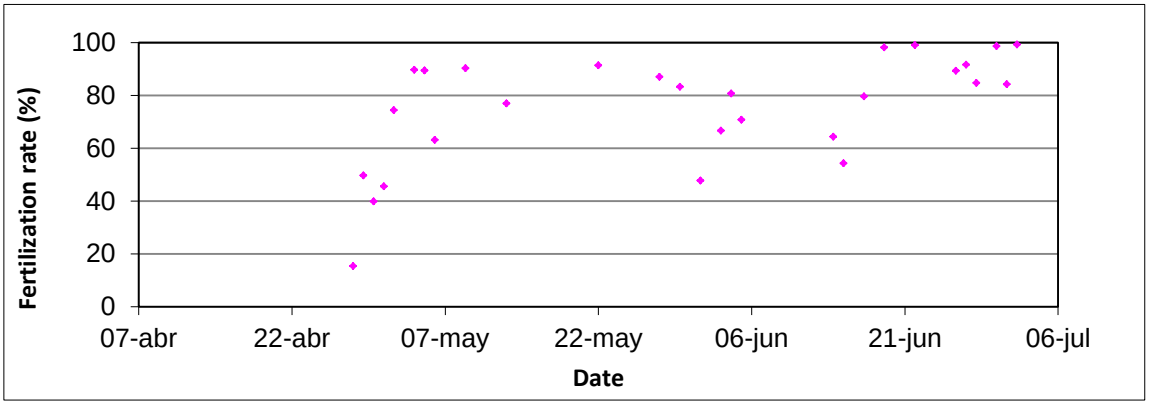

Figure 5: Fertilization rates of Broodstock A during 2010 spawning season.

Broodstock B:

After fishing, 46 bonitos were placed in Z4 tank (outdoor 55 m^3 black cylindrical tank, 6 m diameter and 2.2 m in depth), and a low mortality occurred (only 3 fishes, which means a 6.5% mortality) due to transport and handling during the first week. During 2010 and spring 2011 a total of 8 bonitos died, so only 35 bonitos reached their first spawning season in IEO, in May 2011, but they did not spawn. Bonitos were moved in October 2011 to A8 tank (an indoor 80 m^3 rectangular tank), and at the onset of the following spawning season, only a total of 26 bonitos remained in the tank (9 bonitos died during that year). They were by then three years old, and started to spawn on the 27th of May. The last day of July, after several days without spawning, bonitos were transferred to Z3 tank (indoor 55 m^3 tank). Four bonitos died soon after handling, and another 5 bonitos died throughout the year, so at the beginning of the next spawning

season (June 4th 2013), only 17 bonitos were still alive. During this spawning period and mainly during the following autumn, most of the fish died. In December only remained two bonitos, which were sacrificed.

Taking into account that 43 bonitos comprised the initial number of this broodstock lot and not counting initial deaths caused by transport and handling, results are summarized in Table I.

Initial number= 43 (Bonitos born in May 2009) and yearly classes	Dead fish	Mortality rate	Cummulative survival rate
1^+ (05-2010 to 04-2011)	8	19%	81%
2^+ (05-2011 to 04-2012)	9	26%	60%
3^+ (05-2012 to 04-2013)	9	35 %	40%
4^+ (05-2013 to 12-2013)	15	88%	5%

Table I: Evolution of survival rates in broodstock B during the trial.

In 2011, ABs did not spawn. In spite of bonitos seeming healthy, eating satisfactorily and some males showed courtship behaviour sometimes after females, no eggs were spawned and/or collected. A female died during this period and presented no signs of maturation.

In 2012, just after a sudden drop in the temperature (from 20.2ºC to 15.5ºC) and a further great increase up to 20.7ºC, bonitos started to spawn. This reproductive season drew out by 58 days (from 27th May to 23rd July), and a total of 1,996,000 eggs (Fig. 6) were obtained in 27 spawning batches, with an average of 73,900 eggs/batch.

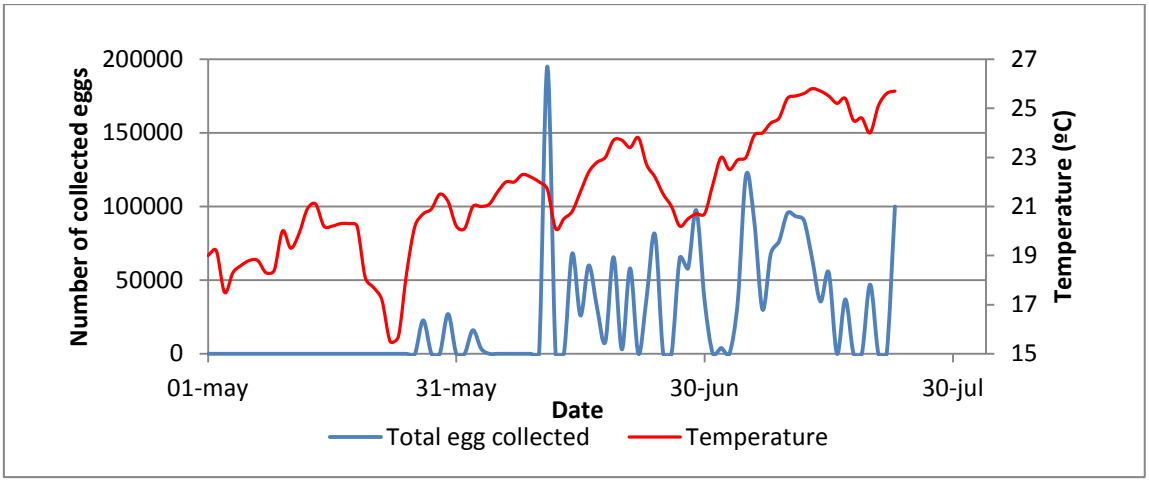

Figure 6: Total eggs collected from Broodstock B in 2012 spawning period.

Maximum daily egg production was 195,000, and mean fertilization rate was 73.9 ± 29.1 %. Nevertheless, and taking into account that some bonitos died, sex ratio was not determined and there was not a reliable estimation of the weight, we could not calculate fecundity in this reproductive season.

Egg diameter was 1,301.5 ± 37.5 µm, and the trend was also to decrease as the spawning season progressed.

During the following maturation season (March to June 2013), water temperature gradually increased from 13 to 20ºC and feeding ration was also increased from 2.5 to 6% /day, accordingly. At the onset of the spawning period 17 bonitos remained in the tank: 9 females with an average weight of 2.5 kg and 8 males with an average weight of 2.1 kg. Bonitos started to spawn on the 4th of June, just after one degree increase of the temperature, from 19 to 20ºC. Last spawned eggs were collected on the 9th of September, with a water temperature of 25.8ºC. Spawning period occurred for more than three months (98 days). From the 4th of June to the 14th of July, when temperature reached 25.5ºC, bonitos spawned every day. From then onwards, spawning events were becoming more irregular, with only 18 spawns in 54 days. As the spawning period progressed, the temperature reached 26.5ºC by the end of the season. Total amount of eggs collected was 11.6 million, showing a maximum peak of 880,000 eggs collected on the 13th of July (Figure 7). Average of eggs obtained per female was 1.3 million, with 510.85eggs g^{-1}of femaleBody weight.

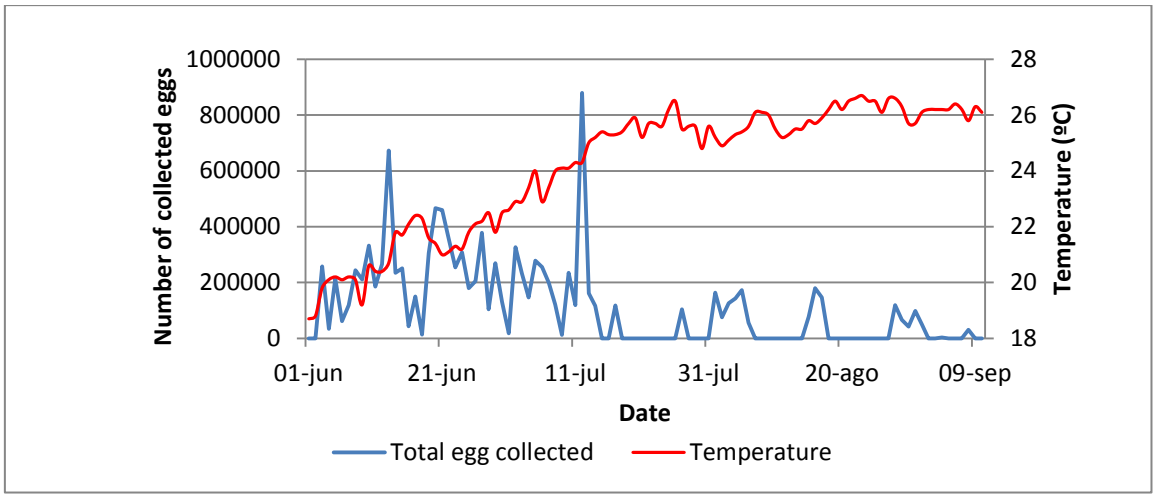

Figure 7: Total eggs collected from Broodstock B in 2013 spawning season.

Fertilization rates were not as high compared to other spawning seasons. Mean value recorded was 47.2 ± 31.4 % and highly depended on temperature: when temperature was below 24ºC, fertilization rates were about 56 %, but when temperature showed higher, fertilization rates only reached about 31 %. Figure 8 shows fertilization rates vs. temperature during the 2012 and 2013 spawning seasons.

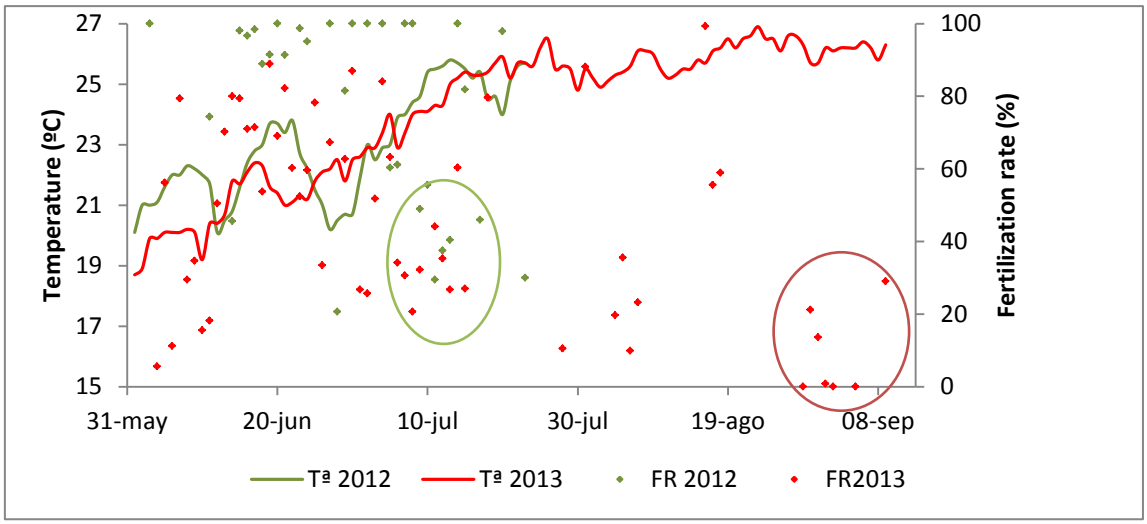

Figure 8: Fertilization rates from broodstock B in relation to temperature during 2012 and 2013 spawning seasons.

Egg diameter was 1,293.5 ± 32.9 µm, and the number of oil globules average value was 3.4 ± 0.6 which remained constant throughout the spawning seasons. Average hatching rate observed was 78.5 ± 10.7 %.

Discussion

Mortality rate in broodstock A was 35.7% during the first year in captivity but 72.7% on the second one. Bonitos were close to 2 years old when they were captured, which means that former mortalities occurred during their third and fourth years of life.

Mortality rate in broodstock B were lower than for broodstock A and remained stable during the first three years in captivity, but from this point onwards increased highly. Mortality was increasing slowly year by year, and by the end of their third year of life (two years in captivity) was about 40%. During their fourth year mortality rate was 35% and suffered even a higher increase in the following one.

Mortalities, mainly during last year, could have been related with injuries by collisions, handling and health problems. Some of the older fishes showed blindness in one or both eyes that could have been due to physical contact with the wall of the tanks, and some malformations probably caused by a forced swimming in relation with the tank dimensions and the consequent difficulties in maneuverability. Another malformations in the head could have been related to the so called "*puffy snout syndrome"*. Puffy snout is a malformation presented when tissues of the snout begin to swell and become edematous, and it could be related with the stress due to confinement in small tanks. When this syndrome progress fishes are unable to close its jaws and die by inanition. Nakamura (1975) cited this syndrome in skipjack tuna (*Katsuwonus pelamis*), mackerels (*Scomber japonicus* and *Scomber scombrus*) and yellowfin tuna (*Thunnus albacares*). More recently, Benetti *et al.*, (2009) also mentioned it in blackfin tuna (*Thunnus atlanticus*). Fish with some of these malformations presented signs of low feed consumption, showing poor growth and health conditions. Wexler *et al.*, (2003) observed similar problems in yellowfin tuna, and recommended the use of larger tanks and decrease the density from 0.75 to 0.5 kg/m^3 as the grow of the fishes progressed.

Survival results obtained with *Sarda sarda* in these experiments were poorer than those obtained during broodstock maintenance of another fish species like sea bream (*Sparus aurata* L.) (personal observation), but 40% survival rate in brodstock B after three years in captivity were much better than 10% survival obtained in the same period with larger size scombrid fish like *Thunnus*

albacares (Wexler *et al.*, 2003) or *Thunnus orientalis* (Mimori, 2006) kept in captivity in tanks.

Fish stocking densities in the present study always remained below 1.4 kg/m^3. This biomass density, perhaps could not be low enough for a scombrid fish. During the first three years (maximum total weight per fish below 2.3 kg) densities up to 1-1.2 kg/m^3 appeared to be tolerable, but when bonitos grew over this weight, density should have been reduced by using greater tanks or reducing the number of fishes per tank. This is in agreement with recommendations for other scombrid broodstocks belonging to the *Thunnus* genus that warns about maintaining fish stocking densities below 0.4-1 kg/m^3 according to the species (Wexler, 2003; Benetti, 2009; Masuma, 2011).

Bonito broodstock needs some time to adapt to confinement and rearing conditions after recovering from the stress caused by fishing, handling and transport. This is the reason why both experimental broodstock did not spawn during the first season, one or two months after capture. But the following years they were able to spawn in captive controlled conditions as it was reported by Ortega *et al.*, (2008). On the other hand, broodstock B was unable to spawn in 2011: an involuntary modification of the photoperiod due to external lights used by watchmen during night time shift could have been the cause.

Bonito is a multiple spawner with asynchronous gamete development. As Hunter *et al.*, (1985) reported, annual fecundity should be estimated from the batch fecundity, the spawning frequency, and the duration of the spawning season. Some studies developed by Rey *et al.*, (1984) and Mayorova and Tachvea (1959) suggested that bonitos have a determinate fecundity and only three or four spawn batches per year. Batch fecundity was estimated about 24-62 oocytes g^{-1}. However, Macias *et al.*, (2005 a, b) pointed out that AB probably is a species with an indeterminate fecundity, and some environmental factors (such as temperature, photoperiod and food availability), affect sexual maturation condition factors and in consequence might influence the batch fecundity of this species. They stated that mean relative bath fecundity was about 65 oocytes g^{-1}. A further study developed by Pascual (2010) increased slightly this estimation to 77 oocytes g^{-1}.

Bonitos in captivity spawn during a 2-3 months period, usually from May to July. In the four years of the present study, this period ranged between 58 and 97 days. As reported for reproduction in the wild (Rodriguez-Roda, 1966; Macias *et al.*, 2005) most important spawning events are concentrated between June and mid July, but spawning season can be enlarged by manipulation of environmental factors as it can be deduced from our trials. Spawning season usually started when temperature reached 18-19ºC, but former temperatures can affect the onset of this season. So, spawning environmental events came earlier in broodstock A than in B, specially in 2010, when water temperature

was 1-2ºC warmer during February and March and, as a consequence, bonitos started to spawn by the end of April instead of later on.

It is important to point out that, if bonitos are maintained in good conditions, the spawning season can be extended until September. This happened in 2013, and these results are in agreement with findings in other several cultured fish species, which are able to spawn in confinement during longer periods than in the wild. It is critical that fishes are stocked at an optimal low density and fed with an adequate diet (good nutritional quality and quantity) in order to influence a good health condition. Water quality parameters must also be maintained within most favorable values, particularly the optimal spawning temperature range, which in bonito is considered between 18 and 25ºC.

Our results support that bonitos perform reproduction on indeterminate fecundity basis. Broodstock A showed a relative annual fecundity of 512 eggs g^{-1} in 2009 and 198 eggs gr^{-1} in 2010. Moreover, broodstock B presented in the second spawning year a fecundity of 511 eggs g^{-1} (as mentioned in the results section, fecundity in 2012 could not be reported because some dead bonitos were not sexed and a not reliable estimation of the weight). All these data support that fecundity largely depends on external factors and can vary between less than 100 to more than 500 egg g-1, while annual fecundity fluctuated between 485 and 1290 egg g^{-1}. According to Rey *et al.*, (1984), the estimation of annual fecundity for this species in the western Mediterranean varied from 220,000 to 1.500,000 oocytes. Macias *et al.*, (2005) found that fertility (estimated by the sum of hydrated, yolked and atretic follicles) ranged from 304,000 to 1.150,000. However, caution should be taken when comparing, these results. In the present study we made the estimations based on the numbers of released floating eggs, and not by means of gonad histological numeric studies, so, in consequence, our practical approach to fertility data show underestimated values to those reported by other authors. Moreover, data on batch fecundity have not been reported, taking into consideration that without genetic studies and markers for individual fish is not possible to know how many females and which ones are spawning every day and contributing to reproduction.

Our trials also support that females are able to attain several spawning events per year, at least 10-15 spawning batches. In 2009, if we assume that every day only one female is spawning, it can be estimated an average of 9 spawning batches per female. However, as some days egg production was very high (batches over 200-250,000 collected eggs, surely indicates that more than one female was participating in the spawning event that day) we can deduce that several females spawned sometimes the same day.

In 2010, we obtained 29 spawning batches from only two females, being difficult to assess in how many spawning evens participated each female in

these conditions. In a very simplistic approach and considering that each day only a female spawned, we have a minimum average value of 14.5 spawning events per female during the spawning period. Results obtained in 2013 also pointed out to 8-10 spawning events at least, but considering the high amount of eggs collected some days, this value should be considered as underestimated. All these data are in agreement with Macias (2005) who suggested the possibility of more than only 3 spawning batches as reported by Mayorova and Tkacheva (1959).

With regard to the time between two consecutive spawns, an estimation can be inferred from 2010 data. That year two females in the tank spawned seven consecutive days at the beginning of the season; which support the possibility that bonitos are capable to ovulate every 24-48 hours.

Fertilization rates ranged between 45 and 70%, and seems to be related with the temperature; when temperature reach values over 24-25ºC, fertilization rates show lower (Figure 8). Temperature not only affects fertilization rates but also viability rate of the eggs and hatching rates as well. Considering that eggs are collected several hours after fertilization, generally at gastrula stage, and taking into account that fertilization rates were measured as a percentage of buoyant eggs, the real fertilization rates not only are lowered by higher temperatures but their values could also be underestimated by the higher mortality rate during first stage of embryonic development. What we can conclude in that high temperature affect viability of eggs, but it is difficult to discern if affect to fertility or hatching rate.

It can also be noted that in those years with a higher production of eggs, egg viability and in consequence the estimation of fertilization rates showed lower. The reasons are not clear but could be related with the egg collection system: a larger amount of eggs could be easily damaged in the egg collector during the night time (before being collected) due to collisions among eggs and against the mesh screen. Possibly the use of larger egg collectors might mitigate this problem if those are the only causes, but further research is needed in order to detect other adverse factors affecting egg viability to fully explain contributing negative factors and in consequence, act to enhance the results.

Average egg diameter of bonito was 1,301.4 ± 36.3 µm, and varied between 1,293.5 ± 32.9 µm in 2013 and 1,326.0 ± 33.2 µm in 2010 but not statistical significant differences were observed throughout the whole experimental period. However, as shown in Figure 9, egg diameter tended to decrease throughout the spawning season. This also has been reported for other species like ABFT (*Thunnus thynnus*) (Anon 2010), and chub mackerel (*Trachurus japonicus*) (Murata, 2005).

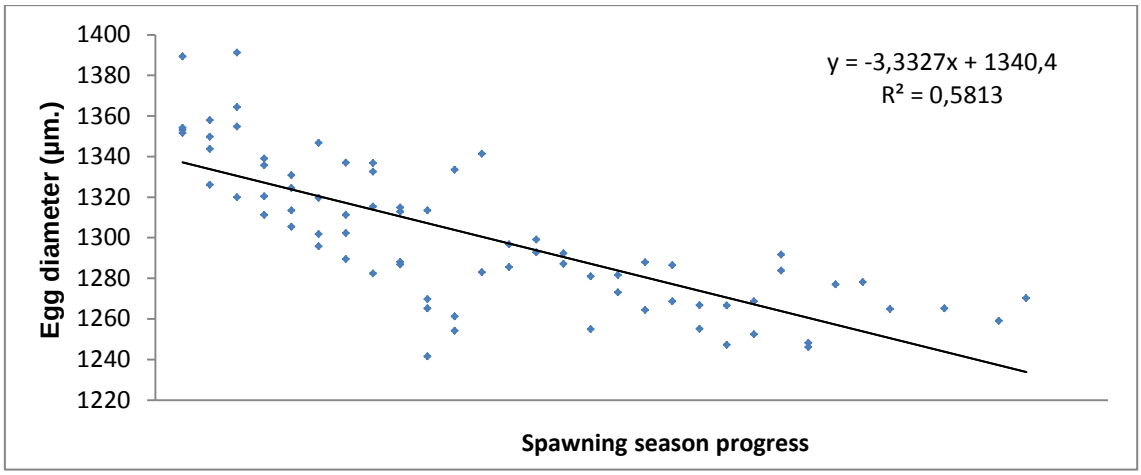

Figure 9: Evolution of egg diameter throughout the spawning season.

Others researchers have found an indirect relationship between egg diameter and temperature (Masuma *et al.*, (2006) for Pacific bluefin tuna and Margulies *et al.*, (2007) for yellowfin tuna). Figure 10 shows this relationship (egg diameter vs temperature) in AB, and as these authors stated, we have also founded this negative correlation. This might be due to an imbalance between nutrient supply and the incorporation rate of yolk reserves in the oocytes at higher temperatures while vitelogenesis and final oocyte maturation because of higher metabolic rates and depletion of reserves in the gonads of the fish.

Temperature increases along the spawning season, and this can be the main reason of delivering smaller eggs by the end of the natural spawning season (not controlled and stabililized but environmental temperature regime in the tanks). However, results reported by Masuma *et al.*, (2006) dealt with a longer spawning period (until November) and it can be noticed that in those last days, when temperatures decreased, egg diameter increased again, which demonstrated that egg diameter and temperature are negatively correlated. This is also supported by results obtained in other fish species like turbot (Bronley *et al.*, 1986) and sole (Devauchelle *et al.*, 1987).

Egg diameter decreased to 1,240-1,280 µm and then oscillated around these values. This happened at the end of the spawning season, when temperature and metabolic rate rise, reserves grow shorter and the period between two consecutive ovulations extend. This is the reason why females may need an extra time to incorporate the amount of yolk needed in the oocytes before final maturation under these conditions.

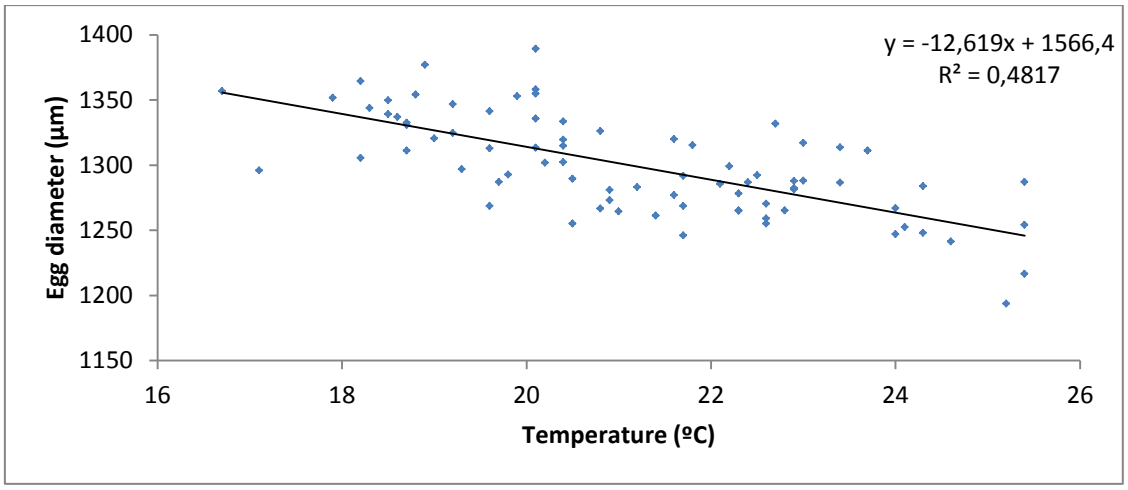

Figure 10: Relationship between egg diameter and temperature

In this study, bonito eggs presented a number of oil globules ranging between 1 and 12, with an average of 4.4 ±1.2. This appeared to be characteristic of bonito eggs as previously reported by Ortega *et al.*, (2007a) and ANON (2010). The number of oil globules slightly increased along 2009 season, but it did not vary significantly throughout the spawning season. However, as it has been pointed out in our results, some differences among spawning seasons can be observed (figure 11). On the other hand, these differences were not significantly related to hatching rates.

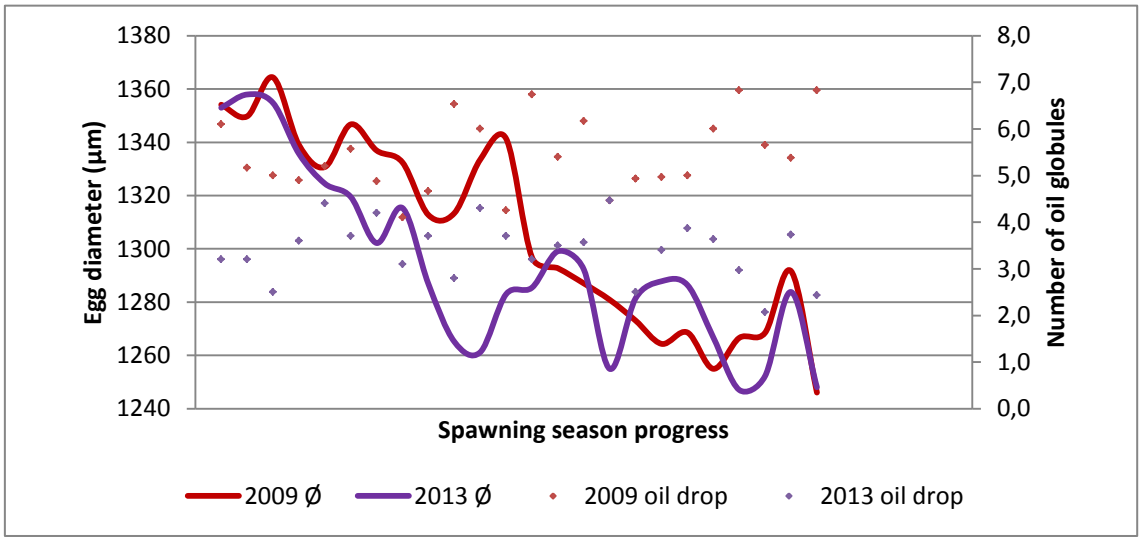

Figure 11: Number of oil drops and egg diameter in two different spawning seasons

In brief, we can conclude that AB is a small scombrid with an indeterminate fertility. Puberty and sexual maturation occur by the end of the first year of life, capable to spawn after some months of confinement and if stocked with suitable environmental conditions, spawning events can happen over fifteen times (per female) and spawning season could be prolonged to more than three months. If so, relative fertility could exceed 500 egg g^{-1} female.

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1.3. Completion of the Atlantic Bonito (*Sarda sarda*, Bloch 1793) life cycle in captivity

Resumen

Cierre del ciclo vital del Bonito Atlantico (Sarda sarda, Bloch 1793) en cautividad

Con la excepción del Atún rojo del pacífico (*Thunnus orientalis*), especie en la que se ha completado el ciclo vital, la acuicultura de las especies pertenecientes a la familia de los escómbridos está basada en la captura de especímenes adultos con capacidad de madurar sexualmente y reproducirse en condiciones controladas de confinamiento. El manejo (captura,trasporte, manipulación y estabulación) de reproductores en tanques y el desarrollo de una tecnología de criadero para estas especies, constituye un paso decisivo adelante para mejorar la diversificación sostenibilidad de la acuicultura. El presente estudio revisa la tecnología aplicada en el centro Oceanográfico de Murcia para completar y cerrar el ciclo biológico del Bonito Atlántico, *Sarda sarda* Bloch, 1758). Estos estudios y ensayos tienen el valor añadido de la mejora en la adaptación al confinamiento y la domesticación de una especie con un alto valor comercial para el consumo humano. Además, los beneficios del conocimiento derivados de cerrar su ciclo de vida implican la mejora de las técnicas de cultivo mediante el manejo y control de parámetros ambientales y biológicos. En el caso del bonito Atlántico, *Sarda sarda*, otro campo de interés podría ser explorado: estudios recientes sobre el uso de técnicas para utilizar especies substitutas como reproductores se han empezado a desarrollar con varias especies de peces. El Bonito Atlántico como especie filogenéticamente cercana al atún rojo del Atlántico (*Thunnus thynnus L.),* con un menor tamaño corporal y tiempo generacional más corto, Si estas técnicas tienen éxito, los reproductores de bonito podrían usarse como sustituto de reproductores de atún rojo Atlántico para la producción de gametos funcionales viables mediante el implante de células germinales primordiales de atún rojo en el tejido reproductor del bonito.

Abstract

With the exception of Pacific Bluefin tuna (*Thunnus orientalis*) whose biological life cycle has been completed in captive conditions, aquaculture of species belonging to the Scombridae family is based on capture of wild specimens capable of attain sexual maturation and reproduction under controlled conditions in confinement. Handling (comprising capture, transport, manipulation and housing) of broodfish in tanks and the development of reliable breeding and culture technologies for these species would be a major step forward in improving diversification and sustainability of aquaculture. The present study overviews the technology used in Centro Oceanográfico de Murcia to complete the life cycle of AB, *Sarda sarda.* These trials and studies have the added value of improving the adaptation to captivity and domestication of an important commercial species for the human consumer. Moreover, the benefits of the knowledge derived from closing the life cycle implies the improvement of the culture techniques by mean of the manipulation and control of environmental and biological parameters. In the case of AB, *Sarda sarda,* another field of interest could be explored: recent studies on surrogate broodstock have started to be developed in several species, and AB is a species phylogenetically close related to Atlantic Bluefin tuna (*Thunnus thynnus* L.) with a much smaller body size and shorter generation time. If these techniques success bonito could become a surrogate broodstock for the production of functional ABFT gametes via primordial germinal cells implantation.

Introduction

AB (AB) (*Sarda sarda*) is a fish belonging to Scombridae family, less valuable than some other members of this family like Atlantic bluefin tuna (ABFT) (*Thunnus thynnus* L. 1758), Pacific bluefin tuna (PBFT) (*Thunnus orientalis*, Temminck & Schlegel, 1844), Southern Bluefin tuna (SBFT) (Thunnus maccoyii, Castenau, 1872), yellowfin tuna (YFT) (*Thunnus albacares*, Bonnaterre, 1788) or albacore (*Thunnus alalunga,* Bonaterre, 1788), but still well commercially appreciated by the human consumer. The Scombridae family is characterized by comprising a vast group of fast growing species which can represent an important potential to aquaculture. However, their rearing in captivity and domestication processes are being developed not long past and except some species of tunas (PBT, ABT, SBT and YFT) there are not commercial rearing of these species to date. AB has the special feature of reaching puberty and sexual maturation by the end of the first year of life, which implies an interesting critical added value to aquaculture purposes.

Since 2005, the Murcia Oceanographic Centre (COMU), a research Centre belonging to Spanish Oceanographic Institute placed in Mazarrón, Murcia, SE Spain, has carried out a research program comprising several fishing campaigns to capture ABs to be stocked as broodstock batches and to be housed in their facilities (Ortega y de la Gándara, 2007a) in order to a) achieve the reproduction in captivity under controlled conditions (Ortega *et al.*, 2007b), b) obtain viable embryos, larvae and juveniles to be grown up to adults (Ortega and De la Gándara, 2007c; 2009) and c)close the life cycle by mean of fertile mature adult born in captivity.

Regardless aquaculture of Scombridae is not far developed yet, there are several studies dealing with sexual maturation and spawning in captivity conditions, not only for aquaculture production but also for display these species in aquarium facilities. Spawning in captivity of ABFT, *Thunnus thynnus*, (Anon, 2010), PBFT, *Thunnus orientalis*, (Miyashita *et al.*, 2000), SBFT, *Thunnus maccoyii*, (Woolley *et al.*, 2013), YFT, *Thunnus albacares*, (Wexler *et al.*, 2003; Margulies *et al.*, 2007), AB, *Sarda sarda*, (Ortega *et al.*, 2008), Stripped bonito, *Sarda orientalis* (Shimizu *et al.*, 1998), Pacific bonito, *Sarda chiliensis*, (McFarlane *et al.*, 2000) or Chub mackerel, *Scomber japonicus*, (Masuda *et al.*, 2002) have been carried out with unpredictable success. However, researchers have only been able to close the whole vital cycle in captivity of Pacific bluefin tuna, *Thunnus orientalis* (Sawada, 2005) in Japan and also, as recently reported by Yazawa *et al.*, (2015), the Eastern little tuna (*Euthynnus affinis*, Cantor, 1849).

ABFT, *Thunnus thynnus,* is a very valuable species and their culture has been and still is one of the main goals of the European aquaculture with regard to species diversification programs. Some EU DG XIV projects financed in the last decade studies to achieve the reproduction and domestication in captivity of Atlantic Bluefin tuna (acronyms for these projects REPRODOTT, SELFDOTT and TRANSDOTT). Hatchery and husbandry techniques have been recently developed (Ortega *et al.*, 2011, Morais *et al.*, 2011) in order to improve larval and juvenile growth and survival. Recently, a new land based facility has been built by Instituto Español de Oceanografia in order to house and maintain Atlantic Bluefin tuna adult fish as broostock to produce viable fertilized eggs in controlled condition tanks. Closing the life cycle of larger Bluefin tuna species and particularly Atlantic Bluefin tuna (*T. thynnus*) in captivity has always been acknowleged as a complicated and difficult task for obvious reasons due to the minimum size of the fish to assemble a broodstock lot in dependence of the minimum size of this species to reach sexual maturity and reproduction. New techniques for using a surrogate broodstock species to produce donor-derived gametes are currently in the scope of researchers considering these circumstances and have started to develop innovative investigations in this field with some potential surrogate species according to this initiative. As references

regarding this subject in marine aquaculture with the Carangidae and the Scombridae families recent works by Morita *et al.*, (2012) and Yazawa *et al.*, (2010) can be cited. Yazawa *et al.*, (2015a) have recently reported to reproduce Eastern little tuna (*Euthynnus affinis*) in captivity to be used in the future as a surrogate species as broodstock for PBFT. If the technique shows to be successful, AB could also be a candidate to be used as surrogate broodstock specie for ABFT or any other large sized tunid.

Material and Methods

Culture and food of broodstock:

ABs with an average total weight of 1.8 kg were fished in La Almadraba trap in La Azohía (Cartagena, SE Spain) in early May 2008 according to the techniques described by Ortega *et al.*, (2007a). Regarding to growth data estimated for this species by Rey *et al.*, (1984), Santamaria *et al.*, (1998) and Valeiras *et al.*, (2008) in the Mediterranean Sea, it is suggested that captured bonitos were about 22-24 months old, belonging to the 1^{+} yearly class. ABs were transported to COMU and placed in a 20,000 l. cylindrical tank. The tank was provided with an open flow filtered sea water system, with water inlet placed at the surface and water outlet in the bottom at the centre of the tank. Water flow was regulated in order to maintain dissolved oxygen over 90% saturation level. As the water source pumped was oceanic seawater, the temperature in the tank varied according to local ambient seawater temperatures and ranged between 13ºC in the middle of the winter to 28ºC in the middle of the summer. Artificial lights were adjusted to provide the tank photoperiod according to natural day length illumination time.

Bonitos started to feed about 2 weeks after having been captured. Food ration consisted in a mixture of different defrosted fish, mainly European anchovy (*Engraulis encrasicolus*), European pilchard (*Sardina pilchardus*) and Round sardinella (*Sardinella aurita*) and mackerel (*Scomber scombrus*), supplied to satiation. Fishes were feed once per day, five days per week.

From the beginning of 2009, and in order to improve food quality when approaching sexual maturation and spawning season, mussels and squids were added to diet once per week. Cephalopods were used to vehicle the supply of extra fat-soluble vitamins and pigments inside the mantle cavity, in order to enrich the diet in these essential nutrients which are known to improve fecundity, fertility and spawning performance, as reported for different fish species by Watanabe and Vasallo-Agius in 2003.

Egg incubation and larval rearing:

ABs started to spawn in captivity in May 2009, when they were 3 years old. Spawning occurred at nightfall and eggs were collected with a mesh screen (500 µm) early morning every day. On the 18th of May 2009, a batch comprising 13.000 eggs was collected and after cleaning, disinfecting and counting was placed in a 400 l cylindrical tank. Average egg diameter was 1,364.3 ± 28.3 µm and the mean number of oil drops was 5.0 ± 1.2. Eggs in the incubator tank were kept in a flow through water system, at a temperature of 18.5 ± 0.5 ºC and 24 h continuous illumination photoperiod by mean of artificial lights. After hatching, the first generation of larvae (F1) were placed in a 5 m^3 cylindrical tank and reared according to the methodology reported by Ortega and de la Gándara (2007c). Feeding schedule consisted on enriched rotifers *Brachionus plicatilis* from 2 to 9 days post hatch (dph) at a concentration of 10 rotifers/ml, enriched Artemia nauplii from 7 to 14 dph, at a concentration of 1-2 nauplii per ml and gilthead sea bream yolk sac larvae from 10 dph onwards at increasing concentrations according to the ABs larvae demand and consumption rates. Nutritional enrichment of the live food was achieved by using a commercial product, DHA Selco Inve ® (fish oil based-emulsion enriched in DHA), following the manufacturer instructions for the enrichment procedure.

Weaning from live feeds (rotifers, Artemia nauplii and sea bream yolk sac larvae) to inert diet (minced frozen fish) started at 20 dph with a mixture of frozen yolk sac larvae and minced fish (anchovy and sardine). Live yolk sac larvae were also added to tanks until 25 dph. From this time point onwards, only minced fish was offered to AB fingerlings to apparent satiation 7 days per weeks. At the beginning, fish were fed once per hour, but as fish grew larger and water temperature decreased, ration was also diminished accordingly. From November onwards, only one feeding dose was offered per day. In this period, food mainly consisted of minced round sardinella and mackerel, but in some occasions anchovy and pilchard were also offered.

F1 Generation as broodstock in captivity: closing the cycle

In November 2009, 24 cultured Abs, considered as the first experimental broodstock reared in confinement under controlled conditions, with an average total weight of 600 gr were moved to an outdoor 55 m^3 cylindrical tank covered with a shadow net to prevent from direct sun light. From this moment until the end of the experiment, the broodstock was kept in this tank. The diet used was the same one (based on minced fish) with the exception of the addition once

per week of, squids (*Loligo vulgaris*) to boost the nutritional value of the dietary regime previously to maturation and spawning. During all the time the tank was provided with continuous flow through filtered seawater system, which was regulated in order to maintain dissolved oxygen concentration over 90% of the saturation level. Artificial light from two fluorescent tubes was used, and photoperiod regime adjusted according to the natural day length in the region, ranging from 9.5 hours of light period at the end of December to 15.5 hours at the end of June. Temperature was registered daily.

By the end of April 2010, a total of 15 bonitos still remained alive in the tank. Then the water flow was forced to drain to a surface water outlet. A container provided of a 500 µm mesh screen net was placed in the outlet as an egg collector to gather floating spawned eggs flowing away from the broodstock tank. The egg collector was checked daily for eggs to be incubated. ABs started to spawn on the 8^{th} of May 2009, when fish were 11.5 months old. Spawning occurred at nightfall and eggs were collected early morning every day. After cleaning and separating viable - buoyant from non viable - non-buoyant eggs and counting both fractions, viability and fertilization rate was estimated. A sample of floating eggs was used to measure egg diameter and number of oil globules per egg. Another sample was taken out to calculate hatching rates: A sub sample of 72 eggs coming from every spawning event was incubated in three different multiwell plates. Each plate contained 24 wells of 2 ml capacity, and one egg was placed in each well. As it is reported by Ortega et al (2008), the duration of the embryonic development was temperature dependent and at the normal incubation temperature (20-23ºC) usually ranged between 48 and 60 hours, which means that eggs placed in the plates hatched about 2 days after recollection.

Some of the fertilized eggs were incubated and newly hatched larvae were cultured in the same way above described and once weaned they were kept in a 20 m3 cylindrical tank.

In December 2010, broodstock was comprised by only 11 fish (bonitos 1^{+} born in captivity in May 2009), so in order to get a greater stock, we added 68 new fishes coming from the eggs collected in May and cultured in the 20 m^3 tank above mentioned (bonitos belonging to the 0^{+} class with an average total weight of about 400 gr). This batch suffered a severe mortality post handling, and only a total of 40 ABs reached the onset of spawning season by April 2011. ABs started to spawn on May the 5^{th} and continued spawning during a period of 32 days. Fertilization and hatching rates as well as egg diameters were also measured and reported.

Results

Larvae hatched in May 2009 were cultured according to the methodology reported above. Hatching rate was found to be 83.7 %, and a total number of 10,880 dph larvae were placed in a 5 m^3 cylindrical tank, so that initial density was set at 2.2 larvae l^{-1}. During larval rearing, until the onset of weaning, survival rate was 6.7% and their growth performance based in total length measurements is shown in Figure 1.

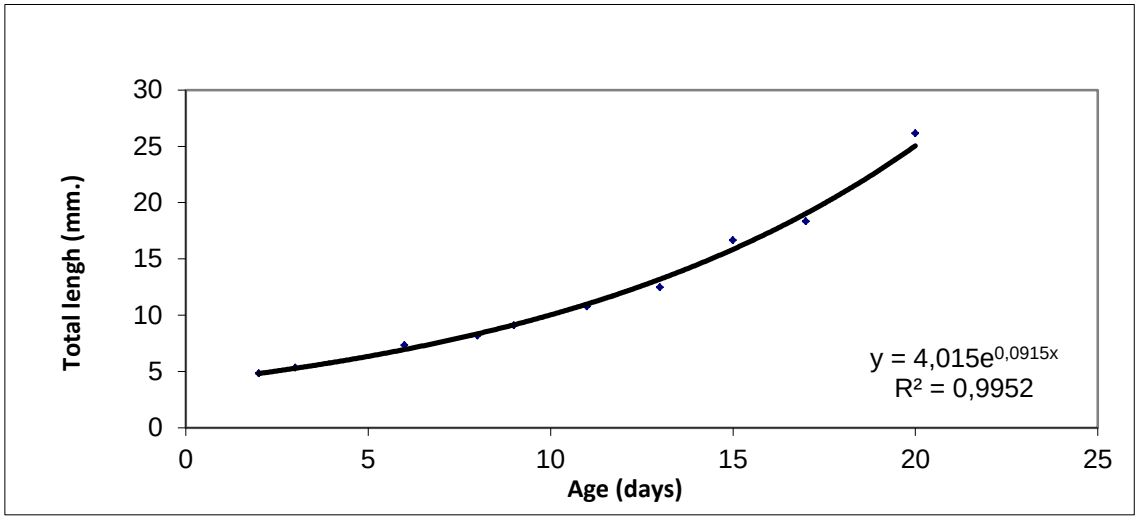

Figure 1: Growth of bonito larvae (total length in mm) during the first 20 dph.

Weaning took ten days to be completed and mortality was slightly above 50 %. So, total cumulative survival until the onset of preongrowing was 2.98 %. At this point, 30 dph, AB fingerlings presented an average total weight of about 1 gr. These fishes were kept in an indoor 20 m^3 tank until November 2009, when they were transferred to the broodstock tank. Growth during this period is shown in Figure 2.

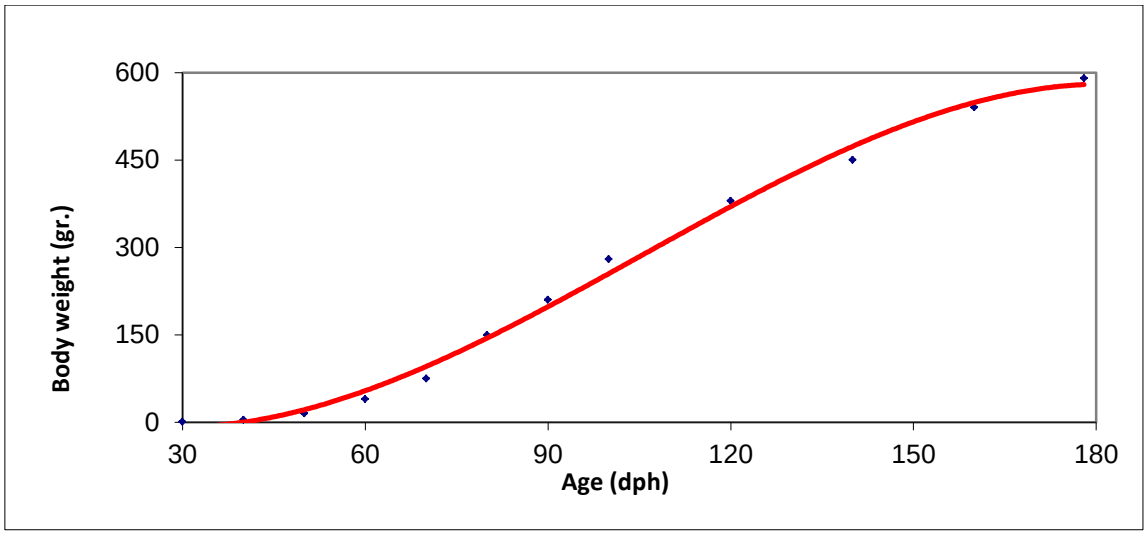

Figure 2: Growth of bonito during the first six months

Temperature was daily registered and displayed in Figure 3.

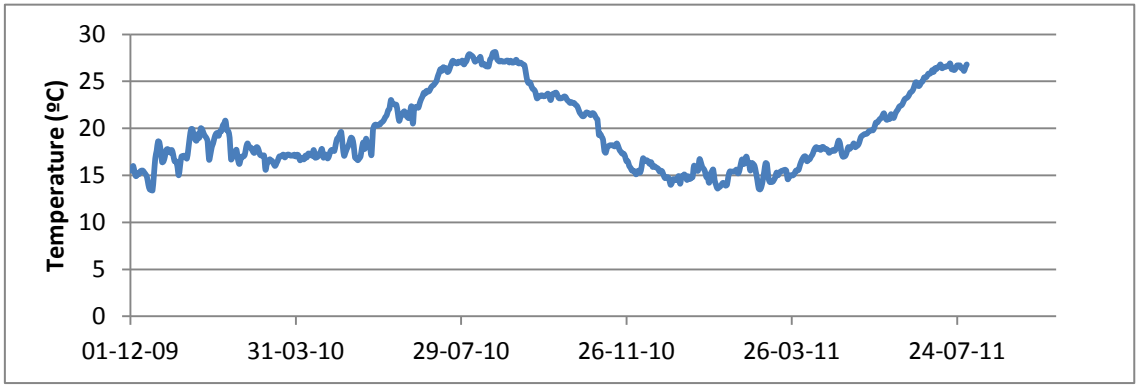

Figure 3: Evolution of temperature during the experiment.

In 2010, bonitos started to spawn spontaneously on the 8th of May, at 355 dph. Temperature during spawning season varied between 16.8 and 22.1ºC. A total of 1,1 million eggs were collected during 31 days, in the period between the 8th of May and the 7th of June 2010, with batches between 7,200 and 135,000 eggs spawned daily (Fig. 4). Spawning occurred most of the days during this period (25 days within a total of 31 days) with an average of 42,000 eggs spawned per day. Mean fertilization rate success during the whole period was 83.2 ± 17.1 %, and hatching rate was 65.2 ± 14.5. Mean egg diameter was 1241.5 ± 35.9 µm.

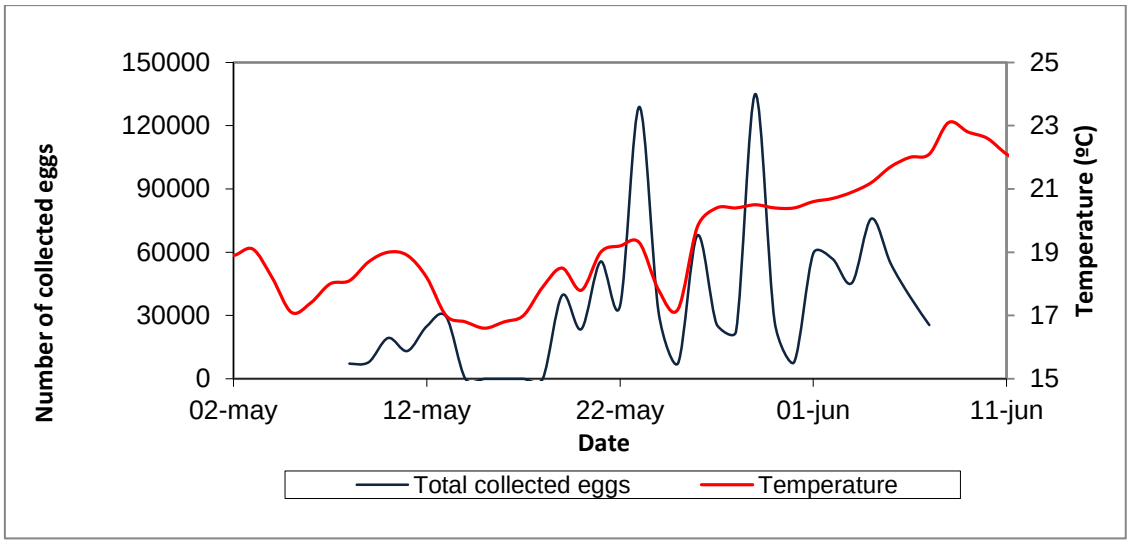

Figure 4. Daily egg production of bonito broodstock during 2010.

In 2011, fishes started to spawn on May the 5th and were spawning for a 32 days period. The total number of eggs collected was 1,926,000 eggs. Fishes spawned for 27 days, with an average of 71,300 eggs per day and a maximum daily spawning of 162,500 (Fig. 5). Overall fertilization rate success was 81.1 ± 8.1 % and hatching rate was 77.4 ± 12.9 %. Figure 6 shows fertilization rate results in both spawning season. Temperature during spawning season varied between 18.0 and 21.6ºC and mean egg diameter was 1,293.7 ± 30.3 µm.

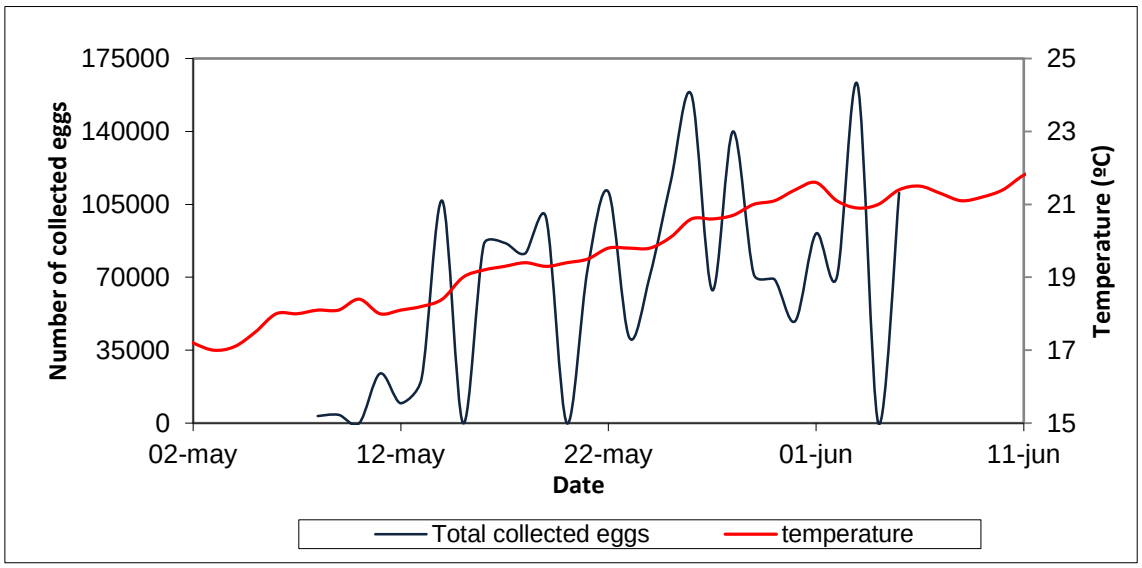

Figure 5. Daily egg production and water temperatures of bonito broodstock during 2011 spawning season.

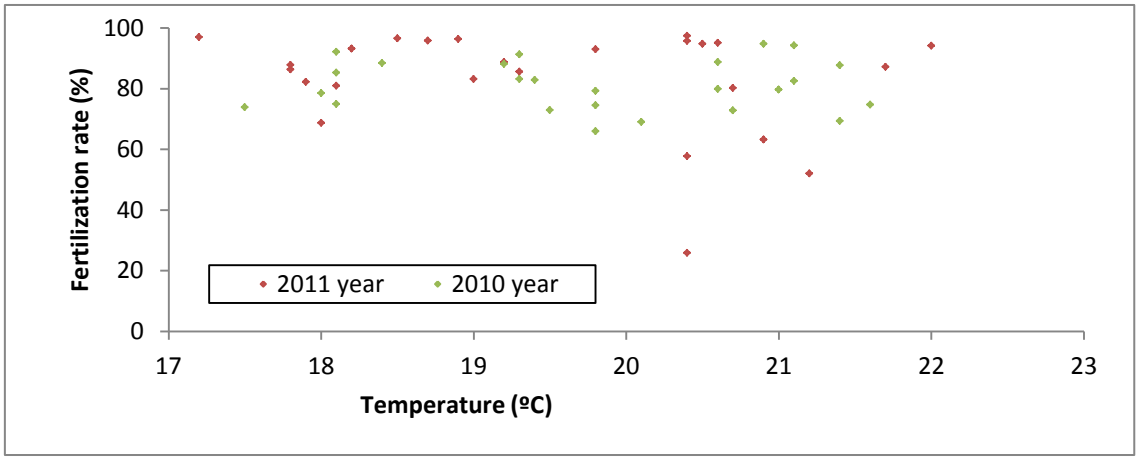

Figure 6: Fertilization rates and temperature during 2010 and 2011 spawning seasons.

Evolution of egg diameter throughout 2010 and 2011 seasons is displayed in Figure 7.

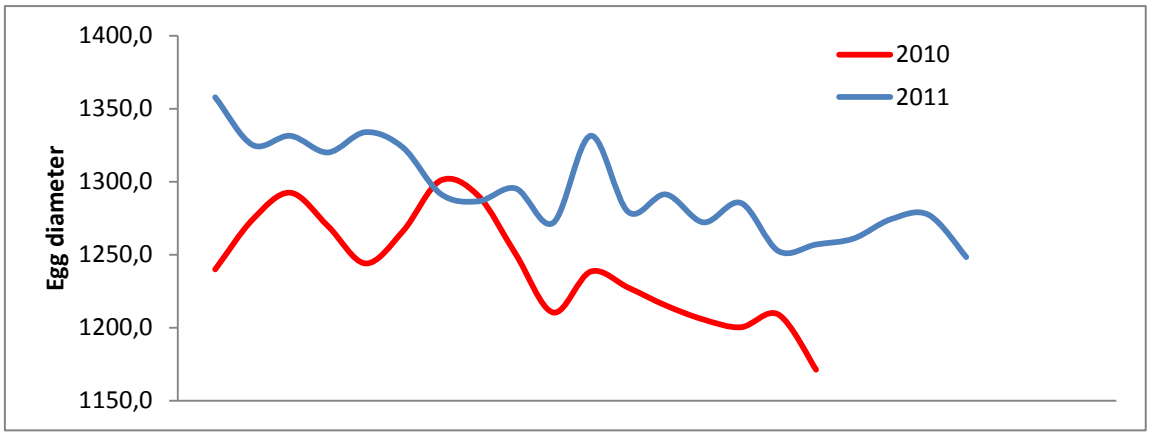

Figure 7: Evolution of egg diameter throughout 2010 and 2011 seasons

With regard to hatching rates, average value was 74.4 ± 14.9 %., which showed a higher value in 2011 (77.4 ± 12.7 %) than in 2010 when mean hatching rate value was only65.2 ± 14.5 %.

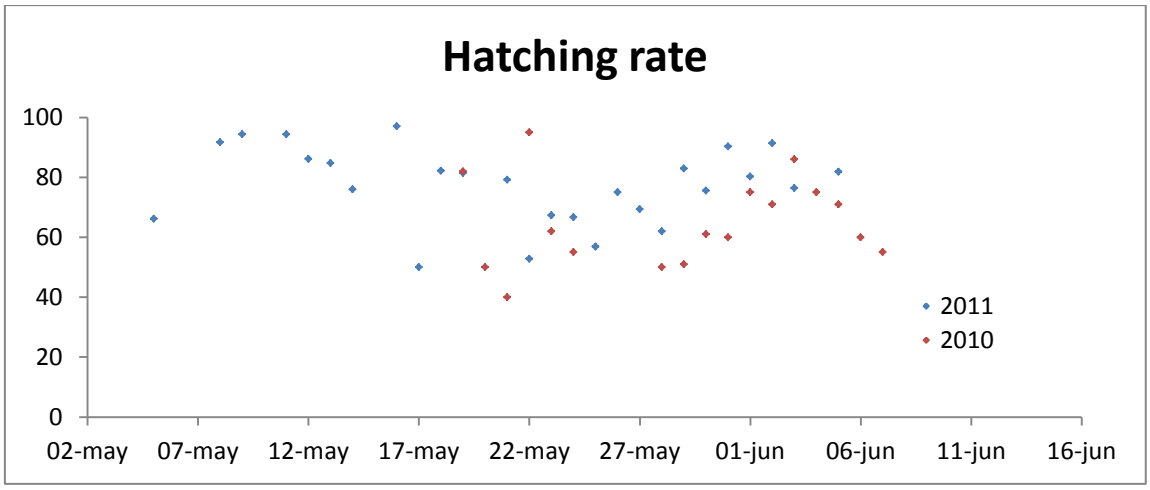

Figure 8: Evolution of hatching rate throughout 2010 and 2011 seasons

Discussion:

Besides the fact of a nearly full control of reproduction, independently of captures, the completion of the life cycle in species able to spawn at the end of their first year of life offers the researcher the possibility to obtain faster results from genetic improvements than with longer span life species. In this study, we have obtained growth rates of ABs lower than those reported by Santamaria *et al.*, in 2005 for wild fish in southern Italian seas. ABs kept in captivity, in this experiment, reached 600 gr in 180 days while the same species gained this average total weight in only 120 days in the study of Santamaria *et al.* (2005). AB, as species such as Atlantic Bluefin tuna,*Thunnus thynnus*, (see chapter 1.4), gilthead sea bream , *Sparus auratus*, (Ortega 2009), and some others, reduced their growth rate during the following months, corresponding with winter lower temperatures, and they started to increase the growth rate again on the onset of the next spring. Slow growth during the winter period is a disadvantage to the establishment of commercial aquaculture of any species. However, if AB growth rate could be improved during the fast growing warmer period and if ABs with more than 1 kg in total weight can be produced in less than 6-7 months, a potential market could be open for this species by means of aquaculture technology. In addition to nutritional studies and feeding technology, perhaps the improvement of zootechnics in the rearing conditions together with the improvements that can be gained with broodstock genetic selection (throughout generations) could help to reach this aim.

According to literature, AB is able to reproduce by the end of the first year of life in the wild. Different authors such as Rey *et al.*, (1984), and Ates el al., (2008) estimated the age of bonito according to otholite measurement studies. Other

author (Valielas, 2008), estimated the age of the fish based on measurements of annual rings of sagittal cuts of the first dorsal spine. This author reported size-age tables and equations which have been used by Rey *et al.*, (1984), Macias *et al.*, (2005) or Pascual (2010), to assess the age of the first maturation in AB. Our results are in fully agreement with these observations, so that, bonitos born in May 2009 were able to spawn viable eggs by the beginning of May 2010, when they were only 11 months old.

Nevertheless, results in terms of quantity of eggs (fecundity) in this study are lower than those obtained with older and larger fish (see previous chapter). Results by Pascual (2010) pointed out that the age of first sexual maturation in females of AB is around 1.4 years. This means that not all the females reach sexual maturity during the first year of life, so taking into account this point of view, the broodstock lot belonging to the 0^{+} yearly class would present a lower fecundity than the expected for the following older age class lots, when most or the totality of females would be participating in spawning events. In addition, larger females with larger ovaries may spawn a larger number of eggs per season than smaller ones.

In 2011, total number of spawned eggs was greater but at the same time, appeared to be lower than expected. It was not possible to determine the relative fecundity because we did not know the sex ratio and we were unable to sex the fish by visual/morphological sexual dimorphism, so at least two hypothesis could be considered: i) on one side, an unbalanced sex ratio could have occurred when selecting/forming the broodstock lot, which led to a lower female presence (it is also well known that, for several fish species, sex ratio could be modified by raising conditions like reported by Blázquez *et al.*, 1998, Ospina-Álvarez and Piferrer, 2008); ii) on the other hand, the smaller size of the ABs added at the end of 2010 to constitute the broodstock lot (only average 400 gr total weight by mid December) could be the cause of a high presence of sexually immature fishes during 2011 season. Further research is needed to sex identification and sex ratio determination occurring in broodstock lots in order to determine the total and relative fecundity of reared ABs females and if some particular rearing conditions can affect the sex ratio determination in this species.

The spawning pattern during the first year was quite constant: ABs spawned every day during the spawning season except for five days in the middle of May, just when water temperature decreased below 17ºC. As spawning in nature occurs mainly in June and July, it is possible that 17ºC could be considered as the low temperature threshold for reproductive activity. Spawned eggs would be viable (see chapter 2.5) but ABs stopped courtship activity and eggs spawning was paused until temperature increased again above 18ºC. As it was pointed out in section 1.2. of this thesis, bonito egg diameter tends to

decrease through spawning season. In 2010, by the end of the spawning season, the decrease was greater than expected, and values went below 1,200 µm. This could be related with the low initial total weight of the broodfish, and as the spawning season progressed, the reproductive condition of the ABs worsened and egg diameter decreases.

Average egg diameter was significantly larger in 2011 than in 2010. That could be due to the age or to the size of the broodstock: in 2010 broodfish were in their first spawning season and their weight was close to 1 kg. As Bromage reported in 1995, generally, as fish size increases so does the diameter of the eggs produced. During 2011, in the second reproductive season, as Abs increased their total weight to 1.5-2.0 kg, they spawned larger eggs with improved results regarding hatching rates, which could be related to a better quality of the eggs spawned with richer yolk reserves which would imply a better performance of the embryo and yolk sac larvae developments.

Aquaculture of scombrids has been mainly limited to tunas and primarily to Bluefin tunas, but in the last years a promising progress is starting to be developed with the culture of another species. Research on AB, as it is reported in this article, and Eastern little tuna (Yazawa *et al.* 2015b), have achieved the completion of their life cycle in captivity, and some other species could be soon coming. Their commercial interest, but also the chance of using them like surrogates broodstock for larger Bluefin tunas, are increasing the interest on aquaculture of these small scombrids.

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1.4. Maturation and spawning of Atlantic Bluefin tuna (*Thunnus thynnus,* L.) maintained in sea cages

Resumen

Maduración y puesta de Atún rojo del Atlántico (*Thunnus thynnus,* L.) mantenido en jaulas.

Hasta hace unos pocos años, el cultivo de Atún Rojo del Atlántico (*Thunnus thynnus* L.) se limitaba al engorde y engrase en jaulas marinas. Estas actividades comprenden la captura mediante métodos de pesca industrial (cerco o trampas) de ejemplares adultos vivos para su mantenimiento y alimentación en jaulas en el mar, durante varios meses, antes de su comercialización. Este semicultivo industrial no solo representa una actividad de gran interés comercial sino que ha sentado las base tecnológica en la que se fundamentan una actividad acuícola mas completa, y que tiene como finalidad la domesticación de esta especie icónica en condiciones controladas.

Esta acuicultura integral implica la reproducción y obtención de embriones viables y el desarrollo de técnicas de cultivo de larvas, juveniles y adultos hasta completar y cerrar el ciclo de vida en confinamiento de esta especie.

En los últimos años, la Unión Europea (DG XIV, Pesquerías) ha financiado dentro del programa para de diversificación de especies en acuicultura, varios proyectos de investigación (acrónimos REPRODOTT y SELFDOTT) para la domesticación del Atún Rojo del Atlántico en condiciones controladas. En este capítulo se muestran los resultados más relevantes obtenidos en estos proyectos y durante los años posteriores con tres lotes de reproductores mantenidos en jaulas marinas, pertenecientes al Instituto Español de Oceanografía y a la empresa Caladeros del Mediterráneo, en aguas de la costa de Murcia.

Abstract

Until a few years ago, the culture of Atlantic Bluefin tuna (*Thunnus thynnus*) was limited to ongrowing and fattening in sea cages. These activities comprises the capture alive of big adult tunas by industrial fisheries (purse-seine and/or traps) transport to offshore farms and the maintenance and feeding in sea cages for a few months before commercialization However, this semi-culture activity means much more than a very profitable industry, it has settle the foundations of a much more complete aquaculture activity in order to achieve

the domestication of Bluefin tuna under controlled conditions in confinement. This integral aquaculture system implies the reproduction and obtention of viable embryos, the development of larval, juvenile and adult rearing techniques, and finally closing the life cycle in captive conditions for this species.

In the last years, the European Union (DG XIV, Fisheries) has financed, within a program for the diversification of aquaculture species, several research projects (acronyms REPRODOTT and SELFDOTT) to achieve the domestication of the Bluefin tuna under controlled conditions in confinement. In this chapter are explained the most relevant results obtained in these projects and the following years, with three broodstock lots maintained in sea cages, belonging to IEO and Caladeros del Mediterraneo, off the Murcia region coast.

Introduction

Bluefin tunas are among the most valuable fish species. Three different species, Atlantic bluefin tuna (ABFT) *Thunnus thynnus* L. (1758), Pacific Bluefin tuna (PBFT) *Thunnus orientalis* Temminck and Schlegel (1844) and Southern Bluefin tuna (SBFT) *Thunnus maccoyii* Castelnau (1872) are target of important commercial fishery activities. Moreover, during the last years semi-culture practices have been developed for ongrowing these species in sea cages for the so-called “fattening” before commercialization. Fattening is a seasonal activity that involves the capture of big adult fish alive from the different spawning areas and transported to sea pens at offshore coastal farms and maintained alive for periods between 3 months to 2 years (Mylonas *et al.*, 2010; de la Gándara & Ortega, 2013).

This semi-culture activity is establishing the foundations of a step forward complete aquaculture activity, the breeding and rearing of these species under controlled conditions in confinement, aiming to completely close the life cycle for commercial production purposes. In this context, three different metholological approaches must be employed according to tuna life cycle stage: reproduction, larval rearing and ongrowing to adults, in order to be commercialized or used as breeders.

Since the decade of the eighties in the last century, Japanese fishery scientists have been considering reproduction in captivity of PBFT, *T. orientalis*, (Kumai, 1997; Miyashita *et al.*, 2000; Masuma, 2006a; Seoka, 2007; Mimori *et al.*, 2008), and finally they have succeeded to complete the life cycle (Kumai &

Miyashita, 2003; Sawada, 2005). More recently, other researchers have also been able to reproduce other tuna species like yellowfin tuna, *T. albacares*, Bonaterre (1788) (Wexler *et al.*, 2003) and Southern bluefin tuna, *T. maccoyii*, (Woolley *et al.*, 2009; Bubner *et al.,* 2012). With ABFT these studies started a few years ago, at the beginning of the 21st Century. In the last years, several funded European Union research projects (acronyms for these projects REPRODOTT, SELFDOTT, TRANSDOTT, etc…) have studied reproduction in captivity and larval rearing techniques of ABFT (Corriero *et al.*, 2007; Mylonas *et al.*, 2007; Anon., 2007, 2010, 2012; De la Gándara *et al.*, 2009; De Metrio *et al.*, 2010a,b; Delaguara, 2011; Ortega *et al.*, 2011). In the present chapter, results obtained in the EU SELFDOTT project, coordinated by IEO, and during trials performed the following years with three broodstock lots belonging to IEO and Caladeros del Mediterraneo SL in SE Spain, are presented and discussed.

Scombridae, and more concretely tuna species, are large migrating pelagic fishes which demand and need large water volume to swim and live. Handling of these species is usually difficult due to their physiological characteristics and non-invasive methods have to be used in order to avoid stress, injuries, infections or even death. Because of this, broodstock are usually maintained in large or very large sea cages, and one of the main challenges is to collect the eggs, specially when the cages are placed in offshore unprotected areas with occasionally mild to strong currents and winds, like it happens in Spain. To solve this problem, special egg collectors have been designed, but to face future activities, the main choice and feasible solution is to construct special inland facilities to have a more complete control of reproduction events. In the last years, big efforts have been carried out to design large on shore tanks, with recirculation system, capable to accomodate these big fishes in a healthy state and proportionate the adequate environmental conditions to achieve reproduction. These facilities have been built in Achotines bay (Panama) for *T. albacares*, Arno Bay (Australia) for *T. maccoyii*, Yokohama (Japan) for *T. orientalis* and recently in Cartagena (Spain) for *T. thynnus*. The design and description of this facility is also the objective of the last chapter of this thesis.

Matherial and Methods

Three broodstock lots were used during the period of study:

1. The elder broodstock lot was placed in cage nº1 and it was formed by 37 bluefin tunas caught in the Balearic Sea in June 2007, whith an estimated average total weight of 30 kg. These fishes were transferred from fattening cages to cage nº1 by the end of 2007. This lot spawned from 2009 to 2012, when after the reproductive season we lost due to a sabotage.

2. The second broodstock lot was also captured in the Balearic Sea in June 2008. This lot was maintained and fattened during seven months and then, in early 2009, 25 bluefin tunas were moved to cage nº2. Fishes weighted about 40-45 kg in this moment and spawned from 2010 to 2013

Both cages were placed in El Gorguel (Cartagena, Spain) and their dimensions were 25 m diameter and 20 m in depth. The cages were fitted with a 2 cm mesh screen net to restrict the entry of opportunistic small pelagic fish species that seasonally feed on spawned Bluefin tuna eggs released in the cages.

3. A broodstock lot coming from a commercial cage was formed and used during 2015 season. Tunas from this lot were also captured off Balearic Island waters in May 2014 and moved to San Pedro del Pinatar where Caladeros del Mediterraneo SL has a second fattening facility. Fish were placed in cage nº 3, a 50 m diameter and, 16 m in depth cage (about 25 m depth in the center) and fishes were fattened during 11 months. From January a few fishes were slaughtered once or twice per week. In early May 2015, still about 400 fishes remained in the cage, and Caladeros del Mediterraneo SL decided to stop slaughtering operations for a few months. By the end of May, an egg collector was placed in this cage and spawned eggs were collected throughout June and July months. At this point, average total weight of the fishes was about 230 kg.

Broodstock were fed to saciety once per day, five times per week on a mixture of frozen fresh fish consisting mainly of mackerel (*Scomber scombrus*) and Spanish mackerel (*Scomber japonicus*). Other bait fish species like sardinella, jack mackerel and herring were sometimes offered as food.

Bluefin tunas kept in San Pedro (cage nº 3) were fed similarly to cage nº1 and 2 but six times per week. Food was supplied to saciety, and daily ration offered ranged between 1 and 3 % of the broodstock lot weight biomass.

Figure 1: Broodstock cages at El Gorguel, Cartagena (SE Spain).

Egg collector:

In order to gather and collect released eggs, a special device was designed and constructed by Centro Tecnológico y Naval del Mar supported by several scientific partners in the frame of SELFDOTT EU project. The system (see Plan in Annex II) consisted of a plastic curtain which surrounds the inside entire perimeter of the cage and hangs from the surface to 2 m down into the water. Eleven net cones protrude outwards from the curtain and each cone has a cylindrical collector at the end of it, which is where the BFT eggs are collected. To avoid opportunistic fish species entering inside the collector and predate on ABFT eggs, an “anti predator mesh” was placed at the entrance of the cones. The entire system is made with a polyethylene 500 µm mesh screen size. Egg collection was enhanced by placing a solid PVC sheet (tarpaulin) which surrounded the inside perimeter of the cage from 0.5 m above the surface to 1.5 m underwater. This allowed the floating eggs to be maintained within the cage in case of light currents. This system was used during 2009 in cage nº1 (Figure 2)

Improvements were necessary because some eggs—despite being buoyant—were transported under the curtain and out the cage. Besides, final collectors were quite difficult to manage and it offered a strong resistance to the water flowing through and therefore for carrying the eggs. As a consequence it was necessary to design another system and build a new egg collector for cage nº 2

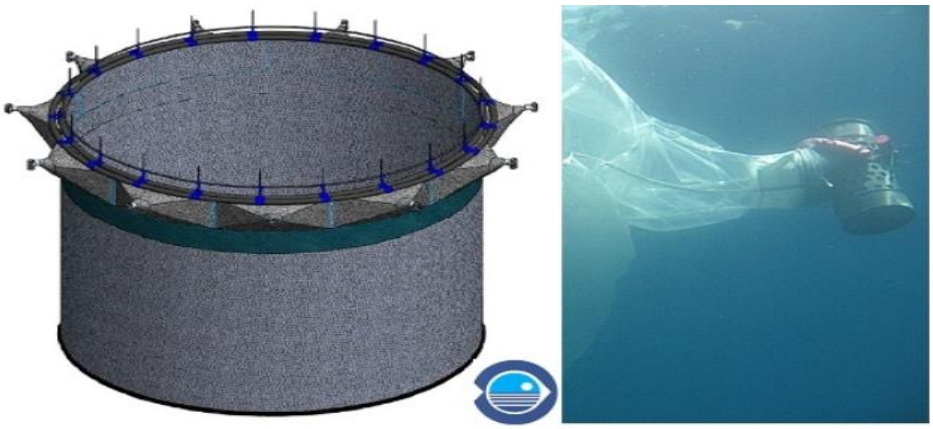

Figure 2: Egg collector system used in 2009.

So, some changes were carried out in the new design: the new collector increased the vertical extent of the curtain, from 50 cm above water to 6 m deep and not surrounding the entire perimeter of the cage. As the main currents in the area were of W-E directions (Fig. 3), only the area to which stronger currents flowed was covered as it was inefficient to put net and collector cones in the N-S axis. So the cones were used to construct two complete systems for both cages with a different final collector device (Fig 4); The cones were placed in the W-E axis, replacing cylindrical PVC collectors with soft mesh tubular collectors which improved access to secure eggs. The N-S sides of the cages were covered just with the surface PVC tarpaulin.

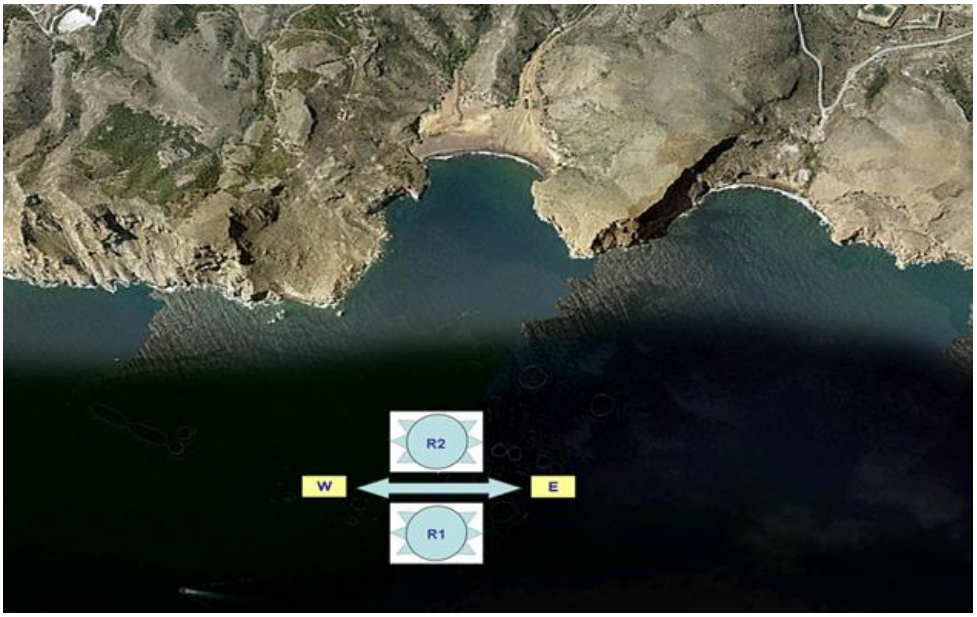

Figure 3: Prevalent currents and disposition of egg collectors in cages.

Figure 4: Improved final collectors used in 2010

Initially, a water temperature of 24ºC—considered optimal for ABFT spawning—occasioned the deployment of egg collectors. However, monitoring agencies in 2010 documented fish spawning at 20ºC in the Balearic Sea; thus, the decision was made for an early deployment (June 14th, 2010), even though water temperature had not yet reached 24ºC. Calm seas and lack of currents on those dates caused the majority of fertilized eggs to reach the water surface and a dip net (500 µm) was used to skim the eggs directly from the perimeter curtain with only a minor fraction of the eggs actually reached the collectors, as these require a weak current to be transported to the collection tube. As practically no eggs were collected inside the final collector that year, even in presence of stronger currents, in 2011 we decided to change the system to place only tarpaulins (Figure 5) to 6 m depth. This system was used practically unchanged during 2012 and 2013 spawning seasons.

Figure 5: PVC tarpaulin used in 2011

In 2009, the egg collector was placed just after induction of the fishes to spawn. In 2010, the collecting systems were placed in the cages on 14th – 16th June, when the mean water temperature exceeded 20ºC regularly (Fig. 13). From 2011 onwards, egg collector was placed in the first week of June.

After sabotage suffered in summer 2012, which implied the loss of the broodfish inside cage nº1, the company Caladeros del Mediterraneo, SL owner of the cages, decided to move the cage inside the same bay to a more watched but less sheltered and more exposed to currents area. The same collector was used.

In 2015, the collector was placed in cage nº3 in San Pedro. As the cage was larger only a half of the cage could be covered with the tarpaulin. This was placed in the direction of the predominant current, which are quite stable in this area. Besides the tarpaulin, the water area surrounding the cage was also checked every night for the presence of eggs.

In order to measure temperatures, some HOBOS pendant sensors were placed at three different depths (2m, surface; 10m, middle; 18 m, bottom) in the cages during the 2009 to 2011 period. From 2012 onwards, only surface temperatures were registered.

To measure the speed of the current, a current meter (Aquadopp profiler ® Nortek) was placed in the area between 2009 and 2010. During 2009 spawning season was situated out of the cages, but in 2010 a second device was also placed inside the cage, so that it was suppose that cage net could diminish the speed of the current when it went through the cage.

Spawning induction:

On the 26th and 27th of June 2009, 15 captive-reared ABFT from cage 1 were implanted with gonadotropin releasing hormone analog (GnRHa) at a dose of 6 mg GnRHa fish^{-1}. This procedure has been described in the REPRODOTT project report (Anon, 2007).

As natural spawning were obtained in 2010, 2011 and 2012, no hormonal induction techniques were used. In 2012, 6 tunas from cage nº 1 were hormonally induced early July to try to increase the number of spawned eggs.

At the end of the spawning season in 2012, cage nº 1 was sabotaged and all the tunas in this cage were lost, so during 2013, cage nº2 was the only one remaining.

Collection and transport of the eggs:

In order to collect the eggs, an auxiliary boat sailed every night to the cage facility. During night time (from 02:00 AM to dawn) they checked the cages for the presence and collection of eggs firstly from the tarpaulin helped with a small net device (see figure 5). Then, a diver checked all the egg collectors.

Netted fertilized eggs were placed in 20 or 500 l plastic tanks supplied with pure oxygen and transported by boat to Cartagena and then by van to the IEO facilities. Total transport time was about 2 hours, and density was always kept below 40,000 eggs/liter.

When they arrive and after cleaning and separating buoyant from non-buoyant, eggs were counted. A small sample of floating eggs was extracted to measure total diameter, oil globules and general appearance for quality assessment. Another sample was taken out to calculate hatching rates: 24 eggs were incubated during 24-36 hours in a 24 wells (2 ml capacity each) plate, and one egg was placed in each well.

In June 2009, a few thousands of newly fertilized ABFT eggs were collected from a cage in Balearic Sea. The cage had several hundred just caught ABFT adults and they spawned spontaneously during nights. Eggs were collected with a Bongo net (Gordoa *et al.*, 2009) and they were place in a 10 l. transport box with oxygen and then transported during 8 hours up to COMU. When arriving, eggs were measured to compare with eggs coming from cultured broodstock.

Sampling

As handling of tunas is problematic, it was decided to use non-invasive method for sampling. So, data from dead fish and a stereo video device named VICASS (Video Image Capture And Sizing System) was used to estimate individual total weight. This underwater stereo–video is a research tool that is capable of making measurements of fish length. The VICASS is a device composed of two submersible video cameras in underwater housings mounted on a steel frame as a stereo pair (Figure 6). During the sampling, the dual camera is placed in the cage and the system is linked to a portable computer. The operator captures images of fish at different depths. A computer program then calculates weight of individual fish based on fork length and girth measurements by means of algorithms. However, due to technical reasons, VICASS could not be used during all the experimental period.

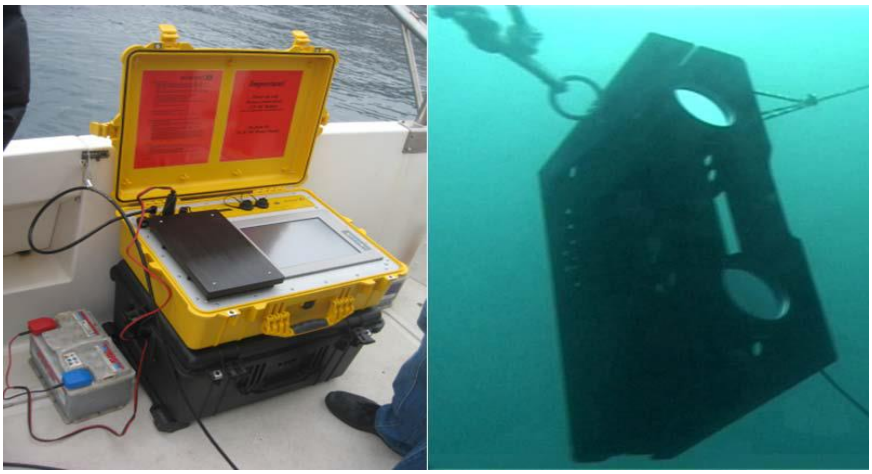

Figure 6: Computer and underwater stereo-camera of VICASS

RESULTS

Number and estimated average individual total weight of both broodstock are displayed in Tables I and II

Table 1: Cage 1 during 2008-2012 period

Date	Estimated Weight (kg)	Number	Spawning season
January 2008	55	37	-
January 2009	75	37	June 29th – July 17th
January 2010	100	35	June 20th – July 21st
January 2011	120-130	30	June 11th – July 11th
January 2012	150-160	28	June 8th – July 13th
July 2012	160-170	26	

Table 2: Cage 2 during 2009-2013 period.

Date	Estimated Weight (kg)	Number	Spawning season
January 2009	45	25	-
January 2010	65	24	June 20th – July 15st
January 2011	85-90	22	June 11th – July 11th
January 2012	110-120	20	June 8th – June 27th
January 2013	140-150	18	June 23rd – July 15th

On the 26th and 27th of June 2009, 15 captive-reared BFT from cage 1 were administered an implant loaded with gonadotropin releasing hormone analog (GnRHa) at a dose of 6 mg GnRHa $fish^{-1}$. Spawning in cage R1 began 48-72 h later, and massive spawning occurred, from the 29th of June every day for 17 days, with a daily maximum fecundity of 34 million eggs (Figure 7).

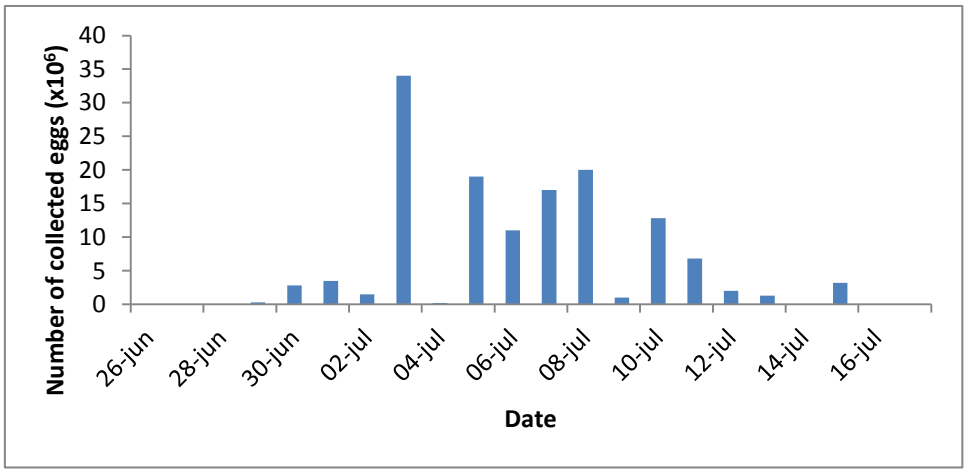

Figure 7: ABFT spawning and collected eggs in 2009 spawning season.

The water temperature ranged between 22 and 28ºC on the surface and between 19 and 27ºC at the bottom of the cage (Figure 8). Spawned eggs were observed from 0300 h until after sunrise.

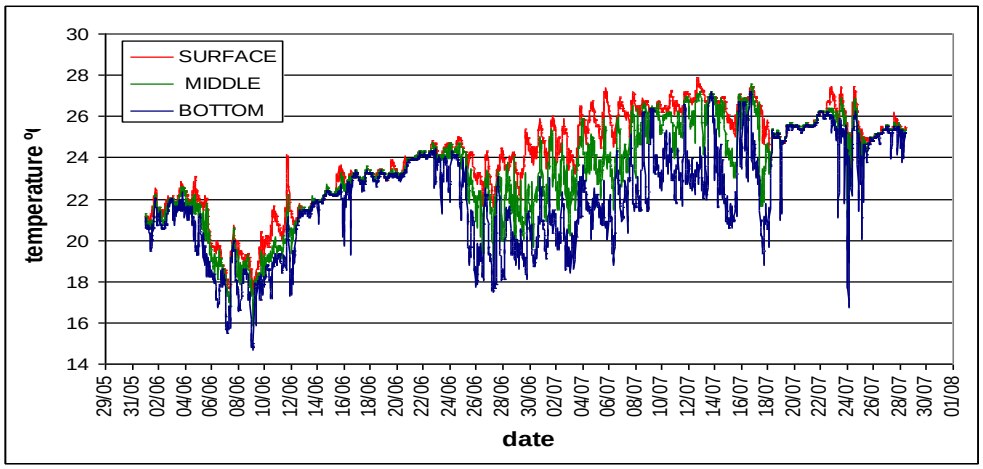

Figure 8: Water temperature in cages during 2009 spawning season

Due to the low currents throughout the spawning period (Figure 9), most of the eggs spawned were collected at the surface, held by the plastic tarpaulin. Only a small amount of eggs was captured by the egg collectors, which were designed to operate under current situations.

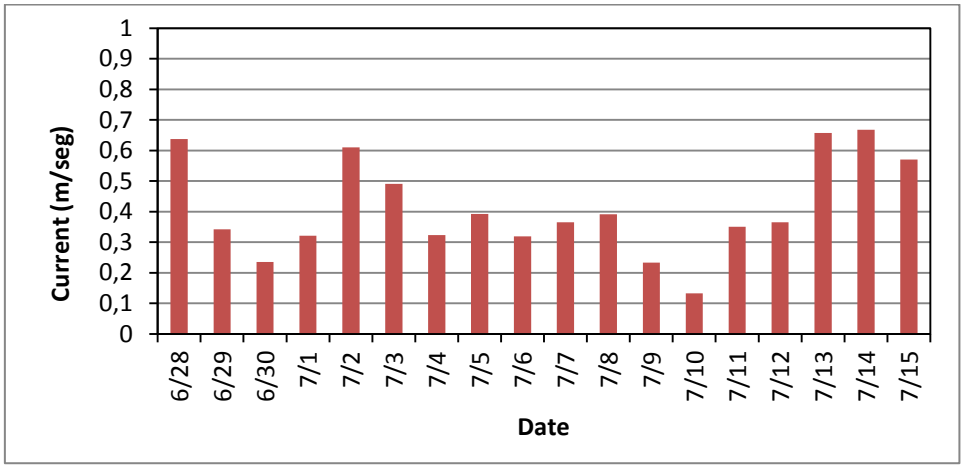

Figure 9: Mean current intensity values (m/s) at 7m depth between 03 h and 08 h AM.

Total eggs collected were 136 millions, with an average hatching rate of 84.4 % ± 13.5 %. Egg diameter was 1030.6 ± 15.7 µm, and egg dry weigh was 64.6 ± 4.2 µg. Both values diminished throughout the spawning season (figure 10). The number of oil globules was 1.27 ± 0.1 and without significant changes during spawning season.

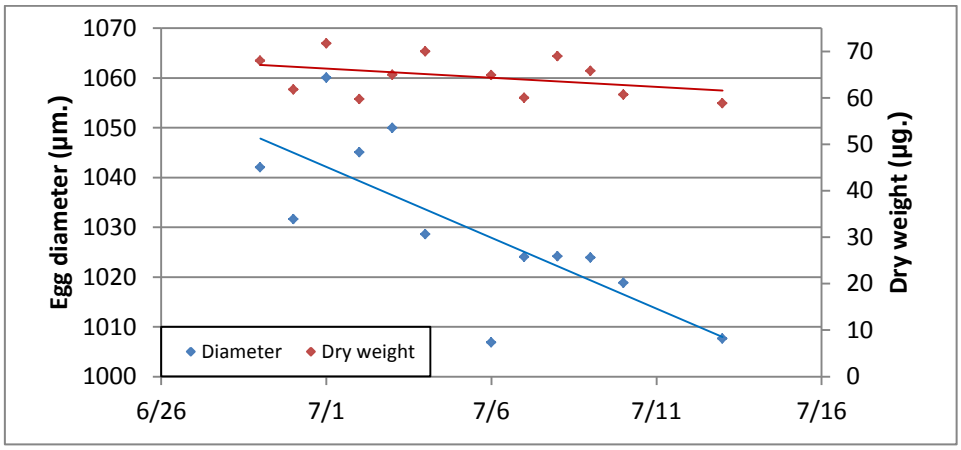

Figure 10: Egg diameter and dry weight throughout the spawning season.

In 2010 we placed the egg collector in the cages on the 16th of June. A day after, and in an unexpected way, we collected eggs from, apparently, a spontaneous spawn in cage R2. From this day onwards viable eggs were obtained, from the two cages, without the need to induce maturation by means of hormonal implants. Fishes spawned intermittently for a period of 34 days, collecting a total of 60 millions eggs, with more than 80% coming from cage nº 1. During two periods of strong northeast wind (between 24th and 26th June and between 3rd and 7th July) eggs could not be collected because of the rough seas.

The daily spawning in both cages are shown in Figure 11. As it happened in 2009, almost all the eggs were collected at the surface, held by the plastic PVC curtain. Only a small amount of eggs were captured by the egg collectors, which were designed to operate under stronger current conditions. Data from currents are shown in figure 12.

During the spawning period, the water temperature ranged between 21ºC and 27ºC (Fig 13).

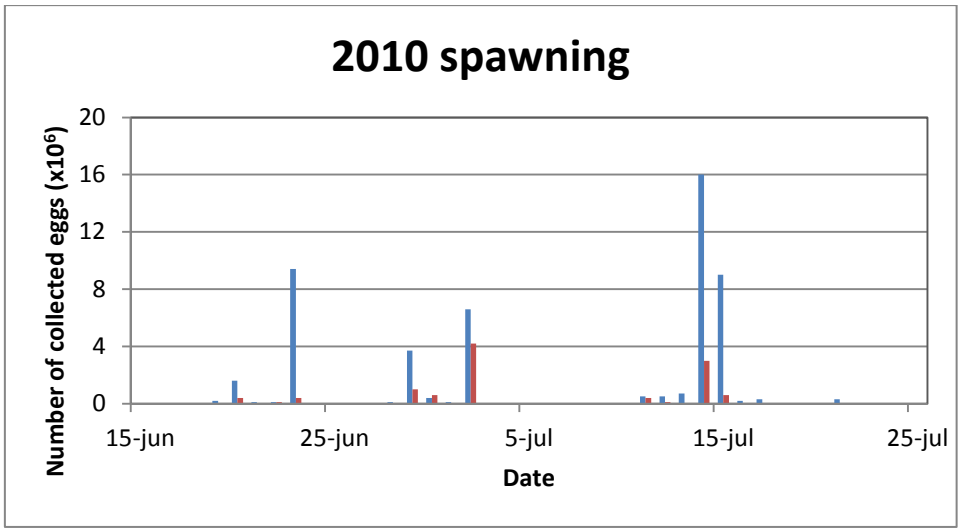

Figure 11: Eggs collected in 2010 from both cages

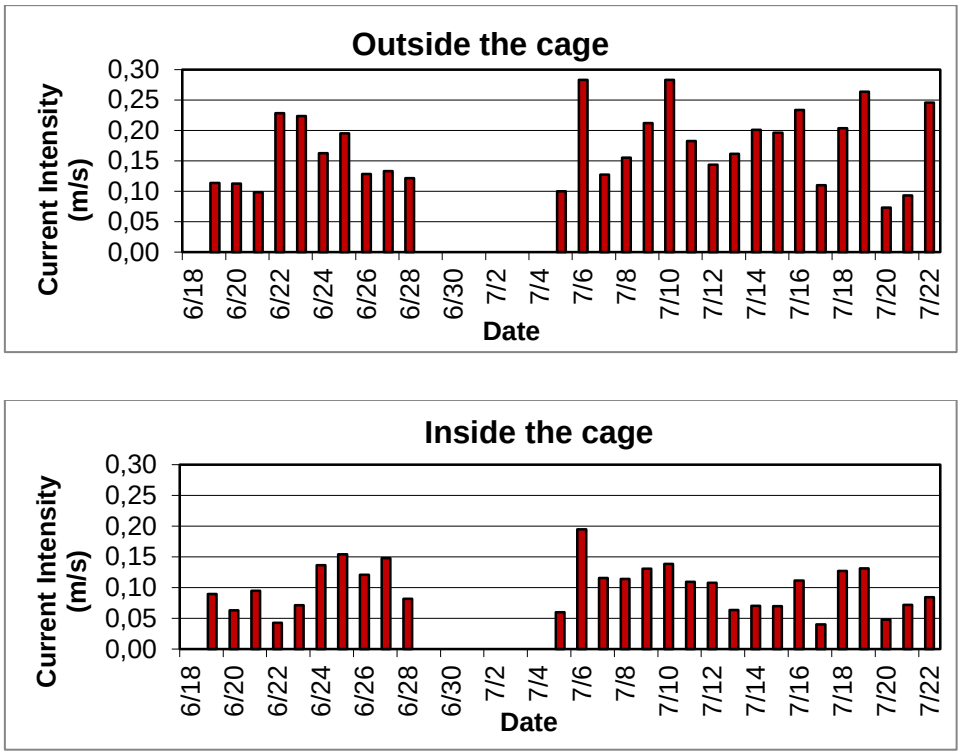

Figure 12: Mean current intensity (m/s) during all the day in the spawning area, outside and inside the cage. Due to a battery failure, from 29/06 to 05/07 current values couldn't be recorded.

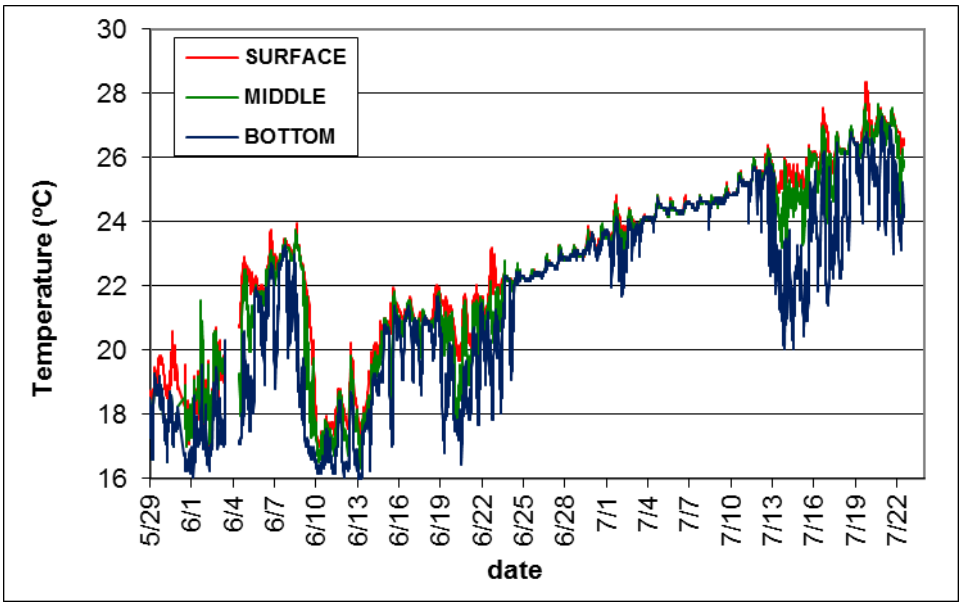

Figure 13: Water temperature during the spawning period in 2010.

Egg diameter was 1046.0 ± 16.5 in cage 1 and 1063.3 ± 19.4 in cage 2. Both values decreased throughout the season. Hatching rate was 74.5 ± 15.1 in cage nº 1 and 48.3 ± 16.5% in cage nº 2. Their evolution is showed in Figure 14.

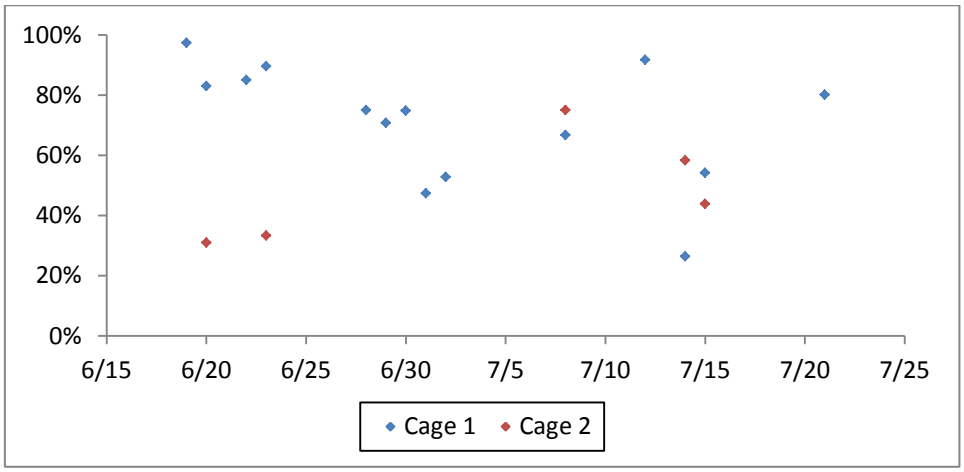

Figure 14: Evolution of hatching rate in eggs from both cages during 2010.

In 2011, it was the first time that was possible to exactly determine when spawning started, as we put the egg collecting system in place much earlier (2nd June). In previous years eggs were collected immediately after the collecting

system was set up, so existed the possibility that spawning had already started some days before.

Fertilized eggs were first collected one week after placing the egg collector, on 9th June 2011, and spawning continued for 37 days (Fig 15). The total number of eggs collected in cage R1 was 161 million and in cage R2 it was 18 million. It is necessary to point out that a technical problem caused that the complete egg collector system in cage 2 could not be set in place, so the total number of eggs collected was much lower due to many of the spawned eggs drifted out and away of the cage. Because of this problem, the number of eggs collected from cage R2 is not represented. During the spawning period, the water temperature ranged between 21ºC and 26ºC (Fig. 16). As occurred in 2009 and 2010, most of the time there were not strong currents, and only some strong winds from the East were reported between 30th June and 2nd July which prevented egg collection.

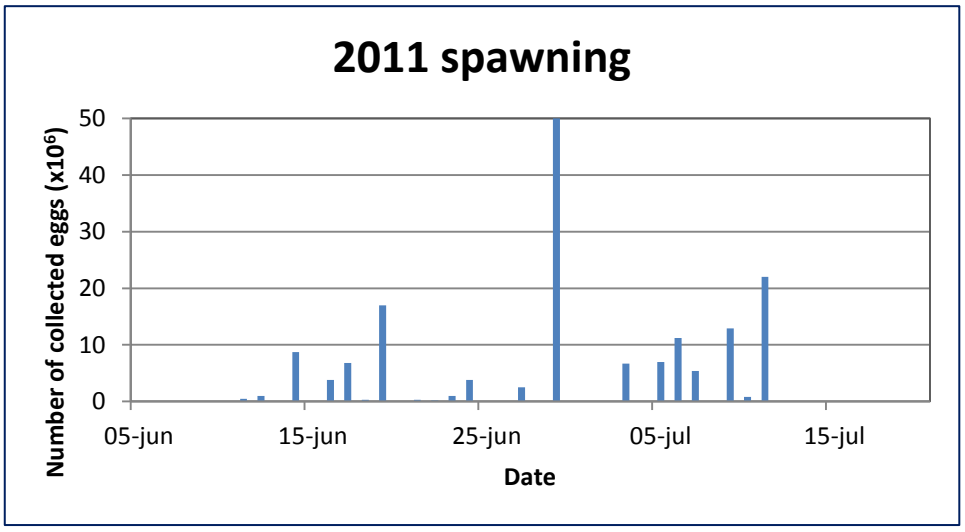

Figure 15: Daily fertilized egg collected during 2011 in Cage 1.

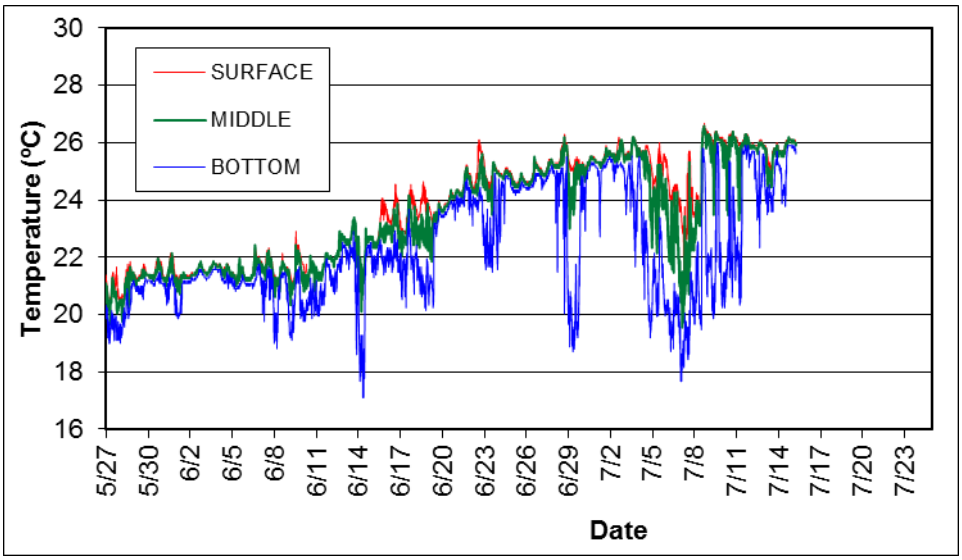

Figure 16: Water temperatures during the spawning period in 2011.

Hatching rates were very similar in both cages: 81.7 ± 12.1 % in cage 1 and 87.5 ± 10.2 % in cage 2.

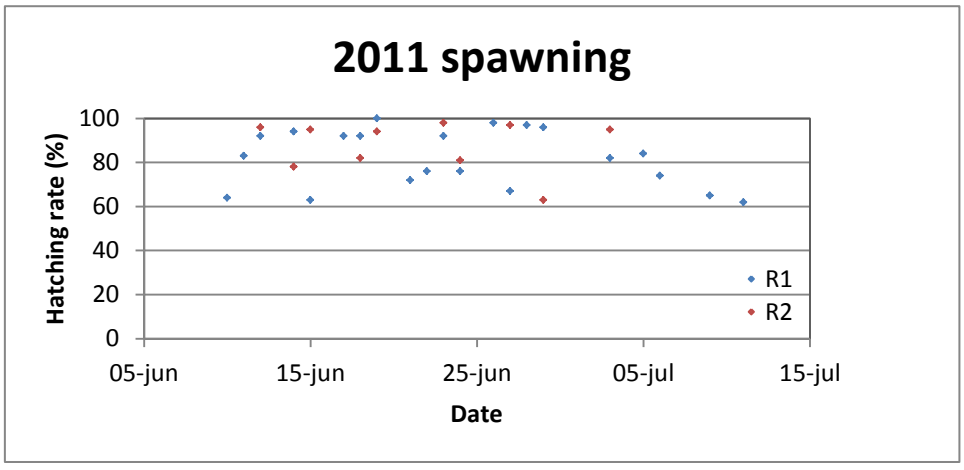

Figure 17: Hatching rates throughout 2011 season in both cages.

In 2012 season, first fertilized eggs were collected a couple of days after placing the egg collector, on the 8th of June, and last spawning was collected on the 13th of July, 35 days later (Fig 18). The total number of eggs collected in cage R1 was 39.2 million and in cage R2 it was only 9 million. It is important to point out that spawning practically finished on the 27th of June. From this moment, in

spite of six tunas from cage nº 1 were implanted on the 7th of July, as it had been done in 2009, only 600.000 eggs were collected.

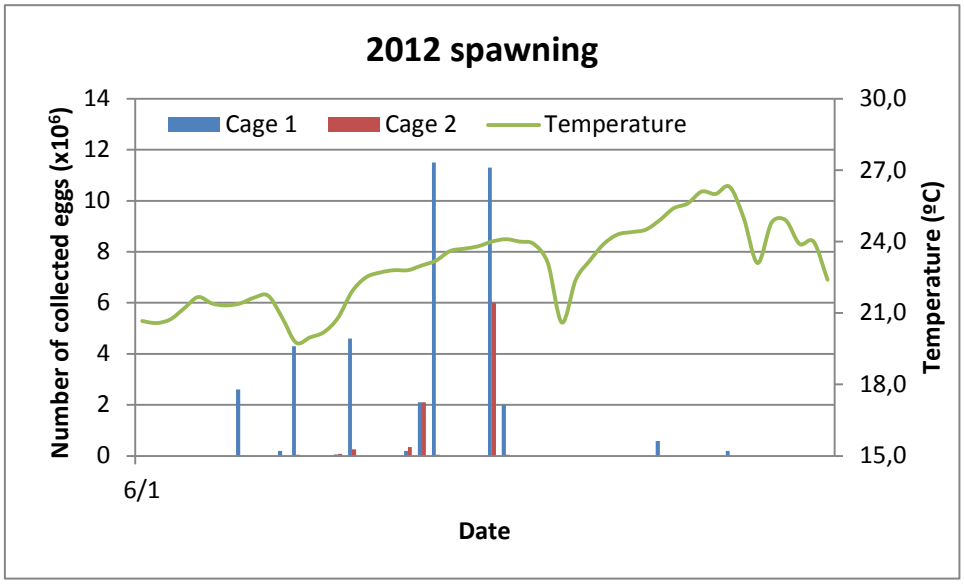

Figure 17: Temperature and number of eggs collected in both cages during 2012 season.

In 2012 a very sharp decrease in the temperature happened by the end of June. This temperature drop came accompanied with bad weather conditions and strong currents. During the spawning period, the water temperature ranged between 19.8ºC and 26.3ºC, and hatching rates were similar in both cages: 79.3 ±1 5.0 % and 74.5 ± 7.6 %, respectively in cages 1 and 2.

Spawning season in 2013 was delayed because of the unusual low temperatures registered in the area during June. Even when the egg collectors had been set in place at early June and checked every day, first spawning was not collected until the 23rd of June. Temperature was 21.5ºC

Total collected eggs only comprised 7.5 millions (figure 18), a lower number than those collected in 2011 and 2012 seasons. This could be due to a delay in the spawning season onset, but also to the stronger currents that hit the cages in the new location, which diverted the eggs out of the cages most of the days.

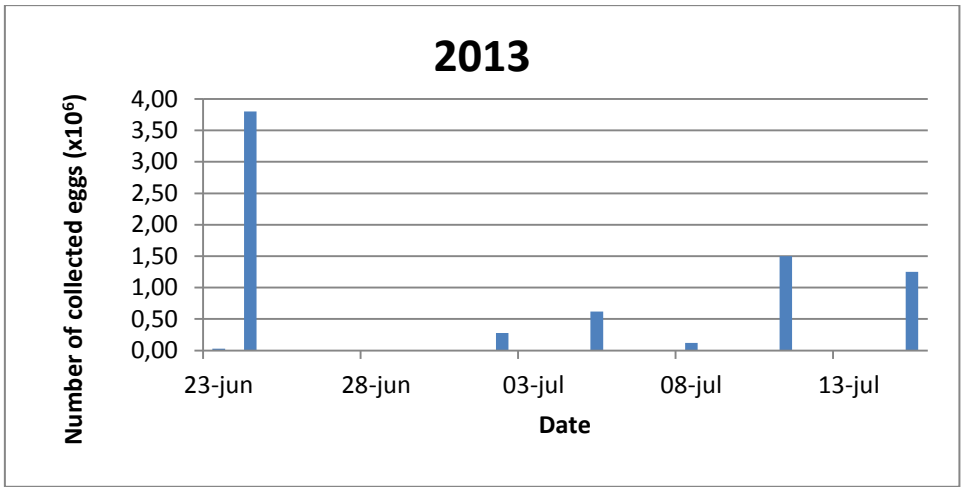

Figure 18: Number of eggs collected from cage 2 in 2013.

In 2015 season, egg collection started on the 1st of June. Eggs were collected from this very first day, so may be that tunas had started to spawn some days earlier. From this moment and due to bad weather conditions and a noticeable decrease in water temperature, eggs were not collected during ten days. From the 10th of June onwards, egg collection was resumed and it lasted until the 21st of July. Besides not only the tarpaulin did not cover the entire perimeter of the cage but also it got broken several times, and we had to collect the eggs in the water surrounding the cages. This means that surely we only caught a low amount of the released eggs. Data are showed in Figure 19

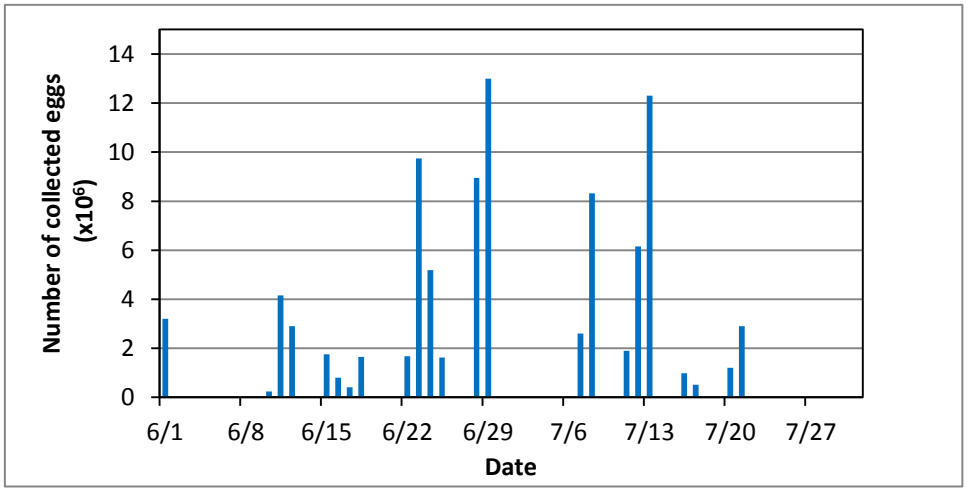

Fig 19: Number of eggs collected in 2015 spawning season.

With regard to feeding, the ration offered daily to broodstock lots was between 1 and 8% of the weight biomass, decreasing year by year with the growth of the tunas. In one year, as feeding ration is dependent on temperature, maximum intake used to be after spawning season, with a peak in August and a minimum on December-January. From this moment started to increase quickly and it decreased before the beginning of spawning season.

The following table shows some biometric characteristics and proximate composition of eggs coming from both cages. The only significant difference was the one related to fat content.

Table III. Biometric data and proximate gross composition of eggs coming from both broodstock in 2012.

	R1	R2
Diameter	1071.6 ± 22.8	1098.1 ± 20.6
Nº oil globules	1.00	1.03
Length of 0 DPH larvae	3846.2 ± 158.4	4042.7 ± 99.9
Hatching rate	87.8 ± 4.3	86.8 ± 5.3
Survival after 24 hours	83.9 ± 12.6	78.3 ± 9.2
Dry matter, %	9.6	8.4
Protein (% in dry mass)	63.8	63.5
Fat (% in dry mass)	19,9	27.3
Ash (% in dry mass)	10.6	11.3

Average diameters of fertilized eggs coming from both cages throughout the experimental period are shown in the table IV. Eggs coming from cage nº 2 were larger but not statistically significantly different. On other hand, eggs are larger as females grow; this tendency is clearer in cage 1, and there are significant differences in egg diameters between eggs spawned by smaller tunas (average 85 kg in 2009 spawning season) and larger ones (170 kg in 2012).

Table IV. Evolution of egg diameter throughout the experimental period.

	Cage 1	Cage 2	Cage 3
2009	1030,3 ± 15,7		
2010	1045,7 ± 17,0	1060,5±19,6	
2011	1056,7 ± 14,0	1064,0±17,4	
2012	1061,6 ± 9,8	1075,4±11,2	
2013		1071,5±20,8	
2015			1059.7±14.9
Mean	1048,6	1067,85	
Standard deviation	12,0	5,9	

A comparison between two batches of eggs collected same week in June 2009 from wild broodfish and cultured broodfish is shown in Table V

Table V: Biometric parameters of eggs coming from wild and cultured broodstock

	Wild eggs	Cultured eggs
Diameter (µm.)	1025.4±23.2	1027.4±18.1
Wet weight (mg)	0.81±0.09	0.79±0.17
Dry weight (µg)	65.2±5.0	63.4±4.2
Number of oil globules	1.23±0.52	1.21±0.51
Hatching rate	65.0 ± nd	84.4 ± 13.5

Discussion

The reduction in the captures of Atlantic bluefin tuna from 2006, has made to redoubled the efforts to control reproduction under controlled conditions of this species. So, during the last years, several research projects (Selfdott, Allotuna, Transdott) and companies (Caladeros del Mediterraneo, Malta Fish Farming, Mare Nostro Farm, Kalituna, Kilic and Ballfego Group) have dealt with reproduction in captivity of ABFT. All of these efforts have been developed in cages in the sea, and one of the main problems has been the collection of the spawned eggs.

We have used a special collector design to operate under medium current conditions and with a vertical surface of tarpaulin to keep the eggs when currents are not so strong. In the first location of the cages (2009 to 2011) we were capable to collect eggs during most of the days. If we compare the current inside and outside the cages, we can observe that the reduction in the current speed is about 40%. So, when currents are about 20 cm/seg. outside the cages, it seems that the tarpaulin sheet operates adequately. However, when currents are stronger the number of collected eggs is quite lower either in the collection cones used in 2009 and 2010 or in the tarpaulin panel. This also happened in Malta Fish Farming facilities were currents were stronger (ANON, 2010 and 2012). In these conditions, it is advisable that special devices should be placed out of the cage to collect the eggs.

ABFT is a gonochoric species with an asynchronous development of the gonads. According to Bagli (1982), is a multiple spawner, with a spawning frequency of 1.2 (Medina *et al.*, 2002) days. In the Balearic Island, reproduction starts at the end of May or beginning of June, with a maximum between the 15th and the 30th of June (Gordoa & Carreras, 2014). Spawning starts when temperatures reached 19-20ºC y it last until middle of July. Batch fecundity is estimated to be 45.6 eggs/g (Knapp *et al.* 2014), and most of females become mature when they are 4 o 5 years old (Corriero *et al.*, 2005)

PBFT has a spawning frequency of 2 – 4.5 days and spawning season also takes about 45 days (Chen *et al.* 2006). Miyashita *et al.*, (2000) also consider a length of the spawning season of 45 days, but further studies carried out in captivity by Masuma (2006a) revealed that although spawning extended between June and July, sometimes could start in May and finish in November. A single female could spawn eggs several consecutive days and then take a break (sometimes more than a month) before start a new spawning batch, being able to spawn closely to 20 times per season.

With regard to temperature, 23-24ºC it was noticed to be as a threshold to onset spawning (Block *et al.*, 2001; Corriero *et al.*, 2007). But recent studies have demonstrated that ABFT is able to spawn at temperatures below 23ºC; Alemany (2010) got fertilized eggs from 20.5ºC and Gordoa & Carreras (2014) also got eggs from tuna cages in the Balearic Island at 19ºC. In this study, the onset of spawning activity happened at 21ºC in 2010 and 2011, 21.5ºC in 2012 and 2015. But since we did not try to collect eggs in May, there are no evidences on spawning events occurring below 21ºC in our cages.

With regard to the end of the spawning season, it seems to be determined by temperature in most of the tunas. YFT is a tropical species which spawns between 24 and 30ºC. In Achotines laboratory, YFT stop to spawn when temperatures go below 24ºC, so except for 3 or 4 months, they are spawning during all the year round (Wexler *et al.*, 2003). In PBFT and ABFT temperatures also determine spawning season, but their duration is quite shorter. PBFT spawns between 21.5 and 29ºC in Amami and Kushimoto, Japan (Miyashita, 2002; Masuma *et al.*, 2006b). Above this temperature do not spawn. In a similar way, ABFT spawns between 19 and 26ºC. In both cases, minimum temperature is related with the minimum temperature required by the eggs to hatch. However, maximum temperatures cannot be considered as the maximum temperatures for hatching, so that both species have fine hatching rate at those temperatures (see chapter 2.5.).

It has been suggested that high temperatures are favorable for the development of early life stages but physiologically stressful for the adults (Block *et al.*, 2005). If we had to explain the spawning temperature because of offspring survival, minimum temperatures should be higher and maximum temperatures should be kept or to be a bit higher. But taken into consideration the suggestion of Block *et al.*, (2005), the condition of broodstock could be the most important aspect to determine spawning season.

According to the data of ABFT, a female of 100-150 kg would be able to spawn between 4.5 and 7 million eggs per spawning event (Gordoa & Carreras, 2014), and should spawn several times during the reproductive season. So, the number of collected eggs in this experiment has not really been high enough. There is not a clear explanation of this. Even when sex ratio could not have been well balanced and some females could have not contributed every year and skip some spawning season (Miyashita *et al.*, 2000, reported this situation for PBFT spawners). A more plausible explanation could be related to the high loss of the spawned eggs by the standard collection procedures. In 2015, even when currents were low, a lot of eggs were also collected outside of the cage. So, even when broodstock lots would have spawned a few hundred millions of eggs, only a small fraction could have been collected in the actual experimental

conditions. A similar hypothesis was contemplated in Malta during the Selfdott project (Anon, 2012).

Results were even worse in R2 cage. A lower number of fishes with greater size dispersion could have been the cause that very few females were spawning eggs. When comparing proximate gross composition of eggs coming from both cages are quite similar with the exception of fat content. This could be due to a higher fat content of the bloodstock diet given to lot 2.

In 2009 tunas only spawned during for short period of time. This has also been described by Corriero *et al.*, (2010), with only 5 days of spawning activity. It seems than GnRHa implants produce shorten spawning seasons, perhaps due to the stress produced to the breeders at a very critical period, although this point is not clear enough. In any case, from 2010 onwards, natural spontaneous spawning events occurred and hormonal implants were not used any more longer. In the 2012 season, egg collection practically finished on the 27th of June. The main reason why fishes stopped spawning and egg collection was detained could have been a sharp decrease by 4ºC in the water temperature at the end of June, which is in agreement with the results of Gordoa & Carreras in 2014, who founded that after storms which caused a decrease in the water temperature, ABFT stopped spawning for several days. So, after ten days without collecting eggs at the beginning of July 2012, we decide to induce six tunas, but only a very low amount of eggs were obtained the following days after hormonal implanting.

Gordoa (2010) founded spontaneous spawning during June 2009 in two broodstock groups: one composed by just recently fished tunas, and another one composed by tunas kept in captivity during one year and then moved to the Balearic Islands area when reproductive season was approaching. In the present study we also achieved to collect eggs without hormonal induction from 2010 onwards, in this case out of their spawning area. These spontaneous spawns have also been reported in Japan for PBFT (Miyashita *et al.*, 2000; Masuma, 2006a).

Spawning occurred during the night, between 2:00 and 5:30 h A.M. (personal observation). At the beginning of the season, spawning started between 2:00 and 3:00 h A.M. and as the spawning season progressed, spawning event time delayed, mainly in cages 1 and 2, and fertilized eggs were not observed and collected until about 5:00 am (this fact could not be confirmed in cage 3). This is in agreement with Gordoa & Carreras (2014), who reported that ABFT is the only tuna species reported to spawn in full darkness. Miyashita *et al.*, 2000 and Masuma, 2006a, in PBFT and Margullies *et al.*, 2007 in yellowfin tuna, reported that these species spawned at sunset. These authors also reported the tendency in both species to change the time of spawning according to the water temperature. When temperature rose, spawning time was delayed. This is in

agreement with our results, since water temperature increased during the spawning season and spawn time delayed as spawning season progressed. According to Margullies *et al.,* (2007) this could be an strategy to hatching of the eggs at the same day time, but this could not be the only reason in bluefin tuna, so that an increase from 22 to 26ºC in the water temperature can cause an advance of 10-12 hours in the embryonic development and the time of hatching (see chapter 2.4.) which is not compensated by a 2-3 hours delay in spawning time.

With regard to egg diameter, an increase throughout continuos spawning seasons can be observed. This happened in both cages, but mainly in cage nº1. In this cage, significant differences ($p<0.05$) were detected when comparing results along 2010 and 2013. This has also been reported for other fish species, as stated by Bromage (1995).

There is an inverse relationship between egg diameter and temperature (Figure 20). This has been also reported for other tuna species like PBFT (Miyashita *et al.*, 2000; Masuma *et al.*, 2006b) and yellowfin tuna (Margullies *et al.*, 2007). Smaller size eggs developed normally, and no decrease in hatching rates has been reported. But according to Shirota (1970), the size of the hatching yolk sac larvae and first feeding larvae is directly related to the egg size and this is probably advantageous for the survival of the larvae (Hunter, 1981). However, since we have only measured just hatched larvae from a few batches, we have not been able to prove this fact in ABFT.

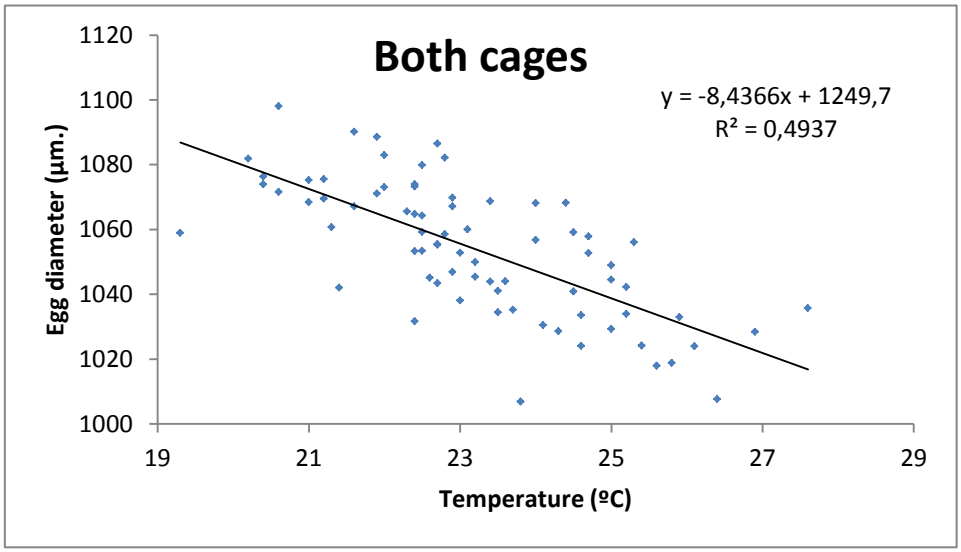

Figure 20: Relationship between egg diameter and temperature in ABFT.

There is not any significant difference between parameters measured in ABFT eggs coming from wild or cultured broodstock. Different in hatching rate could

be due to the differences in collection and transport. Wild eggs suffered additional stress with the collection with Bongo nets and with a larger transport time.

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CAPITULO 2:

Desarrollo temprano y cultivo larvario

Early development and larval rearing

2.1. Comparison of the lipid profiles from wild caught eggs and unfed larvae of two scombroid fish: northern Bluefin tuna (*Thunnus thynnus* L., 1758) and Atlantic bonito (*Sarda sarda* Bloch, 1793)

Aurelio Ortega and Gabriel Mourente

Fish Physiol. Biochem. (2010) 36, 461-471.

Resumen

Comparación de los perfiles de lípidos de huevos obtenidos de individuos salvajes y larvas mantenidas en ayuno de dos especies de escómbridos: Atún rojo del Atlántico (*Thunnus thynnus* L., 1758) y Bonito Atlantico (*Sarda sarda* Bloch, 1793)

Los lípidos y los ácidos grasos esenciales son determinantes de los procesos reproductivos en peces marinos, afectando la fecundidad, calidad de los huevos, rendimientos de la eclosión, pigmentación y malformaciones larvarias. Se han analizado y caracterizado los lípidos de los huevos y de las larvas mantenidas en ayuno de dos escómbridos capturados del medio, el Atún rojo del Atlántico (*Thunnus thynnus*) y el Bonito Atlantico (*Sarda sarda*). La materia seca y el contenido total en lípidos, las clases de lípidos, totales y neutros, y los contenidos de ácidos grasos fueron determinados en huevos de atún rojo del Atlántico y huevos y larvas mantenidas en ayunas durante el desarrollo de Bonito Atlántico. Los huevos de atún rojo tenían valores ligera pero significativamente mayores en materia seca que los huevos de bonito, pero mostraban un contenido lipídico muy similar. Sin embargo los huevos de atún rojo presentaban un mayor contenido en lípidos polares debido a las mayores proporciones de fosfatidiletanolamina (PE), fosfaditil serina (PS) y fostaditilinositol (PI). Los huevos y larvas de bonito presentaron un incremento en materia seca y un descenso en el contenido lipídico a lo largo del desarrollo. La proporción de lípidos polares se incrementó debido al incremento de PE, PS y PI, mientras que los lípidos polares que contenían colina (fosfaditilcolina y esfingomielina) permanecían relativamente constantes. El colesterol libre también se incrementó, mientras que los niveles de otros lípidos neutros, especialmente las fracciones de triglicéridos y de ésteres de esterol, decrecían

presumiblemente debido a su utilización como energía para llevar a cabo el desarrollo. Los huevos de atún rojo tenían niveles mas altos de ácidos grasos altamente insaturados del tipo n-3 y n-6 debido respectivamente a los mayores contenidos en ácido docosahexanoico y araquidónico, que los huevos de bonito. Se discuten los resultados en relación a los requerimientos de lípidos y ácidos grasos de las larvas de escómbridos en relación a otras larvas de peces marinos en condiciones de cultivo.

Palabras clave: ácidos grasos, atún rojo, bonito, clases de lípidos, huevos, larvas.

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Comparison of the lipid profiles from wild caught eggs and unfed larvae of two scombroid fish: northern bluefin tuna (*Thunnus thynnus* L., 1758) and Atlantic bonito (*Sarda sarda* Bloch, 1793)

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Abstract Lipids and essential fatty acids are determinants of the reproductive process in marine fish, affecting fecundity, egg quality, hatching performance, pigmentation and larval malformation. We have analyzed and characterized the lipids of eggs and unfed larvae of two wild caught scombroid fish, the Atlantic northern bluefin tuna (*Thunnus thynnus*) and Atlantic bonito (*Sarda sarda*). Dry matter and total lipid contents, polar and neutral lipid classes and total lipid fatty acid contents were determined in the eggs of bluefin tuna and eggs and unfed larvae during the development of Atlantic bonito. Bluefin tuna eggs had slightly but significantly more dry mass than bonito eggs but very similar lipid content. However, bluefin tuna eggs presented a higher polar lipid content due to increased proportions of phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI). Bonito eggs and larvae showed increasing dry mass and decreasing lipid content with development. The proportion of polar lipids increased due to increased PE, PS and PI, whereas choline-containing polar lipids (phosphatidylcholine and sphingomyelin) remained relatively constant. Free cholesterol also increased, whereas the levels of other neutral lipids, especially triacylglycerol and steryl ester fractions, decreased, presumably due to utilization for energy to drive development. Bluefin tuna eggs had higher levels of $n-3$ and $n-6$ highly unsaturated fatty acids due to higher docosahexaenoic and arachidonic acid contents, respectively, than bonito eggs. The results are discussed in relation to the lipid and fatty acid requirements of larval scombroid fish in comparison to those of other larval marine finfish species under culture conditions.

Keywords Bluefin tuna · Bonito · Eggs · Fatty acids · Larvae · Lipid classes

Abbreviations

ARA	All-*cis*-5,8,11,14-eicosatetraenoic acid (arachidonic acid, 20:4$n-6$)
BFT	Bluefin tuna (*Thunnus thynnus*)
C	Free cholesterol
CL	Cardiolipins
DHA	All-*cis*-4,7,10,13,16,19-docosahexaenoic acid (22:6$n-3$)
EPA	All-*cis*-5,8,11,14,17-eicosapentaenoic acid (20:5$n-3$)
FFA	Free fatty acid
HPTLC	High-performance thin-layer chromatography

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HUFA	Highly unsaturated fatty acids ($\geq C_{20}$ and with ≥ 3 double bonds)
LA	Linoleic acid
LNA	Linolenic acid
PA	Phosphatidic acid
PBT	Pacific bluefin tuna (*Thunnus orientalis*)
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PI	Phosphatidylinositol
PS	Phosphatidylserine
PUFA	Polyunsaturated fatty acid(s)
SE	Steryl ester
TL	Total lipids
TNL	Total neutral lipids
TPL	Total polar lipids
WE	Wax ester

Introduction

The composition of the broodstock diet and in particular its lipid composition is believed to have profound effects on the reproduction and egg quality of marine fish (Watanabe 1985; Izquierdo et al. 2001). The importance of lipids for the growth and development of fish is based on their key roles as sources of metabolic energy and as essential components of tissue and cell membranes. In most fish species, lipid is the preferred energy source in comparison to protein and carbohydrate and is also characterized by its high content of the long chain highly unsaturated fatty acids (HUFA) that are essential for cell membrane structure and function. Fish also show seasonal variations in lipid levels that are related to the reproductive cycle, accumulating large lipid deposits prior to gonad development (Sargent 1995; Wiegand 1996; Bell 1998; Sargent et al. 2002). Lipid reserves in teleost fish eggs are stored as lipoprotein in yolk reserves and, in some species, as an oil globule(s). Yolk lipoproteins contain primarily polar lipids (PL), mainly in the form of phosphatidylcholine (PC) and phosphatidylethanolamine (PE), with high levels of $n-3$ polyunsaturated fatty acids (PUFA), particularly the $n-3$ HUFA eicosapentaenoic acid (20:5$n-3$; EPA) and docosahexaenoic acid (22:6$n-3$; DHA). Eggs can contain a single discrete oil globule or several fragmented droplets; in both cases, these contain neutral lipids (NL), primarily triacylglycerol (TAG), steryl ester (SE), and/or wax ester (WE), which are rich in both monounsaturated fatty acids (MUFA) and $n-3$ PUFA (Wiegand 1996).

The lipid reserves of fish eggs are used by the developing embryo and subsequent larvae as substrates for energy metabolism, structural components of biomembranes, and precursors of lipid-derived hormones (Sargent 1995; Wiegand 1996; Sargent et al. 1989, 2002). It has been well demonstrated that the lipid content and composition of broodstock fish can consequently influence the lipid content and composition of the eggs and embryos produced, affecting hatching, larval development, growth, and survival (Sargent et al. 1989, 2002; Sargent 1995; Rainuzzo et al. 1997). Lipid is delivered to the oocyte in the form of lipoproteins, particularly vitellogenin, which is rich in polar lipids and $n-3$ HUFA, and very low-density lipoproteins rich in neutral lipids, particularly TAG (Sargent 1995; Wiegand 1996; Sargent et al. 2002). All lipid classes, polar and neutral, can be used as energy fuel during embryogenesis and larval development. Some cold water fish species, such as Gadiformes (cod, haddock, saithe, or whiting), produce eggs with no oil globule and consume primarily PL (PC and PE) as energy substrates, while temperate and warm water species, such as Pleuronectiformes (turbot, sole) and Perciformes (sand eel, mullet, red drum, sea bass, and sea breams), produce eggs with oil globule(s) consisting of NL (TAG and SE/WE) that are used as the primary energy source (Wiegand 1996; Sargent et al. 2002). Thus, eggs of the former group have a high content of polar lipids [>60% of total lipids (TL)] that are used partly as fuel for energy and partly as structural components of biomembranes whereas, in contrast, the eggs of the latter group have a high content of NL (>60% of TL), which mostly comprise energetic lipid reserves, although the fatty acids can also be structural components. Moreover, the egg lipid class composition, either polar or neutral, varies considerably among fish species, and studies of their fatty acid composition have shown that PL are richer in $n-3$ HUFA, arachidonic acid (20:4$n-6$; ARA), and saturated fatty acids, whereas NL are richer in MUFA (Wiegand 1996; Sargent et al. 2002). Thus, the

Note: PC and PE represent diradyl (diacyl + alkenilacyl + alkylacyl) glycerophosphocholine and glycerophosphoethanolamine, respectively.

essential fatty acids vital for early survival and development of newly hatched larvae in both wild and farmed fish, are directly derived from the broodstock lipid reserves built up in the period preceding gonadogenesis (Sargent 1995; Bell 1998; Sargent et al. 2002).

Data obtained from studied of the lipid content, lipid class composition, and fatty acid composition of fish eggs have provided important nutritional clues not only on the lipid requirements of the early developing larvae but also on their influence on the quality, performance, and survival of wild and farmed fish species (Sargent et al. 2002; Cejas et al. 2004; Salze et al. 2005; Gimenez et al. 2006). Within the Perciformes, Scombridae fish include a number of species of great commercial value, such as bonitos and tunas. In recent years, high market prices and consequent overfishing—mainly due to high market demand—considerable research efforts have been undertaken to develop a viable aquaculture protocol for these large pelagic migratory fish species (Wexler et al. 2003; Margulies et al. 2007; Masuma et al. 2008). In this context, we have studied and characterized the lipid content, lipid class composition, and total lipid fatty acid composition of fertilized eggs and of 20- and 60-h posthatch unfed larvae of wild captured Atlantic bonito (*Sarda sarda* Bloch, 1793) and fresh fully ripe eggs of the Atlantic bluefin tuna (*Thunnus thynnus* L., 1758; hereafter BFT). Our results were compared to those from other fish species, and the metabolic utilization of lipids in tunids during early development is discussed accordingly.

Materials and methods

Sample collection

Eggs from Atlantic BFT were collected on board a tuna purse seiner operating in the Mediterranean Sea close to the Balearic Islands in mid-July 2005. The eggs were extracted by evisceration and dissection of the ovary from a fully mature BFT female that died during fishing operations. The eggs were hydrated and fully ripe, with an average diameter of 1 mm, and contained a single oil globule about 0.2 mm in diameter. The BFT eggs were rinsed in distilled water and blotted on filter paper before being frozen in liquid nitrogen. Wild mature Atlantic bonito were captured in a tuna trap near Tiñoso Cape, La Azohía, Murcia (southeastern Spain). About 30 fish were transported to the aquaculture facilities of the Instituto Español de Oceanografía (IEO) in Mazarrón, Murcia southeastern Spain. The fish were stripped, and hydrated eggs from one female were fertilized with the sperm of two males under dry conditions. After 10 min, sea water at 21°C was added to the container. The first cleavage cells were observed 2 h after artificial fertilization; about 60% of the eggs were fertilized and subsequently separated from non-viable eggs by flotation. A total of 21,900 viable eggs were obtained. These were incubated in a 400-l cylindro-conical tank containing sea water under conditions of 21°C, 38‰ s.u., and continuous illumination (intensity 100 lux). A sample of fertilized eggs was collected in an appropriately sized mesh screen (500 μm), rinsed in distilled water, and blotted on filter paper before being frozen in liquid nitrogen and stored at −80°C until analysis. The remaining eggs were allowed to hatch, about 50 h after fertilization, and the same treatment was used for sampling unfed larvae at 20 and 60 h posthatch.

Materials

Potassium bicarbonate, potassium chloride, butylated hydroxytoluene (BHT), and tricosanoic acid (>99% pure) were from Sigma Chemical (St. Louis, MO). The high-performance thin-layer chromatography (HPTLC) (10 × 10 cm × 0.15 mm) and TLC (20 × 20 cm × 0.25 mm) plates precoated with silica gel 60 (without fluorescent indicator) were obtained from Merck (Darmstadt, Germany). Glacial acetic acid, sulphuric acid, and all solvents (HPLC grade) were purchased from Fluka Chemicals (Deisenhofen, Germany).

Dry weight and biometric values

Replicates of pre-weighed samples (approximately 50 mg wet weight) were maintained at 110°C for 24 h. The dry weights were determined after cooling in vacuo for at least 1 h. Individual dry weight was determined by weighing counted individual eggs or larvae ($n = 15$–30) on previously tared glass slides (triplicates) after maintaining the samples in the oven

at the conditions described above. Biometric determinations were carried out by micrometric analysis under light microscopy.

Total lipid extraction, lipid class separation, and quantification

Total lipid was extracted from eggs and larvae samples by homogenization in 20 volumes of ice-cold chloroform/methanol (2:1, v/v) containing 0.01% BHT as antioxidant, basically according to the method of Folch et al. (1957), and non-lipid impurities were washed with 0.88% KCl. The amount of lipid extracted was determined gravimetrically after solvent evaporation under a stream of oxygen-free nitrogen and desiccation in vacuo overnight. Lipid classes were separated by HPTLC on silica gel 60 plates using a single-dimension double-development method described previously (Henderson and Tocher 1992). The classes were quantified by charring (Fewster et al. 1969) followed by calibrated densitometry using a Shimadzu CS-9001PC dual-wavelength flying spot scanner (Olsen and Henderson 1989). The identification of individual lipid classes was confirmed by comparison with reference to the Rf values of authentic standards run and developed under the same chromatographic conditions.

Total lipid fatty acid analyses

Fatty acid methyl esters (FAME) from TL were prepared by acid-catalyzed transmethylation for 16 h at 50°C, using tricosanoic acid (23:0) as the internal standard (Christie 1989). The FAME were extracted and purified as described previously (Mourente and Tocher 1994) and were separated in a HP 5890A series II gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a chemically bonded (PEG) Supelcowax-10 fused silica wall-coated capillary column (30 m × 0.32 mm inner diameter; Supelco, Bellefonte, PA), an "on column" injection system, and flame ionization detection. Hydrogen was used as the carrier gas with an oven thermal gradient ranging from an initial 50–180°C at 25°C per min to a final temperature of 235°C at 3°C per min. The final temperature was maintained for 10 min. Individual FAME were identified by comparison with known standards and by reference to published data (Tocher and Harvie 1988). Data were collected and processed by means of a direct-linked PC and Hewlett–Packard ChemStation software.

Statistical analysis

Results are presented as means ± standard deviation (SD) ($n = 3$ or 4). The data were checked for homogeneity of the variances by the Bartlett test and, where necessary, arc-sin transformed before further statistical analysis. Differences between mean values were analyzed by one-way analysis of variance (ANOVA) followed, when pertinent, by a multiple comparison test (Tukey). Differences were reported as statistically significant when $P < 0.05$ (Zar 1984).

Results

The water content of BFT and bonito eggs was around 93%. Relative to bonito eggs, those of BFT had slightly but significantly more dry mass but a similar lipid content, about 20% of the dry mass. However, BFT eggs had a higher TPL content due to a higher PC content, with concomitant lower total neutral lipid (TNL) content due to a lower TAG content. The content of other polar classes, such as PE, phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidic acid/cardiolipin (PA/CL), sulfatides, cerebrosides, sphingomyelin and pigments, and neutral classes, such as free cholesterol (C) and free fatty acids (FFA), were identical in BFT egg and bonito egg lipids (Table 1). In contrast, the SE/WE fraction was significantly higher in BFT eggs than in bonito eggs.

Unfed bonito larvae 20 h posthatch had the same dry matter content as the egg, but 27.7% less than that of unfed bonito larvae 60 h posthatch. The TL content decreased by 7.7% from the bonito egg to unfed larvae 20 h posthatch and by 5.6% from 20 to 60 h posthatch unfed larvae. A net significant decrease of 13.3% in the TL content occurred from the bonito egg to 60 h posthatch unfed larvae (Table 1). The proportion of TPL in bonito eggs increased significantly, by 10.8%, in 20-h posthatch larvae and by 18.3% in 60-h posthatch larvae. A significant increase in TPL, 27.1%, occurred from the developing egg to 60-h posthatch unfed larvae. This was mainly due to significant increases in PE (47.9%), PS (75.5%), and PI (57.6%). However, the

Table 1 Dry mass percentage, total lipid content (as dry mass %) and lipid classes (as total lipid %) from eggs of *Thunnus thynnus* L. and eggs and yolk sac stages larvae from *Sarda sarda* L.

	Tuna egg	Sarda egg	Sarda larvae 20 h	Sarda larvae 60 h
Dry mass (%)	7.8 ± 0.2 [a]	7.2 ± 0.2 [b]	7.3 ± 0.3 [ab]	10.1 ± 0.4 [c]
Total lipid (dry mass %)	19.5 ± 1.2 [a]	19.4 ± 0.2 [a]	17.9 ± 1.7 [ab]	16.9 ± 1.2 [b]
Lipid classes (%)				
Sphingomyelin	1.1 ± 0.1	1.2 ± 0.4	0.8 ± 0.1	1.1 ± 0.1
Phosphatidylcholine	20.2 ± 0.5 [a]	16.4 ± 0.9 [b]	17.5 ± 0.1 [b]	16.6 ± 1.4 [b]
Phosphatidylethanolamine	4.5 ± 0.5 [a]	4.9 ± 0.8 [a]	6.8 ± 0.6 [b]	9.4 ± 0.1 [c]
Phosphatidylserine	0.9 ± 0.2 [a]	1.1 ± 0.2 [a]	2.2 ± 0.1 [b]	4.5 ± 0.5 [c]
Phosphatidylinositol	2.3 ± 0.3 [a]	1.4 ± 0.1 [a]	2.1 ± 0.3 [a]	3.3 ± 0.6 [b]
Phosphatidic acid/cardiolopin	1.5 ± 0.1 [ab]	1.4 ± 0.1 [ab]	1.1 ± 0.2 [b]	1.9 ± 0.3 [a]
Sulfatides	0.5 ± 0.2	0.5 ± 0.1	0.4 ± 0.1	0.7 ± 0.3
Cerebrosides	0.2 ± 0.1 [a]	1.1 ± 0.4 [b]	0.6 ± 0.1 [ab]	1.0 ± 0.5 [ab]
Pigments	1.3 ± 0.3 [a]	1.4 ± 0.1 [a]	1.6 ± 0.1 [ab]	2.1 ± 0.2 [b]
Total polar lipids	32.7 ± 0.8 [a]	29.8 ± 0.4 [b]	33.4 ± 0.2 [a]	40.9 ± 1.8 [c]
Cholesterol	7.4 ± 0.9 [a]	6.9 ± 0.4 [a]	10.3 ± 0.8 [b]	12.3 ± 0.3 [c]
Free fatty acid	1.4 ± 0.4 [ab]	0.8 ± 0.6 [a]	2.6 ± 0.1 [bc]	3.8 ± 0.7 [c]
Triacylglycerol	25.5 ± 0.5 [a]	31.6 ± 0.5 [b]	27.2 ± 0.2 [c]	18.9 ± 0.3 [d]
Sterol ester	32.8 ± 0.6 [a]	30.7 ± 0.6 [b]	26.6 ± 0.7 [c]	23.8 ± 0.9 [d]
Total neutral lipid	67.3 ± 0.7 [a]	70.2 ± 0.4 [b]	66.7 ± 0.4 [a]	59.1 ± 1.9 [c]
PC/PE	4.5 ± 0.4 [a]	3.3 ± 0.3 [b]	2.6 ± 0.2 [b]	1.8 ± 0.2 [c]
Triacylglycerol/cholesterol	3.5 ± 0.5 [a]	4.6 ± 0.3 [b]	2.7 ± 0.2 [a]	1.5 ± 0.1 [c]

Results are means ± standard deviation ($n = 3$). Values followed by different superscript lowercase letters are significantly different at $P < 0.05$

level of PC did not vary during bonito embryogenesis and yolk sac larvae development up to 60 h posthatch. Moreover, the content of PE in the bonito egg increased significantly from the egg to 20-h posthatch larvae (×1.4) and from 20- to 60-h posthatch larvae (×1.4), and that of the PS fraction increased significantly from the egg to 20-h posthatch larvae (×2) and from 20- to 60-h posthatch larvae (×2). In addition, the PI content in the bonito egg increased significantly from the egg to 20-h posthatch larvae (×1.5) and from 20- to 60-h posthatch larvae (×1.6). No significant differences were found in the proportions of PA/CL and/or sulfatides among the BFT egg, bonito egg, and posthatch larvae. The proportion of pigments was quite similar in all samples, with the exception of the 60-h posthatch bonito larvae, in which the pigment content was significantly higher (Table 1). The proportion of TNL in bonito eggs was 70.2% of the TL and decreased significantly by 3.5% in the 20-h posthatch larvae and by 7.6% in the 60-h posthatch larvae; there was a net significant decrease of 11.1% from the egg to 60-h posthatch larvae. However, the free C level increased significantly in the 20-h and 60-h posthatch unfed larvae—by 33.0 and 16.3%, respectively—most likely due to the utilization of SE as a source of fatty acids, thereby liberating free C for bio-membrane assemblage. Consistent with this, the level of SE/WE diminished significantly during embryogenesis, and yolk sac larvae development used free C and free fatty acids as a source. However, the proportion of TAG also diminished significantly, by 13.9 and 30.5%, 20- and 60-h posthatch, respectively, indicating that TAG is also a major reserve of fatty acids for energy (β-oxidation and assemblage of membrane components) during larval development.

The proportions of EPA (20:5$n-3$), total saturated fatty acids, and total monoenoic fatty acids were similar in TL from BFT and bonito eggs. However, TL 16:0, 18:1$n-9$, LA (18:2$n-6$), ARA (20:4$n-6$), DHA (22:6$n-3$), total $n-3$ PUFA, and total PUFA were all significantly higher in BFT eggs than in bonito eggs, and 16:1$n-7$ was higher in bonito eggs. The ratio DHA/EPA/ARA was 18.3/4.9/1.0 in BFT eggs and 21.1/7.7/1.0 in bonito fertilized eggs (Table 2).

Total lipid 16:0 content was similar in fertilized bonito eggs and 20-h posthatch larvae but it decreased significantly from 20- to 60-h posthatch larvae. However, no significant statistical differences were found among TL 18:0 contents from samples of bonito egg and early larvae developmental stages. Overall, a downward trend was observed in total saturated fatty acids during bonito development, with a significant decrease occurring from fertilized eggs to 60-h posthatch larvae. Moreover, TL 16:1$n-7$ and 18:1$n-9$ content decreased significantly from 20- to 60-h posthatch larvae. Total $n-6$ PUFA showed similar values in all bonito samples analyzed, with little change during bonito egg and early larval development. Linoleic acid (LA) content decreased significantly from fertilized eggs to 60-h post-hatch larvae, whereas ARA content remained constant during bonito eggs and yolk sac unfed larvae development. Total $n-3$ PUFA content showed a similar trend to that presented by its major component, TL DHA. During development, only a small decrease in content was observed, with total polyenoic fatty acid content showing a similar trend to that of total $n-3$ fatty acids. Total lipid linolenic acid (LNA) content showed identical values in all samples, with the exception of 60-h posthatch larvae, which showed significantly lower values. Total lipid EPA and DHA contents showed a decreasing trend from bonito fertilized eggs to 60-h posthatch unfed larvae (Table 2).

Discussion

The moisture contents of the BFT and bonito eggs analyzed in this study are identical to that found for cultured Pacific bluefin tuna (PBT) *Thunnus orientalis* (Takii et al. 1997). They are also very similar to those of eggs from other cultured teleost fish species, such as red sea bream (Watanabe et al. 1985), European sea bass (Devauchelle and Coves 1988), gilthead sea bream (Almansa et al. 1999), common dentex (Mourente et al. 1999), and white sea bream (Cejas et al. 2004), all containing a single oil globule, and higher than eggs of cultured Senegal sole, which contain multi-globules (Vázquez et al. 1994; Mourente and Vazquez 1996).

Few studies report TL content in spawned eggs from broodstock fish caught in the wild, and most of the relevant data published to date were obtained on captive cultured animals. The TL content of the BFT eggs and bonito eggs from wild sources studied here account for about 200 μg of the TL content per milligram of dry mass (20% on a dry mass basis). According to Takii et al. (1997) and as referred to in Kamler (2005), the caloric value of freshly spawned (cultured) eggs of PBT is 30.1 J/mg dry mass (or its equivalent 7.2 cal/mg dry mass). Consequently, the energetic value corresponding to lipids of BFT and bonito eggs is 1.9 cal/mg dry mass, which is 26.4% of the total caloric value of the egg. Compared to the values obtained in our study, data published to date report higher egg total lipid content values for cultured fish species, such as European sea bass (Devauchelle and Coves 1988), gilthead sea bream (Mourente and Odriozola 1990; Almansa et al. 1999), common dentex (Mourente et al. 1999), and cobia (Faulk and Holt 2008), or lower values, as in Senegal sole eggs (Vázquez et al. 1994; Mourente and Vazquez 1996). It is also well documented that egg TL content may vary depending on the broodstock diet and/or the period of the spawning season (Devauchelle and Coves 1988; Mourente and Odriozola 1990; McEvoy et al. 1993; Sargent 1995; Evans et al. 1996).

In bonito eggs and developing unfed larvae, there was a significant decrease in TL content between hatching (eggs) and 60 h after hatching (larvae) (Table 1), indicating an average net total lipid consumption of 75 μg TL per hour and per milligram dry mass during the first 20 h of larval development and 50 μg total lipid per hour and per milligram dry mass during the following 40 h of larval development. The caloric consumption due to the use of lipids as fuel (9.5 cal/mg) was 142.5 mcal/mg dry mass during the first 20 h (average 7.1 mcal/h per milligram dry mass) and 94.5 mcal during the following 40 h (average 2.4 mcal/h per milligram

Table 2 Fatty acid content (μg fatty acid/mg total lipid) of the bluefin tuna egg and bonito egg and larvae 20 h and 60 h after hatching

	BFT egg	Bonito egg	Larvae 20 h	Larvae 60 h
Fatty acid (%)				
14:0	17.2 ± 2.8 a	26.4 ± 2.1 b	24.0 ± 1.3 b	10.8 ± 2.5 c
15:0	4.7 ± 0.8 a	6.1 ± 0.2 a	5.4 ± 0.7 a	3.0 ± 0.6 b
16:0	161.6 ± 10.6 a	131.9 ± 3.3 b	128.6 ± 3.4 b	99.9 ± 1.4 c
18:0	40.9 ± 2.6	39.9 ± 3.0	46.4 ± 2.5	44.9 ± 6.1
20:0	2.4 ± 0.7	2.9 ± 0.8	2.0 ± 0.4	1.9 ± 0.3
Total saturated[a]	233.7 ± 14.4 a	212.5 ± 4.6 a	211.7 ± 4.6 a	167.5 ± 18.1 b
16:1*n*−9	1.8 ± 0.2	1.6 ± 0.1	1.6 ± 0.3	1.6 ± 0.1
16:1*n*−7	22.5 ± 1.2 a	34.1 ± 1.3 b	32.2 ± 0.4 b	19.8 ± 2.1 a
18:1*n*−9	123.9 ± 5.7 a	92.1 ± 3.4 b	92.3 ± 2.8 b	64.4 ± 9.1 c
20:1*n*−9	6.5 ± 1.2	6.7 ± 0.6	6.6 ± 0.7	6.7 ± 0.8
20:1*n*−7	5.1 ± 0.6	5.9 ± 0.1	5.9 ± 0.4	6.2 ± 0.5
22:1*n*−11	5.0 ± 0.7 a	3.1 ± 0.9 b	2.7 ± 0.2 b	1.9 ± 0.1 b
22:1*n*−9	1.8 ± 0.4	1.6 ± 0.3	1.6 ± 0.4	1.5 ± 0.3
Total monoenes[a]	212.5 ± 18.3 a	190.9 ± 7.5 a	189.1 ± 5.8 a	140.9 ± 4.1 b
16:2	6.8 ± 0.3	7.6 ± 0.6	7.7 ± 0.6	6.7 ± 0.3
16:3	4.6 ± 0.5	6.4 ± 1.2	6.2 ± 0.6	4.7 ± 0.6
16:4	5.0 ± 0.4 a	3.9 ± 0.1 b	4.0 ± 0.1 b	4.9 ± 0.3 a
18:2*n*−6	17.3 ± 0.7 a	14.5 ± 0.4 b	14.2 ± 0.2 b	13.4 ± 0.8 b
18:3*n*−3	7.6 ± 0.8 a	8.5 ± 1.2 a	7.7 ± 0.4 a	2.4 ± 0.3 b
18:4*n*−3	25.3 ± 1.3 a	15.7 ± 1.8 b	15.8 ± 1.2 b	15.0 ± 2.5 b
20:3*n*−6	ND	1.2 ± 0.2 a	1.2 ± 0.4 a	2.2 ± 0.2 b
20:3*n*−3	0.4 ± 0.1 a	0.3 ± 0.0 ab	0.2 ± 0.0 b	0.2 ± 0.0 b
20:4*n*−6	13.2 ± 1.2 a	8.1 ± 0.3 b	10.4 ± 0.2 b	12.6 ± 1.6 a
20:4*n*−3	7.5 ± 0.5 a	5.8 ± 0.9 b	3.7 ± 0.2 c	5.7 ± 0.7 b
20:5*n*−3	65.3 ± 5.2 a	61.8 ± 1.4 a	59.9 ± 1.3 ab	51.9 ± 1.7 c
22:5*n*−6	2.4 ± 0.8	1.4 ± 0.2	2.1 ± 0.4	2.4 ± 0.2
22:5*n*−3	9.5 ± 0.6	10.5 ± 0.6	10.3 ± 0.2	9.2 ± 1.4
22:6*n*−3	243.2 ± 18.3 a	168.1 ± 2.8 b	160.6 ± 3.1 b	150.7 ± 3.5 b
Total polyenes[a]	428.5 ± 28.7 a	328.6 ± 4.4 b	323.1 ± 6.7 b	295.5 ± 4.0 b
Total *n*−9	143.4 ± 5.9 a	102.1 ± 4.3 b	101.5 ± 3.1 b	81.7 ± 3.9 c
Total *n*−7	51.3 ± 2.1 a	73.2 ± 2.8 b	64.6 ± 1.2 c	46.8 ± 2.5 a
Total *n*−6	52.2 ± 1.8 a	43.0 ± 0.4 b	45.6 ± 1.2 b	45.9 ± 4.4 ab
Total *n*−3	375.2 ± 26.8 a	285.6 ± 4.7 b	277.5 ± 6.3 b	249.5 ± 3.5 b
HUFA *n*−6	16.2 ± 1.2 a	10.8 ± 0.4 b	13.7 ± 0.8 ab	17.3 ± 2.0 a
HUFA *n*−3	332.1 ± 24.8 a	251.1 ± 4.5 b	241.6 ± 4.6 b	222.3 ± 3.1 b
n−3/*n*−6	7.2 ± 0.3 a	6.6 ± 0.3 ab	6.1 ± 0.2 bc	5.5 ± 0.4 c

Results are given as the mean ± SD ($n = 3$). A SD of 0.0 implies an SD of <0.05. Values followed by different lowercase letters are significantly different at $P < 0.05$

HUFA, Highly unsaturated fatty acid; ND, not detected

[a] Totals include some minor components not shown

dry mass). However, this net balance of TL consumption includes the conservation and synthesis of total polar lipids (TPL), which showed a significant net increase of 29.1% from the egg to 60-h posthatch unfed larvae, and a concomitant catabolic decline, mostly for energetic purposes, of TNL by 11.1%. This two phenomena can be considered to be common features of fish eggs, observed either in polar lipid-rich (no oil globule) and neutral lipid-rich (with oil globule) developing fish embryos and yolk sac larvae, as observed with the conservation and synthesis of PE primarily in newly formed tissues at the structural and energetic expenses of neutral lipids (Tocher et al. 2008). The PC/PE ratio was significantly higher in BFT eggs than in bonito eggs, but the PC/PE ratio diminished significantly during bonito larval development. In the case of the bonito eggs and larvae data presented in this study, net increases were observed in PE, PS, PI, and free C fractions, while the PC remained constant, indicating a rapid and intense synthesis of new tissues in the fast larval growing stage of this scombroid fish species (Table 1). Moreover, PE and PS are prominent in neural tissue, indicating rapid retinal and neural development in the new emerging embryo and larvae, as required in a fast-growing visual marine aquatic predator (Mourente and Tocher 1992; Mourente 2003). Furthermore, PI, a lipid class particularly rich in ARA, has also been found to be positively correlated with egg and larvae performance parameters (Pickova et al. 1997; Salze et al. 2005).

Neutral lipid content and class distribution varies considerably in eggs of Perciformes, with TAG and SE being the major lipid fractions. The relative amounts of TAG and SE also vary within this class of teleost fish (Wiegand, 1996). We found a TAG/SE ratio of 0.77 for BFT eggs and 1.03 for bonito eggs, indicating the presence of similar proportions of these two neutral lipid classes among the egg reserves of these two scombroid fish species. However, during bonito larval development, the TAG/SE ratio decreased to 1.02 and 0.79 at 20 h and 60 h posthatch, respectively, indicating a preferential use of TAG over SE. Any lipid class containing fatty acids can act as source of energy, but TAGs are the primary class for lipid storage and energy provision in most fish species with neutral lipid-rich eggs, followed by SE and WE (Sargent 1995; Wiegand 1996; Tocher et al. 2008). The TAG content was 11.6 μg/mg dry mass higher in bonito eggs than in BFT eggs. The TAG content decreased by 12.6 μg/mg dry mass from the bonito egg to 20-h posthatch larvae and by 16.7 μg/mg dry mass from 20- to 60-h posthatch larvae; this is a total loss of 29.3 μg/mg dry mass from fertilized bonito eggs to 60-h posthatch larvae. In caloric values, TAG produces 119.7 mcal/mg dry mass up to 20-h the posthatch unfed larval stage and 158.6 mcal/mg dry mass from the 20- to 60-h posthatch unfed larval stage; this is a total of 278.3 mcal/mg dry mass. Triacylglycerol was also detected as a main egg constituent in PBT eggs and showed a rapid decrease in content after gastrulation, which indicates that TAG plays an important role as a primary energy fuel during embryonic and larval development (Takii et al. 1997). Triacylglycerol is of paramount importance as an endogenous energy reserve and serves to maintain basal metabolism when catabolized. Moreover, the storage of TAG corresponds to the physiological condition of the embryo and subsequent larval stages, and it can be correlated with larval condition when expressed in the form of the TAG/free C ratio, a relation or nutritional index between energetic reserves (TAG) and structural components (free C content is highly positively correlated to embryo or larval dry mass) (Fraser 1989). The nutritional index TAG/C was significantly lower in BFT eggs than in bonito eggs. Moreover, the TAG/C index decreased by 41.3% from the bonito egg to 20-h posthatch larvae and by 44.4% from 20- to 60-h posthatch larvae, denoting the use of TAG mainly for energetic purposes despite C accretion due to tissue assemblage.

The fatty acid composition of the oocyte and egg TL varies with species, partly reflecting differences in lipid class composition, but it is relatively more resistant to dietary changes than the fatty acid compositions of other tissues in broodstock fish (Sargent et al. 1989, 2002). The catabolism of lipids, whichever class, results in the release of FFA, which can be either be utilized for energy or re-acylated back into lipid classes for other uses, such as the formation of rapidly developing larval tissues during embryogenesis and larval development (Sargent et al. 2002). It is noteworthy that the fatty acid contents (expressed as μg fatty acid/mg total lipid) determined in the eggs of the two scombroid fish species analyzed in our study were higher than those found in species such as Senegal sole (Mourente and

Vazquez 1996) or the common dentex (Mourente et al. 1999). Total saturated fatty acid content decreased significantly from 20- to 60-h posthatch larvae by about 10 μg/mg dry mass. Based on the data obtained in our study, 16:0, as a major saturated fatty acid, can be a good energy β-oxidation substrate since an important significant decrease was observed from 20- to 60-h posthatch bonito larvae. In contrast, 18:0 content remained stable and so may be preferentially retained in phosphoglycerides for structural membrane functions. Total monoene content decreased significantly along bonito larval development, as denoted by decreases in the major components (16:1$n-7$ and 18:1$n-9$), indicating their major role as an energy source. The monoene 22:1$n-11$, which is abundant in zooplankton (mostly copepods) and zooplanktivorous fish from cold marine waters of the northern hemisphere (Sargent et al. 1989, 2002), was scarce in total lipid fatty acid composition of the eggs and larvae analyzed in our study, and its content declined during bonito larval development, possibly due to it being oxidized for metabolic energy production.

It has been well established that fish eggs, in general, are rich in PUFA and particularly rich in $n-3$ HUFA (Bell 1998; Sargent et al. 2002; Tocher 2003). However, relatively less attention has been paid to $n-6$ HUFA, such as ARA (Bell 1998). Although fatty acids such as EPA and DHA are largely retained as part of tissue structural components in embryos and new emerging larvae, the absolute amounts of any individual HUFA are probably less important, both functionally and physiologically, than the proportions among them; this is particularly true for the ratio DHA/EPA/ARA, since most fish larvae species have requirements for $n-3$ and $n-6$ HUFA (Sargent et al. 2002). Essential fatty acids, especially ARA and the $n-3$ HUFA EPA and DHA, have been shown to have a significant effect on hatching and fertilization rates and survival in the early larval stages of gilthead sea bream (Fernandez-Palacios et al. 1995; Rodriguez et al. 1998) and European sea bass (Bruce et al. 1999). Moreover, $n-3$ HUFA, the DHA/EPA ratio, and ARA content have been found to be related to blastomere morphology, hatching rate, and larval survival (Pickova et al. 1997; Furuita et al. 2000). In our study, the DHA/EPA ratio in BFT eggs was 3.7/1, whereas in bonito eggs it was 2.7/1, a ratio that remained constant during bonito larvae development. In contrast, the EPA/ARA ratio was lower in BFT eggs than in bonito eggs (4.9/1 vs. 7.7/1), but this ratio was lower in bonito unfed larvae at 20-h posthatch (5.9/1) and 60-h posthatch (4.3/1). This last ratio value resembles that found in total lipid fatty acids from BFT eggs. Analysis of eggs and newly hatched larvae from different marine fish species have revealed that the ARA content is several-fold higher than that of other body lipids in these fish (Bell 1998). This finding would seem to indicate that ARA can be specifically concentrated in fish eggs, thereby emphasizing the biological importance of this fatty acid for embryogenesis and early larval development (Bell 1998; Sargent et al. 2002). Several studies have shown that egg quality criteria, including hatching and fertilization rates, as well as survival in larval stages are positively correlated with increased levels of ARA and $n-3$ HUFA in gilthead sea bream (Harel et al. 1992; Fernandez-Palacios et al. 1995; Rodriguez et al. 1998), European sea bass (Bruce et al. 1999), cod (Pickova et al. 1997), and Atlantic halibut (Mazorra et al. 2003).

The high levels of $n-3$ HUFA in scombroid fish found in our study can be explained by the requirement for a rapidly developing brain and retinas in the larvae, since many of the major phosphoglyceride components of these tisues, such as PC, PE, and PI, must be elaborated by acyl exchange reactions to synthesize the di-DHA molecular species that are the principal and major structural constituents of neural tissue development (Sargent et al. 2002).

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2.2. Evolution of lipid composition of unfed Atlantic bluefin tuna (*Thunnus thynnus* L.) larvae during first days of development

Resumen

Evolución de la composición lipídica de larvas de Atún rojo del Atlántico sin alimentar durante los primeros días de desarrollo

La concentración de los ácidos grasos n-3 poliinsaturados de cadena larga (LC-PUFA) en los tejidos neurales es crucial parala efectiva captura de presas desde el momento de la primera alimentación en las larvas de peces marinos. Además, los tejidos de los túnidos, incluyendo al atún rojo del Atlántico, tienen relativamente altos niveles de DHA (ácido docosahexanoico; 22-6n-3) y una elevada relación de DHA:EPA (ácido eicosapentanoico; 20-5n-3) comparado con los niveles de la mayoría de otras especies. Aunque la clave en la bioquímica de los lípidos de la alta relación DHA:EPA es incierta, se asume que deben acumular y retener selectivamente DHA en sus tejidos. En el presente estudio investigamos el metabolismo de los lípidos y los ácidos grasos durante el desarrollo temprano de atún rojo del Atlántico y determinamos los cambios en el contenido lipídico, clases de lípidos y composición de perfiles de ácidos grasos en lípidos totales, fosfolípidos o lípidos neutros en larvas sin alimentar durante la utilización del saco vitelino. El nivel de DHA se mantuvo, pero decreció la proporción de EPA, con lo que la relación DHA:EPA se incrementó. Esto pudo ser el resultado de la relativa retención de DHA durante un periodo de elevada oxidación y utilización de ácidos grasos.

Abstract

The concentration of n-3 long-chain polyunsaturated fatty acids (LC-PUFA) in neural tissues is known to be crucial for effective prey capture from the time of first feeding in marine fish larvae. Furthermore, tissues of tunids, including ABFT, have relatively high levels of DHA (docosahexaenic acid, 22:6n-3) and a high ratio of DHA:EPA (eicosapentaenoic acid; 20:5n-3) compared to most other species. Although the lipid biochemistry underpinning the high DHA:EPA ratio in tuna is unclear, it has been generally assumed that they must selectively accumulate and retain DHA in their tissues. In the present study, we investigated lipid and fatty acid metabolism during early development of ABFT and determined the changes in lipid content, lipid class composition and total, phospholipid and neutral lipid fatty acid profiles in unfed larvae during yolk sac

utilisation. The level of DHA was maintained, but the proportion of EPA declined, and so the DHA:EPA ratio increased. This could be the result of relative retention of DHA during a period of generally high fatty acid oxidation and utilization.

Introduction

Scombrid fish comprise species of high commercial value such as bonitos and tunas. One of the most iconic species is the ABFT (ABFT), *Thunnus thynnus* (Linnaeus 1758), which has relevant ecological, recreational and commercial importance in Atlantic and Mediterranean ecosystems (Rodríguez-Roda, 1964; Rey, 1999; Fromentin and Powers, 2005). The ABFT fishery has become a highly profitable activity with the development of the sushi-sashimi market in Japan increasing the demand for high quality fish, stimulating very high prices and, in consequence, increasing regulated and unregulated fishing effort (Fromentin and Powers, 2005). In recent years considerable research effort has been undertaken in order to develop aquaculture of these large pelagic migratory fish species (Wexler *et al.*, 2003; Margulies *et al.*, 2007; Masuma *et al.*, 2008). Determining nutritional requirements, particularly at larval stages, will be a key task (Mourente and Tocher, 2003, 2009).

The importance of lipids for growth and development of fish is based on their key roles as sources of metabolic energy and as essential components of tissue and cell membranes. In most fish species, lipids are favoured as an energy source compared to proteins and carbohydrates, and are characterized by high contents of long-chain polyunsaturated fatty acids (LC-PUFA) essential for cell membrane structure and function. The reserves of lipid in fish eggs are used by the developing embryo and subsequent larvae for energy metabolism, as structural components of biomembranes and precursors of lipid-derived hormones (Sargent, 1995; Wiegand, 1996; Sargent *et al.*, 1989, 2002). Lipid reserves in teleost fish eggs are stored as lipoproteins in yolk reserves and, in some species, as discrete or fragmented oil globules. Yolk lipoproteins contain primarily phospholipids, mainly phosphatidylcholine and phosphatidylethanolamine, showing high levels of polyunsaturated fatty acids (PUFA), particularly the n-3 LC-PUFA, eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA). Tuna eggs contain a single discrete oil globule, comprised of neutral lipids, primarily triacylglycerol (TAG), steryl ester and/or wax ester, rich in both monounsaturated fatty acids (MUFA) and n-3 LC-PUFA (Wiegand, 1996; Ortega and Mourente, 2010). The LC-PUFA have well-established roles in membrane structure and function as well as energy sources during embryonic and early larval development of marine fish (Sargent *et al.*, 2002). The composition and metabolism of lipid and fatty acids during embryogenesis and yolk-sac larvae development can give useful information of

the nutritional requirements during early larval stages (Sargent *et al.*, 2002). This approach can provide insights that can be applied in the development of live feed enrichments and/or artificial feeds (Tocher, 2003).

Tuna tissues have a relatively high level of DHA and a high DHA:EPA ratio (Tocher, 2003; Mourente and Tocher, 2009). However, all marine fish studied to date have only very limited ability to biosynthesize LC-PUFA and so have an absolute requirement for dietary EPA and DHA (Tocher, 2003). The fatty acid profile of lipids from eggs of wild-caught tunas shows high levels of DHA, possibly suggesting that tunas may have a high requirement for this fatty acid (Nichols *et al.*, 1998; Mourente and Tocher, 2009; Ortega and Mourente, 2010). Although the lipid biochemistry underpinning the high level of DHA and the high DHA:EPA ratio in tuna is unclear, it has been generally assumed that tuna must selectively accumulate and retain DHA in their tissues (Ishihara and Saito, 1996; Saito *et al.*, 1996; Mourente and Tocher, 2009). Tuna are top predators of the benthic-pelagic trophic web and the concentration of n-3 LC-PUFA in neural tissues is known to be crucial for effective prey capture from the time of first feeding (Bell *et al.*, 1995; Mourente and Tocher, 2009).

Long-chain PUFA can be biosynthesized from short-chain (C_{18}) PUFA in reactions catalysed by fatty acyl desaturase (Fad) and elongase (Elovl) enzymes. Understanding of the biochemical and molecular mechanisms of LC-PUFA biosynthesis in fish has advanced in recent years and several

In the present study, we investigated lipid and fatty acid metabolism during early larval development of ABFT. Specifically, we describe the changes in total lipid content, lipid class composition and total, phospholipid and neutral lipid fatty acid profiles in unfed ABFT larvae during yolk sac utilisation.

Materials and Methods

1. ABFT larvae

ABFT eggs came from a broodstock composed of 35 fish with an estimated mean body weight of 100 kg. The broodstock were kept in captivity for 2 years in a floating cage located at El Gorguel, off Cartagena coast, SE Spain and were fed on frozen fish, mainly mackerel. At the end of June, 15 captive-reared ABFT broodstock fish were induced to mature and reproduce by means of GnRHa implants, and an egg collector, 500 µm mesh screen size, was placed around the cage. Tunas spawned every day during the first half of July 2009.

Collected eggs were transported in a 500 l plastic tank supplied with pure oxygen to the IEO – Mazarrón aquaculture facilities and placed in 100 L tanks with gentle oxygenation and flow through sterilized sea water in order to clean them. After 1 h, aeration and water flow were stopped to separate buoyant (viable) from non-buoyant (non-viable) eggs, and fertilized eggs collected and incubated at an initial egg density of 1100 eggs per L in 1000 L cylindro-conical tanks. Incubation was carried out at 25-26 ºC, 37 ppt and continuous photoperiod, with a light intensity of 100 lux. An upwelling flow-through, with gentle aeration in order to keep oxygen levels next to saturation, was employed.

2. Sample collection and dry weight determination

On 8th July a batch of 1.1 million eggs was separated and incubated in a 1000 L cylindro-conical tanks Eggs hatched 9th July (0 days post-hatch, 0 dph) and the larvae were maintained in the conditions described above. Hatching rate in this batch was close to 94 %, and survival of hatched larvae after 1, 2, 3 and 4 days was 69 %, 46 %, 12 % and 4 %, respectively. Thus, the final sample was obtained at 4 days post-hatch (4 dph) as survival was too low at 5 dph to obtain viable samples. Each day, three samples of 5-10,000 larvae were collected from the incubator. Water from the incubator was filtered with a 250 µm mesh screen net in order to concentrate the larvae. Larvae were washed with distilled water and excess water blotted through the screen with filter paper. Sampled larvae were collected in cryotubes, frozen in liquid nitrogen and stored at -80ºC until analysis. Replicates of preweighed samples (approximately 50 mg wet weight) were maintained at 110°C for 24 h. The dry weights were determined after cooling *in vacuo* for at least 1 h.

3. Lipid content, lipid class composition and fatty acid analysis

Total lipid of ABFT larvae was extracted from triplicate pooled samples according to the method of Folch *et al.* (1957). Approximately 1 g of ABFT larvae was placed in 20 ml of ice-cold chloroform/methanol (2:1, by vol) and homogenized with an Ultra-Turrax tissue disrupter (Fisher Scientific, Loughborough, U.K.). The non-lipid and lipid layers were separated by addition of 5 ml of 0.88 % (w/v) KCl and allowed to separate on ice for 1 h. The upper non-lipid layer was aspirated and the lower lipid layer dried under oxygen-free nitrogen. The lipid content was determined gravimetrically after drying overnight in a vacuum desiccator.

Lipid class composition was determined by high-performance thin-layer chromatography (HPTLC) using 10 x 10 cm plates (VWR, Lutterworth,

England). Approximately 2 mg of total lipid was applied as 2 mm streaks and the plates developed in methyl acetate/isopropanol/ chloroform/methanol/0.25 % aqueous KCl (25:25:25:10:9, by vol.) to two-thirds up the plate. After desiccation for 20 min, the plate was fully developed with isohexane/diethyl ether/acetic acid (85:15:1, by vol.). The lipid classes were visualized by charring at 160 ^{o}C for 15 min after spraying with 3 % (w/v) aqueous cupric acetate containing 8 % (v/v) phosphoric acid and quantified by densitometry using a CAMAG-3 TLC scanner (version Firmware 1.14.16) (Henderson and Tocher, 1992). Scanned images were recorded automatically and analyzed by computer using winCATS Planar Chromatography Manager (version 1.2.0).

Fatty acid methyl esters (FAME) were prepared from total lipid, total phospholipids and total neutral lipids by acid-catalyzed transesterification at 50 ^{o}C for 16 h according to the method of Christie (1993). Phospholipids and neutral lipids were separated by TLC, and extraction and purification of FAME was carried out as described by Tocher and Harvie (1988). The FAME were separated and quantified by gas-liquid chromatography (Carlo Erba Vega 8160, Milan, Italy) using a 30m x 0.32 mm i.d. capillary column (CP Wax 52CB, Chrompak, London, U.K.) and on-column injection at 50^{o}C. Hydrogen was used as carrier gas and temperature programming was from 50 ^{o}C to 150 ^{o}C at 40 ^{o}C min^{-1} and then to 230 ^{o}C at 2.0 ^{o}C min^{-1}. Individual methyl esters were identified by comparison with known standards and by reference to published data (Ackman, 1980; Tocher and Harvie, 1988). Data were collected and processed using Chromcard for Windows (version 1.19)

4. Statistical analysis

Results are presented as means ± SD (n = 3) except for gene expression data, where a single pool of larvae was analysed. The data were checked for homogeneity of variances using the Bartlett test and, where necessary, arc-sin transformed before further statistical analysis. Differences between mean values were analyzed by one-way analysis of variance (ANOVA), followed when pertinent by a multiple comparison test (Tukey). Differences were reported as statistically significant when $P < 0.05$ (Zar, 1999).

Results

1. *Dry mass, lipid content and lipid class composition of unfed ABFT larvae*

Yolk sac larvae of ABFT showed a significant decrease of around 29 % in dry mass from hatching (dph0) to dph4 (Table 1). During the same period, total lipid content decreased by approximately 27 %, on a dry mass basis. The lipid class

composition of ABFT yolk sac larvae at hatching was predominantly neutral lipids (~77 %), primarily steryl ester/wax ester, TAG and cholesterol, with 23 % total polar lipids, primarily phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol. The proportion of neutral lipids declined to 59 % by 4 dph due to decreased percentages of both TAG and steryl ester/wax ester. Over the same period, the proportions of membrane lipid increased with total polar lipid and cholesterol rising to 41 % and 22 %, respectively (Table 3). Consequently, the nutritional index (TAG/cholesterol) decreased significantly from 1.9 to 0.3.

2. Fatty acid composition

Total lipids of ABFT yolk sac larvae at hatching were characterized by 26 % saturated fatty acids (primarily 16:0, followed by 18:0 and 14:0), 33 % MUFA (primarily 18:1n-9) and 34 % PUFA, primarily the n-3 LC-PUFA, DHA (19 %) and EPA (6 %) with a DHA:EPA ratio of 3.0 (Table 2). From hatching to dph4, the proportion of saturated fatty acids increased to 32 % whereas total MUFA decreased to 27 %, mainly due to increased percentages of 18:0, and decreased percentages of almost all MUFA. There were no significant changes in the proportions of n-3, n-6 or total PUFA during this period of development but the percentages of arachidonic acid (ARA; 20:4n-6) and DHA significantly increased, whereas the proportion of EPA decreased (Table 2), such that the DHA:EPA ratio in total lipid increased to 5.0 by dph4 (Fig.5).

Total polar lipids of ABFT yolk sac larvae at hatching were characterized by 36 % saturated fatty acids (primarily 16:0 and 18:0), 19 % MUFA (primarily 18:1n-9) and 39 % PUFA, primarily DHA (27 %) and EPA (6 %) with a DHA:EPA ratio of 4.7 (Table 3). There were few major effects of development on polar lipid fatty acid composition from hatching to dph4, other than a slight but significant increased proportion of total n-6 PUFA, and decreased percentage of EPA (down to 3.7 %) with a trend for increased DHA that resulted in the DHA:EPA ratio significantly increasing to 7.9 (Table 3, Fig.1). In contrast, there were no overall changes in the proportions of total saturated fatty acids, MUFA or PUFA, including EPA and DHA, in total neutral lipids between dph0 and dph4 (Table 4). Similarly, the DHA:EPA ratio was constant at around 2.4-2.5 in neutral lipids (Fig.5).

Table 1. Dry mass (percentage), total lipid content (percentage live mass and dry mass) and lipid class composition (percentage of total lipid) of ABFT (*Thunnus thynnus* L.) unfed yolk sac larvae from 0 to 4 days post hatch (dph).

	dph0	dph1	dph2	dph3	dph4
Dry mass (%)	8.4 ± 1.8 ^{ab}	8.9 ± 0.3 ^{a}	9.8 ± 0.1 ^{b}	8.4 ± 0.3 ^{a}	6.3 ± 0.9 ^{c}
Total Lipid (% live mass)	2.4 ± 0.4 ^{a}	2.3 ± 0.2 ^{a}	2.4 ± 0.1 ^{a}	1.5 ± 0.1 ^{b}	1.3 ± 0.1 ^{b}
Total Lipid (% dry mass)	29.9 ± 2.3 ^{a}	25.9 ± 1.9 ^{ab}	22.8 ± 1.8 ^{b}	19.2 ± 0.3 ^{b}	19.3 ± 0.8 ^{b}
Lipid Class					
Phosphatidylcholine	10.4 ± 0.3 ^{a}	13.4 ± 0.8 ^{bc}	12.1 ± 1.4 ^{ab}	13.9 ± 1.1 ^{bc}	15.9 ± 0.8 ^{c}
Phosphtidylethanolamine	4.6 ± 0.2 ^{a}	7.4 ± 0.3 ^{b}	7.0 ± 0.5 ^{b}	10.0 ± 0.3 ^{c}	11.1 ± 0.3 ^{d}
Phosphatidylserine	2.1 ± 0.4 ^{a}	4.1 ± 0.4 ^{b}	3.7 ± 0.2 ^{b}	5.8 ± 0.2 ^{c}	7.7 ± 0.2 ^{d}
Phosphatidylinositol	2.6 ± 0.1	2.0 ± 1.0	2.6 ± 0.2	2.6 ± 0.1	2.6 ± 0.2
Phosphatidic acid/cardiolipin	1.0 ± 0.3	1.8 ± 0.8	1.0 ± 0.4	1.5 ± 0.2	1.7 ± 0.1
Sphingomyelin	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.7 ± 0.2
Lyso- Phosphatidylcholine	0.7 ± 0.1 ^{a}	0.9 ± 0.2 ^{a}	0.5 ± 0.1 ^{b}	1.1 ± 0.2 ^{ab}	0.9 ± 0.3 ^{ab}
Total polar	22.6 ± 1.3 ^{a}	30.7 ± 1.5 ^{b}	27.8 ± 1.6 ^{b}	35.9 ± 1.5 ^{c}	40.8 ± 2.0 ^{d}
Total neutral	77.4 ± 1.2 ^{a}	69.2 ± 1.5 ^{b}	72.2 ± 1.6 ^{b}	64.1 ± 1.5 ^{c}	59.1 ± 2.0 ^{d}
Cholesterol	11.8 ± 0.8 ^{a}	15.2 ± 0.2 ^{b}	16.1 ± 1.6 ^{b}	21.2 ± 0.9 ^{c}	22.2 ± 0.8 ^{c}
Triacylglycerol (TAG)	23.4 ± 1.5 ^{a}	18.9 ± 0.7 ^{b}	19.3 ± 2.2 ^{b}	9.0 ± 1.2 ^{c}	7.3 ± 0.3 ^{c}
Steryl/wax ester	37.2 ± 0.5 ^{a}	29.7 ± 1.2 ^{b}	31.6 ± 1.1 ^{b}	29.1 ± 1.0 ^{b}	23.5 ± 0.6 ^{c}
Free fatty acid	4.7 ± 0.5	5.3 ± 0.9	4.8 ± 1.3	4.7 ± 0.9	6.0 ± 0.5
TAG:Cholesterol	1.9 ± 0.2 ^{a}	1.2 ± 0.0 ^{b}	1.2 ± 0.2 ^{b}	0.4 ± 0.1 ^{c}	0.3 ± 0.0 ^{c}

Results are means ± SD (n = 3). An SD of 0.0 implies an SD of < 0.05. Values bearing different superscript letter are significantly different (P<0.05).

Table 2. Fatty acid composition (percentage of total fatty acids) of total lipid from unfed ABFT (*Thunnus thynnus* L.) yolk-sac larvae from 0 to 4 days post hatch (dph).

Fatty acid	dph0		dph1		dph2		dph3		dph4	
14:0	2.5 ± 0.1	a	2.2 ± 0.2	ab	2.0 ± 0.4	abc	1.6 ± 0.1	bc	1.5 ± 0.2	c
16:0	16.8 ± 0.8		18.8 ± 1.0		19.1 ± 0.8		18.7 ± 0.8	a	19.3 ± 1.4	
18:0	5.6 ± 0.5	c	6.9 ± 0.5	bc	7.4 ± 0.3	bc	9.0 ± 0.6	ba	10.2 ± 1.5	a
Total saturated[1]	25.6 ± 1.4	b	28.6 ± 1.7	ab	29.2 ± 1.2	ab	30.1 ± 1.3	b	31.9 ± 2.8	a
16:1n-7	4.9 ± 0.5	a	4.3 ± 0.2	a	4.1 ± 0.2	a	3.2 ± 0.2	b	2.9 ± 0.4	b
18:1n-9	18.1 ± 1.4	a	17.8 ± 0.6	b	17.9 ± 0.4	b	16.2 ± 0.4	ba	16.1 ± 1.3	b
18:1n-7	3.9 ± 0.8	a	3.1 ± 0.1	ab	3.1 ± 0.1	ab	2.9 ± 0.2	b	2.6 ± 0.2	b
20:1n-9	3.3 ± 0.4		3.4 ± 0.1		2.9 ± 1.3		3.1 ± 0.1		3.0 ± 0.1	
22:1	2.1 ± 0.3		2.1 ± 0.1		2.0 ± 0.2		1.7 ± 0.1		1.6 ± 0.2	
24:1n-9	0.5 ± 0.2		0.4 ± 0.1		0.4 ± 0.2		0.7 ± 0.5		0.6 ± 0.4	
Total monoenes[2]	33.0 ± 0.7	a	31.2 ± 0.8	a	30.6 ± 1.8	ab	28.0 ± 1.1	b	27.0 ± 2.3	b
18:2n-6	2.0 ± 0.2	a	1.6 ± 0.1	b	1.5 ± 0.1	b	1.5 ± 0.1	b	1.3 ± 0.0	b
20:4n-6	1.2 ± 0.1	c	1.5 ± 0.0	bc	1.6 ± 0.1	b	2.0 ± 0.2	a	2.2 ± 0.2	a
Total n-6PUFA[3]	4.2 ± 0.1		4.1 ± 0.2		4.2 ± 0.2		4.6 ± 0.3		4.6 ± 0.2	
18:3n-3	0.8 ± 0.1	a	0.7 ± 0.1	ab	0.6 ± 0.1	ab	0.5 ± 0.1	b	0.5 ± 0.1	b
18:4n-3	1.1 ± 0.1	a	1.0 ± 0.0	ab	0.9 ± 0.1	ab	0.7 ± 0.1	bca	0.5 ± 0.2	c
20:4n-3	0.7 ± 0.1	a	0.6 ± 0.0	a	0.6 ± 0.0	a	0.5 ± 0.0	b	0.4 ± 0.1	b
20:5n-3	6.4 ± 0.3	a	6.2 ± 0.2	a	5.8 ± 0.2	ab	5.1 ± 0.1	bc	4.7 ± 0.4	c
22:5n-3	1.7 ± 0.1		1.7 ± 0.1		1.6 ± 0.1		1.6 ± 0.1	a	1.5 ± 0.2	
22:6n-3	19.3 ± 0.8	c	20.6 ± 0.5	bc	20.6 ± 0.9	bc	22.7 ± 0.8	b	23.0 ± 0.9	a
Total n-3PUFA[4]	30.2 ± 1.3		30.8 ± 0.8		30.3 ± 0.8		31.2 ± 0.8		30.8 ± 1.4	
DHA:EPA	**3.0 ± 0.1**	**c**	**3.3 ± 0.0**	**bc**	**3.6 ± 0.3**	**b**	**4.4 ± 0.1**	**a**	**5.0 ± 0.5**	**a**
Total PUFA	34.4 ± 1.3		34.9 ± 1.0		34.5 ± 0.9		35.8 ± 1.0		35.4 ± 1.4	

Results are means ± SD (n = 3). An SD of 0.0 implies an SD of < 0.05. Values bearing different superscript letters are significantly different (P<0.05). [1], Totals include 15:0, 20:0 and 22:0; [2], Totals include 16:1n-9 and 20:1n-7; [3], Totals include 18:3n-6, 20:2n-6, 20:3n-6 and 22:5n-6; [4], Totals include 20:3n-3; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acid.

Table 3. Fatty acid composition (percentage of total fatty acids) of total polar lipids from unfed ABFT (*Thunnus thynnus* L.) yolk-sac larvae from 0 to 4 days post hatch (dph).

Fatty acid	dph0		dph1		dph2		dph3		dph4	
14:0	1.6 ± 0.2		1.3 ± 0.2		1.3 ± 0.3		1.2 ± 0.5		1.3 ± 0.1	
16:0	21.1 ± 0.6		22.5 ± 0.2		21.9 ± 2.5		19.5 ± 1.6		19.7 ± 0.0	
18:0	11.7 ± 1.6	b	12.6 ± 0.6	ab	14.1 ± 0.8	a	11.9 ± 0.5	ab	14.0 ± 0.1	a
Total saturated[1]	35.8 ± 1.8	ab	37.3 ± 0.9	ab	38.8 ± 2.9	a	33.5 ± 0.2	b	36.3 ± 0.1	ab
16:1n-7	3.6 ± 1.3		2.6 ± 0.4		2.7 ± 0.8		2.2 ± 0.4		2.6 ± 0.0	
18:1n-9	10.0 ± 1.4		9.3 ± 0.0		9.1 ± 0.3		9.1 ± 0.4		9.5 ± 0.1	
18:1n-7	2.0 ± 0.1		2.3 ± 0.2		2.2 ± 0.2		2.1 ± 0.1		2.1 ± 0.4	
20:1n-9	1.3 ± 0.1		1.3 ± 0.1		1.4 ± 0.1		1.4 ± 0.0		1.4 ± 0.1	
22:1	1.5 ± 0.1		0.5 ± 0.1		0.4 ± 0.0		0.7 ± 0.1		0.4 ± 0.0	
24:1n-9	1.1 ± 0.4		1.5 ± 1.1		1.1 ± 0.5		1.9 ± 1.6		1.3 ± 0.4	
Total monoenes[2]	19.4 ± 3.0		17.7 ± 1.2		17.2 ± 1.2		17.3 ± 1.5		17.3 ± 0.6	
18:2n-6	1.2 ± 0.4		1.0 ± 0.1		1.1 ± 0.2		1.0 ± 0.0		1.1 ± 0.0	
20:4n-6	2.3 ± 0.4		2.1 ± 0.3		2.1 ± 0.1		2.4 ± 0.2		2.6 ± 0.0	
22:5n-6	0.5 ± 0.1	b	0.6 ± 0.1	ab	0.6 ± 0.0	ab	0.7 ± 0.0	a	0.7 ± 0.0	a
Total n-6PUFA[3]	4.4 ± 0.1	bc	4.3 ± 0.1	cd	4.1 ± 0.0	d	4.6 ± 0.0	ab	4.8 ± 0.1	a
18:3n-3	0.3 ± 0.1		0.3 ± 0.0		0.3 ± 0.0		0.2 ± 0.0		0.2 ± 0.0	
18:4n-3	0.4 ± 0.1	a	0.3 ± 0.0	ab	0.2 ± 0.0	b	0.2 ± 0.0	b	0.2 ± 0.0	b
20:4n-3	0.3 ± 0.1		0.3 ± 0.0		0.3 ± 0.1		0.3 ± 0.0		0.2 ± 0.0	
20:5n-3	5.8 ± 0.8	a	5.0 ± 0.7	ab	4.0 ± 0.1	b	4.3 ± 0.5	b	3.7 ± 0.1	b
22:5n-3	1.2 ± 0.1		1.3 ± 0.1		1.3 ± 0.1		1.4 ± 0.0		1.3 ± 0.0	
22:6n-3	26.8 ± 4.1		27.8 ± 4.0		27.2 ± 1.1		30.0 ± 2.5		29.1 ± 1.0	
Total n-3PUFA[4]	34.7 ± 5.1		35.1 ± 4.9		33.4 ± 1.2		36.4 ± 2.9		34.8 ± 1.1	
DHA:EPA	**4.7 ± 0.1**	**e**	**5.5 ± 0.0**	**d**	**6.8 ± 0.0**	**c**	**7.1 ± 0.2**	**b**	**7.9 ± 0.0**	a
Total PUFA	39.1 ± 4.9		39.3 ± 4.9		37.5 ± 1.2		41.0 ± 2.9		38.5 ± 1.1	

Results are means ± SD (n = 3). An SD of 0.0 implies an SD of < 0.05. Values bearing different superscript letters are significantly different (P<0.05). [1], Totals include 15:0, 20:0 and 22:0; [2], Totals include 16:1n-9 and 20:1n-7; [3], Totals include 18:3n-6, 20:2n-6, 20:3n-6 and 22:5n-6; [4], Totals include 20:3n-3; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acid.

Table 4. Fatty acid composition (percentage of total fatty acids) of total neutral lipids from unfed ABFT (*Thunnus thynnus L*.) yolk-sac larvae from 0 to 4 days post hatch (dph).

Fatty acid	dph0		dph1		dph2		dph3		dph4	
14:0	3.3 ± 0.1		3.3 ± 0.1		3.2 ± 0.3		3.3 ± 0.2		3.5 ± 0.2	
16:0	14.5 ± 0.8		15.5 ± 0.6		15.5 ± 0.1		15.0 ± 1.2		14.3 ± 0.4	
18:0	4.2 ± 0.4	c	5.6 ± 0.1	a	5.7 ± 0.3	a	5.8 ± 0.1	a	4.9 ± 0.0	b
Total saturated[1]	22.8 ± 1.6		25.1 ± 0.6		25.3 ± 0.3		24.8 ± 1.4		23.6 ± 0.8	
16:1n-7	6.1 ± 0.1	ab	6.1 ± 0.1	ab	6.2 ± 0.1	ab	5.8 ± 0.0	b	6.5 ± 0.4	a
18:1n-9	22.7 ± 1.3	ab	23.6 ± 0.5	a	24.6 ± 0.5	a	23.3 ± 0.5	ab	21.5 ± 0.4	b
18:1n-7	3.1 ± 0.0		3.5 ± 0.5		3.2 ± 0.3		3.2 ± 0.1		3.6 ± 0.1	
20:1n-9	3.9 ± 0.1	b	4.5 ± 0.1	a	4.7 ± 0.1	a	4.7 ± 0.1	a	4.1 ± 0.1	b
22:1	2.7 ± 0.1	b	3.1 ± 0.0	a	3.0 ± 0.0	a	3.1 ± 0.0	a	2.8 ± 0.1	b
24:1n-9	1.0 ± 0.8		0.8 ± 0.4		0.8 ± 0.2		0.7 ± 0.1		0.6 ± 0.1	
Total monoenes[2]	39.7 ± 0.6	b	41.7 ± 0.9	a	42.6 ± 0.5	a	41.0 ± 0.6	ab	39.4 ± 0.3	b
18:2n-6	2.0 ± 0.1		1.9 ± 0.0		2.1 ± 0.1		2.0 ± 0.1		2.3 ± 0.3	
20:4n-6	0.6 ± 0.0	c	0.8 ± 0.0	b	0.8 ± 0.1	b	1.0 ± 0.0	a	1.0 ± 0.1	a
Total n-6PUFA[3]	3.4 ± 0.0	b	3.6 ± 0.1	b	3.8 ± 0.4	b	4.0 ± 0.1	b	5.0 ± 0.6	a
18:3n-3	0.9 ± 0.0		0.9 ± 0.0		1.0 ± 0.1		0.9 ± 0.1		0.9 ± 0.0	
18:4n-3	1.5 ± 0.1	a	1.3 ± 0.0	b	1.2 ± 0.0	b	1.3 ± 0.1	b	1.3 ± 0.0	b
20:4n-3	1.0 ± 0.1		0.8 ± 0.0		0.8 ± 0.1		0.8 ± 0.0		0.8 ± 0.1	
20:5n-3	6.0 ± 0.7	a	5.4 ± 0.1	ab	5.0 ± 0.0	b	5.3 ± 0.2	ab	5.9 ± 0.3	ab
22:5n-3	2.0 ± 0.2	a	1.8 ± 0.1	ab	1.6 ± 0.0	b	1.7 ± 0.1	ab	1.8 ± 0.1	ab
22:6n-3	15.2 ± 1.2	a	12.5 ± 0.4	b	11.8 ± 0.0	b	13.6 ± 1.3	ab	14.0 ± 0.5	ab
Total n-3PUFA[4]	26.5 ± 2.3	a	22.8 ± 0.7	ab	21.4 ± 0.1	b	23.6 ± 1.8	ab	24.7 ± 0.9	ab
DHA:EPA	**2.5 ± 0.1**		**2.3 ± 0.1**		**2.4 ± 0.0**		**2.6 ± 0.1**		**2.4 ± 0.0**	
Total PUFA	29.9 ± 2.3	a	26.4 ± 0.6	ab	25.2 ± 0.5	b	27.5 ± 1.8	ab	29.7 ± 0.4	a

Results are means ± SD (n = 3). An SD of 0.0 implies an SD of < 0.05. Values bearing different superscript letters are significantly different (P<0.05). [1], Totals include 15:0, 20:0 and 22:0; [2], Totals include 16:1n-9 and 20:1n-7; [3], Totals include 18:3n-6, 20:2n-6, 20:3n-6 and 22:5n-6; [4], Totals include 20:3n-3; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acid.

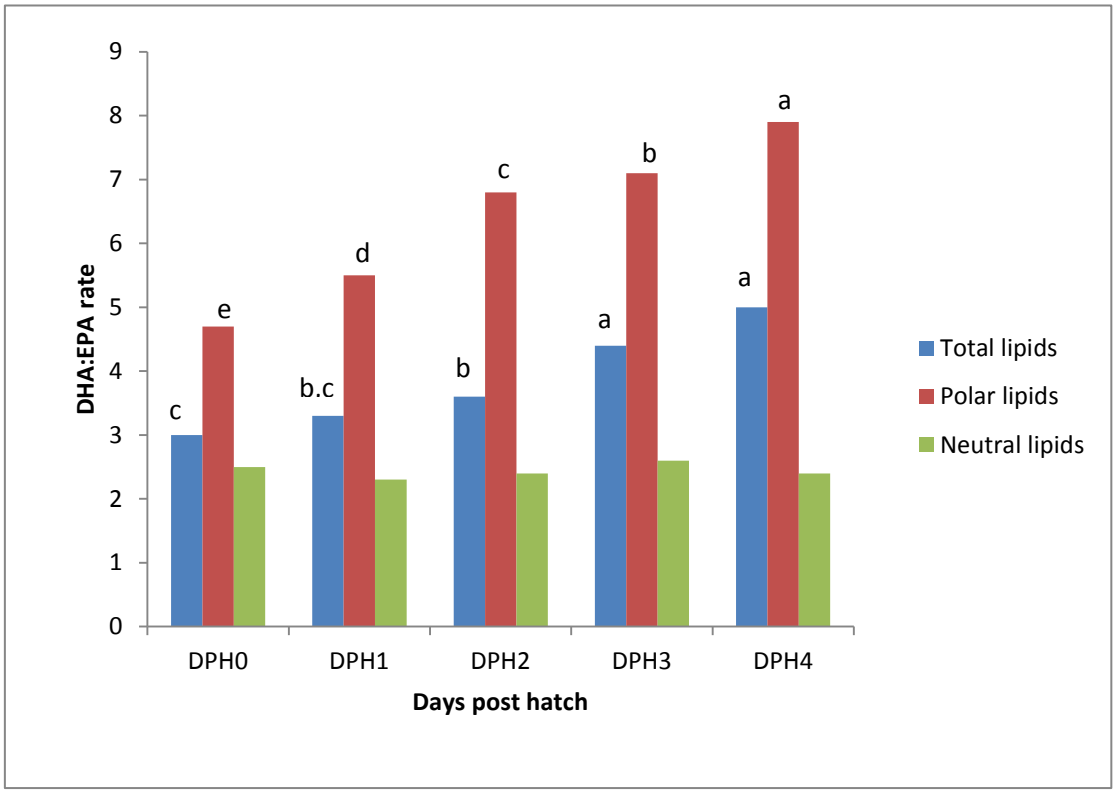

Figure 1: Evolution of DHA:EPA ratio in total, neutral and polar lipids through the time. Values bearing different superscript letter are significantly different ($P<0.05$).

4. Discussion

The present study is the first report of lipid and fatty acid metabolism in ABFT and has focussed specifically on a critical time in marine fish development, such as larvae during the period of yolk sac utilization. Although nutrient utilization and metabolism during early larval development has been a relatively well-studied area in fish there are few comparative data on scombrid species (Weigand, 1996). Fish of the order Perciformes, to which *T. thynnus* (Perciformes, Scombridae) belongs, generally produce eggs with oil globules, showing variability in the egg or oocyte lipid class compositions but generally with neutral lipids predominating over polar lipids (Wiegand, 1996). Previously, we have reported lipid and fatty acid compositions of wild ABFT eggs (Ortega and Mourente, 2010). The newly-hatched yolk sac larvae obtained from captive ABFT showed higher dry mass and total lipid content, and a higher proportion of

total neutral lipids, and lower polar lipids, than wild ABFT eggs. Importantly, however, the nutritional index (TAG/cholesterol) was almost 50 % lower in yolk sac larvae from the captive ABFT than in wild ABFT eggs (Ortega and Mourente, 2010). The ABFT yolk sac larvae at hatching also showed higher proportions of monoenes and lower proportions of PUFA and a lower DHA:EPA ratio than the total lipid fatty profile of eggs of wild caught ABFT (Ortega and Mourente, 2010). These differences in apparent quality parameters (TAG/cholesterol, PUFA level and DHA:EPA ratio) of the larvae from captive ABFT compared to eggs from wild ABFT suggest that this is an area worthy of future research effort.

The decreasing total lipid content, and the decreased proportions of neutral lipids, particularly TAG but also steryl ester/wax ester, showed that lipid was utilized during this early stage of yolk sac larval development in ABFT. Phospholipid, TAG or wax ester, exclusively, sequentially or in combination, are all used as energy sources by fish embryos with the pattern varying with species (Sargent *et al.*, 1989, 2002; Wiegand, 1996). Thus, ABFT showed a pattern of lipid metabolism during early development similar to that of marine larval fish from temperate waters whose eggs contain high levels of total lipids, including an oil globule, and which preferentially utilize neutral lipids as the primary energy source (Weigand *et al.*, 1996; Sargent *et al.*, 2002). Studies of fatty acid depletion in developing embryos and early larvae of a range of Perciform species have found preferential catabolism of MUFAs along with preferential retention of DHA, ARA and specific saturated fatty acids, usually 16:0 or 18:0 (Mourente and Vazquez, 1996; Wiegand *et al.*, 1996; Mourente *et al.*, 1999; Sargent *et al.*, 2002; Ortega and Mourente, 2010). This reflects the essential structural role of DHA in membranes, the importance of ARA in eicosanoid production and specific roles of saturated fatty acids in the sn-1 position of structural phospholipids.

In the present study the ABFT larvae were not fed and so were, in effect, starving and the changes in lipids have to be interpreted in that context. Therefore, one explanation for the changes observed in the larval fatty acid composition, showing an increasing DHA:EPA ratio, can be advanced simply in terms of differential oxidation and retention of DHA. The results show total lipid and neutral lipid decreased during the development period and so fatty acids were undoubtedly being utilized for energy. Although it is known that both EPA and DHA can be oxidized in fish, at least salmon, when in dietary excess (Stubhaug *et al.*, 2007), DHA is known to be the fatty acid that is most preferentially retained (Sargent *et al.*, 2002). Whether this is a true active retention or whether it is due to DHA being more slowly and inefficiently oxidized is not clear (Tocher, 2003). The results with the ABFT larvae showed that the proportion of polar lipid increased with time and, as polar lipid had a higher DHA content than neutral lipid, the proportion of DHA in total lipid would

consequently increase, as observed. In addition however, whereas DHA content in neutral lipid remained constant there was a trend for increased proportions of DHA in polar lipids suggesting that DHA released by hydrolysis of TAG could be then reesterified in polar lipid as observed in previous studies on larval development in marine fish (Tocher *et al.*, 1985; Fraser *et al.*, 1988). So the fact that the content of DHA relative to total fatty acids tends to increase in both total lipid and polar lipid would be consistent with the known phenomenon of retention of DHA.

A possible explanation for the increasing DHA:EPA ratio observed in total lipid in the ABFT larva could be the retention of DHA through relatively lower oxidation and channeling towards reesterification in polar lipid. It was previously speculated that the high DHA:EPA ratio in tuna tissues was consistent with selective metabolism of EPA by catabolism via β-oxidation (Tocher, 2003). Another explanation suggested by Tocher (2003) is the conversion of EPA in DHA via elongation and desaturation. This via has been recently studied by Morais *et al.* (2011), and she found an increasing expression of the genes of LC-PUFA biosynthetic pathway, Δ6fad and elovl5, which could offers an alternative mechanism.

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2.3. Perfomance of Atlantic bluefin tuna (*Thunnus thynnus* L.) larvae at first feeding on live preys: influence of dietary lipid content and composition

RESUMEN

Respuesta de las larvas de Atún rojo del Atlántico, (*Thunnus thynnus* L.) a la primera alimentación con presas vivas: influencia del contenido y composición de los lípidos en la dieta

En este capítulo se muestran y discuten los resultados obtenidos en la primera alimentación de larvas de atún rojo (Thunnus thynnus, L. 1758) con diferentes presas vivas durante los periodos de reproducción de 2013 y 2014: i) copépodos, nauplius de *Acartia tonsa*, ii) rotíferos enriquecidos, *Brachionus plicatilis*, y iii) una dieta mixta de ambas presas vivas. En el ensayo de 2013, las larvas fueron alimentadas únicamente con dos tratamientos, copépodos o rotíferos enriquecidos; las larvas de atún no presentaron diferencias con respecto al crecimiento pero las alimentadas con copépodos mostraron una mejor supervivencia. Con respecto al ensayo de 2014, las larvas alimentadas con copépodos presentaron un mejor crecimiento, aunque las larvas alimentadas con rotíferos y copépodos mostraron los mejores resultados de supervivencia en esta etapa y valores intermedios de crecimiento frente a las alimentadas únicamente con copépodos o rotíferos enriquecidos. En ambos casos, estos resultados parecen estar más relacionados con los niveles de ácidos grasos polinsaturados de cadena larga (ácidos eicosapentaenóico (EPA) 20:5n-3 y docosahexaenóico (DHA)) de la serie omega 3 de la dieta, que con la composición en clases de lípidos, cuyo contenido parece ser suficiente para satisfacer las necesidades en estos nutrientes. Los beneficios resultantes de usar dietas mixtas (rotífero-copépodo) presentan el valor añadido de reducir la cantidad requerida de copépodos como alimento.

ABSTRACT

In this chapter are presented and discussed the results achieved at first feeding of ABFT (*Thunnus Thynnus*, L. 1758) larvae with different live preys in trials

during 2013 and 2014 spawning seasons: i) copepods, *Acartia tonsa* nauplii, ii) enriched rotifers, *Brachionus plicatilis*, and iii) a mix diet of both live preys. In 2013 trial, Bluefin tuna larvae were only fed with two dietary treatments; copepods or enriched rotifers. In this case, larvae did not show significant differences regarding to growth but presented a better survival those fed on enriched copepods. Regarding 2014 trial, larvae fed on copepods showed a better growth, although those co-fed on enriched rotifers and copepods presented the best survival results at this stage, with intermediate growth results in comparison with larvae fed only with rotifers or copepods. In both cases, results seem to be more related to the level of long chain n-3 polyunsaturated dietary fatty acids (eicosapentaenoic (EPA) 20:5n-3 and docosahexaenoic (DHA) 22:6n-3 acids) than to live food lipid class composition and content which seem to cover the requirements for these nutrients. The benefits resulting of using mixed live diets (copepod-rotifer) has the added value of reducing the amount of copepods required

Introduction

The improvement in the production of ABFT larvae and juveniles is essential to establish the full-cycle culture technology. In recent years considerable research effort has been undertaken in order to develop aquaculture of Pacific bluefin tuna (PBFT), *Thunnus orientalis, (Temminck and Schlegel)* and some important improvements, mainly at larval rearing stages, have been achieved in the last years. Different husbandry, management and feeding studies have deal dealing with larval rearing methods in order to reduce the high mortalities ocurring during the first days of life in PBFT such as sinking death or surface death (Miyashyta *et al.*; 2006, Kato *et al.*, 2008), and ABFT larval rearing methodologies are getting benefits from those findings.

However mortality continue to be high and, up to date, standard live feeds and artificial diets feeding protocols for larvae and juveniles are giving poor survival, growth and stress resistance results. Moreover, dispersed sizes and malformations are common not only in ABFT culture but also in other bluefin tunas species. Thus, to get an adequate nutritional value of live preys (rotifers, *Artemia*, copepods, fish yolk-sac larvae) and juvenile diets are paramount to improve the yield of the cultures.

Use of copepods as live preys for marine fish has been reported to improve the results in larval rearing of marine fish (Witt *et al.,* 1984; Toledo *et al.*, 1999; Holmvaag, 2011; Barroso *et al.*, 2013). Only a few experiences have been carried out with tunas (Evjemo *et al.*, 2014), but due to the large presence in the

sea, together with a very high nutritional value, copepods can be considerer as the msot adequate food prey for marine fish larval rearing.

In fish, lipids and their constituent fatty acids, play essential roles in maintaining optimum growth, survival, feed efficiency, health, neural and visual development, and response to stressors in addition to be the main energy source (Sargent *et al.*, 1989; 2002; Tocher, 2003; 2010). Among the lipids and their constituents, phospholipids and highly unsaturated fatty acids (HUFA) with lipid storage as the source of energy have denoted their importance due to their critical roles in the biological processes cited above. Appropriate uptake and accumulation of lipids improve growth and survival of fish but, particularly, lipids are much more important and key compounds in highly active migratory fish species such as tunas (Mourente and Tocher, 2003; 2009).

Another key characteristic of lipid metabolism and tissue FA compositions in large-sized tuna species is that they display high DHA contents (> 20 %) and high DHA/EPA ratios (Mourente *et al.*, 2002; Mourente and Tocher, 2003; 2009; Ortega and Mourente, 2010). Thus, in ABFT and other "warm blooded" active-migratory species, the DHA content and, DHA/EPA ratio of muscle is much higher than in non-migratory ones, due to selective utilization of monoenoic and saturated FAs oxydized as energy sources relative to PUFA such as DHA (Nakamura *et al.*, 2007; Osako *et al.*, 2009). Thus, the high DHA/EPA ratio may also be partly due to the selective catabolism of EPA relative to DHA (Mourente and Tocher, 2003). In consequence, it also suggest that tunas may have a high requirement for dietary DHA and a high DHA/EPA ratio. In any case, the lipid biochemistry underpinning the high level of tissue DHA and the high DHA/EPA ratio in this species is unclear and their capacity for endogenous synthesis of EPA and DHA limited (Gregory *et al.*, 2009; Morais *et al.*, 2011).

In the former article of this thesis, lipid and FA metabolism during early development of yolk-sac ABFT larvae were investigated. In unfed larvae, the level of DHA was maintained, but the proportion of EPA declined, and so the DHA/EPA ratio increased during yolk sac utilization. As described above, this could have been due to relative retention of DHA during a period of high FA oxidation and utilization. But as Morais *et al.*, (2011) reported, expression of genes for high polyunsaturated fatty acids in ABFT, D6Fad and Elovl5 was also increased with larval development. This argue that increased activity of these enzymes could be crucial for normal development of ABFT larvae related to the provision of enough DHA for the formation of membranes, particularly in neural tissues (Mourente, 2003).

Yet another key factor in the biology of tuna species that has consequences for nutrition is that they have a higher requirement for dietary lipid than many other marine fish species due to large lipid stores being required as the major energy source during migration and reproduction (Mourente and Tocher, 2009). Lipid is

required in the diet of fish to supply both metabolic energy and essential nutrients such as essential fatty acids (EFA) and, in early life stages, intact phospholipid and possibly cholesterol (Sargent *et al.*, 2002). Thus, defining a single dietary lipid requirement for any species as this will vary depending upon the other dietary components supplying energy, such as protein and carbohydrate (Biswas, 2010; NRC, 2011).

The regulation of lipid homeostasis in fish is a complex balance between lipid uptake, transport, storage, energy utilization and biosynthesis. Each single process needs to be controlled independently and also in conjunction with other processes (Tocher, 2003). Thus, recent studies looking at global gene expression using transcriptomic and proteomic approaches have shown that dietary lipid content can have significant effects on gene expression in salmonids (Kolditz *et al.*, 2008; Panserat *et al.*, 2008; Martinez-Rubio *et al.*, 2013). In this context, will be relevant the study of the effects of dietary lipid on lipid and FA metabolism to interpret lipid nutrition in ABFT and issue an assessment new tool in the formulation of practical diets for this species.

Materials and Methods

1. ABFT larvae rearing conditions

Bluefin tuna larvae used in this work correspond to two consecutive larval rearing trials performed in July 2013 and July 2014, respectively. ABFT eggs came from a broodstock composed of 20 fish with an estimated mean body weight of 150 kg. They were kept in captivity for several years in a floating cage located at El Gorguel, off Cartagena coast, SE Spain. Captive-reared ABFT broodstock fish spawned naturally and spontaneously (during its natural spawning season in June-July) and floating eggs were collected inside the cage by means of a net of 500 µm mesh screen size. A 2.0 m depth polivynil sheet was placed inside around the cage to avoid eggs floating away from the cage. Collected eggs were transported in a 500 L cylindrical plastic tank supplied with pure oxygen to the IEO – Mazarrón aquaculture facilities and placed in 100 L cylindro-conical tanks with gentle oxygenation and flow through sterilized sea water system in order to clean them. After 1 h, aeration and water flow were stopped to separate buoyant (viable) from non-buoyant (non-viable) eggs. After washing and counting, part of the eggs, were incubated in 1500 L cylindrical tanks at a density of about 5 eggs L^{-1}. Incubation was carried out at 22.5 - 23.5 ºC, 37 ppt salinity and continuous photoperiod, with a light intensity of 1000 lux. An upwelling flow-through, with gentle aeration in order to keep oxygen levels next to saturation, was employed.

Larvae hatched in the afternoon of the next day (aprox. 34-36 hours after fertilization), with a hatching rate of aprox. 90%, and started to be fed, with rotifers or copepod nauplii, when they were 2 days old (2 days post hatch hereinafter dph). As green water, the microalgae *Isochrysis galbana* (T-Iso) was added to tanks, at a density of 100-120.000 cells mL^{-1}. During the trials, photoperiod was maintained at 14 h/10 h light/darkness (light intensity about 1000 lux), temperature ranged between 22.9 - 24.8ºC and daily water renewal ranged between 50 - 100%. Incoming sea water was filtered at 10 µm and UV sterilized. Upwelling current was kept during all the experiment in order to avoid sinking of the larvae (mainly at night) and keep oxygen level. As it was reported by Takashi *et al.*, 2006, and Tanaka *et al.*, 2009, tuna larvae swam slowly in the night time and that is the reason because they sank. In order to help the larva to be kept in suspension, aeration was manipulated to be milder during light time and stronger during night time

In order to prevent surface death, some drops of fish oil were added to the tanks from 1 dph to 7 dph. Surface skimmer were used from day 3 onwards in order to keep as clean as possible the surface of the tanks and to allow to the larvae to go to the surface of the tank for gulping an air bubble to do the first inflation of the swim bladder, which is known to be necessary not only in PBFT (Tanaka *et al.*, 2009; Kurata *et al.*, 2012) but also in other species (Chatain & Ounais-Guschemann, 1990; Moretti el at., 1999). Further studies developed by Kurata *et al.* in 2013 revealed that swim bladder inflation took place in the afternoon and evening, and in order to keep the fish oil in the tank, we only turned on the skimmer from 15 h to 21 h

a) *2013 ABFT larval Trial*. Two different feeding treatments were tested from the beginning of exogenous feeding 2 dph to 14 dph; i) L-type rotifers (*Brachionus plicatilis*), cultured with DHA enriched Chlorella (Super Fresh Chlorella SV12 ® Chlorella Industry Co Ltd) and enriched with taurine (500 ppm taurine added to rotifer culture tanks during 18 h before harvesting) and Origreen Skretting® during 3 h at a dose of 0.3 g per million rotifers, ii) nauplii of the copepod *Acartia grani.* To maintain constant live prey concentration (5 rotifer mL^{-1} or 5 copepod nauplii/copepodite mL^{-1}) within each experimental tank, three water samples (10 mL) from each tank were used and counted twice per day before supplying new food.

b) *2014 ABFT larval Trial.* In this case, three feeding tratments were tested from 2 dph to 14 dph: i) enriched L-type rotifers (*Brachionus plicatilis*) with taurine and Origreen Skretting ®, ii) nauplii of the copepod *Acartia tonsa*, and iii) co-feeding of enriched rotifers and *Acartia* nauplii at aprox 50% concentration of both live preys. Density of rotifers was maintained at 5 rotifers mL^{-1}, *Acartia tonsa* at 5 nauplii mL^{-1} and co-feeding of rotifers and *Acartia* at 5 individual mL^{-1}

in a 50% mixture of both organisms. In order to maintain prey concentration, new rotifers and copepods were added twice per day.

2. Acartia culture

In 2013, Acartia grani was cultured. Cultivation was made according to Calbert & Alcaraz (1997), and Dos Santos Sumares (2012). Adults were kept at 37 ppt salinity and temperature ranged between 23 and 25ºC in 200 l. cylindrical tanks, with a water renewal rate of 30% day. Algal feeding constisted on a 50% mixture of *Tetraselmis suecica* and *Rhodomonas baltica*. Resting eggs were obtained daily by siphoning and they were incubated at the same temperature and salinity than breeders. After hatching, *Acartia* nauplii were fed on *Rhodomonas baltica* and *Isochrysis aff. galbana* T-Iso during 48 hours, and then offered to ABFT larvae.

In 2014, the copepod species used as food was *Acartia tonsa*, cultured according to Stottrup (2006) and Marcus & Wilcow (2007). Adults were kept at 30 ppt salinity and temperature ranged between 23 and 25ºC in 5,000 l. cylindrical tanks, with a water renewal rate of 25% day. Algal feeding consisted on *Rhodomonas baltica*. Resting eggs were obtained daily by siphoning and cold preserved at 2ºC according to Drillet (2006) and Hagemann (2011) until their use. *Acartia tonsa* eggs were incubated at 30 ‰ salinity and 24 - 26ºC and once hatched fed on *Rhodomonas baltica* and *Isochrysis aff. galbana* T-Iso during 2 - 4 days, and then offered to ABFT larvae.

3. Sampling for biometrics and biochemical analysis.

Larvae (25 individuals for each rearing condition) used for length determination were anaesthetized (0.02% phenoxyethanol) and total lengths were measured. Then they were maintained at 110°C for 24 h and the dry weights were determined after cooling *in vacuo* for at least 1 h.

Triplicate samples of the live feeds rotifers and copepods (*Acartia*) were washed and filtered, and excess water drained and blotted with filter paper and immediately frozen inside cryovials in liquid N_2 and preserved at -80ºC until lipid analysis.

Triplicate samples of 14 dph ABFT larvae that had been fed on the different live preys used in 2013 and 2014 feeding trials were collected inside cryovials, frozen in liquid N_2 and preserved at -80ºC until lipid analysis.

At the end of the experiment, all the larvae in the tanks were extracted and counted to estimate survival rates, and a sample of 50 larvae was collected to be measured and weighted.

All procedures were carried out according to the national and present EU legislation on the handling of experimental animals.

4. Lipid content, lipid class composition and fatty acid analysis

Total lipid of live feeds (enriched rotifers and copepods) and ABFT larvae fed on the different feeding treatments was extracted from triplicate pooled samples according to the method of Folch *et al.* (1957). Approximately 1 g of ABFT larvae was placed in 20 ml of ice-cold chloroform/methanol (2:1, by vol) and homogenized with an Ultra-Turrax tissue disrupter (Fisher Scientific, Loughborough, U.K.). The non-lipid and lipid layers were separated by addition of 5 ml of 0.88 % (w/v) KCl and allowed to separate on ice for 1 h. The upper non-lipid layer was aspirated and the lower lipid layer dried under oxygen-free nitrogen. The lipid content was determined gravimetrically after drying overnight in a vacuum desiccator.

Lipid class composition was determined by high-performance thin-layer chromatography (HPTLC) using 10 x 10 cm plates (VWR, Lutterworth, England). Approximately 1 µg of total lipid was applied as a single spot and the plates developed in methyl acetate/isopropanol/ chloroform/methanol/0.25 % aqueous KCl (25:25:25:10:9, by vol.) up to two-thirds up the plate. After desiccation for 20 min, the plate was fully developed with isohexane/diethyl ether/acetic acid (85:15:1, by vol.). The lipid classes were visualized by charring at 160^{o}C for 15 min after spraying with 3 % (w/v) aqueous cupric acetate containing 8 % (v/v) phosphoric acid and quantified by densitometry using a CAMAG-3 TLC scanner (version Firmware 1.14.16) (Henderson and Tocher, 1992). Scanned images were recorded automatically and analyzed by computer using winCATS Planar Chromatography Manager (version 1.2.0).

Fatty acid methyl esters (FAME) were prepared from total lipid by acid-catalyzed transesterification at 50^{o}C for 16 h according to the method of Christie (1993). The FAME were separated and quantified by gas-liquid chromatography (Carlo Erba Vega 8160, Milan, Italy) using a 30 m x 0.32 mm i.d. capillary column (CP Wax 52CB, Chrompak, London, U.K.) and on-column injection at 50^{o}C. Hydrogen was used as carrier gas and temperature programming was from 50 ^{o}C to 150 ^{o}C at 40 ^{o}C min^{-1} and then to 230 ^{o}C at 2.0 ^{o}C min^{-1}. Individual methyl esters were identified by comparison with known standards and by reference to published data (Ackman, 1980; Tocher and Harvie, 1988).

Data were collected and processed using Chromcard for Windows (version 1.19)

5. Statistical analysis

Results for biometrics, lipid classes and fatty acids are presented as means ± SD (n = 20 for biometrics and n = 3 for survival, individual lipid classes and fatty acids). The data were checked for homogeneity of the variances by the Bartlett test and, where necessary, arc-sin transformed before further statistical analysis. Differences between mean values were analyzed by t-test and one-way analysis of variance (ANOVA), followed when pertinent by a multiple comparison test (Tukey). Differences were reported as statistically significant when $P < 0.05$ (Zar 1984).

Results

1. ABFT larvae biometry and survival in feeding trials

Total length, individual dry mass and survival of 14 dph ABFT larvae are displayed in Table 1. In 2013 trial, ABFT larvae fed on enriched rotifers showed larger than ABFT larvae fed on copepods although no significant differences were detected as for total dry mass. However, survival was nearly two fold higher in ABFT fed copepods. But due to technical problems with the egg copepod hatching, several days we couldn´t complete to 5 copepods/ml the prey concentration in the larval rearing tanks, and we must adjust only to 2 cop/ml., which could have limited the growth of ABFT larvae fed on copepods.

In 2014 trial, all the tanks were adjusted to 5 preys/ml. ABFT total length and total dry mass were highest in larvae fed copepods, showing intermediate values larvae co-fed with rotifers and copepods and the lowest values for those fed on rotifers. However, survival was highest in co-fed larvae followed by single copepod and single rotifer fed larvae, respectively. Results at the end of both experiences are displayed in Table I, and growth throughout all the 2014 experiment are displayed in Figures 1 and 2.

Table I. Rearing performance of 14 days after hatch ABFT (*Thunnus thynnus*) larvae fed on enriched rotifers *Brachionus plicatilis*, *Acartia tonsa* copepod nauplii, and co-feeding rotifer + copepod in 2013 and 2014 feeding trials.

Trial	2013		2014		
	Rotifer	copepod	rotifer	copepod	rotifer + copepod
Total length (mm)	7.7 ± 0.6	7.3 ± 0.5	7.0 ± 0.2 [a]	8.3 ± 0.1 [b]	7.5 ± 0.2 [c]
Dry mass (mg)	0.7 ± 0.1	0.6 ± 0.1	0.3 ± 0.03 [a]	0.8 ± 0.01 [b]	0.5 ± 0.06 [c]
Survival (%)	3.2 ± 1.1	5.9 ± 0.9 *	2.9 ± 1.0 [a]	7.5 ± 1.2 [b]	10.2 ± 3.5 [c]

Results are Mean ± SD (n = 25 for total length and dry mass and n=3 for survival). An * indicates significantly different ($p < 0.05$) for t-tests in 2013 trial. A different superscript letter denotes significantly different ($p < 0.05$) for one way ANOVA and Tukey multiple comparison tests in 2014 trial.

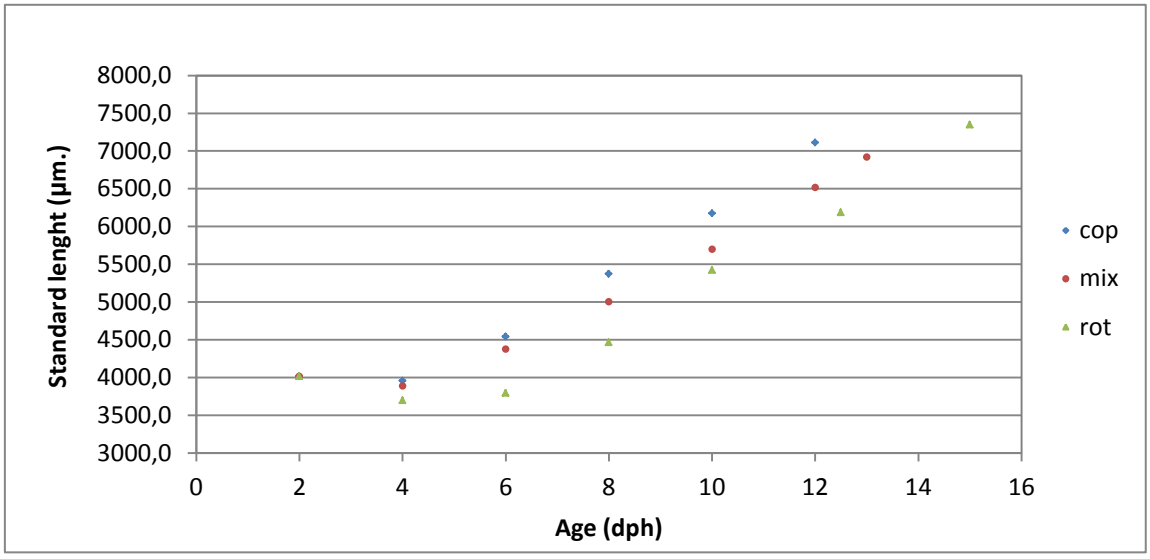

Figure 1: Data on standard length of ABFT larvae fed on three different experimental dietary trials (data corresponding to 2014).

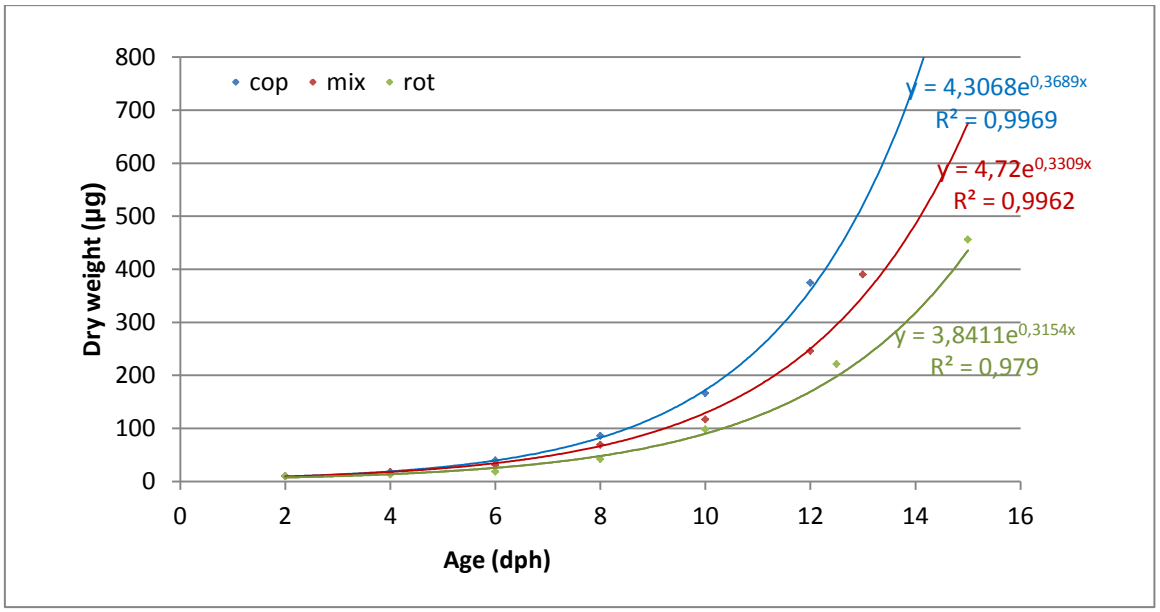

Figure 2: Dry weight evolution between 0 and 14 dph larvae fed with three different live food diets. Every data series are adjusted to an exponential curve.

2. Total lipid content, lipid class and total lipid fatty acid compositions of enriched rotifers B. plicatilis and *Acartia nauplii*

Total lipid content, lipid class and total lipid fatty acid compositions of rotifers and copepod nauplii used in 2013 ABFT dietary trial are shown in Tables 1(2) and 3(4). The lipid class composition of enriched *B. plicatilis* was predominantly polar lipids (~56%), primarily phosphatidylcholine (PC, ~ 15%) and phosphatidylethanolamine (PE, ~ 13%), with total neutral lipids (~ 44%), primarily triacylglycerol (TAG, ~ 25%). Total lipid fatty acid profile was characterized by 25% saturated fatty acids (primarily 16:0 followed by 18:0), 12.7% MUFA (primarily 18:1n-9) and 59.9% PUFA, primarily 18:2n-6 (20.5%) followed by the (n-3) PUFA 18:3n-3 (6.7%), EPA (4.5%) and DHA (12.1%). DHA:EPA ratio was 2.7.

The lipid class composition of *Acartia* nauplii was approximately 50% polar lipids and 50% neutral lipids. Major polar lipid classes were PC (14.2%) followed by PE (6.8%), whereas in neutral lipids predominated TAG (25.5%). Total lipid fatty acids were denoted by 27.7% saturated (primarily 16:0, followed by 14:0); 13.3% MUFA (primarily 18:1n-9 followed by 16:1n-7) and 57.1% PUFA, primarily DHA (24.9%), followed by EPA (8.9%), LA (8.7%) and DHA:EPA ratio of 2.8.

Total lipid content and lipid class composition of rotifers and copepod nauplii used in 2014 ABFT dietary trial are presented in Tables 2(3) and 4(5). The lipid class composition of enriched *B. plicatilis* was predominantly polar lipids

(52.8%), primarily PE, (13.2%) and PC 12.0%), with total neutral lipids (47.4%), primarily triacylglycerol (TAG, 23.6%). Total lipid fatty acids were characterized by 28.1% total saturated, primarily 16:0 (22.7%); 11.6% total MUFA, primarily 18:1n-9 (4.5%) and 55.1% total PUFA, with major components DHA (17.5%), LA (14.8%) and EPA (4.5%). DHA:EPA ratio was 3.8

The lipid class composition of *Acartia* nauplii was, in this case, predominated by total polar lipids (55.8%), with PC (16.2%) followed by PE (7.7%) as predominant classes. Whereas in total neutral lipids (44.2%), predominated TAG (20.6%). Total lipid fatty acids were represented by 26.6% saturated (primarily, 16:0 and 14:0, with aprox. 11.0% each); 11.8% MUFA (primarily 18:1n-9 followed by 16:1n-7) and 59.0% PUFA, primarily DHA (26.0%), followed by LA (8.0%), EPA (3.8%) and DHA:EPA ratio of 6.9

3. Total lipid, lipid class and total lipid fatty acid compositions of ABFT larvae

In 2013 dietary trial, total lipid content, lipid class and total lipid fatty acid compositions of ABFT larvae fed with enriched rotifers and *Acartia* nauplii are presented in Tables 1(2) and 3(4). ABFT larvae (14 dph) fed on enriched rotifers presented a lipid class composition mostly composed of polar lipids (66.4%), primarily PC (25.0%) and PE (11.4%); with total neutral lipids (33.6%), primarily represented by TAG (13.8%) followed by free cholesterol (C) (10.6%). Total lipid fatty acids were composed of 30.1% total saturated, primarily 16:0 (18.4%) followed by 18:0 (9.3%); total MUFA with 14.6%, particularly 18:1n-9 (6.3%) and 51.9% total PUFA, mainly represented by DHA (17.5%), LA (13.1%) and EPA (5.4%). DHA:EPA ratio was 3.2.

ABFT larvae (14 dph) fed on *Acartia* nauplii showed a lipid class composition predominated by total polar lipids (63.2%), primarily PC (22.2%), followed by PE (11.0%) and phosphatidylserine (PS) (9.5%). Total neutral lipids (36.8%) were mainly represented by C (14.9%) and TAG (11.6%). Total lipid fatty acids were represented by 31.9% saturated, primarily 16:0 (19.2%); total monoenes (12.7%), including 18:1n-9 as major component (6.0%) and total PUFA (51.8%), primarily DHA (29.6%), EPA (5.9%), LA (4.5%) and DHA:EPA ratio of 5.0. The nutritional index TAG:C showed higher in larvae fed on rotifers than the ones fed on copepods

In 2014 dietary trial, total lipid content, lipid class and total lipid fatty acid compositions of 14 dph ABFT larvae fed with enriched rotifers, *Acartia* nauplii and a co-feeding of both organisms are presented in Tables 2(3) and 4(5).

The lipid class composition of 14 dph ABFT larvae fed on enriched rotifer *B. plicatilis* was predominantly polar lipids (67.3%), primarily PC, PE, PS and PI

(22.2%, 14.2%, 8.6% and 5.7%, respectively) with 32.7% total neutral lipids, primarily TAG, C and SE (13.6%, 10.0% and 3.6%, respectively). Total lipids of 14 dph ABFT larvae fed on enriched rotifers were characterized by 27.9% saturated fatty acids, primarily 16:0 (16.9%), followed by 18:0 (8.6%); 12.9% MUFA, primarily 18:1n-9 (4.9%), and 53.8% PUFA, primarily the n-3 LC-PUFA, DHA (17.6%), EPA (6.6%) and LA (12.7%). DHA:EPA ratio was 2.7.

The lipid class composition of 14 dph ABFT larvae fed on *Acartia* nauplii presented total polar lipids as major contributors (62.6%), primarily PC, PE, PS and PI (20.4%, 13.9%, 7.5% and 4.4%, respectively), with total neutral lipids (37.4%) mostly composed by TAG, C, and SE (15%, 11% and 6.1%, respectively). Total saturated fatty acids accounted for 31.6%, mostly 16:0 (18.3%) and 18:0 (7.8%); 15.7% represented total monoenes, primarily 18:1n-9 (5.7%) and total PUFA (51.1%) vastly represented by DHA (28.2%), EPA (5.5%), LA (4.2%) and DHA:EPA of 5.1

The lipid class composition of 14 dph ABFT larvae co-fed on enriched rotifer *B. plicatilis* and *Acartia* nauplii presented a similar lipid class composition to the results showed above, total polar lipids (62.8%), primarily PC, PE, PS and PI (21.6%, 13.5%, 6.7% and 4%, respectively); total neutral lipids (37.2%), being predominantly TAG and C (17.6% and 11%, respectively). Total lipids were characterized by 29.8% total saturated fatty acids, primarily 16:0 (17.1%) and 18:0 (8.3%). Total monoenes represented 14% of total fatty acids, primarily 18:1n-9 (5.1%), and total PUFA (53.7%), with DHA (24.4%), LOA (10.3%) and EPA (4.8%) as major components. DHA:EPA ratio was 5.0. The nutritional index TAG:C showed identical value for all treatments of about 1.4.

Table 2. (2013 Trial). Total lipid (% dry mass for preys and % live mass for ABFT larvae) and lipid class composition (% of total lipid) of rotifer *Brachionus plicatilis* enriched with Origreen + Skretting ® (a), nauplii of the copepod *Acartia tonsa* fed on *Isocrysis* T-Iso (b), 14 days after hatch ABFT (*Thunnus thynnus*) (ABFT) larvae fed on rotifers (c) and copepod (d).

	(a) Rotifer	(b) Copepod	(c) ABFT + Rotifer	(d) ABFT + Copepod
Total Lipid (% dry mass)	13.4 ± 0.9	6.5 ± 0.3 *	1.0 ± 0.1	0.8 ± 0.2
Lipid Class				
Phosphatidylcholine	14.7 ± 0.5	14.2 ± 1.5	25.0 ± 0.8	22.2 ± 1.4
Phosphatidylethanolamine	13.3 ± 0.5	6.8 ± 1.4 *	11.4 ± 0.6	11.0 ± 0.3
Phosphatidylserine	4.9 ± 0.2	1.6 ± 0.9 *	7.3 ± 0.9	9.5 ± 0.7 *
Phosphatidylinositol	7.7 ± 0.5	1.3 ± 0.1 *	3.7 ± 1.1	4.6 ± 0.6
Phosphatidic acid/cardiolipin	nd	3.5 ± 0.8	3.1 ± 0.1	2.0 ± 0.2 *
Sphingomyelin	0.5 ± 0.0	5.2 ± 0.1 *	2.3 ± 0.4	2.8 ± 0.3
Lyso-phosphatidylcholine	2.4 ± 0.1	3.5 ± 0.2 *	1.8 ± 0.3	1.6 ± 0.1
Total Polar Lipids	55.9 ± 1.5	50.5 ± 4.9	66.4 ± 2.0	63.2 ± 1.7
Cholesterol	4.1 ± 0.6	14.2 ± 0.7 *	10.6 ± 0.3	14.9 ± 0.9 *
Triacylglycerol	25.5 ± 0.9	25.5 ± 3.9	13.8 ± 0.9	11.6 ± 1.8
Steryl/wax ester	5.4 ± 0.2	1.0 ± 0.1 *	3.1 ± 0.9	5.0 ± 0.5
Free fatty acid	8.9 ± 0.3	8.4 ± 0.4	5.9 ± 0.1	5.0 ± 1.3
Total Neutral Lipids	44.1 ± 1.5	49.5 ± 4.9	33.6 ± 2.0	36.8 ± 1.7
TAG:C	6.2 ± 0.7	1.8 ± 0.2 *	1.3 ± 0.1	0.8 ± 0.2

Results are Mean ± SD (n=3); TAG:C, triacylglycerol/cholesterol ratio; * significantly different ($p < 0.05$).

Table 3. (2014 Trial). Total lipid (% dry mass for preys and % live mass for ABFT larvae) and lipid class composition (% of total lipid) of rotifer *Brachionus plicatilis* enriched with Origreen + Skretting ® (a), nauplii of the copepod *Acartia tonsa* fed on *Rhodomonas salina* (b), and 14 days after hatch ABFT (*Thunnus thynnus*) larvae fed on rotifers (c), copepod (d), and co-feeding rotifer + copepod (e).

	(a) Rotifer	(b) Copepod	(c) ABFT + Rotifer	(d) ABFT + Copepod	(e) ABFT + Rotifer +Copepod
TL (% dry mass)	15.9 ± 1.6	9.0 ± 0.6 *	1.2 ± 0.0	0.8 ± 0.1	0.8 ± 0.3
Lipid Class					
Phosphatidylcholine	12.0 ± 0.5	16.2 ± 0.5 *	22.2 ± 0.7	20.4 ± 0.2	21.6 ± 1.0
Phosphatidylethanolamine	13.2 ± 0.5	7.7 ± 0.3 *	14.2 ± 1.5	13.9 ± 0.5	13.5 ± 1.0
Phosphatidylserine	4.9 ± 0.8	6.2 ± 0.5	8.6 ± 0.4 [a]	7.5 ± 0.6 [ab]	6.7 ± 0.6 [b]
Phosphatidylinositol	8.8 ± 0.6	5.1 ± 0.3 *	5.7 ± 0.3 [a]	4.4 ± 0.2 [b]	4.0 ± 0.4 [b]
Phosphatidic acid/cardiolipin	1.6 ± 0.2	3.9 ± 0.1 *	1.5 ± 0.1	1.6 ± 0.2	1.7 ± 0.2
Sphingomyelin	0.5 ± 0.1	5.6 ± 0.4 *	2.6 ± 0.2	2.1 ± 0.2	2.0 ± 0.2
Lyso-phosphatidylcholine	1.8 ± 0.3	2.4 ± 0.2	0.6 ± 0.1 [a]	0.6 ± 0.1 [a]	0.9 ± 0.1 [b]
Total Polar Lipids	52.8 ± 1.7	55.8 ± 0.3	67.3 ± 1.1 [a]	62.6 ± 0.2 [b]	62.8 ± 1.9 [ab]
Cholesterol	11.2 ± 1.2	10.9 ± 0.3	10.0 ± 0.6	11.0 ± 0.4	11.0 ± 0.3
Triacylglycerol	23.6 ± 2.1	20.6 ± 0.5	13.6 ± 0.7 [a]	15.0 ± 0.4 [ab]	17.6 ± 2.8 [b]
Steryl/wax ester	5.6 ± 0.3	1.7 ± 0.1 *	3.6 ± 0.2 [a]	6.1 ± 0.6 [b]	3.2 ± 0.8 [a]
Free fatty acid	6.7 ± 1.1	10.9 ± 0.4 *	5.5 ± 0.2	5.2 ± 0.2	5.1 ± 0.8
Total Neutral Lipids	47.4 ± 1.7	44.2 ± 0.3	32.7 ± 1.1 [a]	37.4 ± 0.2 [b]	37.2 ± 1.9 [b]
TAG:C	2.1 ± 0.3	1.9 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.6 ± 0.3

Results are Mean ± SD (n=3); TAG:C, triacylglycerol/cholesterol ratio. An * or different superscript letter denote significantly difference ($p < 0.05$).

Table 4. (2013 Trial). Fatty acid composition (weight %) of rotifers *Brachionus plicatilis* enriched with Origreen + Skretting ® (a), nauplii of the copepod *Acartia tonsa* fed on *Isocrysis* T-Iso (b) and 14 days after hatch ABFT (*Thunnus thynnus*) larvae (ABFT) fed with rotifers (c) and copepod (d).

Fatty Acid	(a) Rotifer	(b) Copepod	(c) ABFT + Rotifer	(d) ABFT + Copepod
14:0	0.9 ± 0.1	8.9 ± 0.4*	0.8 ± 0.0	1.8 ± 0.3*
15:0	0.4 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	0.6 ± 0.0
16:0	19.6 ± 0.2	14.9 ± 0.2*	18.4 ± 0.2	19.2 ± 1.1
18:0	3.4 ± 0.1	2.9 ± 0.0*	9.3 ± 0.4	9.5 ± 1.4
20:0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
22:0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.3 ± 0.0
24:0	0.2 ± 0.1	0.1 ± 0.0	0.3 ± 0.1	0.3 ± 0.1
Total SAT	25.0 ± 0.2	27.7 ± 0.6*	30.1 ± 0.3	31.9 ± 2.3
16:1n-9	1.2 ± 0.1	nd	0.7 ± 0.0	nd
16:1n-7	0.8 ± 0.0	5.1 ± 0.1*	1.2 ± 0.3	2.5 ± 0.1*
18:1n-11	1.6 ± 0.1	nd	1.5 ± 0.2	0.1 ± 0.0*
18:1n-9	4.5 ± 0.1	5.2 ± 0.1*	6.3 ± 0.9	6.0 ± 0.2
18:1n-7	0.9 ± 0.1	1.3 ± 0.1	1.6 ± 0.2	2.2 ± 0.1
20:1n-9	2.1 ± 0.3	0.3 ± 0.1*	1.3 ± 0.2	0.4 ± 0.1*
20:1n-7	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
22:1n-11	0.3 ± 0.1	0.1 ± 0.0*	0.3 ± 0.0	0.1 ± 0.0*
22:1n-9	0.6 ± 0.1	0.1 ± 0.0*	0.3 ± 0.1	0.1 ± 0.0*
24:1n-9	nd	nd	1.0 ± 0.3	1.0 ± 0.1
Total MUFA	12.7 ± 0.2	13.3 ± 0.5	14.6 ± 0.9	12.7 ± 0.2
16:2	4.1 ± 0.1	0.2 ± 0.0*	1.1 ± 0.1	0.7 ± 0.1
16:3	1.4 ± 0.1	0.3 ± 0.0*	0.3 ± 0.0	0.4 ± 0.0
16:4	1.0 ± 0.1	0.2 ± 0.0*	1.2 ± 0.1	0.8 ± 0.2
18:2n-6	20.5 ± 0.3	8.7 ± 0.2*	13.1 ± 0.5	4.5 ± 0.3*
18:3n-6	0.1 ± 0.0	0.7 ± 0.0*	0.2 ± 0.0	0.4 ± 0.0*
18:3n-3	6.7 ± 0.1	3.9 ± 0.0*	3.0 ± 0.1	1.7 ± 0.2*
18:4n-3	0.2 ± 0.0	3.4 ± 0.1*	0.4 ± 0.0	0.9 ± 0.2*
20:2n-6	1.4 ± 0.1	0.6 ± 0.0*	1.1 ± 0.1	0.7 ± 0.0*
20:3n-6	0.5 ± 0.0	0.6 ± 0.0	0.4 ± 0.0	0.3 ± 0.0
20:3n-3	0.6 ± 0.0	0.1 ± 0.0*	0.4 ± 0.0	0.2 ± 0.0*
20:4n-6	0.6 ± 0.0	1.2 ± 0.0*	1.3 ± 0.1	1.5 ± 0.1
20:4n-3	1.0 ± 0.1	0.1 ± 0.0*	0.9 ± 0.1	0.6 ± 0.0
20:5n-3	4.5 ± 0.1	8.9 ± 0.1*	5.4 ± 0.1	5.9 ± 0.4
22:4n-6	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.1
22:3n-3	0.6 ± 0.0	0.3 ± 0.0*	0.5 ± 0.1	0.2 ± 0.1*
22:5n-6	0.5 ± 0.0	2.5 ± 0.1*	0.6 ± 0.1	2.2 ± 0.2*
22:5n-3	3.9 ± 0.2	0.3 ± 0.0*	4.3 ± 0.4	1.0 ± 0.0*
22:6n-3	12.1 ± 0.5	24.9 ± 0.9*	17.5 ± 0.9	29.6 ± 1.0*
Total PUFA	59.9 ± 0.8	57.1 ± 0.9	51.9 ± 0.6	51.8 ± 2.0
DHA/EPA	2.7 ± 0.1	2.8 ± 0.1	3.2 ± 0.2	5.0 ± 0.1*
n-3/n-6	1.1 ± 0.1	2.9 ± 0.1*	1.9 ± 0.1	3.9 ± 0.1*
Unknown	2.4 ± 0.3	1.9 ± 0.2	3.4 ± 0.2	3.6 ± 0.4

Results are Mean ± SD (n=3). SD = 0.0 implies that SD < 0.05. SAT, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid. * denotes significant differences ($p<0.05$) between rotifer and copepods or between 14 dph ABFT larvae fed with rotifers or copepods.

Table 5 (2014 Trial). Fatty acid composition (weight %) of rotifer *Brachionus plicatilis* enriched with Origreen + Skretting ® (a), nauplii of the copedod *Acartia tonsa* fed on *Rhodomonas salina* (b), and 14 days after hatch ABFT (*Thunnus thynnus*) larvae fed with rotifers (c), copepod (d), and co-feeding rotifer + copepod (e).

Fatty Acid	(a) Rotifer	(b) Copepod	(c) ABFT + Rotifer	(d) ABFT + Copepod	(e) ABFT + Rotifer + Copepod
14:0	0.7 ± 0.1	11.0 ± 0.2*	0.7 ± 0.1[a]	2.2 ± 0.2[b]	1.6 ± 0.3[b]
15:0	0.9 ± 0.1	1.2 ± 0.3	1.0 ± 0.1	1.4 ± 0.2	1.1 ± 0.1
16:0	22.7 ± 0.9	11.9 ± 0.2*	16.9 ± 0.3	18.3 ± 0.5	17.1 ± 0.4
18:0	2.9 ± 0.1	2.1 ± 0.0*	8.6 ± 0.4	7.8 ± 0.1	8.3 ± 0.4
20:0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
22:0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.0
24:0	0.3 ± 0.1	0.1 ± 0.0	0.3 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
Total SAT	28.1 ± 1.2	26.6 ± 0.6	27.9 ± 0.5	31.6 ± 0.7	29.8 ± 0.3
16:1n-9	0.8 ± 0.1	nd	0.7 ± 0.0	nd	0.5 ± 0.0
16:1n-7	0.7 ± 0.0	3.9 ± 0.1*	1.7 ± 0.3	2.3 ± 0.1	1.4 ± 0.1
18:1n-11	1.0 ± 0.2	0.1 ± 0.0	1.3 ± 0.2	0.3 ± 0.0	0.8 ± 0.1
18:1n-9	4.5 ± 0.3	4.5 ± 0.2	4.9 ± 0.2[a]	5.7 ± 0.2[b]	5.1 ± 0.2[ab]
18:1n-7	0.9 ± 0.1	1.2 ± 0.1	1.9 ± 0.2	2.0 ± 0.1	1.7 ± 0.1
20:1n-9	1.9 ± 0.1	0.3 ± 0.1*	1.2 ± 0.1[a]	0.3 ± 0.0[b]	0.9 ± 0.1[a]
20:1n-7	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.11 ± 0.0	0.2 ± 0.0
22:1n-11	0.4 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
24:1n-9	0.7 ± 0.1	0.2 ± 0.0*	0.4 ± 0.2	0.4 ± 0.1	0.3 ± 0.0
Total MUFA	11.6 ± 0.2	11.8 ± 0.4	12.9 ± 0.3[a]	15.7 ± 0.3[b]	14.0 ± 0.3[ac]
16:2	1.9 ± 0.4	0.3 ± 0.0*	1.3 ± 0.1	0.9 ± 0.1	1.0 ± 0.2
16:3	0.8 ± 0.1	0.2 ± 0.0*	0.6 ± 0.0	0.6 ± 0.2	0.9 ± 0.0
16:4	0.9 ± 0.1	0.6 ± 0.1	1.1 ± 0.1	0.7 ± 0.0	0.9 ± 0.1
18:2n-6	14.8 ± 1.1	8.0 ± 0.2*	12.7 ± 0.7[a]	4.2 ± 0.1[b]	10.3 ± 0.9[a]
18:3n-6	0.1 ± 0.0	1.3 ± 0.3*	0.2 ± 0.0[a]	0.6 ± 0.0[b]	0.3 ± 0.0[a]
18:3n-3	3.9 ± 0.2	5.2 ± 0.3*	2.3 ± 0.1	2.5 ± 0.2	2.4 ± 0.1
18:4n-3	0.3 ± 0.1	8.2 ± 0.6*	0.2 ± 0.0[a]	2.2 ± 0.2[b]	1.0 ± 0.2[c]
20:2n-6	1.2 ± 0.1	0.6 ± 0.0*	1.2 ± 0.1[a]	0.6 ± 0.0[b]	0.8 ± 0.2[ab]
20:3n-6	0.5 ± 0.0	0.6 ± 0.0	0.6 ± 0.0[a]	0.3 ± 0.1[b]	0.5 ± 0.1[ab]
20:3n-3	0.5 ± 0.1	0.1 ± 0.0*	0.4 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
20:4n-6	0.7 ± 0.0	0.6 ± 0.0	2.6 ± 0.3[a]	1.6 ± 0.1[b]	1.4 ± 0.1[b]
20:4n-3	0.7 ± 0.1	0.1 ± 0.0*	0.7 ± 0.1	0.9 ± 0.2	0.8 ± 0.2
20:5n-3	4.5 ± 0.1	3.8 ± 0.1*	6.6 ± 0.4[a]	5.5 ± 0.1[b]	4.8 ± 0.1[c]
22:4n-6	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.2 ± 0.1	0.2 ± 0.0
22:3n-3	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.2 ± 0.1	0.3 ± 0.0
22:5n-6	0.9 ± 0.3	2.6 ± 0.1*	0.4 ± 0.1[a]	2.6 ± 0.2[b]	1.8 ± 0.3[c]
22:5n-3	4.9 ± 0.3	0.3 ± 0.0*	4.6 ± 0.2[a]	0.6 ± 0.0[b]	2.2 ± 0.3[c]
22:6n-3	17.5 ± 3.1	26.0 ± 0.9*	17.6 ± 0.5[a]	28.2 ± 0.3[b]	24.4 ± 0.5[b]
Total PUFA	55.1 ± 1.8	59.0 ± 0.9	53.8 ± 0.6	51.1 ± 2.0	53.7 ± 1.3
DHA/EPA	3.8 ± 0.6	6.9 ± 0.6*	2.7 ± 0.2[a]	5.1 ± 0.2[b]	5.0 ± 0.2[b]
n-3/n-6	1.7 ± 0.3	3.2 ± 0.1*	1.7 ± 0.3[a]	3.0 ± 0.1[b]	2.0 ± 0.2[a]
Unknown	5.2 ± 0.3	2.6 ± 0.2	5.4 ± 0.4	1.6 ± 0.3	2.5 ± 0.3

Results are Mean ± SD (n=3). SAT, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid. * denotes significant differences ($p<0.05$) between rotifers and copepods. Different superscript letter indicates significant differences ($p<0.05$) among bluefin tuna larvae fed with different treatments.

4. Discussion

The present study reports on the effect of different feeding regimes on the growth, survival and lipid content, lipid class and total lipid fatty acid compositions of ABFT larvae. Due to the actual short spawning season of ABFT in natural conditions (only 30-40 days in June-July) and the scarce availability of fertilized viable eggs, trials on the first feeding of ABFT larvae have been split up in two periods corresponding to 2013 and 2014 spawning seasons. Results on survival and mainly on growth in 2013 were influenced by the low feeding rates in the tanks with larvae fed on copepods, presented some days of the experiment because of the low number of available copepods produced, so these results must be interpreted with caution.

But data obtained in 2014 are conclusive. There were significant differences in growth between all the treatments. This is in agreement with results obtained when using copepods as live preys nor only for ABFT (Evjemo *et al.*, 2014) but also in trials with other larval fish species. (Witt *et al.*, 1984; Holmvaag, 2011). Copepods have a more suitable range of sizes, are more conspicuous for the larvae due to their movement (which is mainly important during the first days when their visual acuity is low) and, in the main, they showed a better nutritional value.

Sawada *et al.*, (2000) reported that PBFT preferred smaller rotifers instead larger ones during the first days of feeding (in spite of the capability to feed on larger rotifers from the beginning). In the same way, ABFT larvae in this experiment would predate on smaller copepod nauplii preferentially. So, those larvae feeding actively grow faster in size, not only in weight but also in standard length. Larvae fed on copepods and a mixed diet (copepods and enriched rotifers) started to grow from 4 dph onwards, but the rotifer fed group did not start to grow in length until 6 dph. However, in southern bluefin tuna, Hilder *et al.* (2014) did not notice this selection for smaller size preys.

At the beginning of the experiments, the growth of the larvae was scarce and only a few preys were detected in the stomach (personal remark). In fact, in spite of presenting a light growth in dry weight, 4 dph larvae were, in all the cases, shorter than larvae at 2dph, when they started their exogenous feeding. What clearly a sign of inadequate feeding during the first days of exogenous feeding and survival at the expense of the remaining yolk reserves.

Between day 2 and day 4, PBFT larvae finish the consumption of their yolk sac, their intestine become coiled and they start to develop their digestive capacity (Miyashita *et al.*, 2001), and this is also happen in ABFT larvae (Yufera *et al.*, 2014). The opening size of the mouth widen and visual acuity and hunting

abilities progress. Swim bladder inflation also gives buoyancy and energy saving as an aid to hunting capabilities. From this moment onwards larvae start to grow at higher rates.

By 6 dph, significant differences between larvae fed on rotifers and the other two groups were detected, and this went on during practically the rest of the experiment. However, the differences were maintained, maybe because the higher mortality in the rotifer fed group caused the survival of the larger larvae. On the other hand, the higher survival rate in the mixed diet group can explain the lower growth in this group from 8-10 dph when compared with the copepod fed one.

From 6 dph onwards, growth in all the groups started to speed up, and several preys (often more than ten) can be observed inside the stomach of the larvae, mainly in the groups fed with copepods.

Besides, early consumption of *Acartia* nauplii could report added benefits due to ingestion of preys with high DHA levels and a high DHA/EPA relationship. It is well known that dietary DHA improves larval growth and survival during early larval feeding and enhanced retinal development and visual acuity which could improve their hunting capacity (Sargent *et al.*, 2002; ANON, 2012). Others researchers like Bell *et al.* (1995) have also reported the importance of n-3 PUFA for effective prey capture from the time of first feeding in other species.

It is also important to highlight the fine results obtained with the mixed diet. An experiment carried out co-feeding ABFT with rotifers and ciliates (ANON., 2012) showed that first day of exogenous feeding, ABFT larvae selected and preferred to consume initially only the smaller sized ciliates. Two next 2 days (3 and 4 dph) the preference for ciliated protozoa over rotifers still went on despite the fact that the size of the mouth of the ABFT larvae (more than 300 μm) can easily ingest rotifer sized prey. Moreover, ciliates consumption contributed to improve larval nutritional status and hunting capability in the following days. In our experiments, we have observed, from the beginning of feeding, that both type of preys were detected inside the ABFT gut, but we have not determinate the rate of each ones.

Tuna larvae fed on copepods finished their flexion between day 12 and 13 dph, and those fed with the mixed diet finished between 13 and 14 dph. It is described that in this point PBFT are able to feed on greater preys, even yolk sac larvae. In fact, in the stomach of some larvae fed on copepods it is possible to distinguish some copepodites as well from day 10 dph onwards. As *Isochrysis* t-ISO was added to tanks every day as green water ingredient, those nauplii not ingested by tuna larvae were able to feed, grow and moult to the following naupliar stages, even to copepodite stages. This could give to the ABFT the chance of completing their diet with larger preys.

In 2013 trial, ABFT larvae fed either on rotifers or copepods showed almost identical lipid class composition with about 65% of structural polar lipids (primarily PC, PE, PS and PI). However in neutral lipids there is a difference in the nutritional index (TAG/Cholesterol), which is lower in ABFT fed on copepods, practically a half. This abnormal situation could reflect the copepod shortage during several days which has been mentioned and it caused a delayed growth.

In 2014 trial, ABFT larvae fed with rotifers and copepods had a slight but significant differences in lipid classes: ABFT larvae fed on rotifers present a higher rate of polar lipids and a lower rate of neutral lipids. Higher rate of TAG was registered in ABFT with the mix diet, while there are not differences in TAG/Chloesterol ratio, pointing out that the value watched in 2013 was quite abnormal.

With regard to fatty acids, the relationship PUFA/monoenes was quite stable in all the treatments and years, averaging 3.8. But important differences could be observed in DHA levels and DHA/EPA ratios: DHA represented about 28% of total lipids in ABFT larvae fed on copepods but only 18% when fed on rotifers. DHA/EPA ratios varied from less than 3 in ABFT larvae fed on rotifers in 2014 and 5 in ABFT fed on copepods. Results obtained when copepods are offered as food are higher than results obtained in ABFT eggs by Ortega and Mourente (2010) but similar to the value of 4.5 than Mourente *et al.* (2002) found in ovaries of wild ABFT.

It also can be noticed the differences in 18:2n-6, which is much higher in ABFT fed on rotifers. It is evident that this fatty acid comes from the *Chlorella* algae used to feed and grow rotifers.

Importance of DHA is well documented (Watanabe, 1993; Sargent *et al.*, 1999; Rainuzzo *et al.*, 1997; Matsunari *et al.*, 2012): it is highly represented in tissue membranes and facilitates key intramembranal reactions due to its role in membrane fluidity. Retina of fishes presents a high amount of DHA in their membranes (cones and rods) and it is believed that high dietary DHA levels contribute to enhance visual acuity. In larvae, DHA can also enhance neural and brain function, apart from improved cellular function in general, which might be facilitating hunting efficiency.

In the former article of this thesis, it is reported that starved ABFT larvae at yolk sac stage showed an increase in DHA:EPA ratio as development progressed. This can be explained in terms of differential oxidation and retention of DHA. Although it is known that both EPA and DHA can be oxidized in fish, at least in salmon, when in dietary excess (Stubhaug *et al.*, 2007), DHA is known to be the fatty acid that is most preferentially retained (Sargent *et al.*, 2002). But in the normal feeding situation, DHA must be provided in the diet. In spite of results by

Morais *et al.* (2011) suggesting that endogenous biosynthesis of DHA may be important during normal early development in ABFT larvae, high level should be incorporated from exogenous sources, and after hatching, the DHA of maternal origin must be quickly replaced after yolk absorption through feeding in order to ensure normal neural function, and the best choice is to feed with preys with a high DHA levels as copepods.

Using a small prey like copepod nauplii is an important advantage for the first days of feeding, so that the possibility of high ingestion rates because of the prey size can satisfy the nutrition of the larvae. Besides, ingestion of preys containing high amounts of DHA with high DHA/EPA ratios not only to improve visual acuity but survival and growth as well. The advantage that represents feeding copepods can also be observed when mixed with enriched rotifers as live preys. This fact can be considered as critical due to the great difficulty to obtain a high population of cultured copepods in comparison to rotifers. This mixed diet type can be very advantageous by diminishing the total amount of copepods to be produced without loosing completely their high nutritional value and quality as live preys. Further research is needed to determine the best strategy, ratios and feeding schedules for co-feeding copepods and rotifers, as well as improving the nutritional quality.

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2.4. Size-related differences in growth and survival in piscivorous fish larvae fed different prey types

P. Reglero, A. Ortega, E. Blanco, Ø. Fiksen, F.J. Viguri, F. de la Gándara, M. Seoka and A. Folkvord.

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RESUMEN

Diferencias relacionadas con el tamaño en crecimiento y supervivencia de larvas de peces piscívoras alimentadas con diferentes tipos de presas

El cambio de la dieta planctívora a la dieta piscívora es crítico en la supervivencia de muchos peces. En este estudio examinamos el desarrollo de las capacidades fisiológicas y de búsqueda de alimento en relación a las dietas planctívoras y piscívoras en larvas de peces. Especialmente hemos manipulado el momento de inicio de la piscivoría y hemos registrado sus consecuencias para el tamaño, crecimiento y supervivencia de larvas de Atún rojo del Atlántico (*Thunnus thynnus*) y Bonito Atlántico (*Sarda sarda)* criado bajo condiciones experimentales en laboratorio. Nuestros resultados muestran que una transición temprana a la piscivoría beneficia la supervivencia e incrementa el crecimiento durante los estadios larvarios, cuando la abundancia de comida no es limitante para ambas especies. Para el atún rojo, sólo se pudieron mantener elevados índices de supervivencia cuando las larvas eran incluidas en la dieta. En el bonito, los índices de supervivencia disminuyeron significativamente durante un periodo de seis días, cuando se retrasó tres días la adición de larvas de peces. Estos resultados son importantes para el estudio del desarrollo de sistemas exitosos de producción de juveniles, y revelaron un elemento importante para el éxito del reclutamiento del atún rojo y el bonito en la naturaleza.

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Size-related differences in growth and survival in piscivorous fish larvae fed different prey types

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ABSTRACT

The switch from a planktivorous to a piscivorous diet is critical to survival in many fishes. In this study we examine the development of foraging and physiological capabilities in relation to planktivorous and piscivorous diets in larval fish. Specifically, we manipulated the timing of onset of piscivory and recorded its consequences for size, growth, and survival in Atlantic bluefin tuna (*Thunnus thynnus*) and bonito larvae (*Sarda sarda*) reared under experimental laboratory conditions. Our results show that an early transition to piscivory benefits survival and increased growth during the larval stage, when food abundance is not limiting for both species. For bluefin tuna, high survival rates could only be maintained when fish larvae were included in the diet. For bonito, survival rates significantly decreased over a six-day period if the addition of fish larvae was delayed by three days. These results are important for the study of the development of successful juvenile production systems and reveal an important element for recruitment success of bluefin tuna and bonito in the field.

1. Introduction

Foraging abilities and growth are key elements in fish larval survival and recruitment success (Miller et al., 1988; Munk, 1995; Nunn et al., 2012). Variations in ontogenetic development and growth lead to differences in size-dependent competitive abilities between individuals (Persson and De Roos, 2006). The early life stages in fish are particularly vulnerable to predation, and this generates a selection pressure towards high growth rates (Folkvord, 2005). For fast-growing fish larvae such as tuna in warm water environments high growth rates may not be possible from a planktivorous diet, and a rapid shift to piscivory is key to maintaining growth rates.

Fast growth may be particularly important in species with a piscivorous phase during the earliest ontogenetic stages (Kaji et al., 2002; Puvanendran and Brown, 2002; Shoji and Tanaka, 2006), since the size-structure within cohorts determines what is prey and what is predator. Growth rate, physiology, and foraging capabilities often change with the shift from a planktivorous to a piscivorous diet (Kaji et al., 2002; Sawada, 2006). An increased understanding of the processes that govern the shift from planktivory to piscivory is crucial to understand processes such as recruitment success, density-dependence, and spawning strategies in species where piscivory occurs early in the larval phase, such as tuna and bonito.

The larval phase is the developmental period with the highest rate of change in size-dependent processes. Most fish larvae generally feed on zooplankton. But in many species of large predatory fish, piscivory can already be observed during the larval stage, including cannibalism (Puvanendran and Brown, 2002; Shoji and Tanaka, 2006). To feed on other fish larvae, a piscivorous larva needs to develop morphological and functional structures such as eyes, teeth, jaws, and digestive glands, and attain a larger size than its potential prey (Yúfera et al., 2014).

Atlantic bluefin tuna (*Thunnus thynnus*) and bonito (*Sarda sarda*) larvae are good model organisms for the role of piscivory. For bluefin tuna, the larval phase is a vulnerable life stage critical to recruitment (Reglero et al., 2011). Concerns about the status of the Atlantic population and its conservation demand better knowledge of essential aspects of larval ecology. The growth of tuna larvae is likely to be food limited with a pure zooplankton diet, particularly as the larvae attain larger sizes (Reglero et al., 2011). Though unknown, similar processes are

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expected for bonito, a species that occasionally shares similar larval habitats as tuna species (Torres et al., 2011).

There are no existing specific experimental laboratory studies for Atlantic bluefin tuna and bonito larval development. However, other closely related species such as striped bonito (*Sarda orientalis*) and Pacific bluefin tuna (*Thunnus orientalis*) already show strong piscivory at the larval stage (e.g. Kaji et al., 1996, 2002). Therefore, the initiation of piscivory and the intra- and interspecific predation of tuna and bonito larvae could be a significant source of mortality. The size dependency of predator–prey relationships in larval tuna of different species are relatively well known during the planktivorous phase from stomach data from cruises (e.g. Llopiz et al., 2010). Still, the physiological development of Atlantic bluefin tuna and bonito larvae has not been well investigated and almost nothing is known about the capacity for growth and the role of diets during ontogeny, particularly during the piscivorous phase.

The main objective of this study is thus to assess size-dependency of foraging and physiological developments in piscivorous fish larvae. We do this by studying the consequences of diets in early life history traits of Atlantic bluefin tuna and bonito. We estimate differences in larval size and resulting size-structure, growth rates, and survival of larvae with a piscivorous diet versus a planktivorous diet.

2. Material and methods

Cultures of Atlantic bluefin tuna (*Thunnus thynnus*), bonito (*Sarda sarda*), and seabream (*Sparus aurata*) larvae were taken in June–July 2012 and 2013. Batches of fertilized bluefin tuna eggs were obtained from naturally spawning captive adult tuna in the farming facilities at El Gorguel, Cartagena (SE Spain), owned by Caladeros del Mediterráneo SL. The bluefin tuna eggs were collected and transported to the Spanish Institute of Oceanography (IEO) facilities in Mazarrón (SE Spain) where the experiments were performed. Bonito were obtained from captive broodstocks at the Institute of Marine Oceanography (IEO) in Mazarrón. During the study period successive groups of seabream eggs were obtained almost daily from captive broodstocks at IEO.

Fertilized eggs of seabream and bonito were incubated separately in 400 l tanks and those of bluefin tuna either in 1500 l or 5000 l tanks. Incubation was carried out with an upwelling current (8–10 renewals per day), mild aeration, and a continuous light regime with light intensity close to 300 lx. The incubation temperature varied between 21 and 23 °C for seabream eggs, 20 and 24 °C for bonito, and 21 and 26 °C for bluefin tuna eggs using ambient seawater and salinity 37. The larvae of bonito and seabream remained in the 400 l tanks during the whole yolk-sac larval stage until 2 days post hatching (dph). Then, the bonito larvae were moved to 1500 l cylindrical tanks where they remained until the beginning of the experiments. Bluefin tuna larvae remained in the 1500 and 5000 l tanks until the beginning of the experiments. Seabream larvae were only used for feeding purposes; therefore no further culture was conducted.

The initial larval stocking densities in the 1500 or 5000 l tanks were approximately 20–40 larvae l^{-1} for bonito and 10 larvae l^{-1} for bluefin tuna. The larvae were fed with live prey supplied in excess two times per day. The feeding schedule consisted of enriched rotifers (*Brachionus plicatilis*) from 2 to 8 and 2 to 18 dph for bonito and bluefin tuna respectively, with densities within the tanks maintained at 10 rotifers/ml. Rotifer was enriched on taurine at a dose of 0.5 g/l during 12 h and on an experimental diet at a dose of 0.3 g/10^6 rotifers during 3 h. Density during enrichment was 1000 rotifers/ml. For the bonito larvae used in the second bonito growth experiment we added enriched Artemia (*Artemia salina* instar II) four times a day from 6 dph onwards. Artemia was enriched on the same experimental diet at a dose of 0.4 g/10^6 during 12 h. Density during enrichment was 300 artemia/ml. The enrichment was an experimental diet designed for these experiments by Skretting Ltd. to fortify the live food with maximal doses in protein, DHA and vitC. We added 0.8 g dry weight of paste of concentrated *Chlorella* (Super fresh Chlorella SV-12, Chlorella Industry Co., Ltd., Japan) per m^3 four times per day in each 1500 and 5000 l tanks. Besides, cultivated microalgae (*Nannochloropsis gaditana*) were added two times per day until 18 dph to the bluefin tuna cultures.

All growth experiments were conducted in 150 l volume tanks. The larvae were acclimated to these tanks during 1–3 days. During the acclimation days 3.2 g dry weight of paste of concentrated *Chlorella* (Super fresh Chlorella SV-12, Chlorella Industry Co., Ltd., Japan) per m^3 was added and the larvae were fed with enriched rotifers at densities of 10 rotifers/ml (1.5 million rotifers per tank per feeding time, three feeding times per day at 9:00, 14:00, and 19:00 h). In the tanks with Artemia feeding (see bonito experiment 1) the larvae were also fed with 0.66 Artemia/ml (100,000 Artemia per tank once a day) from dph 3. The light regime was kept constant for all experiments (15L:9D) with light intensity of around 2400 lx during the photophase.

2.1. Bluefin tuna experiment

When bluefin tuna were 15 dph, 1800 larvae were transferred to 15 tanks of 150 l volume (up to 120 larvae per tank) for acclimation, using the same feeding protocol as previously described. The average water temperature was 24.8 °C ± 0.8. At 19 dph corresponding to the experimental day (Eday 0), when the larvae had attained a size around 7–9 mm SL and could initiate piscivory (e.g. Anon., 2010; Sawada et al., 2005; Seoka et al., 2007) the live larvae were counted in each of the 150 l tanks and a subsample of 3 larvae from each tank was taken for morphometric measurements and frozen for subsequent dry weight estimations. The following days, different diets were provided for each group of larvae (Table 1). In five tanks the larvae were fed enriched rotifers from Eday 0 until the end of the experiment (rotifer fed group, *R*). In five other tanks, yolk-sac larvae of seabream together with enriched rotifers were provided from Eday 3 until the end of the experiment (delayed yolk-sac larvae fed group, *DYSL*). In the remaining five tanks, yolk-sac larvae of seabream together with enriched rotifers were

Table 1
Description of the experiments conducted in the present study. Note: Two different experiments were conducted with bonito larvae. All experiments were conducted in 150 l volume tanks with the light regime 15L:9D.

Species	Feeding	Larval group	Replicates	Duration	DPH	Average temperature (°C, ±SD)
Bluefin	Planktivory	Rotifer	5	8	19–27	24.8 ± 0.8
	Planktivory/piscivory	DYSL	5	8	19–27	
	Piscivory	YSL	5	8	19–27	
Bonito	Planktivory	Artemia	3	6	9–15	21.3 ± 0.4
Exp 1	Planktivory/piscivory	DYSL	3	6	9–15	
	Piscivory	YSL	3	6	9–15	
Bonito	Planktivory	Rotifer	3	6	8–14	23.4 ± 0.4
Exp 2	Planktivory/piscivory	DYSL	3	6	8–14	
	Piscivory	YSL	3	6	8–14	

Table 2
Bluefin tuna larvae. Survivorship estimated 4 and 8 days after the onset of the experiment compared to the initial abundance at the onset of the experiment. The five replicates for the three larval groups are shown: rotifer (tuna fed rotifer), DYSL (tuna beginning piscivorous diet 4 days after the onset of the experiment), YSL (tuna beginning piscivorous diet from the onset of the experiment). The letters following the treatment mean survival values indicate significantly different group means (pairwise comparisons t-test, $p < 0.05$).

Larval group	Survival % (0–4 days)	Treatment mean survival	Survival % (0–8 days)	Treatment mean survival
YSL	28.6	53.4	19.0	39.7[a]
	38.2		29.4	
	53.3		40.0	
	66.7		50.0	
	80.0		60.0	
DYSL	54.5	53.0	18.2	21.7[a,b]
	43.2		21.6	
	53.8		15.4	
	82.3		47.1	
	31.2		6.2	
Rotifer	30.0	52.3	5.0	14.3[b]
	62.1		24.1	
	69.6		26.1	
	33.3		16.7	
	66.7		0	

provided from Eday 0 until the end of the experiment (yolk-sac larvae fed group, *YSL*). When allowing piscivorous feeding (*DYSL* from 23 dph (Eday 4) and *YSL* from 19 dph (Eday 0)), bluefin tuna larvae were fed to satiation providing up to 250 seabream yolk sac larvae per individual bluefin tuna larva, ensuring yolk sac larvae remained in the tanks at any time. The duration of the whole experiment was 8 days (terminated 27 dph, Eday 8).

2.2. Bonito experiment 1

When bonito were 8 dph, 1080 larvae were transferred to nine tanks of 150 l volume (up to 120 larvae per tank). Water average temperature was 21.3 °C ± 0.4. At 9 dph, corresponding to the experimental day (Eday 0), when the larvae had attained a size around 7–9 mm SL and could initiate piscivory (Ortega and de la Gándara, 2007, 2009), the live larvae were counted in each of the 150 l tanks and a subsample of 4 larvae from each tank was taken for morphometric measurements and frozen for subsequent dry weight estimations. The following days, different diets were provided for each group of larvae (Table 1). In three tanks the larvae were fed enriched rotifers and Artemia from Eday 0 until the end of the experiment (Artemia fed group, *A*). In three other tanks, yolk-sac larvae of seabream together with enriched rotifers and Artemia were provided from Eday 3 until the end of the experiment (delayed yolk-sac larvae fed group, *DYSL*). In the remaining three tanks, yolk-sac larvae of seabream together with enriched rotifers and Artemia were provided from Eday 0 until the end of the experiment (yolk-sac larvae fed group, *YSL*). When allowing piscivorous feeding (*DYSL* from 12 dph (Eday 3) and *YSL* from 9 dph (Eday 0)), bonito larvae were fed to satiation providing up to 300 seabream yolk sac larvae per individual bonito larva, ensuring yolk sac larvae remained in the tanks at any time. The duration of the whole experiment was 6 days (15 dph, Eday 6).

2.3. Bonito experiment 2

A total of 1170 bonito larvae (7 dph) were transferred to 9 tanks of 150 l volume (up to 130 larvae per tank). The average water temperature was 23.4 °C ± 0.4. At 8 dph, corresponding to the experimental day (Eday 0), when the larvae had attained a size around 7–9 mm SL and could initiate piscivory (Ortega and de la Gándara, 2007, 2009),

Table 3
Bonito larvae. Survivorship estimated 3 and 6 days after the onset of the experiment compared to the initial abundance at the onset of the experiment. The three replicates for the three larval groups are shown: rotifer (tuna fed rotifer), DYSL (tuna beginning piscivorous diet 3 days after the onset of the experiment), YSL (tuna beginning piscivorous diet from the onset of the experiment). NA represents no data available. The different letters following the treatment mean survival values indicate significantly different group means (pairwise comparisons t-test, $p < 0.05$).

Species	Larval group	Survival % (0–3 days)	Treatment mean survival	Survival % (0–6 days)	Treatment mean survival
Bonito Exp. 1	YSL	37.5	52.8[a]	26.7	40.8[a]
		60.8		45.8	
		60.0		50.0	
	DYSL	15.0	11.4[b]	10.0	5.6[b]
		10.0		4.2	
		9.2		2.5	
	Artemia	20.8	17.5[b]	5.0	4.2[b]
		20.0		5.0	
		11.7		2.5	
Bonito Exp. 2	YSL	NA	NA	42.3	45.4[a]
		NA		52.3	
		NA		41.5	
	DYSL	12.3	9.2	2.3	4.1[b]
		5.4		2.3	
		10.0		7.7	
	Rotifer	4.6	6.7	0.0	1.0[b]
		7.7		1.5	
		7.7		1.5	

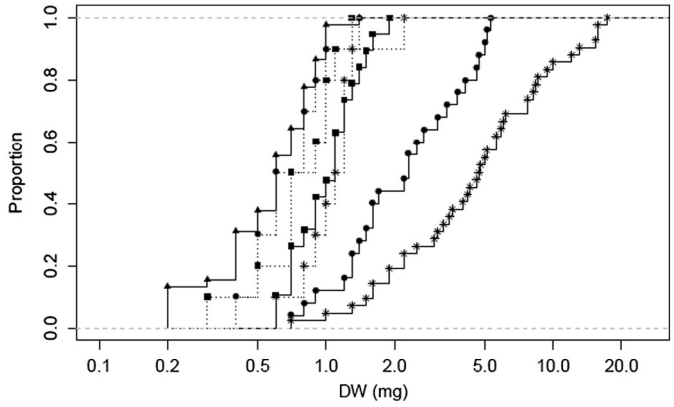

Fig. 1. Bluefin tuna larvae. Observed cumulative larval size distributions in dry weight (DW). Measurements on Eday 0 are represented by triangles and a solid line. The other lines represent measurements on Eday 4 (dashed lines) and the final day of experiment, Eday 8 (solid lines). Different symbols represent differently fed larval groups: squares = rotifer, circles = DYSL, and crosses = YSL.

the live larvae were counted in each of the 150 l tanks and a subsample of 5 larvae from each tank was taken for morphometric measurements and frozen for subsequent dry weight estimations. The different diets that were provided for each group of larvae were identical to the experiment for bluefin tuna. Each experiment consisted of 3 replicates. When allowing piscivorous feeding (*DYSL* from 11 dph (Eday 3) and *YSL* from 8 dph (Eday 0)), bluefin tuna larvae were fed to satiation providing up to 300 seabream yolk sac larvae per individual bonito larva, ensuring yolk sac larvae remained in the tanks at any time. The duration of the whole experiment was 6 days (14 dph, Eday 6).

All the surviving larvae were counted and sampled at the end of the bluefin experiment and the second bonito experiment. For the first bonito experiment, all the larvae were counted and up to 10 larvae from each tank were sampled at the end of the experiment. The larvae were photographed live using a camera (Olympus SC20) connected to a dissecting microscope (Olympus SZ61-TR) and frozen individually in cryotubes at −80 °C for later examination. From images we measured individual standard and total length using the software Image Pro 6.2. The frozen larvae were rinsed in distilled water, dried at 60 °C over 24 h and weighed to estimate dry weight (see protocol in Seljeset et al., 2010). Size distributions were tested for normality using the Shapiro–Wilk test. Cumulative size distributions and daily specific length and dry weight growth rates were estimated as described in Folkvord et al. (2009). Pairwise comparisons using t tests with pooled SD were applied to compare larval size distributions. A Bonferroni correction was applied to the estimates of the probability to avoid type I error when comparing the size distributions from the three treatments within experiments. The percentages of survival were root-squared and arcsine transformed for normality prior to statistical analysis. All statistical analyses were fitted using the R statistical software (Development Core Team, 2011).

3. Results

The survival of bluefin tuna larvae was not significantly different among the rotifer, YSL, and DYSL treatments during the first 4 days of the experiment (Table 2, pairwise comparisons t-test, $p > 0.05$), while the overall survival during the full 8 days of the experiment was significantly higher in the YSL treatment compared to the rotifer treatment (pairwise comparisons t-test, $p < 0.05$).

The survival of bonito larvae from the first bonito experiment (hereafter *bonito E1*) was also higher in the YSL treatment compared to the DYSL and the Artemia treatments during the first 3 days of the experiment (Table 3, pairwise comparisons t-test, $p < 0.05$). The overall survival of the bonito larvae during the full 6 days of the experiment was significantly higher in the YSL treatment compared to the Artemia and the DYSL treatments (Table 3, pairwise comparisons t-test, $p < 0.05$). Similarly, overall survivals in the YSL treatment were significantly higher compared to the rotifer and the DYSL treatment in the second bonito experiment (hereafter *bonito E2*, Table 3, pairwise comparisons t-test, $p < 0.05$).

Overall survivals were similar between the two bonito experiments, and averaged over 40% in the YSL groups and less than 6% in the other

Table 4
Bluefin tuna. a) Specific growth rate of dry weight (DW, % day) and standard length (SL, % day) of small, medium, and large bluefin tuna larvae estimated from CSDs for the three treatments (5, 50, and 95 percentiles respectively in Fig. 2). b) Average DW (mg) and SL (mm) on experimental days 0, 4 and 8 for the three treatments for the small, medium and large size intervals (5, 50 and 95 percentiles respectively in Fig. 2).

a)

Species	Larval group	Size interval	DW		SL	
			(0–4 days)	(4–8 days)	(0–4 days)	(4–8 days)
Bluefin	YSL	Small	0.31	0.16	0.07	0.02
		Medium	0.16	0.35	0.03	0.08
		Large	0.15	0.54	0.02	0.14
	DYSL	Small	0.20	0.16	0.03	0.02
		Medium	0.04	0.30	0.00	0.05
		Large	0.05	0.36	0.01	0.07
	Rotifer	Small	0.17	0.11	0.04	~0
		Medium	0.07	0.08	0.02	~0
		Large	0.05	0.07	0.01	~0

b)

Species	Larval group	Size interval	DW			SL		
			Eday 0	Eday 4	Eday 8	Eday 0	Eday 4	Eday 8
Bluefin	YSL	Small	0.2	0.7	1.3	6.6	8.6	9.4
		Medium	0.6	1.2	4.8	8.2	9.2	12.8
		Large	1	1.8	15.7	9.4	10	17.7
	DYSL	Small	0.2	0.4	0.8	6.6	7.5	8.0
		Medium	0.6	0.7	2.3	8.2	8.3	10.1
		Large	1	1.2	5.1	9.4	9.7	12.7
	Rotifer	Small	0.2	0.4	0.6	6.6	7.7	7.8
		Medium	0.6	0.8	1.1	8.2	8.7	8.7
		Large	1	1.2	1.6	9.4	9.6	9.6

groups (Table 3). The survivorship of bluefin tuna in the DYSL and rotifer treatments at the end of the experiment was more than four times higher compared to comparable bonito groups (Tables 2 and 3). In the YSL treatments the survivorship of bluefin tuna was around 40%, the same magnitude as for the bonito (Table 2 and 3).

Growth rates of bluefin with different size rank on a given day were shown to vary during the experiment since the cumulative size distributions between subsequent sampling days were not parallel (Fig. 1). Length-specific growth rates were 0.01–0.04 (or approximately 1–4% per day) in the rotifer experiment for all size groups during the first 4 days, whereas they were close to 0 in the last 4 days (Table 4a). For the YSL and DYSL larval groups, length and weight-specific growth rates decreased with bluefin tuna larval size during the first 4 days but increased with bluefin tuna larval size during the last 4 days. For example, a smaller increase was observed for the fish in the upper parts of the cumulative size distributions compared to those in the lower during the first 4 days, whereas the opposite pattern was observed during the last 4 days of the experiment (Fig. 1). Furthermore, the growth was higher, both in DW and SL, in the YSL than in the DYSL larval groups for the entire size range (Table 4a–b).

During the first 4 days of the experiments there were no significant differences in the DW and SL distributions between treatments (Table 5, pairwise comparisons t-test, $p > 0.05$). In contrast, at the end of the experiment, YSL-fed bluefin tuna larvae achieved larger DW and SL followed by the DYSL-fed larva whereas rotifer-fed larvae were the smallest larvae and similar in DW but not in SL than the DYSL-fed larvae (Table 5, pairwise comparisons t-test, $p < 0.05$).

The cumulative size distributions between subsequent sampling days were almost parallel in both experiments for bonito larvae (Fig. 2). For each experiment, length-specific growth rates of the different size classes from Eday 0 to 3 were similar within treatments (Table 6). Also length-specific growth rates were similar across the different size classes between Eday 3 to 6 for each treatment though higher compared to the first 3 days of experiment (Table 6a–b). The weight-specific growth rates were higher for the smallest larval sizes compared to larger sizes during the first 3 days of experiment in the rotifer/Artemia and DYSL treatments whereas the opposite pattern was observed during the last 3 days (Table 6a–b). For the YSL treatment weight-specific growth rates were similar across size ranges.

The size distributions of the larvae at Eday 3, measured as DW, were significantly higher in the YSL vs the Artemia treatment in the bonito E1 whereas in the bonito E2 the DW distribution was significantly higher in the YSL treatment compared to both the DYSL and the Rotifer treatment (Table 7, pairwise comparisons t-test, $p < 0.05$). Measured as SL, size distributions at Eday 3 were only significant in the bonito E2, and the YSL treatment yielded the largest sizes compared to both the DYSL and the Rotifer treatment. In Eday 6, both the DW and SL were significantly different across treatments except for the DYSL vs. the Rotifer treatment in the bonito E2. In general, final sizes were highest in the YSL treatment, followed by the DYSL and finally the Artemia/Rotifer treatment.

In general, bonito were showing less variability in length growth rates with size during the two experimental periods compared to bluefin tuna. At the end of the experiment YSL-fed larvae had increased their dry weight 10, 9, and 3 times more than bluefin tuna fed rotifer and bonito fed rotifer and Artemia, respectively. Compared to the larvae that began piscivory later, YSL-fed bluefin larvae had increased their dry weight 3 times more than the larvae from the DYSL group, while the YSL-fed bonito larvae had increased their dry weight 1.5 times more than larvae from the DYSL group.

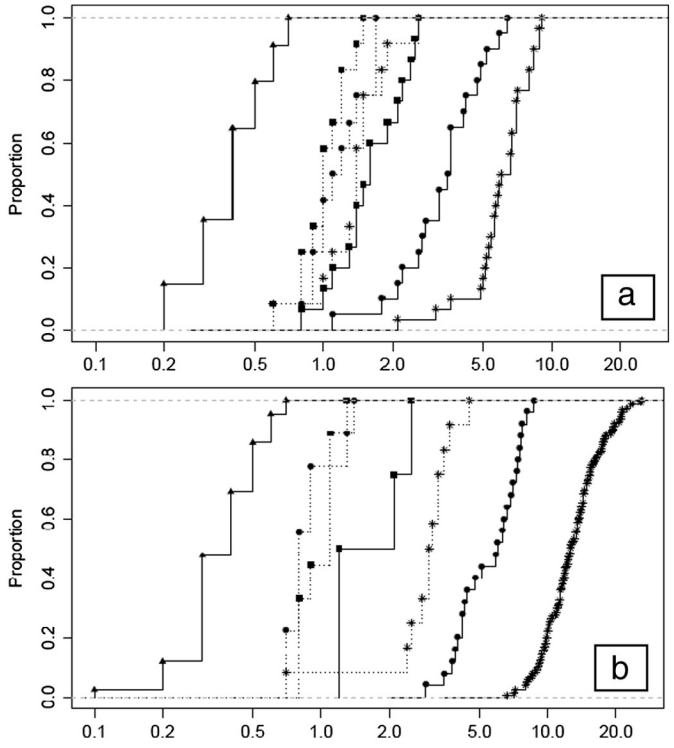

Fig. 2. Bonito larvae. Observed cumulative size distributions in dry weight (DW) of a) bonito E1, and b) bonito E2. Measurements on Eday 0 are represented by triangles and a solid line. The other lines represent measurements on Eday 3 (dashed lines) and the final day of experiment, Eday 6 (solid lines). Different symbols represent different fed larval groups: squares = Artemia in E1 and rotifer in E2, circles = DYSL, and crosses = YSL.

4. Discussion

Studies in marine fish populations have long focussed on the transition from yolk sac to exogenous feeding as one of the most important bottlenecks in larval survival (Hjort, 1914). This critical event lead to the match/mismatch hypothesis relating spawning in temperate waters to the phytoplankton spring bloom and the abundance of micro- and mesozooplankton, the main diet of fish larvae (Cushing, 1969, 1990; Hjort, 1914; Platt et al., 2003). Our results can extend this picture to a second critical window in some species—namely the switch from planktivory to piscivory. Finding fish prey to eat can be a key factor for growth and survival in many of the apex fish predators that undergo a transition between planktivorous and piscivory at the larval stage. The present laboratory experiments show that a transition from a planktivorous to a piscivorous diet can be critical for the growth of bluefin tuna and bonito larvae.

Table 5
Bluefin tuna. Summary table of results from all pairwise combinations between larval size (DW, SL) distributions among the three treatments (Rotifer, DYSL, YSL) on experimental days 4 and 8. We used t tests with pooled SD. A Bonferroni correction was applied to the estimates of the probability to avoid type I error. Significant pairwise combinations (probabilities < 0.05) are marked with an asterisk (*).

Species	Larval group	DW (Eday 4)		DW (Eday 8)		SL (Eday 4)		SL (Eday 8)	
Bluefin		DYSL	Rotifer	DYSL	Rotifer	DYSL	Rotifer	DYSL	Rotifer
	Rotifer	1		0.36		1		0.04	
	YSL	0.05	0.13	<0.001*	<0.001*	0.05	0.10	<0.001*	<0.001*

Table 6
Bonito larvae. a) Specific growth rate of dry weight (DW, % day) and standard length (SL, % day) of small, medium, and large bonito larvae estimated from CSDs for the three treatments (5, 50, and 95 percentiles respectively in Fig. 2). b) Average DW (mg) and SL (mm) on experimental days 0, 3 and 6 for the three treatments for the small, medium and large size intervals (5, 50 and 95 percentiles respectively in Fig. 2).

a)

Species	Larval group	Size interval	DW		SL	
			(0–3 days)	(3–6 days)	(0–3 days)	(3–6 days)
Bonito Exp. 1	YSL	Small	0.47	0.47	0.08	0.12
		Medium	0.42	0.50	0.09	0.11
		Large	0.38	0.46	0.08	0.11
	DYSL	Small	0.48	0.24	0.09	0.05
		Medium	0.35	0.38	0.08	0.08
		Large	0.30	0.42	0.06	0.08
	Artemia	Small	0.42	0.09	0.07	~0
		Medium	0.31	0.16	0.06	0.03
		Large	0.24	0.19	0.06	0.03
Bonito Exp. 2	YSL	Small	0.70	0.54	0.07	0.18
		Medium	0.68	0.47	0.12	0.16
		Large	0.64	0.55	0.13	0.18
	DYSL	Small	0.42	0.54	0.05	0.12
		Medium	0.23	0.67	0.04	0.15
		Large	0.27	0.59	0.05	0.15
	Rotifer	Small	0.46	0.14	0.06	0.03
		Medium	0.34	0.14	0.05	0.03
		Large	0.24	0.23	0.06	0.03

b)

Species	Larval group	Size interval	DW			SL		
			Eday 0	Eday 3	Eday 6	Eday 0	Eday 3	Eday 6
Bonito Exp. 1	YSL	Small	0.2	0.8	3.3	7.2	9.0	12.8
		Medium	0.4	1.4	6.3	7.9	10.3	14.5
		Large	0.7	2.2	8.8	8.8	11.2	15.8
	DYSL	Small	0.2	0.8	1.8	7.2	9.3	10.9
		Medium	0.4	1.2	3.6	7.9	9.9	12.6
		Large	0.7	1.7	5.9	8.8	10.6	13.6
	Artemia	Small	0.2	0.7	0.9	7.2	9	9
		Medium	0.4	1	1.6	7.9	9.6	10.6
		Large	0.7	1.4	2.5	8.8	10.4	11.4
Bonito Exp. 2	YSL	Small	0.2	1.6	8.2	7.4	9.2	16.0
		Medium	0.4	3.0	12.6	7.9	11.4	18.2
		Large	0.6	4.1	21.3	8.3	12.4	21.4
	DYSL	Small	0.2	0.7	3.6	7.4	8.7	12.4
		Medium	0.4	0.8	6	7.9	9	14.0
		Large	0.6	1.4	8.0	8.3	9.8	15.4
	Rotifer	Small	0.2	0.8	1.2	7.4	9.0	9.9
		Medium	0.4	1.1	1.6	7.9	9.3	10.3
		Large	0.6	1.2	2.4	8.3	10	10.9

Bluefin tuna and bonito are among the species that start eating larval prey in the larval period. The initial larval size used in the bonito and bluefin tuna experiments was similar but the larval age was very different. Both species can initiate piscivory at around 7–9 mm SL, but the age of the larvae at this stage varies from 8 to 9 days in bonito to 18 days in tuna larvae. Foraging traits, such as teeth and the digestive system, develop at a very early larval stage in scombrid larvae. Studies in Pacific bonito show the development of traits that allow piscivory to occur earlier than in Pacific bluefin tuna (Kaji et al., 1996, 2002). These important ontogenetic changes during the larval stage can explain why small

Table 7
Bonito larvae. Summary table of results from all pairwise combinations between larval size (DW, SL) distributions among the three treatments (Artemia, DYSL, YSL in bonito experiment 1 and Rotifer, DYSL, and YSL in bonito experiment 2) on experimental days 3 and 6. We used t tests with pooled SD. A Bonferroni correction was applied to the estimates of the probability to avoid type I error. Significant pairwise combinations (probabilities < 0.05) are marked with an asterisk (*).

Species	Larval group	DW (Eday 3)		DW (Eday 6)		SL (Eday 3)		SL (Eday 6)	
Bonito exp 1		DYSL	Artemia	DYSL	Artemia	DYSL	Artemia	DYSL	Artemia
	Artemia	0.72		<0.001*		0.72		<0.001*	
	YSL	0.42	0.03*	<0.001*	<0.001*	1	0.16	<0.001*	<0.001*
Species	Larval group	DW (Eday 3)		DW (Eday 6)		SL (Eday 3)		SL (Eday 6)	
Bonito exp 2		DYSL	Rotifer	DYSL	Rotifer	DYSL	Rotifer	DYSL	Rotifer
	Rotifer	1		0.14			1	<0.001*	
	YSL	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

variations in the timing of onset of piscivory can have such a strong effect in the larval growth and survival of both species.

The survival rates obtained in our study for Atlantic bluefin tuna is in the range obtained in recent experiments for Pacific bluefin tuna feeding on yolk-sac larvae (Seoka et al., 2008). We have supplied yolk-sac larvae in excess to avoid aggressive behavior and cannibalism as suggested for other scombrid larvae (Sawada et al., 2005). In fact, we did not observe such behavior during the experiments. Still, we preferred to shorten the duration of the bonito experiments since aggressive behavior in older larvae may be difficult to control even when supplying food in excess (personal observation). The timing of onset of piscivory is a bottleneck for larval survival but differences are observed between the two species analyzed in our study. In bluefin tuna, high survival rates could be maintained when larval prey was included in the diet. But survival rates in bonito dropped significantly when larval prey in the diet was delayed by only 3 days. Bonito may therefore have a shorter critical window to begin piscivory than bluefin tuna. This may be particularly important in the field, where prey larvae may be difficult to encounter if they are patchy distributed.

The estimated daily ration for wild larvae was about 111–127%, and 90–111% of dry body weight in the piscivorous *Scomberomorus niphonius* larvae (Shoji et al., 2001). Daily rations of 25–30% have been calculated for oceanic tunas *Thunnus* spp up to 10 mm (Young and Davis, 1990). Tuna develop their digestive system earlier, including the digestive enzymes, related to the other non-scombrid species, which could explain the digestive capacity of these species (Buentello et al., 2011). We have not estimated ingestion rates under the prevailing experimental setting, but since the growth rates we have estimated can be up to 60% of body weight we expect the larvae to have similar ingestion rates as *S. niphonius*.

The culture of Atlantic bluefin tuna and bonito is still an emerging field (Mylonas et al., 2010) and diets have not been described in detail yet. In cultured Pacific bluefin tuna larvae, yolk-sac larvae, commonly *Oplegnathus fasciatus*, are supplied routinely after a diet based on rotifer and Artemia (e.g.Sawada et al., 2005). Both Artemia and rotifer are very small compared to the prey found in a natural environment where a mix of larger zooplankton prey is expected. In our experiments, supplying Artemia instead of only rotifer slightly increased survival rates in bonito. But final size distributions for YSL feeding on rotifer prior to the onset of the experiment grew much larger than those feeding on rotifer and Artemia. Our results complement previous investigations into Pacific bluefin tuna with respect to the development of artificial diets and improving survival and growth in the transition between diet types (Seoka et al., 2007, 2008).

We expect that our results will have consequences for the understanding of processes driving survival in natural populations. In a natural environment we expect larvae to find a mix of small and larger zooplankton prey in comparison to Artemia and rotifer. We could not supply the larvae with zooplankton due to technical difficulties in maintaining zooplankton cultures besides those of larval prey, bonito, and bluefin tuna. However, we expect in the future to be able to evaluate how results will differ when a more natural diet of zooplankton is used. The timing of transition to piscivory is crucial for growth and size structure in many piscivorous fish populations (e.g. Mittelbach and Persson, 1998). Our results show that an early transition to piscivory benefits survival and increased growth during the larval stage, even when food abundance is not limiting.

In natural environments there is evidence that early piscivorous fish grow at higher rates than late piscivores (Post, 2003). Most information available from field studies for apex predator larvae focuses on describing their diet (Llopiz, 2013 for review). We show that switching to a piscivorous diet once basic foraging traits have been developed confers survival advantages and improves growth rates, and that this can constitute a critical window for apex fish predators.

In summary, our experiments reveal that the timing of onset of piscivory is critical for the growth and survival of piscivorous larvae. A delay in the shift to piscivory decreases growth rates but survival rates could be maintained as long as larval prey is included in the diet. In our study the inclusion of different prey in the diet depended on both prey species and relative body size. The development of process-based models to understand the recruitment success in bluefin tuna and bonito needs to incorporate piscivory during the larval stage and its influence on growth and survival. We expect similar mechanisms in most apex predators with a piscivorous phase during the larval stage.

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2.5. Effects of temperature on embryonic development of Atlantic bluefin tuna (*Thunnus thynnus,* L 1758) and Atlantic bonito (*Sarda sarda*, Bloch 1793)

RESUMEN

Efecto de la temperatura sobre el desarrollo embrionario del atún rojo del Atlántico (*Thunnus thynnus,* L 1758) y el bonito Atlántico (*Sarda sarda*, Bloch 1793).

Se describe el desarrollo embrionario de huevos de Atún rojo del Atlántico (*Thunnus thynnus, L 1758*) y Bonito Atlántico (*Sarda sarda*, Bloch 1793) obtenidos de puestas espontáneas procedentes de reproductores mantenidos en cautividad, así como los efectos de la temperatura del agua sobre su velocidad de desarrollo, el tiempo requerido para eclosionar, la eclosión y la tasa de eclosión. Los huevos de Atún rojo del Atlántico medían 1,071±20.8 µm y solían mostrar una solo gota de grasa. Los mejores resultados en cuanto a tasas de eclosión se obtuvieron en huevos incubados a temperaturas entre 24 y 25ºC, aunque larvas viables fueron capaces de eclosionar en un amplio rango de temperaturas, entre 19 y 32ºC. El diámetro medio de los huevos de bonito fue de 1,293.5±32.9 µm, y tenían varias gotas de grasas, promediando 3.4±0.6 gotas. Las mejores tasas de eclosión se obtuvieron cuando la temperatura del agua estaba entre 19 y 22ºC, pero los huevos son capaces de desarrollarse y dar larvas viables a temperaturas comprendidas entre 16 y 27ºC

ABSTRACT

The embryonic development of ABFT (*Thunnus thynnus, L 1758*) and AB (*Sarda sarda*, Bloch 1793) eggs, obtained from spontaneous spawning of cultured broodstock is described, as well as the effect of water temperature on their developmental speed, time required to hatch, and hatching rate. ABFT eggs measured 1,071±20.8 µm and usually showed a single oil globule. Best performance in hatching rates were obtained with eggs kept at temperatures between 24 and 25ºC, but viable larvae were able to hatch in a wide range, between 19 and 32ºC. Average diameter of AB eggs was 1,293.5±32.9µm, and they had several oil globules, averaging 3.4±0.6 globules. Better hatching rates were obtained when water temperatures were between 19 and 22ºC, but eggs are able to develop into viable larvae with water temperatures ranging between 16 to 27ºC.

INTRODUCTION

One of the main parameters of the culture of any fish species is the temperature. Embryonic development, survival, presence of abnormal larvae and yolk utilization of larvae is affected by environmental conditions, particularly temperature and salinity (Cook *et al.,* 2005; Kim *et al.*, 2014). In the case of large pelagic fish which migrate through oceans and which spawn in open seas, temperature is a key factor. Temperature is the most important environmental factor that influences the development, growth and survival of marine fishes during early development (Pepin, 1991; Laurel & Blood, 2011). Laboratory-validated data regarding the development, hatching and survival responses of fertilized ABFT and AB eggs exposed to varying temperature treatments are required to analyze their early development.

The temperature response of marine fish eggs is highly variable between species (Houde, 1987; Pepin, 1991), and it is possible that there are stock-specific or population-specific differences (Geffen *et al.*, 2006). As it has been reported in the first chapter of this thesis, range of temperature in which ABFT and AB spawn are wider than expected, and it is necessary to research about the tolerance of eggs and larvae.

ABFT has a confined seasonal spawning (Medina *et al.,* 2002; Corriero *et al.,* 2003), a pattern that is supported by the temporal occurrence of the larvae (Alemany *et al.*, 2006; Reglero *et al.,* 2012). This reproductive strategy has been hypothesized to be related to the thermal tolerance of the species with regard to spawning and egg and larval survival. Particularly, it has been suggested that high temperatures are favourable for the development of early life stages but physiologically stressful for the adults (Block *et al.,* 2005). Field data on adult and larval distributions worldwide indicate that larvae have a narrower and warmer range of temperature preferences than the adults (Boyce *et al.,* 2008). However, only a few experimental researches have been conducted on the thermal tolerances of the ABFT eggs, and with a small range of temperatures (Gordoa & Carreras, 2014). Likewise any study has been developed with AB.

With regards to *scombrids* only a few articles deal with these issues. Miyashita (2002) studied the effect of temperature on survival and time lapsed from different developmental stages in Pacific Bluefin tuna (*Thunnus orientalis*), and it revealed that their eggs are able to hatch in a wide range of temperature. Similar results were founded by Woolley *et al.* (2011) in yellowfin tuna (*Thunnus albacares*). Another authors like Murata *et al.* (2005), Mendiola *et al.* (2007) and Woolley *et al.* (2009), have studied the relationship between temperature and time to embryonic development.

In this study, ABFT eggs were incubated at different temperatures, not only normal spawning temperatures, but also upper and lower extremes. Two main objectives of this study were to determine the effect of temperature on viable hatching rate and the relationship between stage of development and temperature. More than that, study will show the best conditions for aquaculture purposes, so that to achieve the goal of successful hatchery production of ABFT larvae, the temperature ranges must be narrowed to maximize hatching rate and larval survival.

Hatching rate depends on temperature, but as eggs usually have a greater temperature tolerance than larvae, it is important to know the influence of temperature on the incidence of deformities and the rate of abnormal larvae. In this way, we will be able to determine the normal hatching rate, which points out the rate of viable hatched larvae.

With regards to the ontogeny of the development of the eggs, the relationship between the time to arrive to different developmental stages and temperature will be determined. It is intended to estimate the age of the eggs on the basis of the stage of development and water temperature. This regression model will provide an important tool to researchers to determine movements of eggs from spawning field, so that with egg observation and temperature data it will be possible to estimate the exact time of the spawning.

Finally, data on the development, hatching and survival responses of fertilized ABFT and AB eggs exposed to varying temperature is essential not only to understand the processes that determine the recruitment of the larvae, but also to predict the potential response of both species to climate change, mainly to global heating of oceans.

MATERIAL AND METHODS

ABFT:

Four different trials were carried out early July 2013 and late June 2014. Three first trials were made at temperatures ranging between 18-30ºC and last one at temperatures between 18-33ºC.

Fertilized ABFT eggs were collected from spontaneous spawning in the broodstock cages placed at the concession of the company Caladeros del Mediterráneo S.L., in El Gorguel Bay (Cartagena, SE Spain) in 2013 and 2014. Broodfish were fished in Balearic Sea in June 2008. They were fattened during seven months and then, in early 2009, 25 bluefin tunas were moved to cage

nº2. Tunas had about 40-45 kg in this moment. They were monitored and fed from this moment to 2014. Their first spawning season was 2010. In 2013 average weight ranged between 150 and 160 kg, and in 2014 close to 200 kg.

Every night a crew went to the cage to try to collect just spawned eggs. In order to do that, cage was monitored every half hour from 03:00 AM to look forward just fertilized eggs. When some thousands of eggs were watched on the surface of the cage (usually between 3:30 and 4:30 AM) they were collected and transported to Murcia Oceanographic Centre in Mazarrón (COMU). Temperature of sea water during collection ranged between 21.5 and 24ºC. When arriving at COMU, (between 6:00 and 7:00 AM) eggs were in 4-16 cell phase, and we proceeded to distribute among different tanks prepared at wished temperatures. 400 eggs were used to each tank, and they were shared among three 250 ml flasks (50 eggs/flask) to monitor survival and hatching rate, and another 1000 ml flask where 250 eggs were introduced. The flasks were filled previously with filtered sea water. The larger flasks were used to determine the stages of the embryonic development by taking a sample of 10 eggs every 2-3 hours and watching under binocular stereomicroscope.

It was necessary to temperate the eggs in the flask to the wished temperature. In order to get it, the temperature of the water inside the flasks was modified at a rate of 1ºC every half hour. So, in a maximum of 4 hour all the eggs were at the wished temperature and placed in their respective tanks.

In order to keep the temperature constant through all the experimental time, all the tanks were inside a conditioned room (18ºC). Each large tank had a heater and a water thermostat (±0.5C) to control the temperature. Aeration provided homogeneous temperature in all the tanks. Flask inside the tanks were floating and moving by the tank.

When the eggs inside a large flask started to hatch, flask was controlled hourly, and when all the eggs inside had hatched, small flasks placed in the same tank were watched and larvae extracted and counted, identifying normal and abnormal larvae to calculate total hatching rate (rate of total hatched larvae with regards to total inoculated eggs), incidence of abnormal larvae (rate of abnormal larvae with regards to total larvae) and normal hatching rate (rate of normal larvae with regard to total inoculated eggs).

AB:

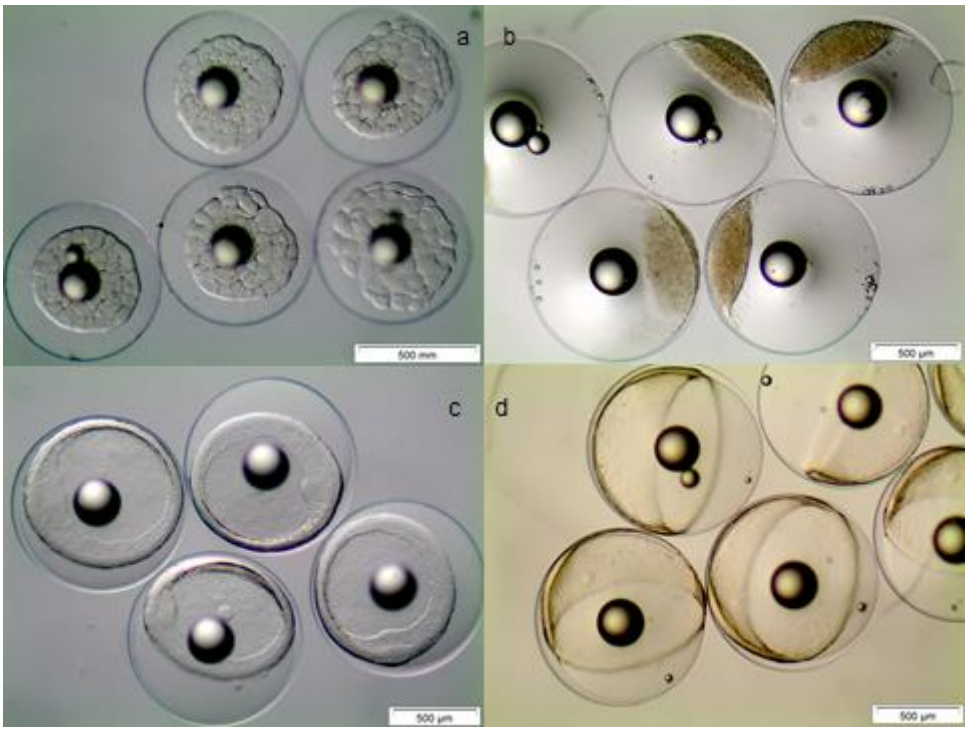
Four different were carried out in June 2013. First one was made between 19 and 25ºC and the remainder trials between 16 and 28ºC.

Fertilized AB bonito eggs were collected from a broodstock consisting in 17 AB broodfish placed in a 55 m^3 tank in COMU. AB were captured in 2010 in this was their second spawning season. Fertilized eggs spawned spontaneously were collected practically every evening from the tank, between 18 and 21 h. Tanks was provided with an egg collector which was checked continuously for floating spawned eggs. When the collector had some thousands of eggs, they were taken to laboratory and place at the wished temperature in the same way than above described. Temperature of sea water during collection ranged between 19 and 23ºC.

The data were analyzed using the software StatGraphic© Plus. A regression analysis was made (exponential). Relation was considered statistically significant when $p<0.01$

RESULTS

Ontogeny of ABFT is shown in Figure nº1. Eggs are spherical, with an average diameter of 1,071.5±20.8 µ. Usually they have a single oil globule, but some eggs can present two or even three globules. They tend to join with the development of the eggs. At hatching larvae had a total length of 3,318±115.67 µm.



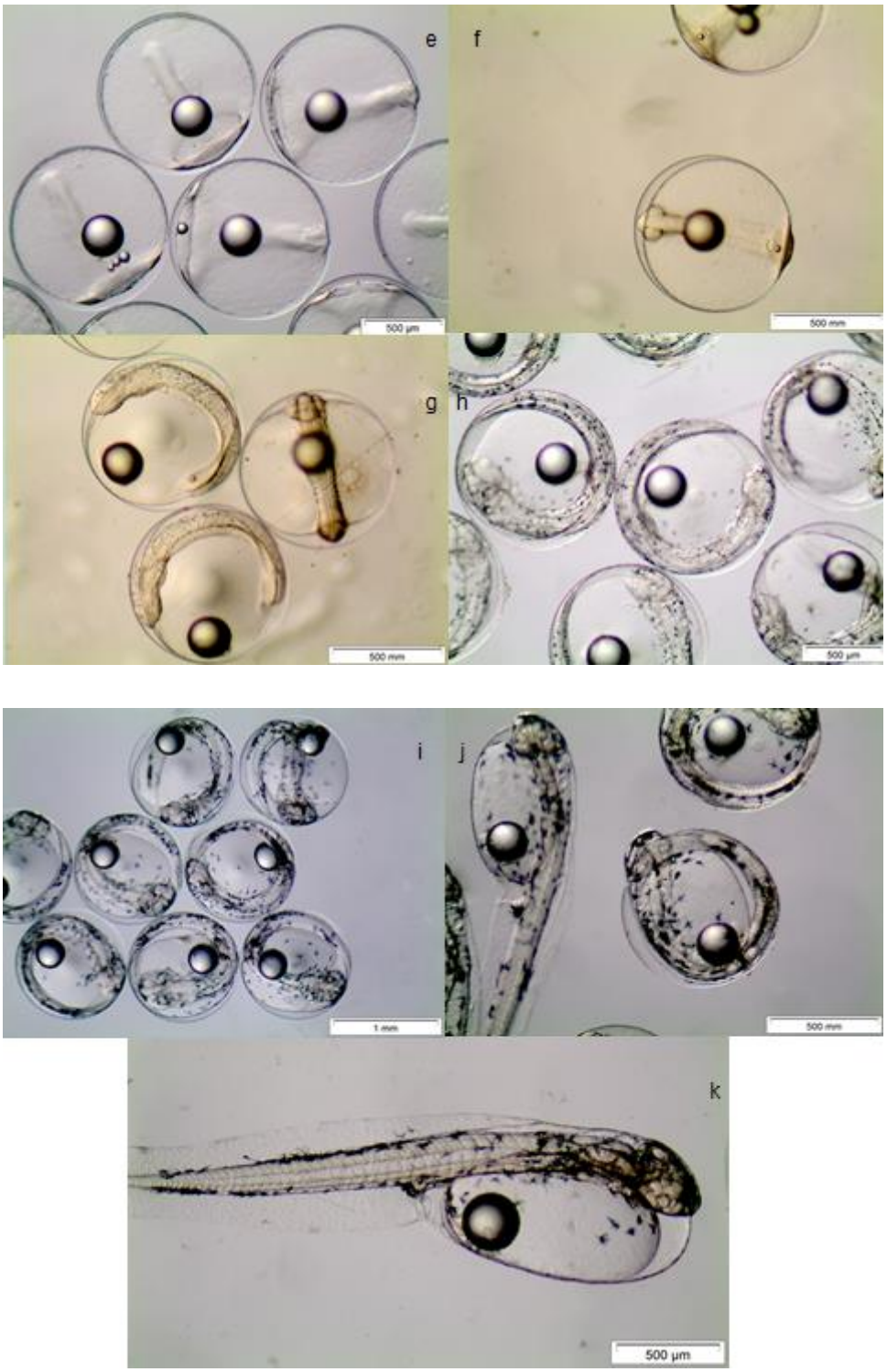


Fig 1: Ontogeny of ABFT: A Morula; B Blastula; C Gastrula; D Embryonic shield; E Appearance of embryonic body; F Kupfer´s vesicle; G Onset of pigmentation; H hearth beating; I Preeclosion; J Beginning of hatching; K Just hatched larvae

In the same way, development of AB eggs is shown in Figure nº2. Eggs had an average diameter of 1,293.5±32.9 µm and the average number of oil globules was 3.4±0.6. At hatching larvae had a total length of 4,268±105.4 µm.

Fig 2: Ontogeny of AB: A Blastula; B Gastrula; C Embryonic shield; D Appearance of embryonic body; E Kupfer´s vesicle; F Onset of pigmentation; G hearth beating; H Preeclosion

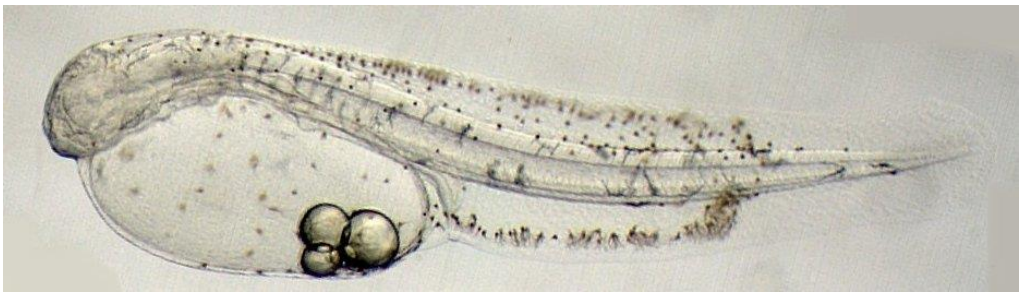


Figure 3: Just hatched larvae of AB

Figures 4, 5 and 6 pointed out respectively the effect of temperature on hatching rate, on the incidence of abnormal larvae (or deformity rate) and on the normal hatching rate of both species. Normal hatching rate is the rate of normal larvae hatched in relation to fertilized eggs; that means rate of larvae able to survive and to have a normal development after hatching. ABFT eggs are able to hatch from 18.5 to 34ºC, but below 19 and above 32ºC, larvae have not any chance of surviving. Something similar happens with AB: eggs are able to hatch up to 29ºC, but incidence of abnormal larvae is very high from 27ºC.

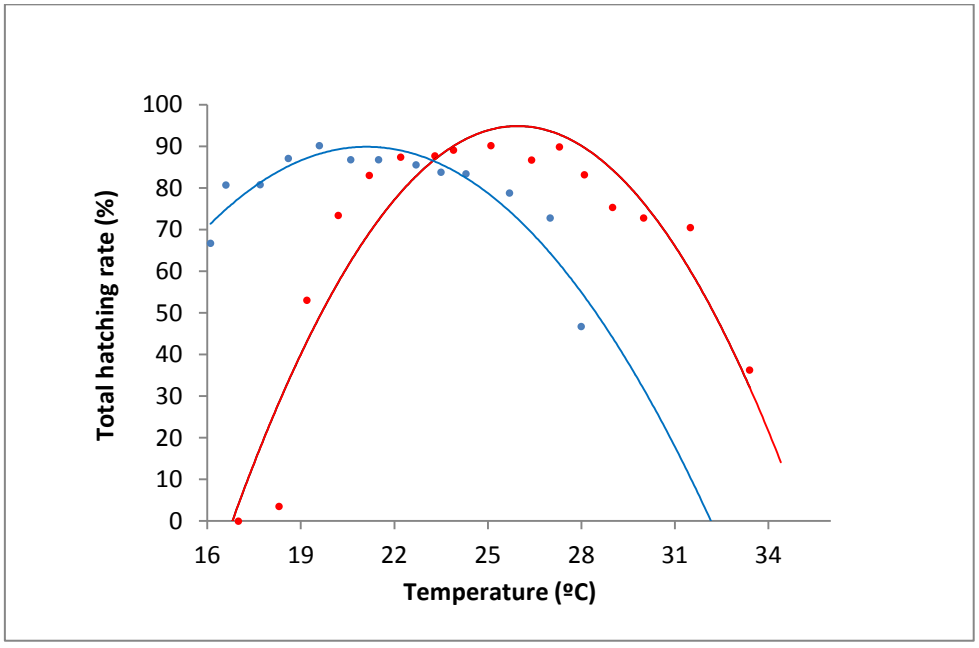


Figure 4: Total hatching rate of ABFT (red dots) and AB (blue dots) eggs incubated at different temperatures

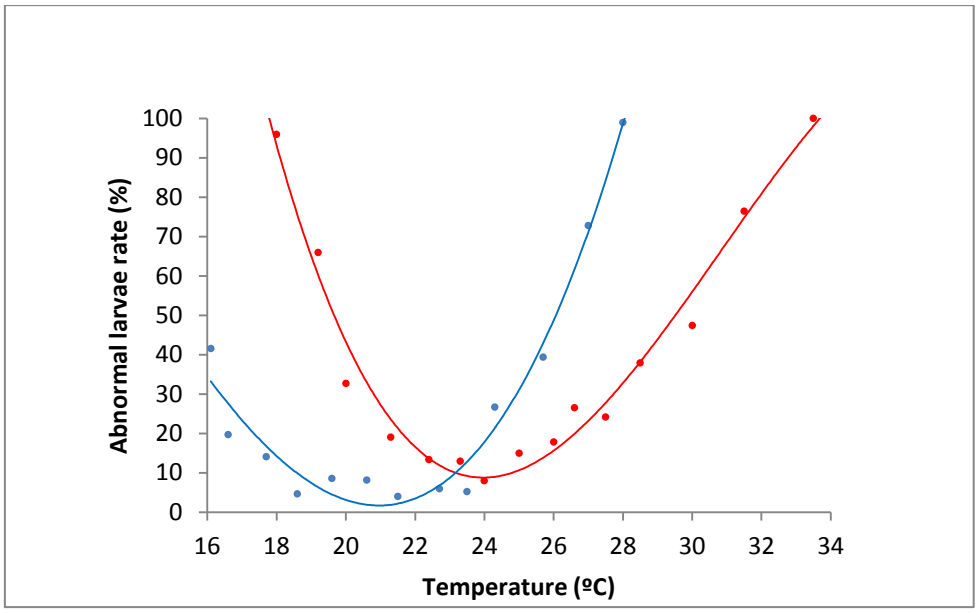


Figure 5: Incidence of abnormal larvae coming from ABFT (red dots) and AB (blue dots) eggs incubated at different temperatures

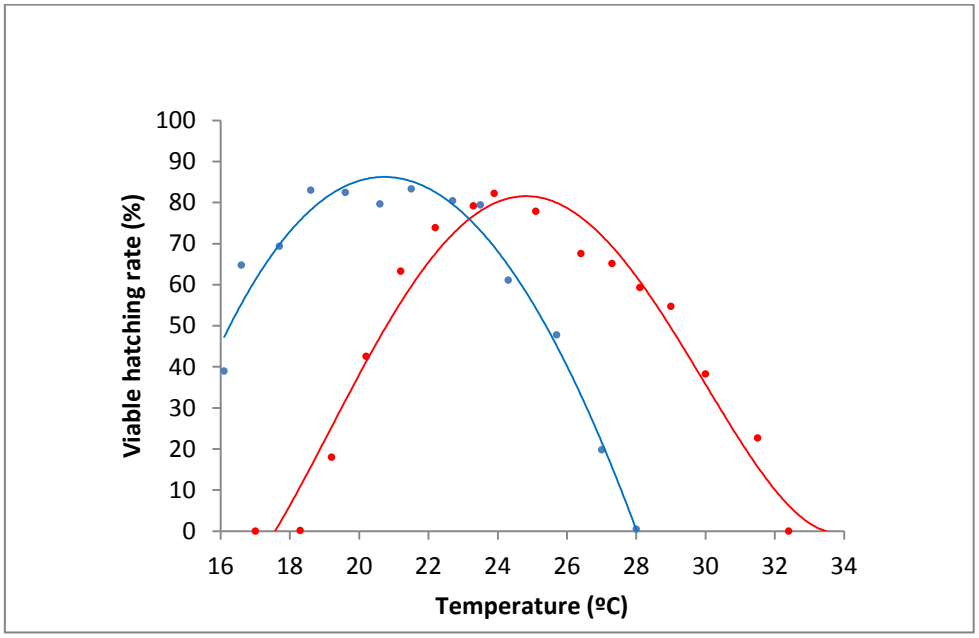


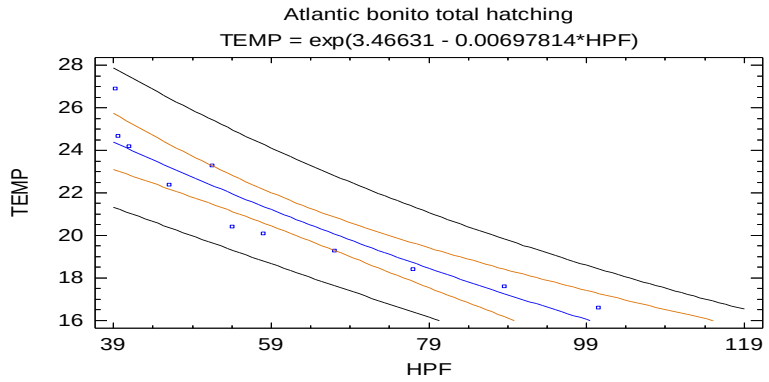
Figure 6: Relationship between water temperature and viable hatching rate of ABFT (red dots) and AB (blue dots) eggs.

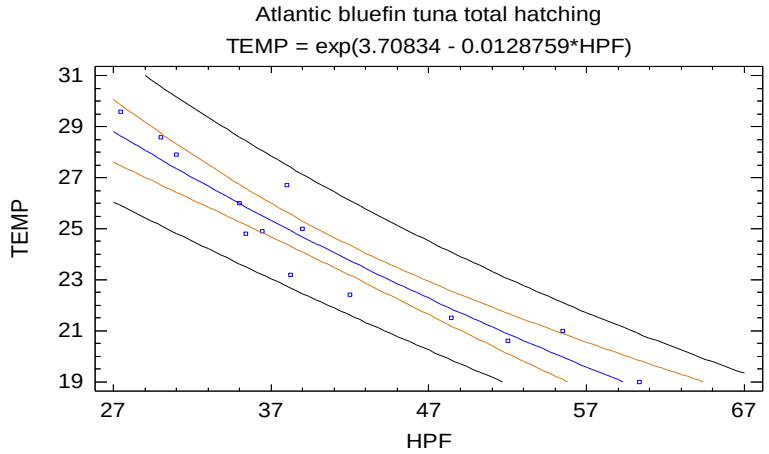
With regards to relationship between development and temperature, Table 1 displays the equations between both parameters in ABFT and B. All the relationships, except of appearance of embryo in ABF, are statistically significant ($p<0.01$).

	ABFT	AB
Blastula	T = exp (3.51044-0.06252*HPF)	T = exp (3.49052-0.05763*HPF)
Gastrula	T = exp (3.70919-0.05719*HPF)	T = exp (3.54791-0.04294*HPF)
Appearance of embryonic shield	T = exp (3.56403-0.03304*HPF)	T = exp (3.47567-0.02747*HPF)
Appearance of Embryo	T = exp (3.66063-0.01806*HPF)	T = exp (3.40426-0.02006*HPF)
Appearance of Kupfer´s vesicle	T = exp (3.5335-0.02010*HPF)	T = exp (3.44357-0.01763*HPF)
Beginning of heart beat	T = exp (3.49853-0.01130*HPF)	T = exp (3.39853-0.00871*HPF)
First hatching	T = exp (3.65883-0.01294*HPF)	T = exp (3.45353-0.00806*HPF)
Total hatching	T = exp (3.70834-0.01288*HPF)	T = exp (3.46631-0.00698*HPF)

Table 1: Relationship between Temperature (ºC) and time (hours post fertilization) from onset of embryonic stage in ABFT and AB.

Hereafter, relationship between temperature and time to the end of the hatching in AB and ABFT are shown. Coefficients of correlation are respectively -0.94238 and -0.95352.





Onset and final of hatching (expressed in time from fertilization –HPF-) depend on temperature, as it is stated for ABFT in the following equations

Onset hatching: time (h) = 282.75 - (Ln T(ºC)/0.01294)
Final hatching: time (h) = 287.91- (Ln T(ºC)/0.0129)

And for AB in the following one:

Onset hatching: time (h) = 478.48 - (Ln T(ºC)/0.00806)
Final hatching: time (h) = 496.61- (Ln T(ºC)/0.00698)

In following figures it can be observed the time lapsed from spawning to total hatching under different temperatures to ABFT and AB.

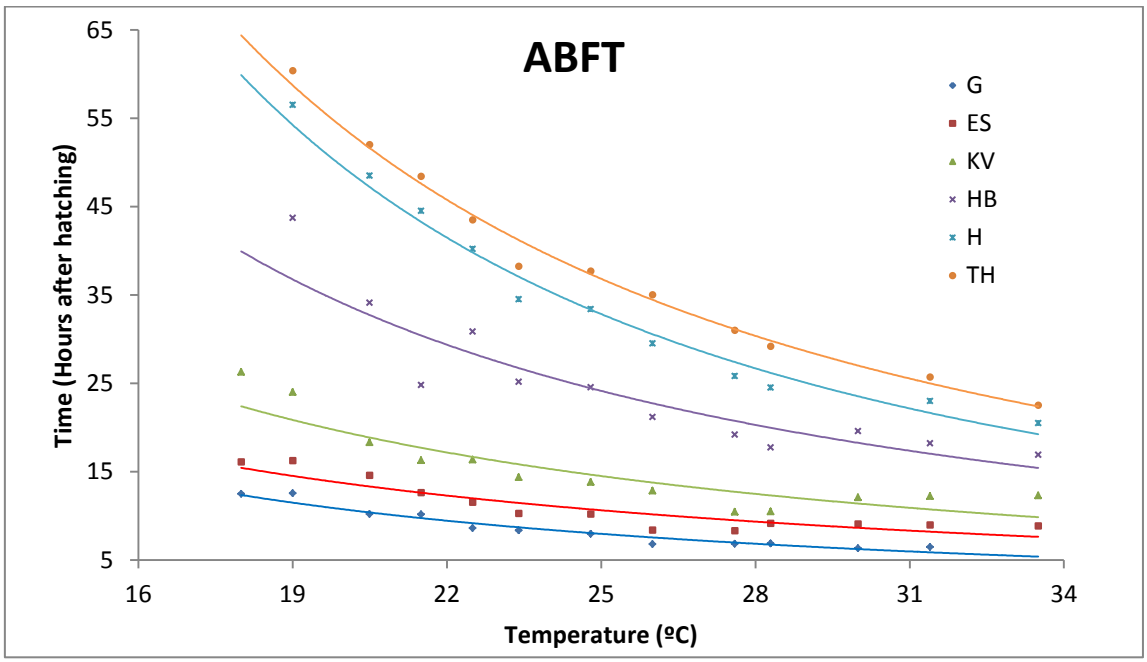


Figure 4: Time required to onset of different development stages in ABFT. G gastrula stage; ES appearance of embryonic shield; VK appearance of Kupfer's vesicle; HB beginning of hearth beat; H beginning of hatching; TH total hatching

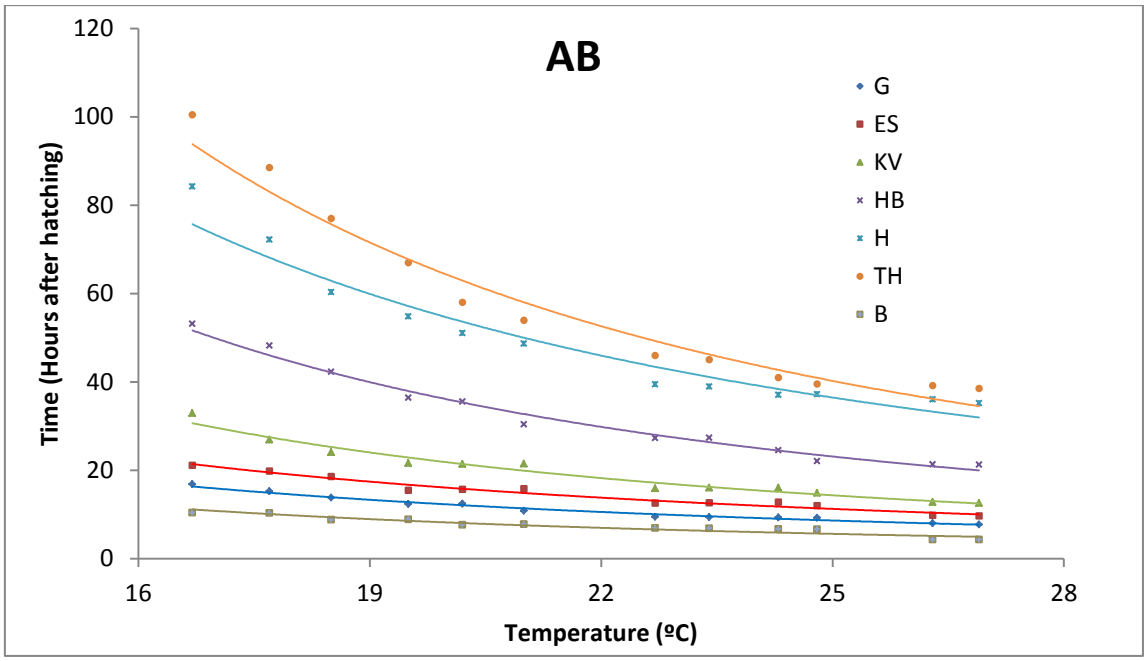


Figure 5: Time required to onset of different development stages in AB. G gastrula stage; ES appearance of embryonic shield; VK appearance of Kupfer's vesicle; HB beginning of hearth beat; H beginning of hatching; TH total hatching, B blastula stage

DISCUSSION

No experimental research has been conducted on the thermal tolerances of the eggs and larvae nor ABFT nor AB, and only one experiment (Gordoa & Carreras, 2014) deal on the effect of temperature on embryonic development of ABFT. This experiment was only conduced at water temperatures between 20 and 26ºC, but we tried a wider range, between 19 and 33ºC for the ABFT.

With regards to thermal tolerance, temperature is known to be the most important environmental factor that influences the development, growth and survival of marine fishes during early development (Pepin, 1991). However, there are many references about a great range in marine fish eggs for different species (Polo *et al.,* 1991; Galloway *et al.,* 1998; Karas & Klingsheim, 1997).

But in spite of this, thermal tolerance is not so high. Abnormality larvae rates are high when water temperature got away not so wide range. Polo *et al.,* (1991), stated that gilthead sea bream, *Sparus aurata,* are able to spawn between 14 and 26ºC, but deformities rates in larvae increased out of 16-22ºC. In the same way, Galloway *et al.*, (1998), found that Atlantic cod (*Gadus morhua*) eggs are able to hatch between -1ºC and 12ºC but incidence if abnormal larvae only remained low between 4 and 8ºC.

With regards to other *scombrids*, Miyashita (2002) and Murata *et al.* (2006) reported that Pacific Bluefin tuna eggs were able to hatch between 19.5 and 34ºC, but in order to obtain a normal hatching rate above 60%, the water temperature range should be between 22 and 30ºC. In the same way, ABFT eggs are able to spawn between 18.5 and 33.5ºC, but the range for a normal hatching rate above 60% is 21.5 to 28.5ºC.

AB required lower temperatures, and eggs require temperatures between 16 (and maybe lower temperatures) and 28ºC to hatch, but the range for a normal hatching rate above 60% is 17 to 25ºC.

Percentage of abnormal larvae (most of them folded larvae) is above 50% when water temperature reached 30ºC, and is 100% at a 33ºC. With AB larva this can be watched at 26 and 28ºC respectively.

Range of temperatures for hatching is wider than range of temperatures to obtain spawning, which is considered between 19.5 and 27ºC and between 17 and 25ºC to ABFT and AB respectively (see chapter 1.2. 1.3 and 1.4 of this thesis). These ranges are more in accordance with the range of normal hatching rate above 60%; so fish only would spawn if eggs or larvae are going to have fine chance of survival. ABFT are able to spawn at 19-20ºC (Gordoa & Carreras, 2014) , but this should be only residual spawns surely obtained in the framework of oscillating temperatures. At the end, situation could be a compromise between warmer temperatures preferred by larvae (Boyce *et al.,* 2008; *Reglero et al.,* 2014) and colder preferred by adults (Block *et al.,* 2005)

Besides that, it is important to point out that both species can resist great variations in water temperature during early development. To temperate the eggs, temperatures in this trials has been modified up to 1ºC each half hour during the morula and blastula stage, and in several trials ABFT and AB have withstood changes of 6-8ºC without remarkable mortality.

Optimum temperature to hatch could be considered between 23 and 26ºC to ABFT. This is very similar to the temperature considered for other bluefin tunas: Miyashita (2002) reported the best results with Pacific bluefin tuna (*Thunnus orientalis*) at 23-27ºC and *Wooley et al.*, (2009) with to Southern bluefin tuna (*Thunnus maccoyii*) at 23-25ºC. Another tropical tunas like yellowfin tuna (*Thunnus albacares*) showed a higher preferred temperature, between 27 and 30ºC (Wexler *et al.,* 2011), although a further study made by *Kim et al.*, (2014) in yellowfin reported a lower preferences, between at 23-26ºC.

With regards to AB, optimum temperatures are lower, between 19 and 22ºC, which agrees with their sooner spawning period.

The time to hatch, as expected, depends on temperature. Provided equations reveal that ABFT eggs take about 43h at 22ºC, 36h at 24ºC and 30 h at 26ºC to

hatch. These time could be reduced in some minutes or even an hour (the higher temperature the best) due to 3-4 hours necessary to temperate the eggs. Gordoa and Carreras (2014) reported lower time to hatch (between 3 and 4 hours according temperature) than our results.

These differences should be investigated, but apart from differences attributed to temperate, another difference could be related with the higher size of ABFT broodstock in our experiment (1.07 mm against 1.0 mm considered by Gordoa). According to Pauliy & Pullin (1988), time to hatch depends on temperature and on egg diameter according to following equation:

$$\text{Log } t = a + b\text{Log}D - c\text{Log}(T+26)$$

Whereas t is time in days, D egg diameter and T temperature.

So time to hatch is directly related to the diameter and inversely related with temperature, and in the same species larger eggs will take an extra time to hatch.

The reason because ABFT egg were greater in this experiment is dealt in chapter 1.4 of this thesis: size of the eggs increase with size of broodfish, and in our experiment ABFT were larger than in the experiments of Gordoa & Carreras (2014), 150-180 kg vs 75-125 kg.

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CAPITULO 3:

Diseño de una instalación en tierra para el control de la reproducción del atún rojo del Atlántico

Design of a land based facility to control Atlantic Bluefin tuna reproduction

3.1. Diseño de una instalación en tierra para el control de la reproducción del atún rojo

Introducción y justificación

Desde hace más de diez años, existen en la Región de Murcia varias empresas que se dedican a cultivar atún rojo. Estas empresas dan empleo a mas de 300 trabajadores, y hasta hace dos o tres años engordaban unas 3-4.000 Tm al año, lo que la convertía en una de las principales actividades exportadoras de la Región de Murcia. Pero en estos dos últimos años, los importantes recortes de cuotas y de esfuerzo pesquero (en cuanto a épocas de veda) que ha sufrido la pesquería de atún rojo en nuestras inmediaciones, ha originado que haya un importante descenso de la producción y de la actividad de estas empresas, algunas de las cuales se han visto forzadas a cerrar.

Como todos los indicios parecen indicar que las medidas impuestas van a seguir existiendo, si acaso, aún más restrictivas, la única salida para este sector a medio y largo plazo es que sus capturas no dependen del medio ambiente, sino que al igual que ocurre con la mayoría de las especies acuícolas cultivadas, los individuos con los que empezar el cultivo provengan de criaderos especializados totalmente independientes de las pesquerías.

Cualquier criadero para peces marinos debe de estar dotado de unas instalaciones que permitan albergar a los reproductores en unas condiciones óptimas de cultivo (elevada calidad del agua, tranquilidad, densidad baja, etc.) y otras instalaciones en las que realizar el cultivo larvario de las puestas obtenidas y el cultivo de las especies acompañantes (fitoplancton, zooplancton, etc.) y la transición a la alimentación inerte y las primeras fases de alevinaje.

El presente proyecto de construcción de una instalación en tierra para la reproducción del atún rojo, es una infraestructura para acometer el cultivo del atún rojo en cautividad que se encuentra realizando el Instituto Español de Oceanografía en unos terrenos próximos a su Planta de Cultivos Marinos de Mazarrón. Esta nueva instalación complementará las instalaciones de cultivo larvario ya existentes y permitirá, por primera vez en Europa, albergar los reproductores de atún en tanques en tierra. Esta infraestructura se realiza por parte del IEO y es financiada al 70% por fondos FEDER, aportando el IEO el 30% restante. Para la selección y ejecución de este proyecto, se firmó un Convenio de colaboración entre el Ministerio de Ciencia e Innovación, la Consejería de Universidades, Empresa e Investigación de la Región de Murcia y el Instituto Español de Oceanografía. Este Convenio fue publicado en el BOE en julio de 2009.

La ubicación de la instalación para la reproducción de atún cerca de las instalaciones que el Instituto Español de Oceanografía posee en el término municipal de Mazarrón, permite la utilización de las mismas para el cultivo larvario y alevinaje de los atunes obtenidos, con lo que la nueva instalación tendría como finalidad única albergar los reproductores de atún, trasladando las puestas obtenidas a la Planta del IEO. Esta elección tiene una doble ventaja: por un lado disminuye sustancialmente el precio de la nueva instalación a construir y por otro lado se aprovecha de las instalaciones y del personal existente en la Planta de Mazarrón.

En la actualidad, la mayoría de las instalaciones de acuicultura marina ubicadas en tierra tienden a usar tecnologías de recirculación, las cuales presentan indudables ventajas sobre las técnicas más tradicionales basadas en bombear elevados caudales de agua nueva que tras su utilización, son devueltos al mar. Entre las ventajas de la recirculación pueden mencionarse que son mas respetuosas con el medio ambiente, ya que consumen una cantidad entre 50 y 1000 veces inferior de agua, que puede ser tratada con facilidad para eliminar gran parte de los residuos producidos por los peces, y que permiten un mayor control de la calidad del agua utilizada así como su tratamiento térmico.

La instalación con un conjunto de equipos electromecánicos cuya función consiste en acondicionar el agua a las necesidades biológicas de los reproductores sobre los volúmenes de recirculación citados con anterioridad. Por una parte la propia recirculación, la filtración, la oxigenación y desinfección, por otra, el acondicionamiento de la temperatura de los tanques a las necesarias al ciclo productivo de los reproductores; y el tratamiento y conservación de la alimentación destinada a los mismos.

Completa el conjunto una pequeña superficie dedicada a salas técnicas y despachos para el personal investigador, de explotación y conservación.

Como se ha mencionado anteriormente, las larvas recién eclosionadas serán transportadas a la Planta de Cultivos que el Centro Oceanográfico de Murcia tiene en Mazarrón, a menos de 1 km. De esta nueva instalación, para el mantenimiento de los reproductores de atún. Dicha Planta de Cultivos está siendo reformada y actualizada para poder acometer con mayor éxito el cultivo de larvas de escómbridos (implantación de tanques de mesocosmos, equipación de sistemas de tratamiento y enfriamiento del agua, etc.). Para completar las obras que se han realizado en este Centro y poder realizar el cultivo larvario y alevinaje de los atunes, se estima necesario mejorar la calidad del agua en dicha instalación y, opcionalmente en el futuro, poder aumentarla, ya que la cantidad de agua que requieren los alevines de atún es elevada.

La utilización de agua de pozo para la captación en la costa mediterránea es una opción ampliamente aceptada por numerosas instalaciones de acuicultura. Entre las numerosas explicaciones que avalan su utilización están:

- Consecución de un agua de gran calidad, prácticamente carente de sólidos en suspensión ya que el agua de mar tiene que pasar a través del lecho de arena hasta llegar al pozo

- Disminución de las oscilaciones térmicas del agua de mar, ya que al pasar a través del fondo marino, el agua atenúa su temperatura, calentándose en invierno y enfriándose en verano, siendo mas patente el primer fenómeno.

- Independencia de las inclemencias climatológicas (temporales, arribazones de algas, etc.) lo que permite mantener un caudal y una calidad estables en la toma de agua.

Descripción funcional

Cada vez son más las instalaciones de acuicultura que se diseñan para funcionar en circuito cerrado, con recirculación de agua. Estos sistemas son efectivos y seguros y permiten un ahorro muy importante en la cantidad de agua nueva necesaria, lo que implica una disminución de los diámetros de las tuberías de captación y vertido y de las electrobombas necesarias. Pero además estos sistemas permiten controlar la Temperatura y calidad del agua, pudiendo blindarse ante cualquier incidencia negativa como contaminaciones puntuales, etc. ya que durante un tiempo limitado se puede prescindir del aporte de agua nueva. A todas estas ventajas hay que añadir un dato de capital importancia en este caso: una desalinizadora perteneciente a Aquamed que se encuentra anexa a los terrenos donde se ubican estas instalaciones, ha ofrecido ceder para uso de esta Planta el agua procedente de unos pozos que realizó a unos 400 metros, cerca de la línea de costa, y cuyo uso descartó debido a su escaso caudal (unos 80 litros/segundo). Además también se ha prestado a ceder parte del agua excedentaria que llega a su cántara de captación; esta cantidad oscilará entre 20 y 70 litros/segundo. La suma de ambas cantidades, que resultan más que suficientes para las necesidades de una instalación como la que se describe en este proyecto de circuito cerrado, resultarían insuficientes para una instalación análoga en circuito abierto.

Así pues, la planta de reproducción de atún en Mazarrón se diseña como una instalación de circuito cerrado con un aporte diario de entre el 25 y el 50% de agua nueva que puede aumentarse en caso de emergencia. La Planta constará de cuatro tanques cilíndricos, de hormigón, denominados respectivamente A, B, C y D con un volumen total de más de 7000 m^3 y cuyo funcionamiento se

integrará en dos sistemas de recirculación independientes y prácticamente iguales en cuanto a dimensiones y caudal a tratar.

El agua nueva será filtrada a 50 micras antes entrar en la Planta, y se añadirá a los dos sistemas de tratamiento, pudiendo mezclarse ambos orígenes a voluntad. Esta agua también llega directamente a los 4 tanques de peces de la instalación para poder usarse si es necesario.

Además del desagüe central que poseen todos los tanques y que conduce el agua por gravedad hacia los sistemas de recirculación, los tres tanques de mayor tamaño poseen también un desagüe supletorio cuya única finalidad es crear una mayor velocidad en el agua dentro del tanque. A tal fin, se instalarán unas bombas que tomarán el agua desde estos desagües, situados en el fondo del tanque y la impulsarán por una tubería que termina en la superficie del tanque, justo debajo de la misma. Esta conducción está permanentemente llena de agua, ya que se encuentra situada por debajo del nivel de agua en el tanque, de tal modo que se las bombas moverán el agua con un consumo energético muy reducido.

A continuación se describen las características de los distintos tanques. En cuanto a dimensiones, se han tenido en cuenta aquellas utilizadas en la reproducción en cautividad de otras especies de atún como el rabil, *Thunnus albacares*, (Wexler *et al*., 2003), el atún rojo del Sur, *Thunnus maccoii, (*Ellis, 2008*)* y el atún rojo del Pacífico*, Thunnus orientalis (*Mimori *et al*., 2006*)* en el Acuario de Tokio, Japón, así como las dimensiones de las jaulas en las que tenemos estabulados los lotes de reproductores que han puesto espontáneamente en cautividad los últimos años en el marco del proyecto SELFDOTT. Estas jaulas tienen unas dimensiones de 25 metros de diámetro y unos 16 metros de profundidad, aunque se ha constatado la reducción de su diámetro en condiciones de fuertes mareas y oleaje así como el hecho de que nadan con mayor frecuencia en los 8-10 metros superficiales.

El proyecto se denominó "*Proyecto de Ejecución EQUIPAMIENTOS DE LA INSTALACIÓN EN TIERRA PARA LA REPRODUCCIÓN DEL ATÚN ROJO EN EL T.M DE CARTAGENA (MURCIA)".* A continuación se describen los tanques y sistemas de tratamiento, con la justificación de su elección. En dos anexos al final de esta tesis se incorporan un anejo de hidráulica, que describe las tuberías dentro de la instalación y las cotas de funcionamiento, un anejo de automatización, que describe los diferentes niveles de control (actuadores y sensores, los controladores y definición del sistema de control) y los planos más significativos.

TANQUES Y TRATAMIENTO DEL AGUA

La Planta constará de cuatro tanques cilíndricos, de hormigón, denominados respectivamente A, B, C y D cuyo funcionamiento se integrará en dos sistemas de recirculación independientes y prácticamente iguales en cuanto a dimensiones y caudal a tratar. El primero de ellos tratará el agua procedente de los tanques A y D, y el segundo tratará a los tanques B y C.

El tanque D funcionará únicamente con peces de pequeño tamaño, y se diseñó teniendo en cuenta que pudiera independizarse del resto de la instalación al objeto de someter a los nuevos individuos a una "cuarentena" al ser introducidos en la planta de cultivos.

Al objeto de optimizar los gastos de bombeo en los tres tanques grandes (A, B y C) y debido a la baja biomasa que se estabulará en los tanques, se ha diseñado dos sistemas hidráulicos diferentes para estos tanques. Esta decisión se basa en el hecho de que no es necesario pasar un elevado caudal de agua a través de los sistemas de tratamiento, pero si es importante mantener una corriente de agua en el tanque es beneficiosa para la natación y orientación de los atunes (referencia). Esta corriente permite disminuir las colisiones contra las paredes del tanque y la tasa de algunas malformaciones (puffy snout). Por tanto, estos tanques presentan dos sistemas de desagüe y de entrada de agua a los tanques:

- Uno de ellos está conectado al desagüe general central del tanque y lleva el agua que sale del tanque por gravedad hasta la zona de tratamiento y desde allí se devuelve después de ser tratada a los tanques por bombeo. La entrada de agua está por encima del nivel de funcionamiento de los tanques. Este sistema se diseñó teniendo en cuenta la biomasa máxima, la temperatura del agua y la máxima cantidad de sólidos en suspensión en cada tanque.
- Otro sistema conectado a otro desagüe situado a un metro del anterior desagüe y que conduce el agua directamente de vuelta a los tanques sin pasar por ningún sistema de tratamiento. La entrada de agua está por debajo del nivel de funcionamiento de los tanques, por lo que el agua no tiene que vencer ningún gradiente de presión sino que fluye por vasos comunicantes. En la conducción se intercala una bomba de hélice que tiene la única finalidad de mover el agua dentro de la tubería para crear una circulación en el tanque.

TANQUE A:

22 metros de diámetro interior. Profundidad: 9.0 m. en las paredes y 9.5 en el centro. Pendiente del fondo: 4.55% Volumen tanque: 3480 m^3

Desagüe por gravedad para filtración biológica: 710 mm de diámetro exterior (655 interior), que proporciona 160 litros/seg.

Desagüe para circulación interior en tanque: 710 mm de diámetro exterior (655 interior), que proporciona 320 litros/seg.

La máxima carga que se mantendrá en el tanque es de 0.7 kg/m^3, lo que implica una biomasa de 2435 kg. Suponiendo un peso medio de unos 80 kg en cada reproductor, podrían estabularse alrededor de 30 atunes en el tanque. Si se estima que las necesidades de oxígeno de un atún adulto están sobre 1 gramos de oxígeno por kg de pez y hora, las necesidades serán del orden de 2.44 kg/hora

Suponiendo una tasa de alimentación del orden del 3 % al día, la máxima cantidad de pescado que se añade a este tanque será de 73 kg al día. Así la cantidad de nitrógeno amoniacal total disuelto excretado estará en torno a 1.7 kg de nitrógeno amoniacal total. Suponiendo una tasa de nitrificación de 0.25 gr/m^2, serán necesarios 6800 m^2. Si se utiliza un lecho fluido, se puede utilizar material plástico con una superficie específica superior a 750 m^2/m^3, con lo que el volumen de biocarrier o biobolas necesarias serán de 9 m^3

TANQUE B:

20 metros de diámetro interior. Profundidad: 8.0 m. en las paredes y 8.5 en el centro. Pendiente del fondo: 5.0% Volumen tanque: 2565 m^3

Desagüe por gravedad para filtración biológica: 560 mm de diámetro exterior (517 interior), que proporciona 120 litros/seg.

Desagüe para circulación interior en tanque: 710 mm de diámetro exterior (655 interior), que proporciona 320 litros/seg.

La máxima carga que se mantendrá en el tanque es de 0.7 kg/m3, lo que implica una biomasa de 1800 kg. Suponiendo un peso medio de unos 80 kg en cada reproductor, podrían estabularse entre 22 y 24 atunes. Si se estima que las necesidades de oxígeno de un atún adulto están sobre 1 g de oxígeno por kg de pez y hora, las necesidades serán del orden de 1.8 kg/hora.

Suponiendo una tasa de alimentación del orden del 3% al día, la máxima cantidad de pescado que se añade a este tanque será de 54 kg al día. Así la

cantidad de nitrógeno disuelto excretado estará en torno a 1.3 kg de nitrógeno amoniacal total. Suponiendo una tasa de nitrificación de 0.25 gr/m^2, serán necesarios 5200 m^2. Si se utiliza un lecho fluido, se puede utilizar material plástico con una superficie específica superior a 750 m^2/m^3, con lo que el volumen de biobolas necesarias serán de 6.9 m^3

TANQUE C:

14 metros de diámetro interior. Profundidad: 5.7 m. en las paredes y 6.0 en el centro. Pendiente: 5.0% Volumen tanque: 892 m^3

Desagüe por gravedad para filtración biológica: 450 mm de diámetro exterior (415 interior), que proporciona 80 litros/s.

Desagüe para circulación interior en tanque: 710 mm de diámetro exterior (655 interior), que proporciona 160 litros/seg.

La máxima carga que se mantendrá en el tanque es de 1.2 kg/m^3, lo que implica una biomasa de 1070 kg. Este tanque albergará atunes creciendo que todavía no han alcanzado la edad reproductora, en torno a los 15 kg de peso medio, con lo que este tanque podría mantener entre 70 y 80 atunes. Si se estima que las necesidades de oxígeno de un atún juvenil están sobre 1.2 gramos de oxígeno por kg de pez y hora, las necesidades serán del orden de 1.29 kg/hora.

Suponiendo una tasa de alimentación del orden del 4% al día, la máxima cantidad de pescado que se añade a este tanque será de 43 kg al día. Así la cantidad de nitrógeno disuelto excretado estará en torno a 1.0 kg de nitrógeno amoniacal total. Suponiendo una tasa de nitrificación de 0.25 gr/m^2, serán necesarios 4000 m^2. Si utilizo un lecho fluido, se puede utilizar material plástico con una superficie específica superior a 750 m^2/m^3, con lo que el volumen de biobolas necesario será de 5.3 m^3

TANQUE D:

8 metros de diámetro interior. Profundidad: 2.8 m. en las paredes y 3.0 en el centro. Pendiente: 4.3% Volumen tanque: 144 m^3

Desagüe por gravedad: 315 mm de diámetro exterior (290 interior), que proporciona 40 litros/seg.

La máxima carga que se mantendrá en el tanque es de 2.0 kg/m^3, lo que implica una biomasa de 288 kg. Este tanque albergará juveniles de primer año,

con un peso medio de 1-2 kg., con lo que podremos mantener entre 150 y 300 atunes. Si se estima que las necesidades de oxígeno de un atún 0+ (de menos de un año de vida) están sobre 1.5 gramos de oxígeno por kg de pez y hora, las necesidades serán del orden de 0.43 kg/hora

Suponiendo una tasa de alimentación del orden del 8% al día, la máxima cantidad de pescado que se añade a este tanque será de 23 kg al día. Así la cantidad de nitrógeno disuelto excretado estará en torno a 0.7 kg de nitrógeno amoniacal total. Suponiendo una tasa de nitrificación de 0.25 gr/m^2, se necesitarán 2200 m^2. Si se utiliza un lecho fluido, se puede utilizar material plástico con una superficie específica superior a 750 m^2/m^3, con lo que el volumen de biobolas necesario será de 3.0 m^3

TRATAMIENTO DEL AGUA

Agua nueva

La instalación de atunes tiene capacidad para tratar un máximo de 40 litros/segundo procedente de la poceta de captación de la desaladora o de los pozos –máximo de 80 litros/segundo-, caudal superior al máximo que se necesitaría de tener los dos sistemas de recirculación con el máximo aporte de agua nueva (20 litros/segundo a cada sistema) y el tanque D funcionando en circuito abierto (25 litros/segundo). No obstante, las bombas que impulsan el agua nueva hasta la Planta están dimensionadas para poder aumentar cada uno de los dos aportes hasta los 60 litros/segundo si fuera necesario.

Sistemas de recirculación

Existen dos sistemas de recirculación, cada uno de los cuales consta en línea de: filtro de tambor, filtro biológico, depósito regulador de 180 m^3 de capacidad, bombeo de vuelta a los tanques y tratamiento UVA. Una parte del agua será oxigenada antes de volver a los tanques de cultivo de peces.

Además, del depósito de regulación surgen dos bucles que devuelven el agua al mismo tanque: uno de ellos tratará térmicamente el agua (la calentará o enfriará para mantener la temperatura deseada) y el otro realizará un tratamiento del agua consistente en hacerla pasar a través de un espumador e inyectarle ozono. Cada uno de estos 2 bucles está dotado de su sistema de bombeo

Sistema I:

Tratará el agua de los tanques A y D, es decir, un volumen total de 3624 m^3. Se pretenden recircular 200 litros/segundo, esto es, 720 m^3/h. Esto supone alrededor de 4.77 movimientos del agua a través del filtro biológico a lo largo del día.

El aporte de agua nueva a este sistema oscilará normalmente entre un 4.2 y 8.4 litros/segundo, lo que significa entre un 10 y un 20% de agua nueva al día. Pero en caso de necesidad puede aumentarse hasta un máximo de 20 litros/segundo (1728 m^3/día), lo que supone un 48% de renovación diaria.

La máxima carga es de 0.7 kg/m3 en el tanque A y 2.0 kg/m^3 en el tanque D, lo que significa una biomasa máxima de 2725 kg de atún.

Las necesidades de oxígeno serán en los tanques son de 2.4 kg/hora para el tanque A y 0.4 kg/hora para el tanque D. Además, para paliar las necesidades de oxígeno del filtro biológico, Wheaton (1971) estima en 4.57 gr de oxígeno la cantidad necesaria para oxidar 1 gr de N amoniacal. Como entre los tanques tanques A y D producirán 2,4 kg de N amoniacal, se requerirán 10.8 kg de oxígeno al día, es decir, unos 0,45 kg a la hora, por lo que se preveerá un aumento ligeramente superior, de un 20% de los requerimientos de oxígeno, que entonces serán de 3.3 kg/hora, es decir, alrededor de 29 Tm. O_2 al año. Además, el oxígeno que proviene del aire que se inyectará con el soplante, supone un aporte de oxígeno extra.

Calentamiento y enfriamiento: Será necesario aportar al agua un máximo de 100 Kw. de frío y no es necesario calentar.

Filtro biológico: 28 m^3 de capacidad y sobre 12 m^3 de material filtrante (ocupación del volumen por el sustrato del 40%%, aunque con los modernos materiales de densidad próxima a 1, estas ocupaciones pueden ser mayores al 50%)

Skimmer y ozono: La máquina de ozono debe de producir del orden 200 g de oxígeno a la hora (al 7% en peso/volumen) o una cantidad equivalente a otras concentraciones (p.e. 225 g de ozono al 6% a la hora). El caudal de oxígeno requerido será del orden de 2 Nm^3 a la hora.

El sistema pasará a través del skimmer un mínimo de 360 m^3/hora con un tiempo de retención mínimo de 1.5 minutos. Esto supone que en caso de necesidad el sistema podría aumentar el caudal de agua tratada hasta 560 m^3/h disminuyendo el tiempo de retención a 1 minuto. El caudal de gas aspirado debe ser superior a los 50 Nm3/h. para asegurar un adecuado funcionamiento del sistema

El depósito de regulación tiene unas dimensiones de 180 m^3. Esto supone que este depósito se renueva totalmente cada 18 minutos. El sistema podría aceptar pérdidas de hasta 60 m^3 (un tercio del volumen total) sin que se vean afectados los niveles normales de funcionamiento, pérdidas muy superiores a las estimadas durante la parada y arranque del sistema hasta llegar a su equilibrio dinámico.

Este sistema está preparado para poder independizar ambos tanques si es necesario. Esto ocurrirá en el momento de introducir peces nuevos en la Planta. Los atunes nuevos (juveniles de primer año) se introducirán al tanque D, funcionando este tanque como si un tanque de cuarentena se tratara, evitando mezclar el agua de dichos peces con el resto de la instalación. En este caso, el tanque D funcionará en circuito abierto, con un caudal máximo de 25 litros/segundo.

Sistema II:

Tratará el agua de los tanques B y C, es decir, un volumen total de 3457 m^3, y mueve 200 litros/segundo, esto es, 720 m^3/h. Esto supone alrededor de 5 movimientos del agua a través del filtro biológico a lo largo del día.

El aporte de agua nueva a este sistema oscilará normalmente entre un 4.0 y 8.0 litros/segundo, lo que significa entre un 10 y un 20% de agua nueva al día. Pero en caso de necesidad puede aumentarse hasta un máximo de 20 litros/segundo (1728 m^3/día), lo que supone un 50% de renovación al día.

La máxima carga es de 0.7 kg/m^3 en el tanque B y 1.2 kg/m^3 en el tanque C, esto significa una biomasa máxima de 2865 kg de atún.

Las necesidades de oxígeno serán en los tanques son de 1.8 kg/hora para el tanque A y 1.1 kg/hora para el tanque D. Además, para paliar las necesidades de oxígeno del filtro biológico, Wheaton (1971) estima en 4.57 gr de oxígeno la cantidad necesaria para oxidar 1 gr de N amoniacal. Como entre los tanques tanques A y D producirán 2,3 kg de N amoniacal, se requerirán 10.5 kg de oxígeno al día, es decir, unos 0,44 kg a la hora, por lo que se preveerá un aumento ligeramente superior, de un 20% de los requerimientos de oxígeno, que entonces serán de 3.5 kg/hora, es decir, alrededor de 31 Tm. O_2 al año. Además, el oxígeno que proviene del aire que se inyectará con el soplante, supone un aporte de oxígeno extra.

Calentamiento y enfriamiento: Es necesario aportar al agua un máximo de 80 Kw. de frío y 14 Kw. de calor.

Filtro biológico: 28 m^3 de capacidad y 12 m^3 de material filtrante (ocupación del volumen por el sustrato del 40).

Skimmer y ozono: La máquina de ozono debe de producir del orden 200 gramos de oxígeno a la hora (al 7% en peso/volumen) o una cantidad equivalente a otras concentraciones (p.e. 225 gramos de ozono al 6% a la hora). El caudal de oxígeno requerido será del orden de 2 Nm^3 a la hora.

El sistema pasará a través del skimmer un mínimo de 360 m^3/h con un tiempo de retención mínimo de 1.5 minutos. Esto supone que en caso de necesidad el sistema podría aumentar el caudal de agua tratada hasta 560 m^3/h disminuyendo el tiempo de retención a 1 minuto. El caudal de gas aspirado debe ser superior a los 50 Nm^3/h. para asegurar un adecuado funcionamiento del sistema

El depósito de regulación tiene unas dimensiones de 180 m^3. Esto supone que este depósito se renueva totalmente cada 18 minutos. El sistema podría aceptar pérdidas de hasta 60 m^3 (un tercio del volumen total) sin que se vean afectados los niveles normales de funcionamiento, pérdidas muy superiores a las estimadas durante la parada y arranque del sistema hasta llegar a su equilibrio.

Equipamiento sistemas tratamiento

1. Filtro mecánico

El sistema de filtración del agua recirculada es un sistema que consiste en un tambor giratorio de malla que filtra todo el agua procedente de los tanques de peces antes de llegar a los tanques de filtración biológica y a los depósitos de regulación.

Este filtro está diseñado y construido para separar sólidos suspendidos que se encuentran presentes en el agua, entrando esta sin presión, en forma de columna de agua de entre 10 y 15 cm de altura como máximo. Para el dimensionamiento de estos filtros se ha tenido en cuenta que se trata de una instalación de recirculación con una baja cantidad de sólidos en suspensión, debido fundamentalmente a la baja cantidad de alimento aportada y a la gran calidad del agua nueva aportada. Existen dos tambores, uno por sistema que están preparados para filtrar unos 450 m^3/hora si se colocan con una malla de 60 micras o hasta 630 m^3/hora si se disponen con malla de 90 micras.

Es recomendable que la velocidad de entrada del agua sea menor a 1 m/s, con el fin de disminuir la turbulencia en el interior del tambor. La máxima diferencia de nivel de agua entre el interior y el exterior del tambor es de 30 cm durante

una operación continua. Se recomienda que la diferencia entre los niveles interior y exterior sea como máximo, 20 cm. El mantenimiento de estos niveles, así como los niveles mínimos de funcionamiento, en el caso que nos ocupa, se realiza a través de vertederos.

Además de este sistema de filtración mecánica del agua de recirculación, existen otros dos sistemas que filtran el agua nueva que entra en la planta procedente de la toma de la desalinizadora o de los pozos. Son dos sistemas consistentes en filtros de anillas que tienen capacidad para filtrar el agua hasta 55 micras. Unos de los sistemas tratará el agua procedente de los pozos y el otro el agua procedente de la desalinizadora. Cada uno de ellos tiene una capacidad de filtración de 40 litros/segundo y consta de una batería de 4 filtros de anillas autolimpiables mediante un sistema de baja presión. Los filtros son de polipropileno. Una sonda de presión instalada en los filtros determinará su grado de suciedad al objeto de proceder a su limpieza automática cuando sea requerido.

2. *Filtro biológico*

Se ha diseñado un sistema de filtración biológica del tipo de lecho fluido, que se basa en mantener en suspensión en el filtro un material biológico de elevada relación superficie/volumen (*biocarrier*) que serán los portadores de las bacterias *Nitrosomonas* y *Nitrobacter* que son las responsables de convertir el amoniaco en nitritos y éste en nitratos.

Estos dos procesos requieren oxígeno, por lo que es importante su inyección, bien sea en forma de oxígeno puro bien en forma de aire.

El compuesto que se forma, nitrato, puede ser eliminado y convertido en nitrógeno gas en un proceso que debe realizarse en ausencia de oxígeno, lo que requeriría otro compartimento apartado de la zona donde se realizaban los procesos antes mencionados. No obstante, los peces son resistentes a concentraciones relativamente elevadas de nitrato, por lo que una renovación como la que se realizará en nuestro caso (20-50% al día) mantendrá la concentración de nitrato en valores inferiores a aquellos considerados como tóxicos para los peces, no siendo pues necesario este segundo compartimento.

Sin profundizar en el tema diremos que en el sustrato (bolas de polietileno de diámetro determinado) se desarrolla una colonia de bacterias aeróbicas. Es decir bacterias que necesitan oxígeno para su proceso vital. El oxígeno es aportado permanentemente por el agua que circula a través del lecho. Dentro de ese conjunto de bacterias aeróbicas hay dos o tres especies que son de fundamental importancia en el proceso de transformar esos desechos

orgánicos en nitratos. El movimiento del agua a través del lecho filtrante se consigue gracias al aire introducido por unos sopladores situados en la parte baja del filtro.

El sustrato del filtro biológico consiste en Biobolas de PEHD con una superficie específica de 884 m^2/m^3, peso 165 kg/m^3, diámetro de 9-11,5 mm y una densidad de 0,95 gr/cm^3, con lo que cada uno de los dos sistemas necesita del orden de 10 m^3 de material filtrante, que suponiendo que ocupe un 40% del total del tanque, requerirán ser ubicados en un filtro biológico de unos 25 m^3 de capacidad.

En el fondo existe un sistema de aireación tipo emparrillado conectado a un soplante de aire de 7.5 Kw capaz de aportar un máximo caudal de 250 m3/h a 4.5 metros de altura a cada tanque y que mantiene en suspensión todo el material filtrante.

3. Tratamiento de climatización

El sistema de enfriamiento consta de una bomba que coge agua del tanque de tratamiento y la hace pasar a través de un intercambiador, volviendo al mismo tanque. El sistema contará con una regulación térmica de tal manera que se pare o arranque la bomba de agua o la de calor según el caso y la temperatura deseada.

Para el sistema I, sería necesario aportar al agua un máximo de 100 Kw. de frío sin ser necesario calentar (según un estudio realizado por especialistas teniendo en cuenta las características del terreno y de la nave). Para el sistema II, sería necesario aportar un máximo de 80 Kw. de frío y 14 Kw. de calor. Esta pequeña diferencia se debe a que se pretende poder variar ligeramente la temperatura de este sistema con respecto al otro, al objeto de poder manipular la época de puesta del tanque B, adelantándola dos o tres meses con respecto a la época natural de puesta.

Cualquiera de los dos sistemas puede aportar el calor o frio necesario a un caudal de 30 m^3/h que será el que circule a través de los intercambiadores.

El intercambiador consta de un sistema primario y uno secundario. El primario está conectado con un equipo de producción de agua fría o caliente por condensación de aire (bomba de calor), un grupo motobomba y un depósito de inercia que se continúa hasta el intercambiador constituyendo el circuito primario propiamente dicho. El circuito secundario coge el agua de mar del tanque de tratamiento, la pasa por el intercambiador y la devuelve al tanque.

El sistema constará de una regulación térmica de tal manera que funciona parando o accionando la bomba de agua del circuito secundario y la bomba de calor. Una sonda situada en el interior del depósito regulador y que mide con una precisión de décima de grado es la que manda las bombas. Su regulación hace que cuando la temperatura baje o suba 0.3ºC de la temperatura de consigna, el equipo se ponga en marcha para calentar o enfriar el agua, según se les indique. Al mismo tiempo, la bomba del circuito secundario funciona para pasar agua de mar a través del intercambiador.

4. Sistema de esterilización por ultravioleta

Consistirá en lámparas de radiación ultravioleta protegidas por una funda de cuarzo y que van insertadas dentro de una cámara de polietileno de alta densidad por la que circula el agua que será tratada.

Habrá 4 sistemas, uno para cada tanque, y dimensionados para poder tratar todo el agua que pasa a través del sistema de recirculación antes de llegar de vuelta a los tanques (a saber, 1 equipo de 540 m^3/h, otro de 480, otro de 120 y otro de 60). Estos sistemas asegurarán una radiación mínima de 32 mJ/cm^2 al final de la vida media de las lámparas en toda la columna de agua que pasa a través de ellos siempre y cuando la transmitancia de la misma sea superior al 90%. Esta radiación es capaz de matar a la gran mayoría de las bacterias y muchos de los protozoos que pueden ocasionar problemas en los peces marinos. La lámpara que se sitúa en la entrada al tanque D que contiene los atunes de menor tamaño, asegurará un mínimo de 56 mJ/cm^2

Todos los sistemas irán dotados de medidores de la intensidad de radiación y de limpiadores manuales para las fundas de cuarzo.

5. Sistema de oxigenación

Existe un sistema para cada uno de los cuatro tanques. El sistema de oxígeno consta de una bomba de presión y de un ventury. Después de ser tratada con radiación ultravioleta, se coloca un by-pass, de tal modo que una parte del agua que retorna a los tanques de cultivo es forzada a pasar a través del *ventury* donde se producirá la inyección del oxígeno. El agua oxigenada se unirá de nuevo con la tubería que conduce el agua de vuelta desde tratamiento hasta los tanques de peces. La regulación del oxígeno se realiza mediante un sistema que puede ser manual o automático. Una sonda colocada en el tanque, cerca de la entrada de agua, indicará cual es el nivel de oxígeno en los tanques y determinará cuanto oxígeno debe ser inyectado en el *ventury*. El valor que debe tener el oxígeno a la entrada del tanque se determinará empíricamente,

de tal modo que el agua en el tanque se mantenga en niveles próximos a la saturación.

Una segunda sonda colocada en la salida de los tanques de peces determinará si los niveles de oxígeno se mantienen en los valores adecuados en el tanque y en caso necesario (concentración de oxígeno inferior al 90% de saturación) aportará directamente oxígeno al tanque mediante unas mangueras difusoras. Este sistema se descarta por poco eficiente en las instalaciones normales de acuicultura, pero su eficacia aumenta con la profundidad del tanque, y a partir de 4 metros de columna de agua puede ser colocado con buenos resultados.

6. Sistema de ozonización y espumación

Los *skimmer* o espumadores se emplean para eliminar materia orgánica disuelta y pequeñas partículas en suspensión mediante la producción de espumas.

La descomposición de los restos orgánicos procedentes de la alimentación liberan al medio compuestos nitrogenados (amonio), fosfato orgánico y otros compuestos no deseados como las gilvinas o los silicatos que enturbian, colorean y perjudican la transparencia e equilibrio del medio.

Básicamente su funcionamiento es el siguiente: se introduce aire en el cuerpo del reactor en forma de microburbujas. Una bomba de movimiento dotada de un rotor “multipala” se encarga de romper el aire tomado a través del sistema venturi instalado a la entrada de la bomba. El resultado es la emisión al cilindro que forma el cuerpo del skimmer de millones de microburbujas en forma de turbulencia que ascienden cargadas de electricidad hacia el punto más alto. Por la diferencia de cargas positivas/negativas las burbujas atraen hacia su superficie a muchas de las partículas orgánicas, y el oxígeno del aire oxida y coagula la materia orgánica disuelta. El material solidificado va formando una capa de espuma en la superficie que se traslada a un recipiente que se limpia periódicamente.

Respecto al skimmer, el equipo se compone de los siguientes elementos de material plástico: Tanque para mezcla y reacción, sistema de inyección de aire, vaso de precipitado de espumas y sistema de aclarado.

El ozono es un potente agente oxidante y por ende desinfectante que tiene variados beneficios para la industria acuícola, dado que oxida fácilmente los contaminantes presentes en el agua usada por esta industria, como así también, los contaminantes generados por ésta

El ozono aplicado en la industria de la acuicultura presenta una amplia serie de ventajas, dentro de las cuales cabe mencionar:

- Elimina los elementos sólidos de tipo orgánico disueltos en el medio acuoso: estos elementos se tienden a acumular y la acción del ozono permite que se disuelvan en el agua haciéndolos biodegradables. Al disolverlos, se recobra el aspecto transparente del agua, puesto que su acumulación provoca un aspecto turbio, esta situación por lo general produce estrés en los peces o cultivos de mala calidad debido a la obstrucción de los biofiltros. Estos sucesos se pueden prevenir con la aplicación de ozono.

- Ejerce una acción bactericida además de eliminar gran cantidad de virus: la aplicación del ozono es capaz de eliminar una gran cantidad de microorganismos y sus esporas lo que permitirá una producción óptima en estos medios.

- Produce una disminución de nitritos: Permite la oxidación directa de nitritos a nitratos, regulando la cantidad existente en el agua, puesto que a altas cantidades el nitrito resulta tóxico para los peces, permitiendo todo ello la mejora de la estancia de los animales y consiguiendo una más eficiente biofiltración y nitrificación.

- Produce micro floculación de materia orgánica: ayuda a que se aglutinen las sustancias coloidales presentes en el agua, facilitando la decantación y filtrado posterior. Con este paso se procede a una mejor potabilización de aguas ayudando a la productividad de las mismas.

- Propicia el incremento de oxígeno en el agua: Debido a su corta vida útil, el ozono se descompone y pasa a formar de nuevo átomos de oxígeno permitiendo la oxigenación del medio acuoso con las consecuentes ventajas para los cultivos contenidos.

- El ozono elimina la materia orgánica coloidal: son pequeñas partículas (de 1 a 30 micras) que permanecen en suspensión y resultan difícilmente eliminadas por sistemas convencionales mecánicos Estos compuestos dan al agua un color turbio, no son biodegradables y son acumulativos, limitando la eficiencia de la nitrificación del biofiltro.

El ozono se produce cuando las moléculas de oxígeno (O_2) son disociadas por medio de una fuente de energía produciendo átomos de oxígeno que posteriormente chocan con una molécula de oxígeno para formar un gas inestable, el ozono (O_3). Para mejorar su efectividad se aconseja introducirlo en el sistema a través del *venturi* de entrada al *skimmer*, donde su presencia en concentraciones elevadas hace que actúa como elemento esterilizador. Pero

se debe controlar periódicamente el ozono residual a la salida del *skimmer* para que no sea peligroso cuando el agua retorne a los tanques de peces.

En el caso que nos ocupa, se instalan dos equipos de *skimmer* y dos de ozono, uno para cada sistema. Cada *skimmer* recircula unos 360 m^3/h y además le aporta ozono a dicho caudal. Cada generador de ozono se proyecta para producir unos 200 g/h, lo que equivale a un máximo de 0,56 ppm O_3 (dosis habitual para este tipo de instalaciones dde baja densidad). Si hacemos el cálculo en función del alimento aportado, supondrá un aporte máximo de alrededor de 40 g de ozono por kg de alimento aportado.

La regulación de estas concentraciones es dificultosa debido a la corta vida media de la molécula de ozono. En acuicultura se utiliza la medición del potencial redox, esto es el potencial de oxidación-reducción. Valores del orden de 300-350 Mvoltios se consideran óptimos para el agua de mar y no afectan la viabilidad de los peces.

La producción de ozono está controlada por una sonda de potencial redox que existe en la salida del espumador y que modula la electroválvula que controla la entrada de oxígeno al ozonizador, y que puede apagar la máquina de producción de ozono si el potencial redox pasa de un límite. Una segunda sonda de seguridad situada a la salida de cada uno de los tanques reguladores hacia los tanques de peces, corta la bomba de recirculación que impulsa el agua desde el tanque de regulación hacia el tanque de peces si pasa de otro nivel. Esta redundancia en las medidas de seguridad se explica porque el ozono puede resultar tóxico para los peces, lo que nos obliga a ser extremadamente cautelosos en su uso.

La ubicación del sistema de ozono será exterior para disminuir el posible peligro de fugas de ozono, No obstante, el equipo estará dotado de un sensor de fugas de ozono.

Como anexos al final de esta tesis se incluyen los planos mas representativos de esta obra, así como los anejos de hidráulica y automatización.

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CONCLUSIONES

1. El Bonito atlántico, *Sarda sarda*, es capaz de adaptarse a la cautividad y madurar sus gónadas, realizando puestas espontáneas prolongadas en el tiempo.
2. Las larvas y juveniles de bonito son capaces de crecer en condiciones de cultivo y reproducirse en menos de un año de vida, por lo que se ha conseguido cerrar su ciclo vital en cautividad.
3. El atún rojo del Atlántico, *Thunnus thynnus*, es capaz de madurar y poner espontáneamente en jaulas de cultivo ubicadas en el mar. Si las condiciones son adecuadas, dichas puestas se prolongan durante algo más de un mes al año y pueden recolectarse con sistemas de lonas y otros sistemas diseñados al respecto.
4. Los huevos de atún rojo y bonito tiene una elevada relación DHA/EPA, especialmente en el caso del atún rojo que contienen un 40% mas de DHA que el bonito.
5. A lo largo de su fase de alimentación endógena se produce una disminución de la cantidad de lípidos, especialmente lípidos neutros.
6. La relación DHA/EPA aumenta a lo largo de la fase de alimentación endógena en ambas especies, lo que puede deberse a una retención selectiva del DHA.
7. La utilización de nauplios de *Acartia tonsa* mejora significativamente los resultados del cultivo larvario en términos de crecimiento y supervivencia, observándose parte de estos beneficios con una alimentación mixta de ambas presas.
8. Las larvas alimentadas con *Acartia tonsa* presentan un mayor contenido en TAG y un menor contenido en PUFAs, aunque la cantidad de DHA es mayor y la relación DHA/EPA prácticamente el doble.
9. Una transición temprana a la piscivoría beneficia la supervivencia e incrementa el crecimiento durante los estadios larvarios de ambas especies.
10. Los huevos de atún rojo y bonito atlántico son bastante euritermos. Los valores considerados adecuados para su desarrollo se encuentran entre los 21 y 28ºC en atún y 17 y 25ºC en bonito.
11. Los avances en la tecnología de cultivo del atún rojo, junto con la puesta en marcha de una instalación para el control de su reproducción en cautividad harán posible el desarrollo de la acuicultura sostenible de esta especie a corto plazo.

ANEJO 5: AUTOMATIZACIÓN

EQUIPAMIENTOS DE LA INSTALACIÓN EN TIERRA PARA LA REPRODUCCIÓN DEL ATÚN ROJO EN EL T.M DE CARTAGENA (MURCIA)

PROMOTOR

INSTITUTO ESPAÑOL DE OCEANOGRAFÍA (IEO)

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Por Tragsa: D. Félix Díaz de Rada Santos

MAYO 2012

ÍNDICE

1. OBJETO Y ALCANCE

En este documento se pretende establecer los principios de la automatización de la planta de reproducción de Atún Rojo a ejecutar en Cartagena.

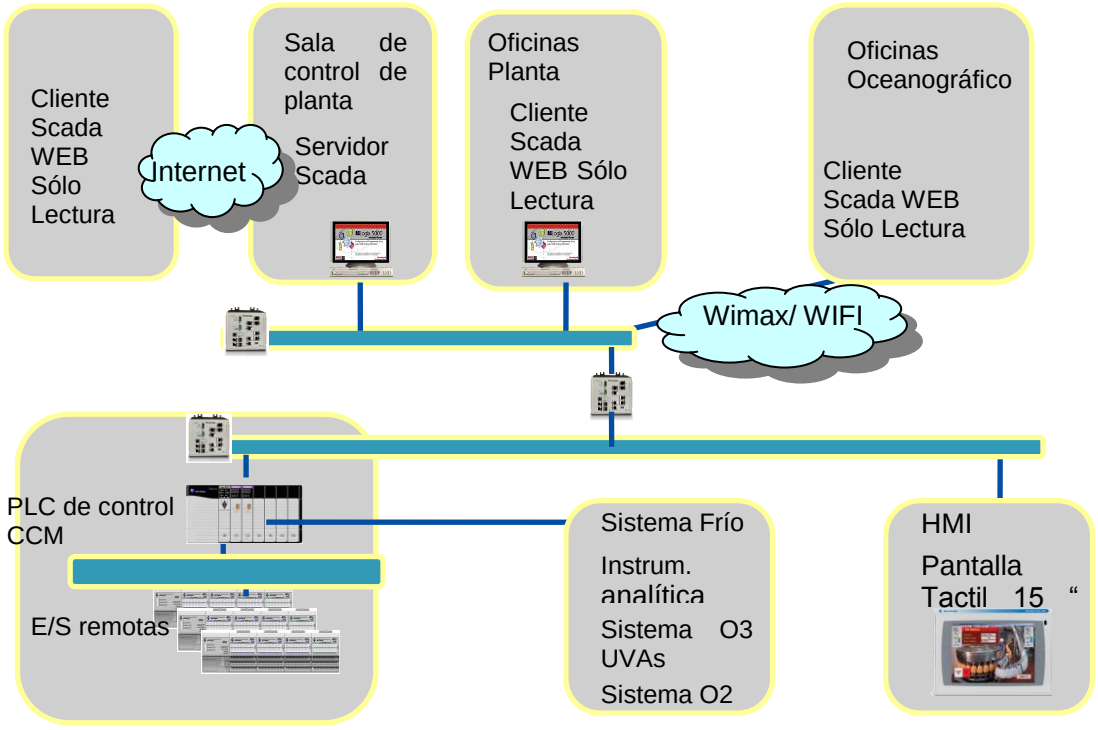
La capacidad de ejecución está dimensionada de acuerdo a la siguiente cantidad de equipos:

Resumen equipos principales	Cantidad
Grupo electrógeno	1
Batería de condensadores	1
Bombeos	16
Agrupaciones de válvulas	2
Cuadros de subprocesos independientes	15
SAI	2
Grupos motobomba con arranque directo	4
Grupos motobomba con arranque mediante variador de frecuencia	16
Grupos motobomba con arranque mediante arrancador electrónico	14
Válvulas motorizadas	13
Instrumentos analógicos	49
Plc	1
Servidor Scada	1
Clientes Scada WEB	3
Controladores de instrumentación	6

2. ARQUITECTURA DE CONTROL

A la hora de definir la arquitectura de control, se analizan los diferentes niveles de control comenzando desde el nivel de los actuadores y sensores, se sigue con el nivel de los controladores y se termina con la definición del sistema de control.

El esquema de control de las instalaciones es el siguiente:

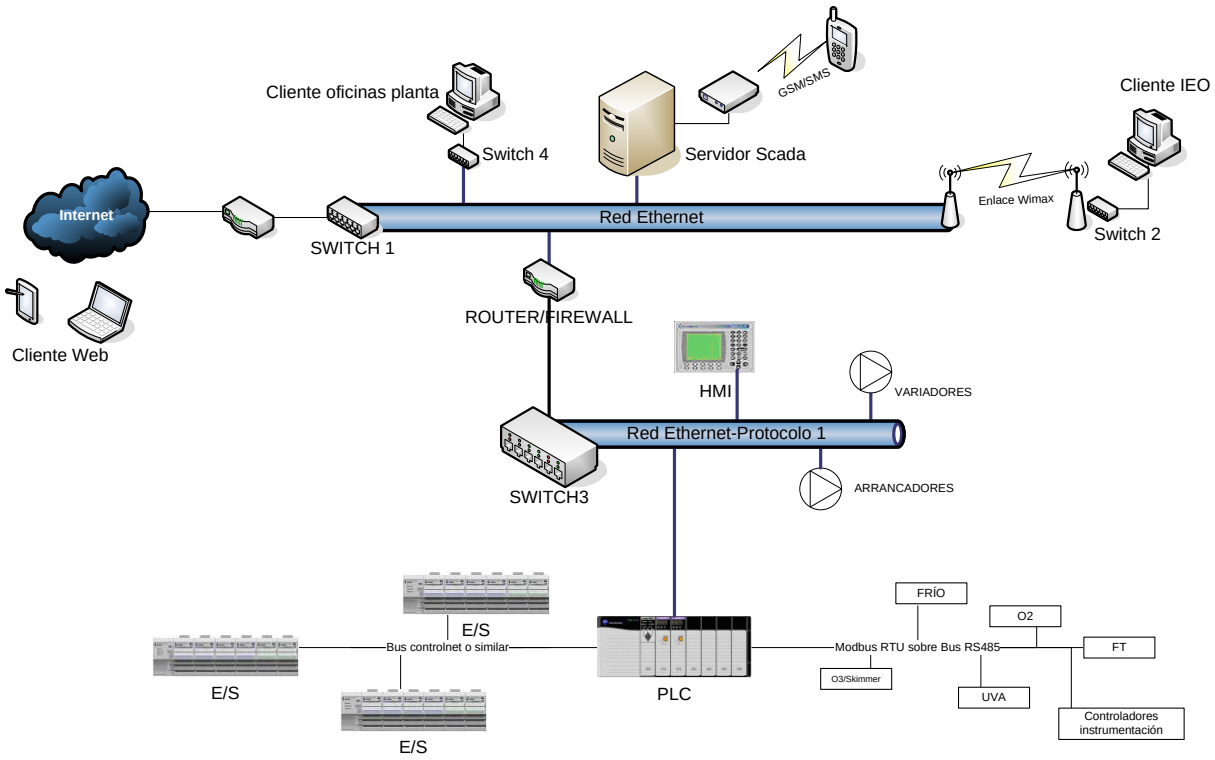


Se instalará un PLC con capacidad suficiente para el control y gestión completa de la planta, de tal forma que recogerá todas las señales existentes y que se corresponden con todos los actuadores y sensores instalados. A su vez, el PLC realizará el control local de todos los procesos y subprocesos que se plantean. Algunos subprocesos se ejecutan de forma autónoma, pues son propios de soluciones particulares y cerradas (UVA, Oxígeno, Ozono, Frío, etc.), no obstante cada una de estas soluciones materializadas en algunos casos mediante controladores propios, deberán mantener al PLC central informado, al menos del estado del subproceso, y la aceptación de una orden de paro y otra de marcha.

Como se aprecia en el esquema, se mantendrán dos redes diferenciadas por su posibilidad de acceso a Internet que pondrán en comunicación los elementos de nivel de gestión y dos bus de campo industriales suficientemente soportados por el autómata con los módulo de E/S y los diferentes sistemas particulares de subprocesos de tratamiento.

2.1. Arquitectura de comunicaciones

Por otra parte y al respecto de la arquitectura del sistema ya planteada, el PLC deberá establecer comunicaciones con diferentes sistemas, a través de los elementos intermedios necesarios, a saber:



Para ello será necesario establecer las diferentes redes en base a los puntos de conexión establecidos, de esta forma:

Punto conexión	Nivel Físico	Nivel Enlace	Protocolo
Cliente oficinas Planta	Par trenzado	Ethernet	Ethernet
Servidor Scada	Par trenzado	Ethernet	Ethernet
Cliente IEO	Par trenzado (WIMAX transparente)	Ethernet	Ethernet
HMI	Par trenzado	Ethernet	Protocolo1*
Variadores	Par trenzado	Ethernet	Protocolo1
Arrancadores	Par trenzado	Ethernet	Protocolo1
PLC	Par trenzado	Ethernet	Protocolo1
Entradas y Salidas	Par trenzado	Controlnet o similar	Controlnet o similar
Controladores de instrumentación	Par trenzado	RS 485	Modbus RTU
Sistema UVA	Par trenzado	RS 485	Modbus RTU
Sistema Skimmer	Par trenzado	RS 485	Modbus RTU
Sistema Frío	Par trenzado	RS 485	Modbus RTU
Sistema oxígeno	Par trenzado	RS 486	Modbus RTU

*El protocolo 1 ha de ser un protocolo sobre Ethernet que independice las redes.

3. MODOS DE FUNCIONAMIENTO

El manejo del sistema se podrá realizar a través de los siguientes modos de funcionamiento distribuidos en dos niveles, que estarán reproducidos a través de los correspondientes conmutadores en la puerta de los armarios. Estos conmutadores serán de dos tipos, el del nivel 1 será el conmutador de manual-0-automático y el del nivel 2 el conmutador de local-remoto.

A su vez, el conmutador de nivel 1 se aplicará a cada actuador (grupo motobomba o válvula motorizada) por separado y el de nivel 2 se aplicará al conjunto de actuadores que precisen de un funcionamiento coordinado, generalmente respondiendo a un mimo proceso como el de bombeo, de tal forma que siempre que esté en local o en remoto, se considerará que se aplica a todos los equipos que estén en modos de funcionamiento en automático.

-

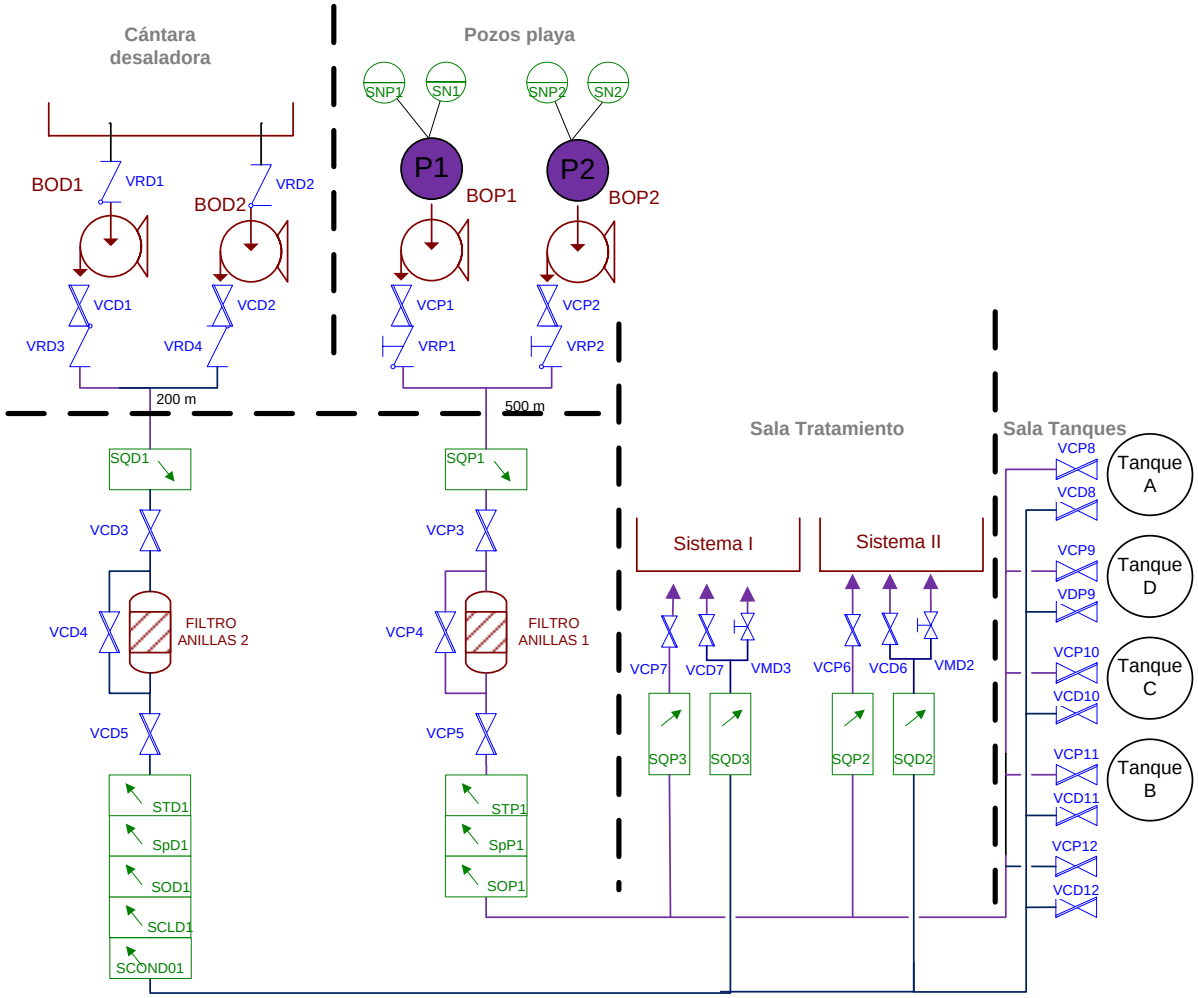
A su vez, en los casos en que el circuito de arranque esté formado por un variador de frecuencia, será posible modificar la frecuencia del variador a través de la acción correspondiente sobre la HMI y que debe proporcionar las funciones de corrección de consigna de frecuencia del variador. Esta función será posible mientras esté dispuesto el selector del grupo correspondiente en automático y local, mientras que si se posiciona en remoto, se hará desde el Scada. En el caso de una acción totalmente manual será necesario actuar sobre el menú propio del variador de frecuencia y modificar los parámetros que se desee.

4. LINEAS DE AGUA

4.1. LÍNEA1: ENTRADA DE AGUA AL SISTEMA

La línea de agua correspondiente a la entrada al sistema incluye las tuberías procedentes de los pozos situados en la playa y las procedentes de las bombas situadas en la cántara de la desaladora, pasando por la caseta de filtrado y distribuyendo a los dos sistemas de tratamiento y a los cuatro tanques de producción.

El esquema es el siguiente:



Como se puede observar está formada por los siguientes sistemas:

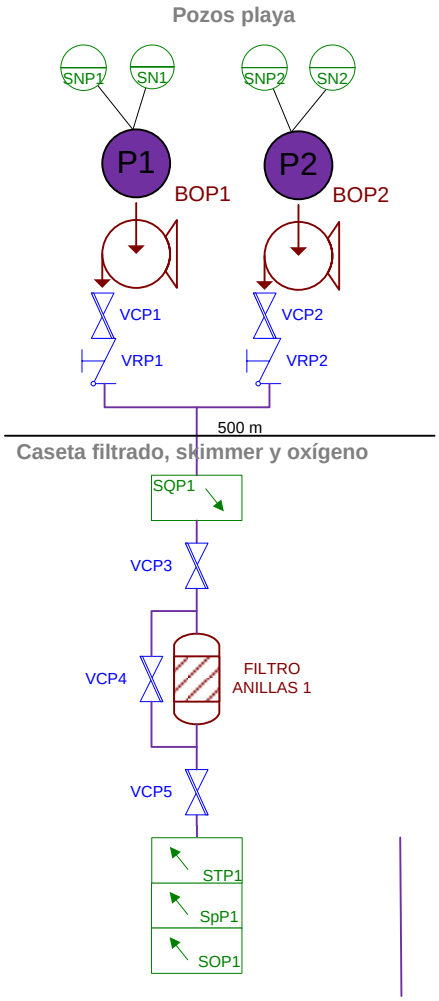
- Impulsión desde pozos de la playa
- Impulsión desde cántara de la desaladora
- Sistema de filtrado y distribución a sistemas de tratamiento y tanques

4.1.1. Impulsión desde pozos de la playa.

La impulsión que se realiza desde los pozos situados en la playa se encuentra a una distancia de 500m de la sala de control donde se alojan los armarios de potencia y el armario de PLC que gobernarán su actuación.

La correlación de bombas responderá a una secuencia tal que el caudal máximo a impulsar sólo será proporcionado si las dos bombas funcionan a la vez.

Tiene el siguiente esquema:

- 

4.1.2. Sensores y actuadores

Los elementos susceptibles de ser automatizados que forman parte de los mismos son:

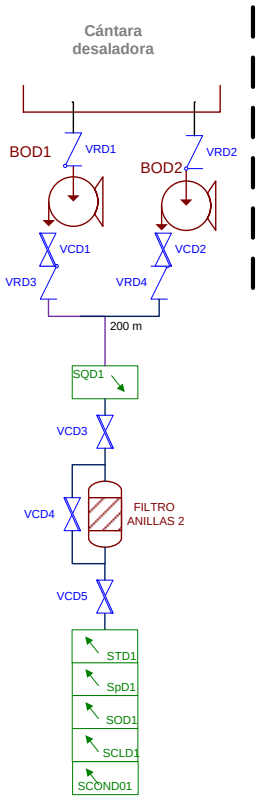
Código equipo	Tipo señal/arrancador	Tipo de equipo	Funcionamiento equipo
SN1	Analógica	Transmisor de nivel hidrostático en pozo 1. Marca en continuo el nivel del agua del pozo, tanto el dinámico como el estático.	Si el nivel desciende de un nivel consignado, para la bomba BOP1 y acciona una señal de alarma.
SN2	Analógica	Transmisor de nivel hidrostático en pozo 2. Marca en continuo el nivel del agua del pozo, tanto el dinámico como el estático.	Si el nivel desciende de un nivel consignado, para la bomba BOP2 y acciona una señal de alarma.
SNP1	Digital	Sensor de control de nivel magnético. Identifica que se ha alcanzado un determinado nivel.	Si se activa el flanco de subida, se habrá alcanzado el nivel consignado y se procederá a parar la bomba BOP1 y a generar una alarma .
SNP2	Digital	Sensor de control de nivel magnético. Identifica que se ha alcanzado un determinado nivel.	Si se activa el flanco de subida, se habrá alcanzado el nivel consignado y se procederá a parar la bomba BOP2 y a generar una alarma .
SQP1	Analógica/digital	Sensor de medida de caudal y de volumen. Proporciona dos medidas.	Localizado en la caseta de filtrado, dado que es de más fácil acceso, se utilizará su medida para realizar un control del caudal bombeado y una vigilancia del correcto funcionamiento del bombeo.
SpP1	Analógica	Sensor de pH.	Localizado en la caseta de filtrado, dado que es de más fácil acceso, se utilizará su medida para realizar un control del pH de agua de entrada. Se generará una alarma si se desvía de los valores deseados. Se podrá desencadenar una parada del grupo correspondiente en caso de desviarse la medida lo suficiente. Así mismo , podrá ponerse en funcionamiento la segunda bomba si así fuera necesario de forma automática.
SOP1	Analógica	Sensor de oxígeno y temperatura.	Localizado en la caseta de filtrado, dado que es de más fácil acceso, se utilizará su medida para realizar un control del oxígeno de agua de entrada. Se generará una alarma si se desvía de los valores deseados. Se podrá desencadenar una parada del grupo correspondiente en caso de desviarse la medida lo suficiente.
BOP1	Arrancador electrónico	Grupo Motobomba sumergido de Potencia: 14 KW Caudal: 30 l/s Hm: 30 mca accionado por medio de arrancador estático con bypass interno y contactor de línea.	Ha de funcionar de forma coordinada con la BOP2, manteniendo un número de arranques a la hora no superior a 4 y un mismo número de horas de funcionamiento. Ha de implementarse un funcionamiento en local y en remoto, éstos a su vez en automático y en manual .
BOP2	Arrancador electrónico	Grupo Motobomba sumergido de Potencia 14 KW Caudal: 30 l/s Hm: 30 mca accionado por medio de arrancador estático con bypass interno y contactor de línea.	Ha de funcionar de forma coordinada con la BOP1, manteniendo un número de arranques a la hora no superior a 4 y un mismo número de horas de funcionamiento. Ha de implementarse un funcionamiento en local y en remoto, éstos a su vez en automático y en manual.

4.1.3. Impulsión desde cántara de la desaladora

La impulsión que se realiza desde los grupos situados en la cántara de la desaladora se encuentra a una distancia de 170m de la sala de control donde se alojan los armarios de potencia y el armario de PLC que gobernarán su actuación.

La correlación de bombas responderá a una secuencia tal que el caudal máximo a impulsar sólo será proporcionado si las dos bombas funcionan a la vez.

Tiene el siguiente esquema:



4.1.3.1. Sensores y actuadores

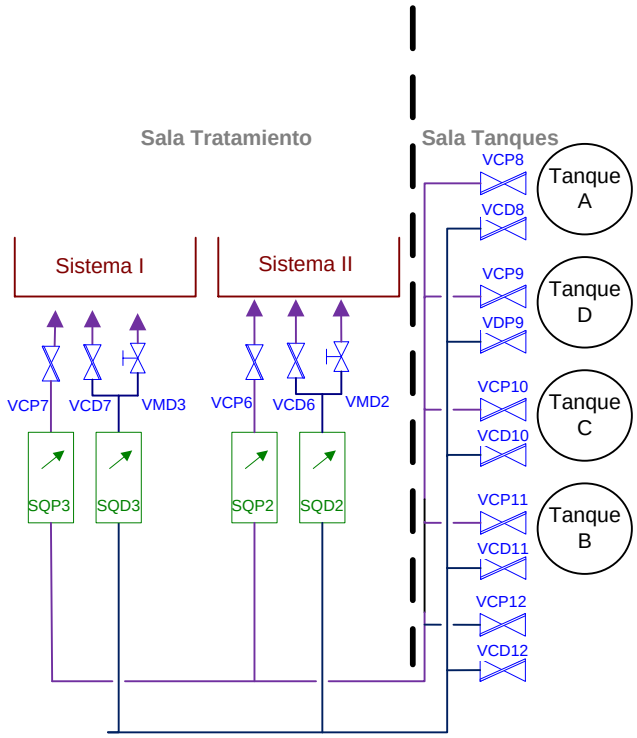
- Los elementos susceptibles de ser automatizados que forman parte de los mismos son:

Código equipo	Tipo señal/arrancador	Tipo de equipo	Funcionamiento equipo
SQD1	Analógica/digital	Sensor de medida de caudal y de volumen. Proporciona dos medidas.	Localizado en la caseta de filtrado, dado que es de más fácil acceso, se utilizará su medida para realizar un control del caudal bombeado y una vigilancia del correcto funcionamiento del bombeo.
STD1	Analógica	Transductor de presión.	Localizado aguas arriba de la batería de filtros su función principal será la de proporcionar la medida de presión en impulsión que se establece como consigna de bombeo.

SpD1	Analógica	Sensor de pH.	Localizado en la caseta de filtrado, dado que es de más fácil acceso, se utilizará su medida para realizar un control del pH de agua de entrada. Se generará una alarma si se desvía de los valores deseados. Se podrá desencadenar una parada del grupo correspondiente en caso de desviarse la medida lo suficiente.
SOD1	Analógica	Sensor de oxígeno y temperatura.	Localizado en la caseta de filtrado, dado que es de más fácil acceso, se utilizará su medida para realizar un control del oxígeno de agua de entrada. Se generará una alarma si se desvía de los valores deseados. Se podrá desencadenar una parada del grupo correspondiente en caso de desviarse la medida lo suficiente.
SCLD1	Analógica	Sensor de cloro.	Localizado en la caseta de filtrado, dado que es de más fácil acceso, se utilizará su medida para realizar un control del Cloro de agua de entrada. Se generará una alarma si se desvía de los valores deseados. Se podrá desencadenar una parada del grupo correspondiente en caso de desviarse la medida lo suficiente.
SCOND01	Analógica	Sensor de conductividad.	Localizado en la caseta de filtrado, dado que es de más fácil acceso, se utilizará su medida para realizar un control de la conductividad de agua de entrada. Se generará una alarma si se desvía de los valores deseados. Se podrá desencadenar una parada del grupo correspondiente en caso de desviarse la medida lo suficiente.
BOD1	Variador de velocidad	Grupo Motobomba sumergido de Potencia:14 KW Caudal: 30 l/s Hm: 30 mca accionado por medio de variador de frecuencia con contactor de línea.	Ha de funcionar de forma coordinada con la BOD2, manteniendo un número de arranques a la hora no superior a 4 y un mismo número de horas de funcionamiento. Ha de implementarse un funcionamiento en local y en remoto, éstos a su vez en automático y en manual. Deberá llevarse a cabo una regulación mano-caudalimétrica que proporcionará un funcionamiento a la demanda de la distribución a la planta a través de sensor STD1 situado aguas abajo de los filtros y el medidor de caudal SQD1.
BOD2	Variador de velocidad	Grupo Motobomba sumergido de Potencia:14 KW Caudal: 30 l/s Hm: 30 mca accionado por medio de variador de frecuencia con contactor de linea.	Ha de funcionar de forma coordinada con la BOD1, manteniendo un número de arranques a al hora no superior a 4 y un mismo número de horas de funcionamiento. Ha de implementarse un funcionamiento en local y en remoto, éstos a su vez en automático y en manual. Deberá llevarse a cabo una regulación mano-caudalimétrica que proporcionará un funcionamiento a la demanda de la distribución a la planta a través de sensor STD1 situado aguas abajo de los filtros y el medidor de caudal SQD1.

4.1.4. Sistema de filtrado y distribución a sistemas de tratamiento y tanques

La distribución y entrada a la planta se bifurca hacia la zona de tratamiento y hacia la nave de tanques.



4.1.4.1. Sensores y actuadores

Los elementos susceptibles de ser automatizados que forman parte de los mismos son:

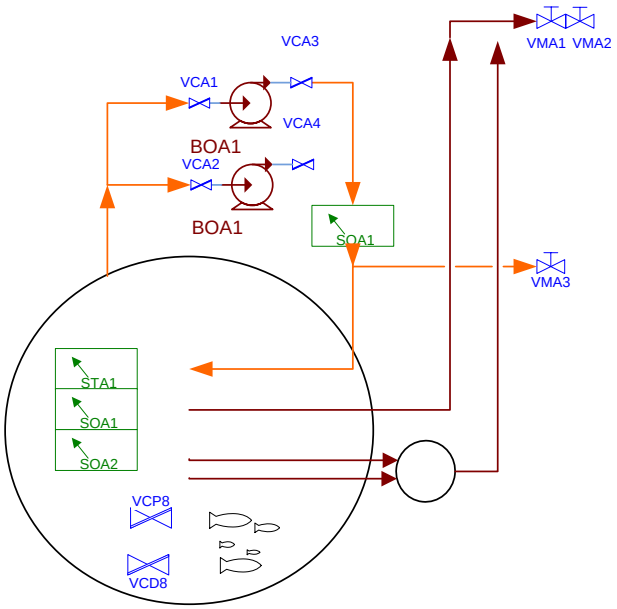
VMD2	Arrancador-inversor	Válvula motorizada.	Localizada en la entrada al sistema de filtrado I, se abre cuando el sensor de nivel STI2 identifica un valor mínimo preconsignado y de esta forma se facilita un llenado rápido del tanque de reserva.
VMD3	Arrancador-inversor	Válvula motorizada.	Localizada en la entrada al sistema de filtrado II, se abre cuando el sensor de nivel STII2 identifica un valor mínimo preconsignado y de esta forma se facilita un llenado rápido del tanque de reserva.

4.2. LÍNEA SALA DE TANQUES

Incluye todo lo relacionado con los tanques y la conducción de agua hasta los sistemas de recirculación o al colector de desagüe.

4.2.1. TANQUE A:

El esquema simplificado de las relaciones hidráulicas del tanque A es es siguiente:



4.2.1.1. Sensores y actuadores

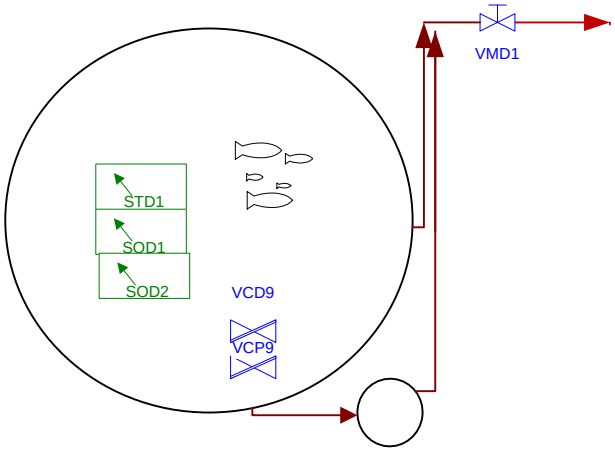
Los sensores y actuadores presentes son los siguientes:

Código equipo	Tipo señal/arrancador	Tipo de equipo	Funcionamiento equipo
STA1	Analógica	Transductor de presión.	Localizado en conducción de salida del tanque, deberá estar parametrizado de forma que informe en continuo del nivel de agua en el tanque. Si sube de un nivel hace sonar una alarma y para las bombas de recirculación del sistema. Si baja de un nivel hace sonar una alarma y cierra la válvula VMA1.
SOA1	Analógica	Sensor de oxígeno y temperatura.	Localizado en el propio tanque, dispuesto en una de las paredes del mismo de forma que no presente un obstáculo al nado de los peces. Se generará una alarma si se desvía de los valores deseados. Si baja de un nivel, abre la electroválvula 1 que inyecta directamente oxígeno al tanque y hace sonar una alarma.
SOA2	Analógica	Sensor de oxígeno y temperatura.	Localizado en el propio tanque, dispuesto en una de las paredes del mismo de forma que no presente un obstáculo al nado de los peces. Se generará una alarma si se desvía de los valores deseados. Si baja de un nivel, abre la electroválvula 1 que inyecta directamente oxígeno al tanque y hace sonar una alarma.

SQA1	Analógica/digital	Sensor de medida de caudal y de volumen. Proporciona dos medidas.	Localizado en la conducción de recirculación, se utilizará su medida para realizar un control del caudal bombeado y una vigilancia del correcto funcionamiento del bombeo. Si baja de un caudal fijado hace accionar una alarma.
VMA1	Arrancador-inversor	Válvula motorizada.	Localizada en aspiración de recirculación, su objetivo es facilitar el vaciado del tanque. Estará funcionando de forma coordinada con la siguiente de índice 2 de forma que si por alguna causa no efectúa la operación de cierre, esta última la realice y no se vacíe el tanque de forma descontrolada.
VMA2	Arrancador-inversor	Válvula motorizada.	Localizada en aspiración de recirculación, su objetivo es facilitar el vaciado del tanque. Estará funcionando de forma coordinada con la anterior de índice 1 de forma que si por alguna causa no efectúa la operación de cierre, esta última la realice y no se vacíe el tanque de forma descontrolada.
VMA3	Arrancador-inversor	Válvula motorizada.	Localizada en conducción de salida de tanque de toma de fondo, su objetivo es facilitar el vaciado del tanque.
BOA1	Variador de velocidad	Grupo Motobomba sumergido de Potencia: 5,5 KW Caudal: 160 l/s Hm: 1 mca accionado por medio de variador de frecuencia con contactor de línea.	Ha de funcionar de forma coordinada con la BOA2, manteniendo un número de arranques a la hora no superior a 4 y un mismo número de horas de funcionamiento. Ha de implementarse un funcionamiento en local y en remoto, éstos a su vez en automático y en manual. Las bombas BOA1 y BOA2 estarán paradas de 10 a 14 horas. Las otras 20 horas funcionan continuamente pero cada dos horas paran 1 minuto. En ese momento se abre la válvula motorizada VMA3, que permanece abierta durante un minuto, cerrándose cuando comienzan a funcionar otra vez las bombas BOA1 y BOA2.
BOA2	Variador de velocidad	Grupo Motobomba sumergido de Potencia: 5,5 KW Caudal: 160 l/s Hm: 1 mca accionado por medio de variador de frecuencia con contactor de línea.	Ha de funcionar de forma coordinada con la BOA1, manteniendo un número de arranques a la hora no superior a 4 y un mismo número de horas de funcionamiento. Ha de implementarse un funcionamiento en local y en remoto, éstos a su vez en automático y en manual. Las bombas BOA1 y BOA2 estarán paradas de 10 a 14 horas. Las otras 20 horas funcionan continuamente pero cada dos horas paran 1 minuto. En ese momento se abre la válvula motorizada VMA3, que permanece abierta durante un minuto, cerrándose cuando comienzan a funcionar otra vez las bombas BOA1 y BOA2.

4.2.2. TANQUE D:

El tanque D tiene en su haber:



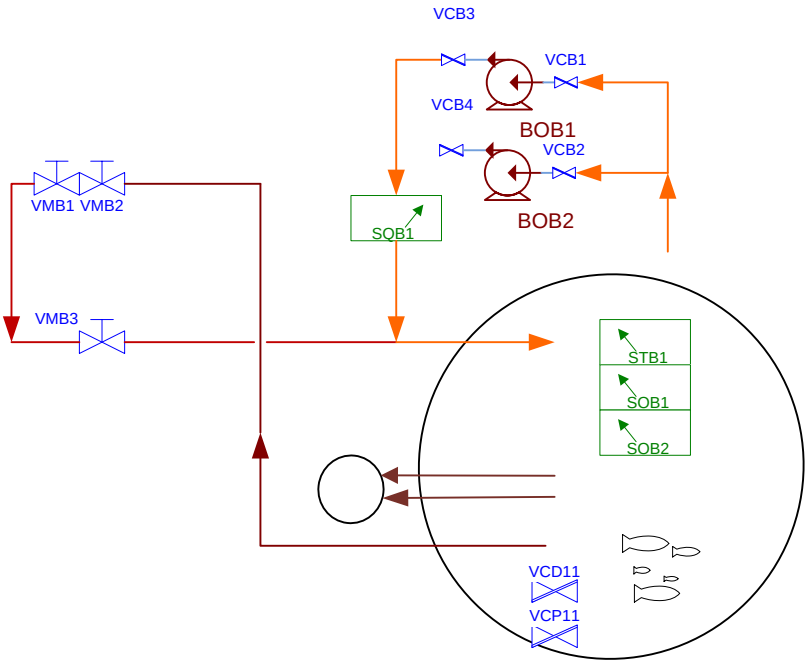
4.2.2.1. Sensores y actuadores

Los sensores y actuadores susceptibles de automatización son:

Código equipo	Tipo señal/arrancador	Tipo de equipo	Funcionamiento equipo
STD1	Analógica	Transductor de presión.	Localizado en conducción de salida del tanque, deberá estar parametrizado de forma que informe en continuo del nivel de agua en el tanque. Si baja de un nivel hace sonar una alarma y cierra la válvula VMD1.
SOD1	Analógica	Sensor de oxígeno y temperatura.	Localizado en el propio tanque, dispuesto en una de las paredes del mismo de forma que no presente un obstáculo al nado de los peces. Se generará una alarma si se desvía de los valores deseados. Si baja de un nivel, abre la electroválvula 1 que inyecta directamente oxígeno al tanque y hace sonar una alarma.
SOD2	Analógica	Sensor de oxígeno y temperatura.	Localizado en el propio tanque, dispuesto en una de las paredes del mismo de forma que no presente un obstáculo al nado de los peces. Se generará una alarma si se desvía de los valores deseados. Si baja de un nivel, abre la electroválvula 1 que inyecta directamente oxígeno al tanque y hace sonar una alarma.
VMD1	Arrancador-inversor	Válvula motorizada.	Localizada en conducción de salida de tanque de toma de fondo, su objetivo es facilitar el vaciado del tanque.

4.2.3. TANQUE B:

El tanque B tiene en su haber:



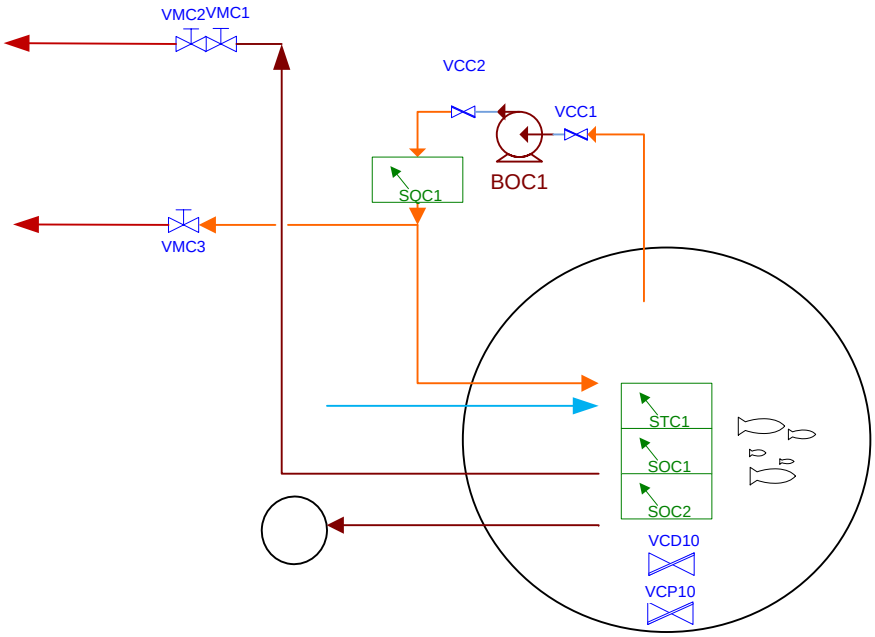
4.2.3.1. Sensores y actuadores

Código equipo	Tipo señal/arrancador	Tipo de equipo	Funcionamiento equipo
STB1	Analógica	Transductor de presión.	Localizado en conducción de salida del tanque, deberá estar parametrizado de forma que informe en continuo del nivel de agua en el tanque. Si sube de un nivel hace sonar una alarma y para las bombas de recirculación del sistema. Si baja de un nivel hace sonar una alarma y cierra la válvula VMB1.
SOB1	Analógica	Sensor de oxígeno y temperatura.	Localizado en el propio tanque, dispuesto en una de las paredes del mismo de forma que no presente un obstáculo al nado de los peces. Se generará una alarma si se desvía de los valores deseados. Si baja de un nivel, abre la electroválvula que inyecta directamente oxígeno al tanque y hace sonar una alarma.
SOB2	Analógica	Sensor de oxígeno y temperatura.	Localizado en el propio tanque, dispuesto en una de las paredes del mismo de forma que no presente un obstáculo al nado de los peces. Se generará una alarma si se desvía de los valores deseados. Si baja de un nivel, abre la electroválvula que inyecta directamente oxígeno al tanque y hace sonar una alarma.

SQB1	Analógica/digital	Sensor de medida de caudal y de volumen. Proporciona dos medidas.	Localizado en la conducción de recirculación, se utilizará su medida para realizar un control del caudal bombeado y una vigilancia del correcto funcionamiento del bombeo. Si baja de un caudal fijado hace accionar una alarma
VMB1	Arrancador-inversor	Válvula motorizada.	Localizada en aspiración de recirculación, su objetivo es facilitar el vaciado del tanque. Estará funcionando de forma coordinada con la siguiente de índice 2 de forma que si por alguna causa no efectúa la operación de cierre, esta última la realice y no se vacíe el tanque de forma descontrolada.
VMB2	Arrancador-inversor	Válvula motorizada.	Localizada en aspiración de recirculación, su objetivo es facilitar el vaciado del tanque. Estará funcionando de forma coordinada con la anterior de índice 1 de forma que si por alguna causa no efectúa la operación de cierre, esta última la realice y no se vacíe el tanque de forma descontrolada.
VMB3	Arrancador-inversor	Válvula motorizada.	Localizada en conducción de salida de tanque de toma de fondo, su objetivo es facilitar el vaciado del tanque.
BOB1	Variador de velocidad	Grupo Motobomba sumergido de Potencia: 5,5 KW Caudal: 160 l/s Hm: 1 mca accionado por medio de variador de frecuencia con contactor de línea.	Ha de funcionar de forma coordinada con la BOB2, manteniendo un número de arranques a la hora no superior a 4 y un mismo número de horas de funcionamiento. Ha de implementarse un funcionamiento en local y en remoto, éstos a su vez en automático y en manual. Las bombas BOB1 y BOB2 estarán paradas de 10 a 14 horas. Las otras 20 horas funcionan continuamente pero cada dos horas paran 1 minuto. En ese momento se abre la válvula motorizada VMC3, que permanece abierta durante un minuto, cerrándose cuando comienzan a funcionar otra vez las bombas BOB1 y BOB2.
BOB2	Variador de velocidad	Grupo Motobomba sumergido de Potencia: 5,5 KW Caudal: 160 l/s Hm: 1 mca accionado por medio de variador de frecuencia con contactor de línea.	Ha de funcionar de forma coordinada con la BOB1, manteniendo un número de arranques a la hora no superior a 4 y un mismo número de horas de funcionamiento. Ha de implementarse un funcionamiento en local y en remoto, éstos a su vez en automático y en manual. Las bombas BOB1 y BOB2 estarán paradas de 10 a 14 horas. Las otras 20 horas funcionan continuamente pero cada dos horas paran 1 minuto. En ese momento se abre la válvula motorizada VMB3, que permanece abierta durante un minuto, cerrándose cuando comienzan a funcionar otra vez las bombas BOB1 y BOB2.

4.2.4. TANQUE C:

El tanque C tiene en su interior:



4.2.4.1. Sensores y actuadores

Código equipo	Tipo señal/arrancador	Tipo de equipo	Funcionamiento equipo
STC1	Analógica	Transductor de presión.	Localizado en conducción de salida del tanque, deberá estar parametrizado de forma que informe en continuo del nivel de agua en el tanque. Si sube de un nivel hace sonar una alarma y para las bombas de recirculación del sistema. Si baja de un nivel hace sonar una alarma y cierra la válvula VMC1.
SOC1	Analógica	Sensor de oxígeno y temperatura.	Localizado en el propio tanque, dispuesto en una de las paredes del mismo de forma que no presente un obstáculo al nado de los peces. Se generará una alarma si se desvía de los valores deseados. Si baja de un nivel, abre la electroválvula que inyecta directamente oxígeno al tanque y hace sonar una alarma.
SOC2	Analógica	Sensor de oxígeno y temperatura.	Localizado en el propio tanque, dispuesto en una de las paredes del mismo de forma que no presente un obstáculo al nado de los peces. Se generará una alarma si se desvía de los valores deseados. Si baja de un nivel, abre la electroválvula que inyecta directamente oxígeno al tanque y hace sonar una alarma.

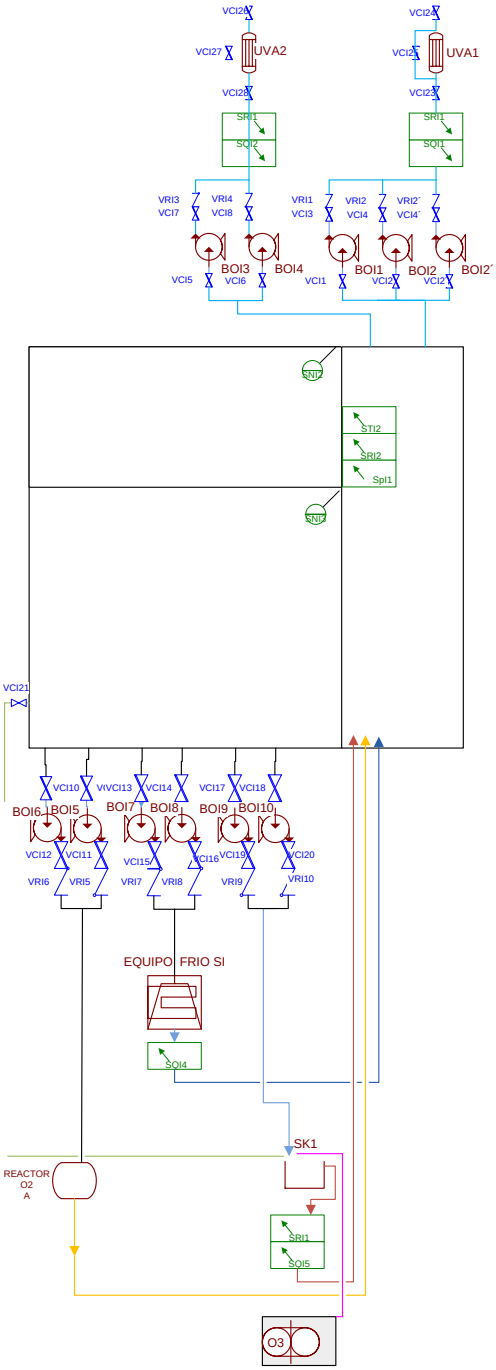
SQC1	Analógica/digital	Sensor de medida de caudal y de volumen. Proporciona dos medidas.	Localizado en la conducción de recirculación, se utilizará su medida para realizar un control del caudal bombeado y una vigilancia del correcto funcionamiento del bombeo. Si baja de un caudal fijado hace accionar una alarma
VMC1	Arrancador-inversor	Válvula motorizada.	Localizada en aspiración de recirculación, su objetivo es facilitar el vaciado del tanque. Estará funcionando de forma coordinada con la siguiente de índice 2 de forma que si por alguna causa no efectúa la operación de cierre, esta última la realice y no se vacíe el tanque de forma descontrolada.
VMC2	Arrancador-inversor	Válvula motorizada.	Localizada en aspiración de recirculación, su objetivo es facilitar el vaciado del tanque. Estará funcionando de forma coordinada con la anterior de índice 1 de forma que si por alguna causa no efectúa la operación de cierre, esta última la realice y no se vacíe el tanque de forma descontrolada.
VMC3	Arrancador-inversor	Válvula motorizada.	Localizada en conducción de salida de tanque de toma de fondo, su objetivo es facilitar el vaciado del tanque.
BOC1	Variador de velocidad	Grupo Motobomba sumergido de Potencia: 5,5 KW Caudal: 160 l/s Hm: 1 mca accionado por medio de variador de frecuencia con contactor de línea.	Ha de funcionar de forma coordinada con la BOC2, manteniendo un número de arranques a lal hora no superior a 4 y un mismo número de horas de funcionamiento. Ha de implementarse un funcionamiento en local y en remoto, éstos a su vez en automático y en manual. Las bombas BOC1 y BOC2 estarán paradas de 10 a 14 horas. Las otras 20 horas funcionan continuamente pero cada dos horas paran 1 minuto. En ese momento se abre la válvula motorizada VMC3, que permanece abierta durante un minuto, cerrándose cuando comienzan a funcionar otra vez las bombas BOC1 y BOC2.

4.3. LINEA DE SISTEMAS DE TRATAMIENTO

Incluye todo lo referente a sistemas de tratamiento del agua recirculada en los dos sistemas –I y II- y su posterior conducción hasta los tanques de peces.

4.3.1. SISTEMA I

El esquema del sistema I es el siguiente:



El agua llega hasta un primer depósito de recepción que está comunicado con el filtro de tambor. Aquí existe una primera sonda de nivel (SNI2) que lo que hace es activar una alarma si el nivel sube por encima de un valor indicado. En realidad lo que hace es indicarnos que el filtro de tambor está tan sucio que el agua lo bypasea por los lados y pasa al sistema sin filtrarse.

El agua después de ser filtrada llega al filtro biológico, donde habrá una segunda sonda de nivel (SNI3), que hace lo mismo que la anterior: activa la alarma si el nivel está por encima de un valor indicado. En realidad lo que hace es indicarnos que el filtro biológico está tan atascado que desborda.

El agua llega ya al depósito regulador. A este depósito le llega el agua de los tanques A y D después de ser filtrada mecánica y biológicamente y le llega también el aporte de agua nueva continuo procedente de los sondeos y/o cántara de la desaladora. Por tanto, este depósito tiene un aliviadero por el que se pierde continuamente una cantidad equivalente al agua nueva que entra al sistema. Las sondas que tiene este depósito regulador son:

-
- STI2: sonda de nivel que da una alarma por nivel máximo. Está justo por encima del aliviadero y tendrá diferentes consignas:
- Consigna de nivel 1: Para la electroválvula de llenado rápido (VMD2) y acciona las bombas de impulsión hacia los tanques A y D (BOI1, BOI2, BOI2', BOI3 y BOI4)
- Consigna de nivel 2: Abre la electroválvula de llenado rápido (VMD2).
- Consigna de nivel 3: Activa una alarma de nivel bajo y detiene las bombas de impulsión hacia los tanques A y D (BOI1, BOI2, BOI2',BOI3 y BOI4)

4.3.1.1. Sensores y actuadores

Código equipo	Tipo señal/arrancador	Tipo de equipo	Funcionamiento equipo
SNI2	Digital	Sensor de control de nivel magnético. Identifica que se ha alcanzado un determinado nivel.	Activar una alarma si el nivel sube por encima de un valor indicado. Indica que el filtro de tambor está tan sucio que el agua lo bypasea por los lados y pasa al sistema sin filtrarse.
SNI3	Digital	Sensor de control de nivel magnético. Identifica que se ha alcanzado un determinado nivel.	Activar una alarma si el nivel sube por encima de un valor indicado. Indica que el filtro biológico está tan atascado que desborda y pasa al sistema sin filtrarse.
STI2	Analógica	Sensor de nivel por ultrasonidos. Identifica en continuo las variaciones de nivel del depósito regulador.	sonda de nivel que da una alarma por nivel máximo. Está justo por encima del aliviadero y tendrá diferentes consignas: Consigna de nivel 1: Para la electroválvula de llenado rápido (VMD2) y acciona las bombas de impulsión hacia los tanques A y D (BOI1, BOI2, BOI2', BOI3 y BOI4) Consigna de nivel 2: Abre la electroválvula de llenado rápido (VMD2). Consigna de nivel 3: Activa una alarma de nivel bajo y detiene las bombas de impulsión hacia los tanques A y D (BOI1, BOI2, BOI2',BOI3 y BOI4)

SpI1	Analógica	Sensor de pH.	Proporcionará medida de pH a corregir con la dosificadora de carbonato
SQI1	Analógica/digital	Sensor de medida de caudal y de volumen. Proporciona dos medidas.	Proporciona una señal que si baja de un caudal determinado activa una alarma y apaga el UVA1
SQI2	Analógica/digital	Sensor de medida de caudal y de volumen. Proporciona dos medidas.	Proporciona una señal que si baja de un caudal determinado activa una alarma y apaga el UVA2
SQI4	Analógica/digital	Sensor de medida de caudal y de volumen. Proporciona dos medidas.	Proporciona la medida del caudal que ha pasado por el subsistema de frío 1
SQI5	Analógica/digital	Sensor de medida de caudal y de volumen. Proporciona dos medidas.	Proporciona la medida del caudal que ha pasado por el subsistema del skimmer 1
SRI1	Analógica	Sonda de potencial redox y de temperatura.	La sonda de potencial redox da una medida y activa una alarma y apaga las bombas BOI1, BOI2, BOI2', BIO3 y BIO4 si pasa de un valor determinado.
SRI2	Analógica	Sonda de potencial redox y de temperatura.	La sonda de potencial redox da una medida y activa una alarma y apaga las bombas BOI1, BOI2, BOI2', BIO3 y BIO4 si pasa de un valor determinado.
SRI3	Analógica	Sonda de potencial redox y de temperatura.	Sonda que pertenece al subsistema del Skimmer y que deberá informar al PLC general de la planta
SRI4	Analógica	Sonda de potencial redox y de temperatura.	Sonda que pertenece al subsistema del Skimmer y que deberá informar al PLC general de la planta

Código equipo	Tipo señal/arrancador	Tipo de equipo	Funcionamiento equipo
BOI1	Variador de velocidad	Grupo Motobomba sumergido de Potencia:7,5 KW Caudal: 75 Hm:7 mca accionado por medio de variador de frecuencia con contactor de línea.	Funciona alternativamente con BOI2 y BOI2´. Si una deja de funcionar, se pone en marcha la otra. Las bombas se paran y se activa una alarma si aumenta el nivel en el tanque A (orden de la sonda SNA1) o desciende en el depósito regulador del sistema I (consigna de nivel 3). Vuelven a funcionar cuando el nivel del depósito regulador alcanza el nivel determinado por la consigna de nivel 1.
BOI2	Arrancador electrónico	Grupo Motobomba sumergido de Potencia:7,5 KW Caudal: 75 Hm:7 mca accionado por medio de arrancador estático de 11kW con bypass interno con contactor de línea.	Funciona alternativamente con BOI1 y BOI2´. Si una deja de funcionar, se pone en marcha la otra. Las bombas se paran y se activa una alarma si aumenta el nivel en el tanque A (orden de la sonda SNA1) o desciende en el depósito regulador del sistema I (consigna de nivel 3). Vuelven a funcionar cuando el nivel del depósito regulador alcanza el nivel determinado por la consigna de nivel 1.
BOI2´	Arrancador electrónico	Grupo Motobomba sumergido de Potencia:7,5 KW Caudal: 75 Hm:7 mca accionado por medio de arrancador estático de 11kW con bypass interno con contactor de línea	Funciona alternativamente con BOI1 y BOI2. Si una deja de funcionar, se pone en marcha la otra. Las bombas se paran y se activa una alarma si aumenta el nivel en el tanque A (orden de la sonda SNA1) o desciende en el depósito regulador del sistema I (consigna de nivel 3). Vuelven a funcionar cuando el nivel del depósito regulador alcanza el nivel determinado por la consigna de nivel 1.
BOI3	Variador de velocidad	Grupo Motobomba sumergido de Potencia:6,5 KW Caudal: 20 l/s Hm: 7 mca accionado por medio de variador de frecuencia con contactor de línea.	Funciona alternativamente con BOI4. Si una deja de funcionar, se pone en marcha la otra. Las bombas se paran y se activa una alarma si aumenta el nivel en el tanque A (orden de la sonda STD1) o desciende en el depósito regulador del sistema I (consigna de nivel 3). Vuelven a funcionar cuando el nivel del depósito regulador alcanza el nivel determinado por la consigna de nivel 1.
BOI4	Arrancador electrónico	Grupo Motobomba sumergido de Potencia:6,5 KW Caudal: 20 l/s Hm: 7 mca accionado por medio de arrancador estático de 11kW con bypass interno y contactor de línea.	Funciona alternativamente con BOI3. Si una deja de funcionar, se pone en marcha la otra. Las bombas se paran y se activa una alarma si aumenta el nivel en el tanque A (orden de la sonda STD1) o desciende en el depósito regulador del sistema I (consigna de nivel 3). Vuelven a funcionar cuando el nivel del depósito regulador alcanza el nivel determinado por la consigna de nivel 1.

BOI5	Contactor	Grupo Motobomba de Potencia: 4 KW Caudal: 20 l/s Hm:10 mca accionado por medio de contactor .	Funciona alternativamente con BOI6. Si una deja de funcionar, se pone en marcha la otra. Impulsan el agua a través de un reactor de oxígeno y la devuelven al depósito regulador.
BOI6	Contactor	Grupo Motobomba de Potencia: 4 KW Caudal: 20 l/s Hm:10 mca accionado por medio de contactor .	Funciona alternativamente con BOI5. Si una deja de funcionar, se pone en marcha la otra. Impulsan el agua a través de un reactor de oxígeno y la devuelven al depósito regulador.
BOI7	Variador de velocidad	Grupo Motobomba de Potencia: KW Caudal: 10 l/s Hm: mca accionado por medio de variador de frecuencia con contactor de línea.	Funciona alternativamente con BOI8. Si una deja de funcionar, se pone en marcha la otra. Impulsan el agua a través de un intercambiador y la devuelven al depósito regulador. Las bombas y el equipo de calor funcionan para mantener una temperatura consignada. Cuando lo consiguen se paran.
BOI8	Arrancador electrónico	Grupo Motobomba de Potencia: 2,6 KW Caudal:10 l/s Hm: 10 mca accionado por medio de arrancador estático de 11kW con bypass interno y contactor de línea.	Funciona alternativamente con BOI7. Si una deja de funcionar, se pone en marcha la otra. Impulsan el agua a través de un intercambiador y la devuelven al depósito regulador. Las bombas y el equipo de calor funcionan para mantener una temperatura consignada. Cuando lo consiguen se paran.
BOI9	Variador de velocidad	Grupo Motobomba de Potencia: 22 KW Caudal: 100 l/s Hm:10 mca accionado por medio de variador de frecuencia con contactor de línea.	Una sonda redox (SRI4) situada en el skimmer controla la adición de ozono desde el ozonizador, bien controlando la entrada de oxígeno o bien controlando la potencia del ozonizador. Para controlar el sistema, en el depósito regulador hay otra sonda de potencial redox (SRI3) que mide el redox y si está por encima de un nivel cierra la entrada de ozono desde el ozonizador hacia el skimmer (cerrando una electroválvula) o bien apaga el ozonizador. Si no obstante se detecta un potencial redox elevado en la tubería que va al tanque A (sonda SRI1), suena la alarma y se paran las bombas BOI1, BOI2, BOI2´ BOI3 y BOI4.

BOI10	Arrancador electrónico	Grupo Motobomba de Potencia: 22 KW Caudal:100 l/s Hm:10 mca accionado por medio de arrancador estático de 22 kW con bypass interno y contactor de línea.	Una sonda redox (SRI4) situada en el skimmer controla la adición de ozono desde el ozonizador, bien controlando la entrada de oxígeno o bien controlando la potencia del ozonizador. Para controlar el sistema, en el depósito regulador hay otra sonda de potencial redox (SRI3) que mide el redox y si está por encima de un nivel cierra la entrada de ozono desde el ozonizador hacia el skimmer (cerrando una electroválvula) o bien apaga el ozonizador. Si no obstante se detecta un potencial redox elevado en la tubería que va al tanque D (sonda SRI2), suena la alarma y se apagan las bombas BOI1, BOI2, BOI2´, BOI3 y BOI4.

4.3.1.2. Subsistemas.

Para cada uno de los subsistemas que desarrollan subprocesos dentro del proceso principal de tratamiento de aguas que se ejecuta en la planta, dado que serán desarrollados por diferentes fabricantes, se opta por mantener una conectividad de bajo nível , pero segura que garantice el completo acceso tanto al control como la información básica del subproceso. De esta forma, se prescribe que todos los subsistemas deben satisfacerla incorporación de un módulo de comunicaciones MODBUS RTU a través del cual se establecerá la comunicación bidireccional con el Autómata general de la planta. Deberá publicarse en las correspondientes direcciones MODBUS todos los estados de funcionamiento, las alarmas y los tipos de fallo que presente el equipo. Así mismo, a la hora de establecer el direccionamiento, para determinar el número de esclavos y direcciones asignadas a cada equipo. Deberá habilitarse la posibilidad de marcha y paro de forma remota a través de las correspondientes direcciones MODBUS RTU y mediante la incorporación de un selector local-remoto que habilite el mando del equipo al Autómata general de la planta en la posición remoto, mientras que en la posición local, se realizará a través de los pulsadores y conmutadores presentes en el propio cuadro. Por otra parte, deberá integrarse de forma independiente al conmutador de local remoto, un paro de emergencia que podrá ser activado tanto en local o en remoto independientemente de la posición del conmutador"

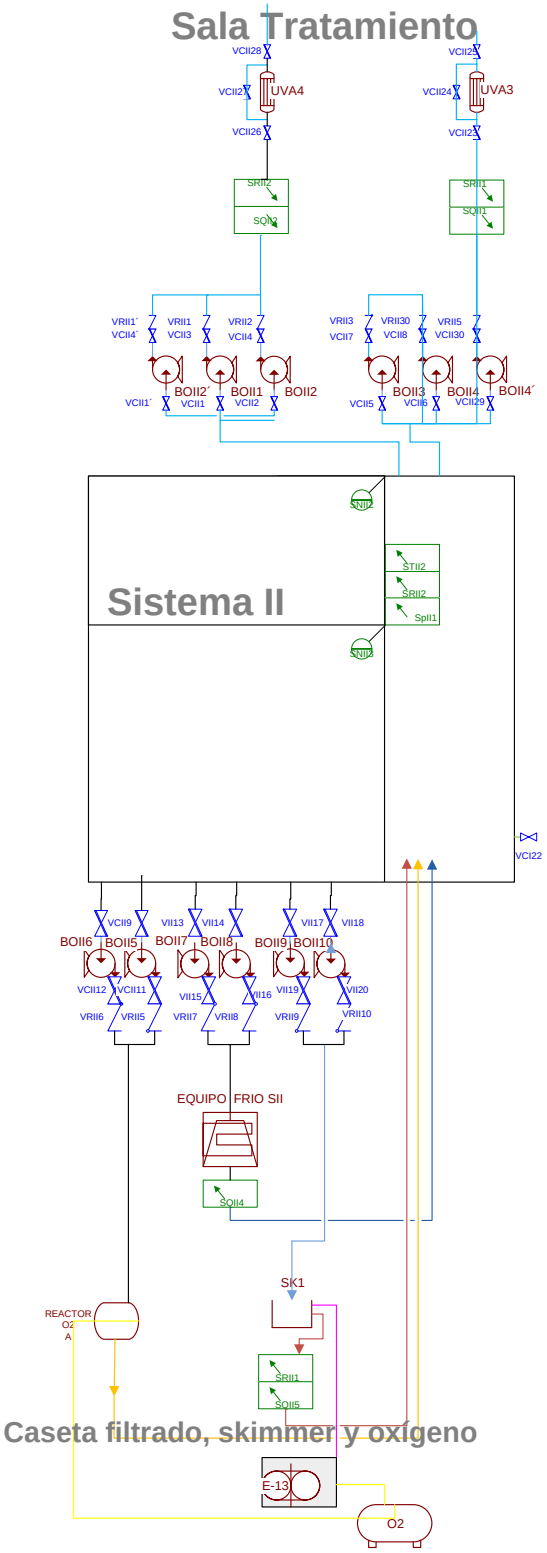
Deberá redactarse una especificación técnica y funcional detallada del funcionamiento del equipo para que se pueda reflejar su funcionamiento en el Scada general de la planta.

En el caso de los subsistemas del sistema 1 , se denominan:

Código equipo
UVA1
UVA2
FT1
SK1
FRÍO 1
O21

4.3.2. Sistema 2

El Sistema 2 responde al siguiente esquema



El agua llega hasta un primer depósito de recepción que está comunicado con el filtro de tambor. Aquí existe una primera sonda de nivel (SNII1) que lo que hace es activar una alarma si el nivel sube por encima de un valor indicado. En realidad lo que hace es indicarnos que el filtro de tambor está tan sucio que el agua lo bypasea por los lados y pasa al sistema sin filtrarse.

El agua después de ser filtrada llega al filtro biológico, donde habrá una segunda sonda de nivel (SNII2), que hace lo mismo que la anterior: activa la alarma si el nivel está por encima de un valor indicado. En realidad lo que hace es indicarnos que el filtro biológico está tan atascado que desborda.

El agua llega ya al depósito regulador. A este depósito le llega el agua de los tanques B y C después de ser filtrada mecánica y biológicamente y le llega también el aporte de agua nueva continuo procedente de los sondeos y/o cántara de la desaladora. Por tanto, este depósito tiene un aliviadero por el que se pierde continuamente una cantidad equivalente al agua nueva que entra al sistema. Las sondas que tiene este depósito regulador son:

- STII2: sonda de nivel que da una alarma por nivel máximo. Está justo por encima del aliviadero y tendrá diferentes consignas:
- Consigna de nivel 1: Para la electroválvula de llenado rápido (VMD3) y acciona las bombas de impulsión hacia los tanques A y D (BOII1, BOII2, BOII2', BOII3, BOII4 y BOII4´)
- Consigna de nivel 2: Abre la electroválvula de llenado rápido (VMD3).
- Consigna de nivel 3: Activa una alarma de nivel bajo y detiene las bombas de impulsión hacia los tanques A y D (BOI1, BOI2, BOI2',BOI3 y BOI4)
- SpII1: sonda de pH
- SRII3: sonda de potencial redox

4.3.2.1. Sensores y actuadores

Código equipo	Tipo señal/arrancador	Tipo de equipo	Funcionamiento equipo
SNII2	Digital	Sensor de control de nivel magnético. Identifica que se ha alcanzado un determinado nivel.	Activar una alarma si el nivel sube por encima de un valor indicado. Indica que el filtro de tambor está tan sucio que el agua lo bypasea por los lados y pasa al sistema sin filtrarse.
SNII3	Digital	Sensor de control de nivel magnético. Identifica que se ha alcanzado un determinado nivel.	Activar una alarma si el nivel sube por encima de un valor indicado. Indica que el filtro biológico está tan atascado que desborda y pasa al sistema sin filtrarse.

STII2	Analógica	Sensor de nivel por ultrasonidos. Identifica en continuo las variaciones de nivel del depósito regulador.	sonda de nivel que da una alarma por nivel máximo. Está justo por encima del aliviadero y tendrá diferentes consignas: Consigna de nivel 1: Para la electroválvula de llenado rápido (VMD3) y acciona las bombas de impulsión hacia los tanques A y D (BOII1, BOII2, BOII2', BOII3 y BOII4) Consigna de nivel 2: Abre la electroválvula de llenado rápido (VMD3). Consigna de nivel 3: Activa una alarma de nivel bajo y detiene las bombas de impulsión hacia los tanques B y C (BOII1, BOII2, BOII2',BOII3, BOII4 Y BOII4´)
SpII1	Analógica	Sensor de pH.	Proporcionará medida de pH a corregir con la dosificadora de carbonato
SQII1	Analógica/digital	Sensor de medida de caudal y de volumen. Proporciona dos medidas.	Proporciona una señal que si baja de un caudal determinado activa una alarma y apaga el UVA3
SQII2	Analógica/digital	Sensor de medida de caudal y de volumen. Proporciona dos medidas.	Proporciona una señal que si baja de un caudal determinado activa una alarma y apaga el UVA4
SQII4	Analógica/digital	Sensor de medida de caudal y de volumen. Proporciona dos medidas.	Proporciona la medida del caudal que ha pasado por el subsistema de frío 2
SQII5	Analógica/digital	Sensor de medida de caudal y de volumen. Proporciona dos medidas.	Proporciona la medida del caudal que ha pasado por el subsistema del skimmer 2
SRII1	Analógica	Sonda de potencial redox y de temperatura.	La sonda de potencial redox da una medida y activa una alarma y apaga las bombas BOII1, BOII2, BOII2', BIIO3, BIIO4 Y BOII4´ si pasa de un valor determinado.
SRII2	Analógica	Sonda de potencial redox y de temperatura.	La sonda de potencial redox da una medida y activa una alarma y apaga las bombas BOII1, BOII2, BOII2', BIIO3, BIIO4 Y BOII4´ si pasa de un valor determinado.
SRII3	Analógica	Sonda de potencial redox y de temperatura.	Sonda que pertenece al subsistema del Skimmer y que deberá informar al PLC general de la planta
SRII4	Analógica	Sonda de potencial redox y de temperatura.	Sonda que pertenece al subsistema del Skimmer y que deberá informar al PLC general de la planta

BOII1	Variador de velocidad	Grupo Motobomba sumergido de Potencia:7,5 KW Caudal: 75 Hm:7 mca accionado por medio de variador de frecuencia con contactor de línea.	Funciona alternativamente con BOI2 y BOI2´. Si una deja de funcionar, se pone en marcha la otra. Las bombas se paran y se activa una alarma si aumenta el nivel en el tanque A (orden de la sonda STA1) o desciende en el depósito regulador del sistema I (consigna de nivel 3). Vuelven a funcionar cuando el nivel del depósito regulador alcanza el nivel determinado por la consigna de nivel 1.
BOII2	Arrancador electrónico	Grupo Motobomba sumergido de Potencia:7,5 KW Caudal: 75 Hm:7 mca accionado por medio de	Funciona alternativamente con BOI1 y BOI2´. Si una deja de funcionar, se pone en marcha la otra.

		arrancador estático de 11kW con bypass interno con contactor de línea.	Las bombas se paran y se activa una alarma si aumenta el nivel en el tanque A (orden de la sonda STA1) o desciende en el depósito regulador del sistema I (consigna de nivel 3). Vuelven a funcionar cuando el nivel del depósito regulador alcanza el nivel determinado por la consigna de nivel 1.
BOII2´	Arrancador electrónico	Grupo Motobomba sumergido de Potencia:7,5 KW Caudal: 75 Hm:7 mca accionado por medio de arrancador estático de 11kW con bypass interno con contactor de línea	Funciona alternativamente con BOI1 y BOI2. Si una deja de funcionar, se pone en marcha la otra. Las bombas se paran y se activa una alarma si aumenta el nivel en el tanque A (orden de la sonda STA1) o desciende en el depósito regulador del sistema I (consigna de nivel 3). Vuelven a funcionar cuando el nivel del depósito regulador alcanza el nivel determinado por la consigna de nivel 1.
BOII3	Variador de velocidad	Grupo Motobomba sumergido de Potencia:6,5 KW Caudal: 20 l/s Hm: 7 mca accionado por medio de variador de frecuencia con contactor de línea.	Funciona alternativamente con BOII4 y BOII4´. Si una deja de funcionar, se pone en marcha la otra. Las bombas se paran y se activa una alarma si aumenta el nivel en el tanque A (orden de la sonda STD1) o desciende en el depósito regulador del sistema I (consigna de nivel 3). Vuelven a funcionar cuando el nivel del depósito regulador alcanza el nivel determinado por la consigna de nivel 1.
BOII4	Arrancador electrónico	Grupo Motobomba sumergido de Potencia:6,5 KW Caudal: 20 l/s Hm: 7 mca accionado por medio de arrancador estático de 11kW con bypass interno y contactor de línea.	Funciona alternativamente con BOII3 y BOII4´. Si una deja de funcionar, se pone en marcha la otra. Las bombas se paran y se activa una alarma si aumenta el nivel en el tanque A (orden de la sonda STD1) o desciende en el depósito regulador del sistema I (consigna de nivel 3). Vuelven a funcionar cuando el nivel del depósito regulador alcanza el nivel determinado por la consigna de nivel 1.
BOII4´	Arrancador electrónico	Grupo Motobomba sumergido de Potencia:6,5 KW Caudal: 20 l/s Hm: 7 mca accionado por medio de arrancador estático de 11kW con bypass interno y contactor de línea.	Funciona alternativamente con BOI3 y BOII4. Si una deja de funcionar, se pone en marcha la otra. Las bombas se paran y se activa una alarma si aumenta el nivel en el tanque A (orden de la sonda STD1) o desciende en el depósito regulador del sistema I (consigna de nivel 3). Vuelven a funcionar cuando el nivel del depósito regulador alcanza el nivel determinado por la consigna de nivel 1.
BOI5	Contactor	Grupo Motobomba de Potencia: 4 KW Caudal: 20 l/s Hm:10 mca accionado por medio de contactor .	Funciona alternativamente con BOI6. Si una deja de funcionar, se pone en marcha la otra. Impulsan el agua a través de un reactor de oxígeno y la devuelven al depósito regulador.
BOI6	Contactor	Grupo Motobomba de Potencia: 4 KW Caudal: 20 l/s Hm:10 mca accionado por medio de contactor .	Funciona alternativamente con BOI5. Si una deja de funcionar, se pone en marcha la otra.

			Impulsan el agua a través de un reactor de oxígeno y la devuelven al depósito regulador.
BOI7	Variador de velocidad	Grupo Motobomba de Potencia: KW Caudal: 10 l/s Hm: mca accionado por medio de variador de frecuencia con contactor de línea.	Funciona alternativamente con BOI8. Si una deja de funcionar, se pone en marcha la otra. Impulsan el agua a través de un intercambiador y la devuelven al depósito regulador. Las bombas y el equipo de calor funcionan para mantener una temperatura consignada. Cuando lo consiguen se paran.
BOI8	Arrancador electrónico	Grupo Motobomba de Potencia: 2,6 KW Caudal:10 l/s Hm: 10 mca accionado por medio de arrancador estático de 11kW con bypass interno y contactor de línea.	Funciona alternativamente con BOI7. Si una deja de funcionar, se pone en marcha la otra. Impulsan el agua a través de un intercambiador y la devuelven al depósito regulador. Las bombas y el equipo de calor funcionan para mantener una temperatura consignada. Cuando lo consiguen se paran.
BOI9	Variador de velocidad	Grupo Motobomba de Potencia: 22 KW Caudal: 100 l/s Hm:10 mca accionado por medio de variador de frecuencia con contactor de línea.	Una sonda redox (SRI4) situada en el skimmer controla la adición de ozono desde el ozonizador, bien controlando la entrada de oxígeno o bien controlando la potencia del ozonizador. Para controlar el sistema, en el depósito regulador hay otra sonda de potencial redox (SRI3) que mide el redox y si está por encima de un nivel cierra la entrada de ozono desde el ozonizador hacia el skimmer (cerrando una electroválvula) o bien apaga el ozonizador. Si no obstante se detecta un potencial redox elevado en la tubería que va al tanque A (sonda SRI1), suena la alarma y se paran las bombas BOI1, BOI2, BOI2´ BOI3 y BOI4.
BOI10	Arrancador electrónico	Grupo Motobomba de Potencia: 22 KW Caudal:100 l/s Hm:10 mca accionado por medio de arrancador estático de 22 kW con bypass interno y contactor de línea.	Una sonda redox (SRI4) situada en el skimmer controla la adición de ozono desde el ozonizador, bien controlando la entrada de oxígeno o bien controlando la potencia del ozonizador. Para controlar el sistema, en el depósito regulador hay otra sonda de potencial redox (SRI3) que mide el redox y si está por encima de un nivel cierra la entrada de ozono desde el ozonizador hacia el skimmer (cerrando una electroválvula) o bien apaga el ozonizador. Si no obstante se detecta un potencial redox elevado en la tubería que va al tanque D (sonda SRI2), suena la alarma y se apagan las bombas BOI1, BOI2, BOI2´, BOI3 y BOI4.

4.3.2.2. Subsistemas

Para cada uno de los subsistemas que desarrollan subprocesos dentro del proceso principal de tratamiento de aguas que se ejecuta en la planta, dado que serán desarrollados por diferentes fabricantes, se opta por mantener una conectividad de bajo nível , pero segura que garantice el completo acceso tanto al control como la información básica del subproceso. De esta forma, se prescribe que todos los subsistemas deben satisfacerla incorporación de un módulo de comunicaciones MODBUS RTU a través del cual se establecerá la comunicación bidireccional con el Autómata general de la planta. Deberá publicarse en las correspondientes direcciones MODBUS todos los estados de funcionamiento, las alarmas y los tipos de fallo que presente el equipo. Así mismo, a la hora de establecer el direccionamiento, para determinar el número de esclavos y direcciones asignadas a cada equipo. Deberá habilitarse la posibilidad de marcha y paro de forma remota a través de las correspondientes direcciones MODBUS RTU y mediante la incorporación de un selector local-remoto que habilite el mando del equipo al Autómata general de la planta en la posición remoto, mientras que en la posición local, se realizará a través de los pulsadores y conmutadores presentes en el propio cuadro. Por otra parte, deberá integrarse de forma independiente al conmutador de local remoto, un paro de emergencia que podrá ser activado tanto en local o en remoto independientemente de la posición del conmutador"

Deberá redactarse una especificación técnica y funcional detallada del funcionamiento del equipo para que se pueda reflejar su funcionamiento en el Scada general de la planta.

En el caso de los subsistemas del sistema 2 , se denominan:

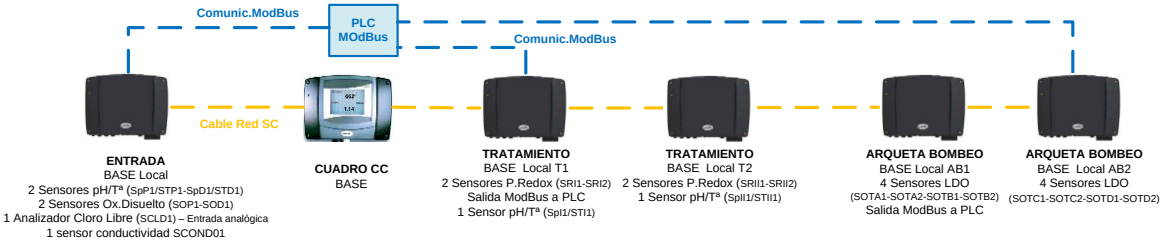
Código equipo
UVA3
UVA4
FT2
SK2
FRÍO 2
O22

5. CONTROL DE LA INSTRUMENTACIÓN

La instrumentación que se utilizará estará dividida en dos grupos, el primero será el correspondiente a las magnitudes físicas principalmente (presión, nivel, caudal) y el segundo grupo se corresponderá con las magnitudes analíticas (Redox, Oxígeno, pH, Temperatura, Conductividad y Cloro)

5.1. Primer grupo de sensores: Magnitudes analíticas.

En el caso de los sensores correspondientes a oxígeno, redox, pH, y Cloro, se establecerá una red independiente que estará debidamente comunicada con el autómata mediante un bus de campo industrial. De esta forma, el esquema planteado es el siguiente:



Los sensores que estarán asociados a esta red son los siguientes:

SpD1	SOP1
SpP1	SOD1
SpI1	SOTA1
SpII1	SOTA2
SCLD1	SOTB1
SRI1	SOTB2
SRI2	SOTC1
SRII1	SOTC2
SRII2	SOTD1
SCOND01	SOTD2

5.2. Segundo grupo de sensores: Magnitudes físicas.

El otro grupo de sensores se cablearán directamente a las entradas y salidas del autómata, dependiendo de si son analógicas o digitales los tipos de señal que generan.

Dentro de este grupo se tendrá los siguientes dispositivos:

SNP1	STA1	SQP1
SNP2	STB1	SQII2

SNI2	STC1	SQP2
SNII2	STD1	SQP3
SNI3	SQA1	SQD2
SNII3	SQB1	SQD3
STI2	SQC1	SQI4
STII2	SQI1	SQII4
SN1	SQI5	SQI2
SN2	SQII1	SQI3
STP1	SQII5	SQII3
STD1	SQD1	

6. CONTROLADOR DE LA PLANTA

El dimensionamiento de las entradas y salidas necesarias de acuerdo a la instalación proyectada se justifica en las siguientes tablas.

El total de señales que se precisan, categorizadas según su naturaleza eléctrica son:

		Nº de señales requeridas	Nº de señales necesarias			
	Nº de señales necesarias		456	60	29	16
	Nº de señales instaladas		480	64	32	16
	Nº de señales reserva		24	4	3	0

Estas se agrupan a su vez en los siguientes elementos:

Nº	Equipo	Grupo	Tipo señal	ED	SD	EA	SA	ED	SD	EA	SA
1	Armarios murales	Generales	Seta emergencia	1				1	0	0	0
			Defecto protección magnetotérmica general	1				1	0	0	0
			Entrada grupo electrógeno	1				1	0	0	0
			Fallo protección sobretensiones general	1				1	0	0	0
			Fallo protección sobretensiones cuadro PLC	1				1	0	0	0
			Fallo sistema climatización	1				1	0	0	0
			Fallo circuito mando 24Vcc	1				1	0	0	0
			Fallo circuito mando 230Vac	1				1	0	0	0
			Fallo protección batería condensadores	1				1	0	0	0
			Antiintrusismo cuadros	1				1	0	0	0
			Alimentación al cuadro de servicios auxiliares	1				1	0	0	0
18	Bombeo pozos playa	Por bombeo o agrupación válvulas	Posición Remoto	1				18	0	0	0
	Bombeo desaladora		Defecto protección diferencial	1				18	0	0	0
	Bombeo soplantes		Defecto protección magnetotérmica	1				18	0	0	0
	Bombeo recirculación tanque A										
	Bombeo a tanque A										
	Bombeo a tanque D										
	Bombeo a reactor oxígeno s1										
	Bombeo a equipos de frío s1										
	Bombeo a skimmer y ozonificador s1										
	Bombeo recirculación tanque B										
	Bombeo recirculación tanque C										
	Bombeo a tanque B										
	Bombeo a tanque C										
	Bombeo a reactor oxígeno s2										
	Bombeo a equipos de frío s2										
	Bombeo a skimmer y ozonificador s2										
	Agrupación válvulas sistema 1										
	Agrupación válvulas sistema 2										
18	Alimentación cuadro UVA1	Por cuadro	Defecto protección diferencial	1				18	0	0	0
	Alimentación cuadro UVA2		Defecto protección magnetotérmica	1				18	0	0	0
	Alimentación cuadro Skimmer s1										
	Alimentación cuadro ozonificador s1										
	Alimentación cuadro oxígeno s1										
	Alimentación cuadro filtro tambor s1										

	Alimentación cuadro UVA3
	Alimentación cuadro UVA4
	Alimentación cuadro Skimmer s2
	Alimentación cuadro ozonificador s2
	Alimentación cuadro oxígeno s2
	Alimentación cuadro filtro tambor s2
	Alimentación cuadro estación filtrado 1
	Alimentación cuadro estación filtrado 2
	Alimentación cuadros grupos de frío
	Alimentación cuadro SAI1
	Alimentación cuadro SAI2
	Alimentación toma camión oxígeno

Nº	Equipo	Grupo	Tipo señal	ED	SD	EA	SA	ED	SD	EA	SA
4	BOI5	Bomba con arranque directo	Manual	1				4	0	0	0
	BOI6		Automático	1				4	0	0	0
	BOII5		Marcha	1				4	0	0	0
	BOII6		Defecto protección magnetotérmica	1				4	0	0	0
			Orden marcha		1			0	4	0	0

16	BOD1	Bomba con variador	Manual	1				16	0	0	0
	BOD1		Automático	1				16	0	0	0
	S1		Marcha	1				16	0	0	0
	BOA1		Defecto protección magnetotérmica	1				16	0	0	0
	BOA2		Defecto variador	1				16	0	0	0
	BOI1		Defecto	2				32	0	0	0
	BOI3		Orden marcha		1			0	16	0	0
	BOI7		Frecuencia				1	0	0	0	16
	BOI9										
	BOB1										
	BOB2										
	BOC1										
	BOII1										
	BOII3										
	BOII7										
	BOII9										
14	BOP1	Bomba con arrancador	Manual	1				14	0	0	0
	BOP2		Automático	1				14	0	0	0
	S2		Marcha	1				14	0	0	0
	BOI2		Defecto protección magnetotérmica	1				14	0	0	0
	BOI2´		Defecto arrancador	1				14	0	0	0
	BOI4		Defecto	2				28	0	0	0
	BOI8		Orden marcha		1			0	14	0	0
	BOI10										
	BOII2										
	BOII2´										
	BOII4										
	BOII4´										
	BOII8										
	BOII10										
13	VMA1	Válvula motorizada consigna posición	Manual	1				13	0	0	0
	VMA2		Automático	1				13	0	0	0
	VMA3		Defecto protección magnetotérmica	1				13	0	0	0
	VMD1		Finales de carrera	2				26	0	0	0
	VMB1		Limitador de par	1				13	0	0	0
	VMB2		Marcha	2				26	0	0	0
	VMB3		Orden marcha		2			0	26	0	0
	VMC1										
	VMC2										
	VMC3										
	VMD2										
	VMD3										
	VMCA1										

Nº	Equipo	Grupo	Tipo señal	ED	SD	EA	SA	ED	SD	EA	SA
29	STI2 STII2 SN1 SN2 STP1 STD1 STA1 STB1 STC1 STD1 SQA1 SQB1 SQC1 SQI1 SQI5 SQII1 SQII5 SQD1 SQP1 SQII2 SQP2 SQP3 SQD2 SQD3 SQI4 SQII4 SQI2 SQI3 SQII3	Instrumentación analógica	Señal analógica			1		0	0	29	0
25	SNP1 SNP2 SNI2 SNII2 SNI3 SNII3 SQA1 SQB1 SQC1 SQI1 SQI5 SQII1 SQII5 SQD1 SQP1 SQII2 SQP2 SQP3 SQD2 SQD3 SQI4 SQII4 SQI2 SQI3 SQII3	Instrumentación digital	Señal digital	1				25	0	0	0

7. SISTEMA DE CONTROL

7.1. Descripción General

El Centro de Control del Sistema se establecerá de forma local en la sala de control anexa a la sala de cuadros CCM. Allí se dispondrá de un sistema de control que permitirá el control local y manejo de todas las instalaciones.

El Sistema de Control a diseñar se basa en un Sistema Scada (Supervisory Control And Data Adquisition). Este se instala sobre una máquina que realiza las funciones de Servidor de Scada, con un monitor TFT de 19" y una impresora.

La comunicación con las instalaciones se realiza a través de una con conexión ethernet. Además se dispone de un modem GSM para el envío de mensajes SMS de alarma.

7.1.1. Software Scada

Se trata de una aplicación software especialmente diseñada para funcionar sobre ordenadores en el control de producción, proporcionando comunicación con los dispositivos de campo (instrumentos de medida, autómatas programables, etc.) y controlando el proceso de forma automática desde la pantalla del ordenador. Además, provee de toda la información que se genera en el proceso productivo a diversos usuarios, tanto del mismo nivel como de otros supervisores dentro de la explotación: control de calidad, supervisión, mantenimiento, etc. Los programas necesarios, y en su caso el hardware adicional que se necesite, se denomina en general sistema SCADA.

Los datos leídos proceden de los PLCs instalados en el sistema, los cuales a su vez la obtienen de la instrumentación de campo (sensores y detectores) y de los cuadros de control de motores.

7.1.1.1. Funcionalidades Básicas

El paquete SCADA que se pondrá a disposición tendrá las siguientes prestaciones:

- Adquisición. Estas funciones requieren trabajar a gran velocidad, con un tiempo de respuesta muy corto si se quiere poder manejar las variables en tiempo real.
- Gestión de alarmas, Incluye el análisis de los valores de las variables adquiridas, la generación de las alarmas que corresponda de acuerdo con la parametrización establecida por el usuario y la transmisión al gestor de datos y a los periféricos de los valores y activaciones adecuados según la configuración establecida.
- Gestión de datos. Aquí se incluye la comunicación con la base de datos y el almacenamiento en la misma de los datos a registrar. También se incluye la comunicación

con la interfase de operación para poder realizar las tareas básicas de supervisión y control por parte del operador.

- Gestión de bases de datos de tiempo real e históricos. Tienen una estructura interna especifica a la que no se puede acceder con un gestor de base de datos (BD) estándar. Esta estructura tiene por objetivo permitir al gestor de datos alcanzar los bajos tiempos de registro y acceso que requieren cate tipo de aplicaciones.
- Presentación. Estas funciones se realizan mediante una interfase de operación e incluyen la representación gráfica y alfanumérica de datos a través de los periféricos de comunicación con el operador, así como la entrada o modificación de datos y órdenes por parte del operador. El módulo especifico para realizar estas funciones, recibe el nombre de software IHM (interfaz Hombre-Máquina) y sus aplicaciones son:
- Creación de sinópticos con objetos estáticos y dinámicos incluyendo mapas de bits de 64 colores entre una paleta de 16 millones de colores.
- Posibilidad de crear paneles de alarma, que exigen la presencia del operador para reconocer una parada o situación de alarma, con registro de incidencias.
- Analizar la evolución de las variables. En las aplicaciones SCADA se incluyen módulos de explotación de datos capaces de analizar la evolución de las variables, tanto el histórico de cada una como en su conjunto, y detectar si el sistema está actuando correctamente, diagnosticar la causa y generar acciones correctivas.
- Creación de gráficos de tendencias en tiempo real e históricos.
- Presentaciones en pantalla, envío de resultados a disco e impresora, etc.
- Creación de informes personalizados.
- Ejecución de programas que modifican la ley de control, o incluso anular o modificar las tareas asociadas al autómata, bajo ciertas condiciones.
- Una librería completa de funciones básicas y avanzadas, de tipo matemático, lógico, de control, de control estadístico, de bases de datos relacionales, etc. Esta librería se puede ampliar con funciones específicas creadas a medida.
- Programación numérica que permite realizar cálculos aritméticos de elevada resolución sobre la CPU del ordenador. Con ellas, se pueden desarrollar aplicaciones para ordenadores (tipo PC, por ejemplo), con captura de datos, análisis de señales…

7.1.1.2. Módulos del Scada

Los módulos o bloques software que deberán instalarse para permitir las actividades de adquisición, supervisión y control son los siguientes:

- Configuración: permite al usuario definir el entorno de trabajo de su SCADA, adaptándolo a la aplicación particular que se desea desarrollar.
- Interfaz gráfica del operador: proporciona al operador las funciones de control y supervisión de la planta. El proceso se representa mediante sinópticos gráficos almacenados en el ordenador de proceso y generados desde el editor incorporado en el SCADA o importados desde otra aplicación durante la configuración del paquete.
- Módulo de proceso: ejecute las acciones de mando preprogramadas a partir de los valores actuales de variables leídas.
- Gestión v archivo de datos: se encarga del almacenamiento y procesado ordenado de los datos, de forma que otra aplicación o dispositivo pueda tener acceso a ellos.
- Comunicaciones: se encarga de la transferencia de información entre la planta y la arquitectura hardware que soporta el SCADA, y entre ésta y el resto de elementos informáticos de gestión.

- Términal Server: Para poder establecer el acceso a las funcionalidades del Scada desde la aplicación Scada desarrollada al efecto en PDAs.

7.1.1.3. Tecnologías del Scada

Se ha considerado como SCADA un producto que soporte las siguientes tecnologías, haciendo de él un sistema abierto y expandidle:

- Tecnología PIug and Solve: Una implementación de la estrategia COM de Microsoft Provee una fácil conectividad con terceras aplicaciones basadas en COM. Esto permite a las aplicaciones ser adaptadas a las necesidades especificas añadiendo las mejores aplicaciones externas en su clase.
- Soporte completo para controles ActiveX: El Workspace de Intellution es un contenedor para controles ActiveX de terceras partes. Estos controles simplemente pueden ser dejados en el Workspace para una fácil integración. En consecuencia, documentos ActiveX como MS Word o Excel, pueden ser dejados en el Workspace mostrando automáticamente los menús y barras de herramientas definidos en esos documentos.
- Secure Containment: Esta tecnología, pendiente de patente por parte de Intellution, asegura que cualquier control ActiveX con un comportamiento erróneo no afecte al sistema, atrapando este control. Costosas paradas en el ámbito de planta son evitadas y ningún dato se pierde ni el control del proceso se interrumpe.
- VBA (Visual Basic for Applications) embebido: el software una el VBA embebido como el leguaje común de script detrás de los objetos. Todas las propiedades, métodos y eventos son expuestos pulsando el botón derecho del ratón. Ejecutar comandos es simple y el uso del editor de VBA permite el desarrollo de código para conectar a bases de datos relacionales y mucho más.
- Soporte completo para OPC (OLE for Process Control) Cliente / Servidor: el software SCADA puede acosar como un servidor OPC para cualquier cliente estándar OPC. Adicionalmente, puede actuar como un cliente OPC sobre cualquier servidor OPC estándar. OPC en el workspace permite a los desarrolladores animar directamente desde cualquier servidor OPC.
- ODBC API: SCADA tiene funcionalidad complete de API ODBC, añadiendo la capacidad de recolectar y escribir datos de proceso en tiempo real a una o más bases de datos relacionables. Los datos almacenados en la base de datos relacional pueden ser leídos o borrados, o escritos de nuevo en la base de datos de proceso de SCADA.
- MYSQL: el software provee una instalación unificada para MYSQL Server 7.0 de Microsoft, permitiendo un rápido y fácil acceso a MYSQL Server 7.0, acortando el tiempo de desarrollo de aplicaciones.

7.1.2. Desarrollo de la Aplicación

El sistema aportará las herramientas software necesarias que permitan implementar una aplicación para que la visualización de la información y la supervisión de las instalaciones sea realizada de forma rápida e intuitiva, para lo cual incluirá las siguientes funciones:

- Interfaz gráfica de usuario. con gráficos interactivos.
- Gestión de bases de datos.

- Gestión de Alarmas e Incidencias.
- Recolección de Históricos.
- Ejecución de órdenes.
- Visualización de gráficos de tendencias en tiempo real e históricos.
- Gestión de informes.
- Control de accesos al sistema.

7.1.2.1. Interfaz Gráfica de Usuario

El sistema facilitará que los usuarios visualicen en tiempo real el estado en el que se encuentra el canal. Sobre gráficos sinópticos representativos de la instalación, el usuario verá representados los parámetros del sistema, tales como medidas del nivel de agua de los depósitos así como el grado de apertura de válvulas, arranque de los bombeos o el estado de las entradas digitales (activo / inactivo) de todas las señales de todos los contactos auxilires de las protecciones con el fin de facilitar las tomas de decisión sobre su funcionamiento, que podrán ser llevadas a cabo directa e interactivamente en el propio gráfico.

Para desplazarse de una pantalla a otra, el usuario dispondrá de menús que le permiten navegar por la aplicación sin que sea necesario aprender complejos comandos de lenguaje operativo.

Simultáneamente, en todas las pantallas se visualizará una lista de las alarmas que se están produciendo, como fallos de la alimentación de los equipos (PLCs, medidores de nivel, caudalímetros...) permitiéndole ejecutar acciones en los mismos gráficos sinópticos sin tener que conmutar a la pantalla de visualización de alarmas e incidencias.

Además de los datos en tiempo real, el sistema permitirá visualizar todos aquellos datos estadísticos enviados por los terminales remotos al Centro de Control que son de gran interés para la correcta explotación de la instalación.

7.1.2.2. Sistema Gestor de Base de Datos

Es la herramienta utilizada por el desarrollador del sistema y el explotador para crear, modificar y gestionar tanto las bases de datos en tiempo real que contienen toda la información del estado del canal, es decir, el valor de sus variables, como aquellas que contienen la parametrización y configuración del propio software.

El sistema gestor de las bases de datos permitirá que, desde el PC con software de desarrollo y durante el proceso de puesta en marcha, el supervisor pueda definir los parámetros necesarios para que los servidores del Centro de Control reconozcan todos y cada uno de los elementos del sistema con la nomenclatura propia de la instalación, es decir, cada uno de los medidores de nivel, caudalímetros, PLCs remotos, etc., queda reconocido por los servidores con un nombre apropiado para su fácil identificación como un elemento del sistema que habrá que supervisar y controlar.

Se configurarán los parámetros básicos de funcionamiento sobre los que se realiza el tratamiento de la información del sistema controlado. Estos parámetros son los rangos de funcionamiento habitual de presiones, caudales o niveles, límites máximos y mínimos de alarma, etc.

La mayoría de los parámetros definidos en estas bases de datos se transmitirán desde el Centro de Control a los terminales remotos con el objeto de que ante un fallo de comunicaciones, estos últimos puedan seguir efectuando las operaciones necesarias para el correcto control y supervisión de las instalaciones. Las circunstancias en las que se efectuará la transmisión de dichos parámetros son:

- Inicialización del programa
- Modificación de algún parámetro por expreso deseo del operador

7.1.2.3. Módulo de Supervisión de Alarmas e Incidencias

Cuando alguna de las variables alcancen valores que se salen de su rango de funcionamiento habitual, o se activan señales de alarma el sistema dispone de un módulo que se encarga de mostrarías al usuario, generando una salida por impresora y alarma acústica según criterio.

Además controlará toda la gestión de aceptación y validación de alarmas, clasificación del nivel de alarmas (leve, severa, muy severa) así como su almacenamiento en archivos históricos.

No solamente se podrán almacenar y controlar las alarmas. Todo cambio de estado de cualquier variable del sistema, o actuaciones y decisiones del operador, puede ser objeto de control y supervisión. Estas incidencias plasman la evolución del sistema de control del canal

El usuario puede configurar qué eventos del funcionamiento del sistema se consideran incidencias, y cuáles de las acciones aplicadas a alarmas (salidas por impresora, visualización en pantalla, alarma acústica) le son asociadas. Las incidencias propias del canal son:

- Cambios de estado de los dispositivos de medida (sensores)
- Alarmas digitales (falta de alimentación de los equipos)
- Alarma por nivel alto bajo de una medida (nivel excesivo o muy bajo, presión baja, etc.)
- Fuera de rango de una medida

7.1.2.4. Curvas de Tendencias Históricas y en Tiempo Real

Para facilitar la gestión y explotación del canal, el usuario verá representadas las evoluciones de las variables características del canal tanto en su estado actual como en el pasado.

Para ello, cualquier variable del sistema podrá ser representada en curvas gráficas como función del tiempo.

El tiempo de refresco de la información de las curvas será el configurado en el programa de comunicaciones como tiempo de "polling".

Como medida de prevención ante la acumulación de datos de archivo histórico en el disco duro, el sistema deberá disponer de la opción de almacenar los datos del mes anterior y del mes en curso en

cd. El operador podrá consultar los datos de un mes anterior al mes pasado de igual forma que lo hace el disco duro, es decir, introduciendo la fecha, el número de terminal remoto y el tipo y el número de sedal.

Los datos almacenados en disco duro del mes en curso pasarán a ser datos del mes anterior en cambio de mes, borrándose los datos de hace dos meses. De cate modo se garantizará el vaciado ordenado del disco duro, puesto que el operador dispondrá de tiempo para guardar todo el archivo histórico del mes anterior en cd.

7.1.2.5. Ejecución de Órdenes

Todos los motores y bombas podrán manejarse desde los ordenadores del Centro de Control. Por ello, el operador seleccionará sobre un gráfico sinóptico el elemento y procederá a ejecutar la orden requerida, En la pantalla, la representación del elemento a accionar será diferente en función del estado en que se encuentre el mismo:

- Estado inicial
- Selección y espera de confirmación de la ejecución de la orden
- Orden ejecutada (cambio de estado del elemento telecontrolado)

7.1.2.6. Módulo de Procesamiento de Informes y Partes

Otra importante función que tiene este sistema de control y supervisión consiste en generar automáticamente informes y partes del funcionamiento y del estado de las instalaciones

Estos informes serán fácilmente configurables por los usuarios, para poder adaptarse a los requerimientos de los explotadores del Sistema.

Mediante este módulo, el usuario puede configurar el formato, tipo de datos a representar, intervalo de tiempo de la representación, etc., de manera que automáticamente se generen los informes de explotación y funcionamiento del sistema. El ordenador tendrá la opción de imprimir diferentes tipos de informe en la impresora.

7.1.2.7. Módulo de Control de Acceso y Seguridad

Puesto que desde la aplicación SCADA instalada en el Centro de Control se puede actuar sobre todo el canal, y acceder a las bases de datos de configuración, es fundamental que se implemente un adecuado sistema de control de acceso y seguridad en la aplicación.

Teniendo en cuenta que puede haber distinto personal como usuario de la aplicación, y que no es necesario que todos los usuarios tengan acceso a todas las aplicaciones o posibilidades de actuación se incluye un módulo que gestione los privilegios que tiene cada operador del sistema.

Con este módulo el supervisor es capaz de configurar la base de datos en la cual se recogen los usuarios que tienen acceso al sistema, niveles de prioridad a los distintos módulos y gestión global del

acceso ya contraseña, para que ningún usuario pueda entrar en módulos o ejecutar acciones para las que no esté autorizada.

7.1.2.8. Módulo de Back-Up

En un sistema de supervisión y control, es muy importante disponer de un sistema de seguridad que garantice el control de las instalaciones en caso de fallo del PC encargado de la tarea.
Es por ello que debe existir un soporte de seguridad que garantice dicha fiabilidad. Para ello se deberá establecer las rutinas de back-up necesarias para preservas la integridad de los datos y el acceso al sistema.