



UNIVERSITAT  
POLITÈCNICA  
DE VALÈNCIA

## **TESIS DOCTORAL**

PROCESOS DE INTERCAMBIO DE MATERIALES  
EN LA INTERFASE AGUA-SEDIMENTO EN  
PISCIFACTORÍAS MARINAS EN JAULAS  
FLOTANTES

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Valencia, julio de 2013



*Nada en el mundo sustituye a la constancia. El talento no la sustituye pues nada es tan corriente como los inteligentes frustrados. El genio tampoco, ya que resulta tópicamente el caso de los genios ignorados. Ni siquiera la educación sustituye a la constancia, pues el mundo está lleno de fracasados bien educados. Solamente la constancia y la decisión lo consiguen todo.*

*Calvin Coolidge*

*Si añades un poco a lo poco y lo haces así con frecuencia, pronto llegará a ser mucho.*

*Hesíodo (S. VIII AC)*

*La dicha de la vida consiste en tener siempre algo que hacer, alguien a quien amar y alguna cosa que esperar.*

*Thomas Chalmers (1780-1847)*

*A los que me han querido  
y me quieren*

## ***AGRADECIMIENTOS***

*Después de 6 años realizando esta tesis doctoral, por fin la he terminado y debo agradecer la participación de personas e instituciones que han facilitado que este trabajo tan laborioso y lleno de dificultades haya llegado a un feliz término. Por ello, es para mí un verdadero placer utilizar este espacio para mostrar mis más sinceros agradecimientos.*

*Debo agradecer de manera especial a Miguel Rodilla y Silvia Falco por aceptarme para realizar esta tesis doctoral bajo sus direcciones. El apoyo y confianza en mi trabajo y la capacidad para guiar mis ideas han sido un aporte invaluable, no solamente en el desarrollo de esta tesis, sino también en mi formación como investigadora. Les agradezco también el haberme facilitado siempre los medios suficientes para llevar a cabo todas las actividades propuestas durante el desarrollo de esta tesis y destacar, que nuestras acaloradas discusiones han redundado benéficamente siempre tanto a nivel científico como personal. Quiero expresar también mi más sincero agradecimiento a Miguel Jover por sus siempre atentas y rápidas respuestas a las diferentes inquietudes surgidas durante el período de realización de esta tesis.*

*Para mis compañeros de grupo, doctorandos/as y becarios, tengo sólo palabras de agradecimiento, especialmente por aquellos momentos en los que pude ser inferior a sus expectativas y por los momentos de charla en que siempre me ayudaron a ver los problemas con un enfoque positivo. Quiero expresar mi agradecimiento especial a Isabel Gadea y Javier Sospedra por haber estado a mi lado, haberme ayudado cuando los he necesitado y por haber compartido momentos difíciles y mostrar su compañerismo y amistad. Gracias también a Julia por haberme prestado su colaboración cuando la he necesitado. Y a mis otros compañeros de despacho Virginia, Rafa y Juan Diego por brindarme sus conocimientos, experiencias profesionales y amistad. También debo agradecer a los alumnos de Ciencias Ambientales Yolanda, Jusep y Puche y especialmente a Pita, quien realizó su proyecto fin de carrera conmigo y a otras personas que también han colaborado en los duros muestreos en mar, como Estanis, Matias y Toni. Sus colaboraciones también han sido importante para que esta tesis llegara a buen puerto, mil y mil gracias! También debo mis agradecimientos a los técnicos de la Escuela Politécnica Superior de Gandía, Alicia, Alex, Javier, Carmen y Ángela por sus servicios prestados.*

*A todos amigos y conocidos.*

*Agradezco de manera especial a la Caja del Mediterráneo (CAM) por haberme concedido una beca predoctoral y permitir de esa manera desarrollar esta tesis doctoral. Quiero extender un sincero agradecimiento a Vicente Sanchis, que es la persona de la CAM con la que he tenido el gusto de contactar para los diferentes asuntos pertinentes, por su amabilidad y atentas y rápidas respuestas a las diferentes cuestiones surgidas durante el periodo de mi beca.*

*También quiero agradecer a Antonio Asunción de la empresa Acuigroup Maremar por las facilidades y apoyo en la realización de esta tesis.*

*Y, por supuesto, el agradecimiento más profundo y sentido va para mi familia. A mis padres, M<sup>a</sup> Dolores y Julio, una vida no sería suficiente para agradecerles todo lo que han hecho por mí. Gracias por el amor, el apoyo, los consejos y por enseñarme el valor de las cosas. A mi abuela Lola por todo lo que ha hecho por la familia y por estar todavía ahí y a mi difunto abuelo Jaime por lo especial que me hacía sentir y la alegría que me transmitió en su día y perdura en mí. A mis hermanas Loli y Yolanda, por ejemplos de valentía, superación, inteligencia y generosidad. A mi apreciada pareja, Vicente Botella, quien desde el inicio ha sido una fuente de motivación, gracias por tu amor, paciencia, comprensión*

*y por apoyarme en los momentos más difíciles. Te amo. Y por supuesto a mi hijo Vicente, que me ha enseñado lo que es realmente importante y me ha dado felicidad y fuerza constante desde que nació...por ellos y para ellos!*



*Gracias por todo*

*Gracias a todos.*

## RESUMEN

La sostenibilidad de la producción piscícola marina en jaulas flotantes requiere, entre otras cuestiones, de la mejora de la gestión ambiental y de la reducción de los impactos ambientales que la actividad genera. Para ello, es importante tener un buen conocimiento de los efectos que el enriquecimiento orgánico tiene en el ecosistema y conocer los procesos de recuperación cuando la actividad cesa.

Este estudio se llevó a cabo en una piscifactoría de dorada (*Sparus aurata*) ubicada en la costa del Mediterráneo español. Se estimaron los flujos bentónicos *in situ* de oxígeno y nutrientes, se midieron parámetros de la columna de agua y de los sedimentos y se determinó la macrofauna bentónica, bajo una jaula ubicada en el centro de la instalación y en una estación control. Se realizaron 8 campañas de muestreo abarcando dos fases: la de funcionamiento y tras el cierre definitivo de la actividad acuícola.

En la fase de funcionamiento se observó que en los sedimentos bajo las jaulas se generó una acumulación de materia orgánica y fósforo total y los sedimentos se volvieron más reductores, mostrando niveles máximos en verano. La macrofauna presentó una menor riqueza específica y una mayor abundancia respecto del control siendo el poliqueto *Capitella capitata* la especie dominante bajo las jaulas. Debido a la mineralización de la materia orgánica, los sedimentos bajo las jaulas funcionaron como un sumidero de oxígeno disuelto, y también como una fuente, hacia la columna de agua, de nitrógeno inorgánico disuelto y fosfato. Sin embargo, los flujos de sílice no parecieron verse afectados por la actividad de la piscifactoría. En la columna de agua se produjo una disminución de la concentración de oxígeno disuelto y un aumento de nitrógeno inorgánico disuelto y fosfato, llegando a modificarse, en ocasiones, la relación estequiométrica de los nutrientes y el nutriente limitante para la producción primaria. A pesar de haber encontrado mayores concentraciones de nutrientes en las inmediaciones de las jaulas, no se apreció una correspondencia con los niveles de clorofila *a* probablemente

debido al papel que juega el biofouling marino de las estructuras sumergidas de las instalaciones piscícolas en el aprovechamiento de nutrientes, partículas y fitoplancton.

Tras el cese definitivo de la actividad, la recuperación de la zona se manifestó, a distintas escalas temporales, mediante síntomas de recuperación parcial de los diversos parámetros abióticos y bióticos del medio. El primer parámetro en recuperarse fueron los flujos bentónicos de amonio, seguido de los de fosfato y oxígeno disuelto y el porcentaje de materia orgánica en los sedimentos, los cuales a los 3 meses del cese de la piscifactoría ya mostraron niveles semejantes a los medidos en la estación control. A los 9 meses del cese también se vieron recuperados el resto de parámetros abióticos de los sedimentos perturbados por la actividad, tales como el porcentaje de la fracción gruesa, la concentración de fósforo total y las medidas del potencial redox. La recuperación de la macrofauna fue más lenta que la de los parámetros abióticos. A los 3 meses del cese de la actividad, la abundancia de *Capitella capitata* ya había descendido drásticamente, en el área que fue afectada por la piscifactoría, pero no se observó hasta el muestro después de 2 años del cierre de la piscifactoría niveles semejantes de riqueza específica en las dos zonas muestreadas.

## ABSTRACT

Sustainability of fish farm production in floating cages requires, between other things, the improvement of environmental management and the reduction of environmental impact that this activity generates. For that reason, it is important to have a good understanding of the effects that organic enrichment has on the ecosystem and understand the recovery processes when the activity ceases.

This study was carried out in a gilthead seabream (*Sparus aurata*) fish farm off the Mediterranean coast of Spain. Measurements were taken under the central cage of the fish farm and in a control station. These included benthic fluxes of oxygen and nutrients *in situ*, different parameters in water column and sediments, and benthic macrofauna. Eight sampling campaigns were carried out covering two phases: one while functioning and the second after the permanent closure of the fish farm.

During the functioning phase, organic matter and total phosphorus were accumulated in the sediment under the cages and the sediment became more reductant, reaching the maximum levels in summer. Macrofauna had a less specific richness and a higher abundance, due to the dominant species *Capitella capitata*. Sediment under the cages functioned as a sink of dissolved oxygen and as a source of dissolved inorganic nitrogen and phosphate due to the mineralization of organic matter. However, silica fluxes did not seem to be affected by the fish farm activity. In the water column, there was a reduction of the dissolved oxygen concentration and an increase of dissolved inorganic nitrogen and phosphate, modifying, on occasions, the stoichiometric ratios between the nutrients, and the limiting nutrient for primary production. Despite having found higher concentrations of nutrients around the cages, there was not an increase in the levels of chlorophyll *a*, probably due to the utilization of nutrients, particles and phytoplankton by marine biofouling attached to the submerged structures of the fish farm.

Recovery was made after the permanent cessation of the activity, through partial recovery signs of the different abiotic and biotic parameters at different time scales. The benthic flux of ammonium was the first parameter to recover, followed by benthic fluxes of phosphate and dissolved oxygen and the % organic matter in the sediments, which 3 months after the cessation of the fish farm already showed levels similar to those in the control station. At 9 months after the cessation, the other abiotic parameters of the sediments disturbed by the activity had recovered, such as the % coarse fraction, total phosphorus concentrations, and redox potential measurements. The recovery of the macrofauna was slower than the abiotic parameters. 3 months after the cessation, the abundance of *Capitella capitata* had descended drastically in the area that was affected by the fish farm, but similar levels of specific richness were not observed between the two sampled zones until 2 years after the cessation of the fish farm.

## RESUM

La sostenibilitat de la producció piscícola marina en gàbies flotants requereix, entre altres qüestions, de la millora de la gestió ambiental i de la reducció dels impactes ambientals que l'activitat genera. Per això, es important tenir un bon coneixement dels efectes que l'enriquiment orgànic té en l'ecosistema i conèixer els processos de recuperació quan l'activitat cessa.

Aquest estudi es dugué a terme en una piscifactoria d'orades (*Sparus aurata*) ubicada en la costa del Mediterrani espanyol. S'estimaren els fluxos bentònics *in situ* d'oxigen i nutrients, es mesuraren paràmetres de la columna d'aigua i del sediment i es determinà la macrofauna bentònica, davall una gàbia ubicada en el centre de la instal·lació i en una estació control. Es realitzaren 8 campanyes de mostreig incloent dos fases: la de funcionament i després del tancament definitiu de l'activitat aquícola.

En la fase de funcionament s'observà que en els sediments davall les gàbies es generà una acumulació de matèria orgànica i fòsfor total i els sediments es tornaren més reductors, mostrant nivells màxims a l'estiu. La macrofauna presentà una menor riquesa específica i una major abundància respecte del control sent el poliquet *Capitella capitata* l'espècie dominant davall les gàbies. Degut a la mineralització de la matèria orgànica, els sediments davall les gàbies funcionaren com un embornal d'oxigen dissolt, i també com una font, cap a la columna d'aigua, de nitrogen inorgànic dissolt i fòsfor. No obstant, els fluxos de sílice no paregueren vore afectats per l'activitat de la piscifactoria. En la columna d'aigua es produí una disminució de la concentració d'oxigen dissolt i un augment de nitrogen inorgànic dissolt i fòsfor, arribant a modificar-se, en ocasions, la relació estequiomètrica dels nutrients i el nutrient limitant per a la producció primària. Tot i haver trobat majors concentracions de nutrients en les immediacions de les gàbies, no es va apreciar una correspondència amb els nivells de clorofil·la *a* probablement degut al paper que juga el biofouling marí de les estructures submergides de

les instal·lacions piscícoles en l'aprofitament de nutrients, partícules i fitoplàncton.

Després del cessament definitiu de l'activitat, la recuperació de la zona es manifestà, a distintes escales temporals, mitjançant símptomes de recuperació parcial dels diversos paràmetres abiòtics i biòtics del medi. El primer paràmetre en recuperar-se foren els fluxos bentònics d'amoni, seguit del fosfat i oxigen dissolt i el percentatge de matèria orgànica en els sediments, els quals als 3 mesos del cessament de la piscifactoria ja mostraren nivells semblants als mesurats en l'estació control. Als 9 mesos del cessament també es veieren recuperats la resta de paràmetres abiòtics dels sediments pertorbats per l'activitat, tals com el percentatge de la fracció grossa, la concentració de fòsfor total i les mesures del potencial redox. La recuperació de la macrofauna fou més lenta que la dels paràmetres abiòtics. Als 3 mesos del cessament de l'activitat, l'abundància de *Capitella capitata* ja havia minvat dràsticament, en l'àrea que fou afectada per la piscifactoria, però no s'observà fins al mostreig després de 2 anys del tancament de la piscifactoria nivells semblants de riquesa específica en les dues zones de mostrejades.

<b>CAPÍTULO 1:INTRODUCCIÓN GENERAL. ....</b>	<b>1</b>
1.1.- ESTADO ACTUAL DE LA ACUICULTURA .....	2
1.2.- PRODUCCION DE PISCICULTURA EN LA UNIÓN EUROPEA.....	3
1.2.1.- <i>Producción de dorada</i> .....	4
1.3.- IMPACTOS AMBIENTALES DE LA ACUICULTURA MARINA .....	5
1.4.- EFECTOS DE LOS APORTES ORGÁNICOS Y NUTRIENTES DE LA PISCICULTURA MARINA7	
1.5.- FLUJOS EN LA INTERFASE AGUA-SEDIMENTO. ....	9
1.6.- BIBLIOGRAFÍA.....	11
<b>CAPÍTULO 2:OBJETIVOS. ....</b>	<b>17</b>
2.1.- OBJETIVO GENERAL Y ESPECÍFICOS .....	18
<b>CAPÍTULO 3:PLAN EXPERIMENTAL Y ORGANIZACIÓN DE LA MEMORIA. ...</b>	<b>19</b>
3.1.- PLAN EXPERIMENTAL .....	20
3.2.- ORGANIZACIÓN DE LA MEMORIA .....	25
3.3.- BIBLIOGRAFÍA.....	27
<b>CAPÍTULO 4.....</b>	<b>29</b>
4.1.- INTRODUCTION .....	31
4.2.- MATERIALS AND METHODS .....	33
4.2.1.- <i>Study area</i> .....	33
4.2.2.- <i>Sampling and sampling points</i> .....	33
4.2.3.- <i>Benthic chamber experiments and analysis</i> .....	34
4.2.4.- <i>Bottom water and sediment</i> .....	35
4.2.5.- <i>Parameters considered and analytical methods</i> .....	35
4.3.- RESULTS.....	36
4.3.1.- <i>Bottom water and sediment</i> .....	36
4.3.2.- <i>Benthic fluxes</i> .....	38
4.4.- DISCUSSION .....	40
4.4.1.- <i>Bottom water and sediment</i> .....	40
4.4.2.- <i>Benthic fluxes</i> .....	42
4.5.- CONCLUSIONS .....	47
4.6.- ACKNOWLEDGMENTS.....	48
4.7.- REFERENCES.....	49



<b>CAPÍTULO 5.....</b>	<b>53</b>
5.1.- INTRODUCTION.....	54
5.2.- MATERIALS AND METHODS.....	57
5.3.- RESULTS.....	60
5.3.1.- <i>Water column</i> .....	60
5.3.2.- <i>Sediment</i> .....	64
5.3.3.- <i>Benthic organisms</i> .....	65
5.4.- DISCUSSION.....	71
5.4.1.- <i>Water column</i> .....	71
5.4.2.- <i>Sediment</i> .....	77
5.4.3.- <i>Benthic organisms</i> .....	79
5.5.- CONCLUSION.....	81
5.6.- ACKNOWLEDGEMENTS.....	82
5.7.- REFERENCES.....	82
<b>CAPÍTULO 6.....</b>	<b>89</b>
6.1.- INTRODUCTION.....	90
6.2.- MATERIALS AND METHODS.....	93
6.3.- RESULTS.....	96
6.3.1.- <i>Sediment physico-chemical variables</i> .....	96
6.3.2.- <i>Benthic fluxes</i> .....	99
6.3.3.- <i>Benthic organisms</i> .....	101
6.3.4.- <i>Relationship between the environmental variables and the distribution of benthic organisms</i> .....	104
6.4.- DISCUSSION.....	106
6.5.- CONCLUSIONS.....	115
6.6.- ACKNOWLEDGMENTS.....	115
6.7.- REFERENCES.....	116
<b>CAPÍTULO 7:DISCUSIÓN GENERAL.....</b>	<b>123</b>
7.1.- FASE 1. EFECTOS CAUSADOS POR LA PRODUCCIÓN DE DORADA, EN JAULAS FLOTANTES EN MAR ABIERTO, EN EL MEDITERRÁNEO OCCIDENTAL SOBRE LOS SEDIMENTOS, LA ESTRUCTURA DE LA COMUNIDAD BENTÓNICA, EL METABOLISMO BENTÓNICO Y LA CALIDAD DEL AGUA. ....	124
7.1.1.- <i>Efectos sobre parámetros físico-químicos de los sedimentos</i> ..	124
7.1.2.- <i>Efectos sobre la macrofauna</i> .....	126
7.1.3.- <i>Efectos sobre los flujos bentónicos</i> .....	128

7.1.4.- Efectos sobre la columna de agua .....	132
7.1.5.- Implicaciones de los flujos bentónicos en la columna de agua. .....	136
7.2.- FASE 2. RECUPERACIÓN DE UNA ZONA QUE FUE AFECTADA POR LA PRODUCCIÓN DE DORADA EN EL MAR MEDITERRÁNEO TRAS EL CESE DEFINITIVO DE LA ACTIVIDAD. ....	145
7.2.1.- Recuperación de los parámetros físico-químicos de los sedimentos.....	145
7.2.2.- Recuperación de la macrofauna .....	147
7.2.3.- Recuperación de los flujos bentónicos .....	149
7.3.- RELACIÓN ENTRE LAS VARIABLES AMBIENTALES DE LOS SEDIMENTOS Y LA MACROFAUNA INCLUYENDO FASE DE FUNCIONAMIENTO Y RECUPERACIÓN .....	152
7.4.- BIBLIOGRAFÍA .....	155
<b>CAPÍTULO 8: CONCLUSIONES .....</b>	<b>159</b>
8.1.- FASE DE FUNCIONAMIENTO DE LA PISCIFACTORÍA .....	160
8.2.- FASE DESPUÉS DEL CESE DE LA PISCIFACTORÍA .....	161

# Capítulo 1: Introducción general

### 1.1.- Estado actual de la acuicultura

En las últimas décadas la cantidad de pescado capturado en los océanos ha disminuido (FAO, 2012; Pauly, 2013), la sobreexplotación se ha presentado generalmente como la principal causa de este descenso, aunque existen evidencias de que las capturas mundiales de pesca han estado afectadas por la tendencia creciente al calentamiento global de las últimas décadas (Cheung et al., 2013). El futuro no es halagüeño puesto que se espera que además del calentamiento; la acidificación y la desoxigenación de los mares y océanos afecten significativamente a los ecosistemas marinos y a la pesca (Gruber, 2011). Este escenario, en el que serán muy patentes las consecuencias negativas sociales y económicas de la reducción en la producción pesquera, puede consolidar la importancia socioeconómica y ambiental de la acuicultura marina ya que ésta se ha multiplicado por 12 en las últimas tres décadas (figura 1.1), con una tasa de crecimiento medio anual de 8.8% (FAO, 2012).

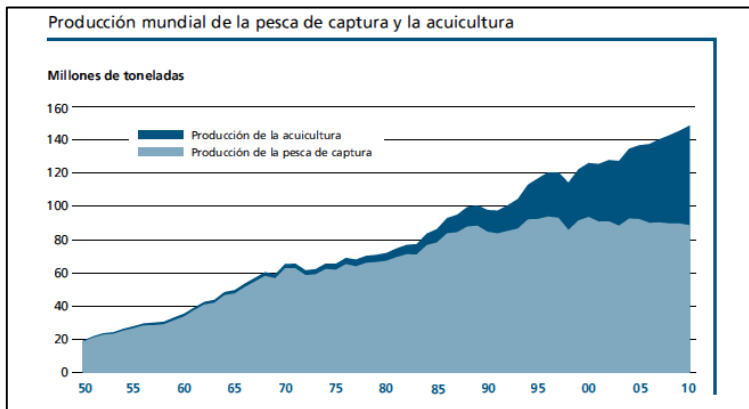


Figura 1.1. Evolución de la producción (pesca y acuicultura) en el mundo en el periodo 1950-2010 (FAO, 2012).

La producción mundial de la pesca de captura se ha estabilizado en la última década, estando cerca de la máxima productividad del ecosistema (NRC, 2006) por lo que no se podría aumentar significativamente en el futuro siendo previsible que se reduzca si no se gestiona adecuadamente. La

acuicultura ha sido el motor de crecimiento constante de la producción pesquera total (figura 1.1), siendo la contribución a la producción mundial de especies comestibles del 47% en 2010 en comparación con el 9% en 1980 (FAO, 2012).

En un escenario donde se espera que la población mundial pase de los actuales 6800 millones de personas a cerca de 9000 millones en 2050 (García y Rosenberg, 2010), el suministro de alimentos y la seguridad alimentaria es de gran preocupación social internacional. Por tanto, a pesar de que la tasa de crecimiento de la acuicultura ha empezado a descender, pasando de un incremento anual del 11.8% en el periodo 1985-1994 al 7.1% en el decenio siguiente, la acuicultura sigue siendo una apuesta de futuro para cubrir la demanda de alimentos de la humanidad a lo largo del siglo XXI (Duarte et al., 2009).

Al igual que cualquier otra industria, la acuicultura presenta impactos ambientales y sociales potencialmente adversos (Porrello et al., 2005). La ubicación de las actividades acuícolas marinas en el litoral suele generar un conflicto con otros usuarios y actividades económicas como son: el turismo, la pesca artesanal, las actividades portuarias, tanto comerciales como recreativas, etc. La evitación de conflictos y la minimización de impactos serán claves para el éxito futuro de la acuicultura marina.

## **1.2.- Producción de piscicultura en la Unión Europea**

La producción de pescado de acuicultura en la Unión Europea fue de 636 000 t en 2010. La trucha arco iris fue la principal especie de crianza (30.3% del total de pescados de acuicultura), la segunda especie fue el salmón atlántico (26.9% del total) y la tercera la dorada (13.8% del total). Por otra parte, las 10 principales especies de peces criadas suponen el 94.4% del total de todas las especies de pescado producidas (APROMAR, 2012).

Los estados miembros de la Unión Europea con una mayor producción de pescado de acuicultura en 2010 fueron: Reino Unido (26.7% del total en peso), Grecia (el 14.3%) y España (el 9.4%) (APROMAR, 2012).

La dorada (*Sparus aurata*), la lubina (*Dicentrarchus labrax*), el rodaballo (*Psetta máxima*) y la corvina (*Argyrosomus regius*) son las especies más relevantes de peces marinos de crianza producidas en los países europeos meridionales (APROMAR, 2012).

### 1.2.1.- Producción de dorada

Existe producción de dorada de acuicultura en 19 países diferentes. Los principales productores se encuentran en el Mediterráneo como son Grecia, con aproximadamente 60 000 t (que representa el 39.6% del total), Turquía con 34 000 t (22.5%) y España con 16 930 t (11.2%) (APROMAR, 2012).

La máxima producción mundial de dorada se produjo en 2008, con 178 554 t. En los últimos años debido a la crisis económica la producción ha disminuido, especialmente en Grecia. A pesar de estas dificultades el sector sigue siendo potente y dinámico y la producción acuícola total de dorada en 2012 fue de 172 830 t, según estadísticas de la Federación Europea de Productores de Acuicultura (FEAP) y APROMAR. La cantidad de dorada procedente de la pesca extractiva permanece a medio plazo prácticamente constante, fluctuando entre 7000 y 8500 t anuales (APROMAR, 2012), mientras la dorada de crianza supone el 94.4% del total.

En España, la Comunidad Valenciana encabeza la producción de dorada de acuicultura (con el 40.8% del total), seguida por Murcia (20.5%), Canarias (19.2%), Andalucía (10.7%) y Cataluña (8.7%). La dorada de crianza en España supone el 95.5% del total producida (APROMAR, 2012).

En España el engorde de la dorada se inició en los sistemas extensivos de las marismas andaluzas, pero estos están limitados por la disponibilidad de grandes extensiones de terreno fácil de inundar. Por otra

parte, las instalaciones intensivas en estanques de hormigón suponen una gran inversión en obra civil e instalaciones hidráulicas y un elevado gasto en bombeo de agua, además de tener problemas en algunas zonas del litoral para encontrar suelo a un coste asequible en una ubicación adecuada (Merinero et al., 2005). Actualmente la fase de reproducción y preengorde de dorada se lleva a cabo en tierra en instalaciones intensivas. En cambio, las etapas finales de cría, generalmente se realizan en jaulas flotantes cerca de la costa (Mediterráneo e Islas Canarias), sistemas de producción intensiva que representan una menor inversión y que disminuyen considerablemente los costes de producción (García-García, 2001).

### **1.3.- Impactos ambientales de la acuicultura marina**

El rápido desarrollo de la acuicultura en las zonas costeras en los últimos 25 años ha causado gran preocupación, tanto a nivel público como científico, sobre sus potenciales impactos sobre el medio ambiente y la sostenibilidad de esta industria en expansión (Hargrave, 2005).

En Europa, las inquietudes por los impactos ambientales de la acuicultura han hecho que existan estudios desde el principio de su desarrollo (Alabaster, 1982; Braaten et al., 1983; Hall et al., 1990).

Los impactos potenciales de la acuicultura son muy diversos, yendo desde los aspectos estéticos y paisajísticos a los problemas de contaminación directa (O'Sullivan, 1992; Midlen y Redding, 1998). Entre los impactos más relevantes se incluyen la dispersión de la materia orgánica (restos de pienso y heces) y nutrientes, tanto en la columna de agua (Pitta et al., 2005; Maldonado et al., 2005) como en los sedimentos subyacentes (Karakassis et al., 1998; Porello et al., 2005; Aksu y Kocatas, 2007). La materia orgánica acumulada bajo las jaulas puede causar cambios importantes en la química de los sedimentos (Hargrave et al., 1997). La acuicultura también causa efectos sobre la fauna y flora: en general se produce una disminución de diversidad (Sanz-Lázaro y Marín, 2011), también puede afectar al plancton (La Rosa et

al., 2002; Neofitou y Klaoudatos, 2008) y a plantas superiores acuáticas, como praderas de *Posidonia oceánica* (Cancemi et al., 2003; Homer et al., 2008). Además se produce atracción de especies salvajes en los alrededores de las jaulas (Dempster et al., 2002; Sánchez et al., 2007) y se ven modificadas las estructuras de las comunidades macrobentónicas (Mazzola et al., 2000; Yucel-Gier et al., 2007). Por otra parte, hay otros impactos asociados a los productos químicos y antibióticos usados como son los efectos a las poblaciones salvajes y resistencia de cepas bacterianas (Chelossi et al., 2003). Además, hay otros impactos consecuencias de los escapes de los peces cultivados como: introducción de especies foráneas (Pérez et al., 2003), introducción potencial de enfermedades (Johansen et al., 2011), interacciones genéticas entre los peces que escapan y los salvajes (Youngson et al., 2001) y amenaza para las especies nativas al competir por el alimento y lugares para vivir.

Por otra parte los efectos ambientales y la extensión de tales alteraciones se relacionan con las características locales (capacidad de asimilación del medio, la topografía, el régimen de mareas, etc.) y con las técnicas de cría (Brooks y Mahnken, 2003).

Es esencial que se estudien los impactos ambientales que generan las explotaciones piscícolas en el medio marino y se evalúen los diferentes parámetros afectados. Si bien es información necesaria para el acuicultor, ya que está relacionada con la salud de los peces y por lo tanto con la rentabilidad de la explotación (Mantzavrakos et al., 2007), además es de vital importancia a nivel político para hacer una gestión correcta de esta industria y tomar decisiones adecuadas, como por ejemplo ver la capacidad de carga en las diferentes zonas aptas para esta actividad en lo referente a número y/o tamaño de instalaciones.

Por otra parte cuando cesa la actividad acuícola, los efectos generados en el medio ambiente pueden persistir periodos de tiempo variables dependiendo principalmente de las características hidrológicas de la



zona y tipo de sedimento (Pereira et al., 2004; Lin y Bailey-Brock, 2008). Además los procesos de recuperación son dependientes de la escala y pueden variar en función de la extensión del impacto (Whithlach et al., 1998; Villnäs et al., 2011). También los factores bióticos, como la composición de la comunidad y sus peculiaridades (dispersión, reclutamiento, ciclo de vida, etc.), y sus relaciones (competencia, depredación, etc.) influyen en los procesos de recuperación (Norkko et al., 2006). Por lo que estudiar el medio después de que cesa la actividad es importante y puede ser útil para predicciones de impacto futuro (Aguado et al., 2012) y para mejorar la gestión de este tipo de actividades en el litoral.

#### **1.4.- Efectos de los aportes orgánicos y nutrientes de la piscicultura marina**

La piscicultura marina intensiva en jaulas flotantes tiende a aportar un exceso de alimentos que al no ser ingerido, principalmente se deposita en el fondo marino. Además, los peces excretan heces y sustancias solubles de nitrógeno y fósforo. Esta disponibilidad de nutrientes y material particulado en suspensión, y sobre todo de nuevo sustrato duro, favorece el desarrollo de incrustaciones biológicas en las que dominan los suspensívoros y filtradores. Periódicamente se realizan limpiezas, para evitar el sobrepeso causado por estos organismos en las instalaciones, en las que finalmente el fouling queda depositado en el fondo. Por otro lado, en determinadas ocasiones, pueden acumularse en el sedimento cadáveres de peces procedentes de la propia instalación. Todos estos materiales orgánicos depositados bajo las jaulas pueden ser aprovechados como alimento por la fauna bentónica y demersal, resuspendidos como consecuencia de la hidrodinámica, enterrados por procesos de bioturbación y/o mineralizados por la acción bacteriana.

La acumulación de los materiales orgánicos en sedimentos bajo las jaulas tiende a crear un ambiente perturbado (Silvert y Sowles, 1996) siendo éste el impacto más evidente de las granjas de peces marinos (Karakassis et

al., 1998). Estos depósitos de materia orgánica provocan una reducción en los niveles de oxígeno que puede dar lugar a la anoxia, lo que modificaría la química de los sedimentos (Hargrave et al., 1997; Ferrón et al., 2009). El enriquecimiento de la materia orgánica por debajo de las jaulas marinas causa una mayor colonización bacteriana en el sedimento que a su vez genera una mayor mineralización de la materia orgánica. Además de intensificar los procesos de transferencia, se reduce la profundidad de penetración de oxígeno en el sedimento y se potencian rutas anaeróbicas de oxidación en los procesos diagenéticos (Ponce, 2002) y estimulan la proliferación de bacterias anaerobias reductoras de sulfato con la consiguiente producción de sulfuros (Holmer y Kristensen, 1992, Kristensen, 2000). Los sedimentos bajo las piscifactorías también se caracterizan por valores negativos de potencial redox y altos contenidos en carbono, fósforo y nitrógeno (Holby y Hall, 1991; Karakassis et al., 1999; Pearson y Black, 2001). Las descargas de residuos sólidos también afectan a la composición y abundancia de las bacterias endémicas y de las poblaciones de fauna y flora. Debido a la alteración física del fondo marino bajo las jaulas (cambios en la distribución del tamaño de grano, de la porosidad, etc.), así como la alteración química (hipoxia, anoxia, pH, sulfuros, niveles de nutrientes en el agua intersticial) y de la composición biológica de los sedimentos, la estructura de las comunidades bentónicas existentes a menudo se ve modificada (Vezzulli et al., 2002; Yucel-Gier et al., 2007).

La tasa de acumulación de materia orgánica y posterior resuspensión tienen una relación directa con las velocidades de la corriente, altas velocidades producen una mayor distribución espacial y por consiguiente disminuye el enriquecimiento orgánico (Black y McDougall, 2002). Además, altas velocidades de la corriente no sólo reduce la acumulación de residuos, sino que también aumenta el suministro de oxígeno, lo que facilita la descomposición aeróbica de la materia orgánica (Yokohama, 2003).

Por otra parte, elevadas concentraciones de sólidos en suspensión pueden reducir la penetración de la luz solar en la columna de agua, alterando la actividad fotosintética y afectando a los macrófitos y fanerógamas. La difusión de estas partículas y los efluentes disueltos está influenciada por los movimientos del agua. Estos, junto con la altura de la columna de agua entre la parte inferior de la jaula y el sedimento por debajo, tienen una influencia fundamental sobre el impacto ambiental del cultivo en jaulas (Porrello et al., 2005).

El aumento de nutrientes inorgánicos solubles (nitrógeno y fósforo) procedentes de la excreción de los peces como de la mineralización de los compuestos orgánicos en el entorno de instalaciones acuícolas (Demirak et al., 2006; Aksu y Kocatas, 2007), estimula el crecimiento del fitoplancton pudiendo llegar a la eutrofización en la columna de agua (FAO 1992; Gowen, 1994). Sin embargo hay estudios, que a pesar de haber encontrado aumento de nutrientes en las aguas de las piscifactorías no encontraron diferencias significativas en la concentración de clorofila *a* entre las granjas y los controles (Pitta et al., 1999; La Rosa et al., 2002; Kaymakci et al., 2010).

### **1.5.- Flujos en la interfase agua-sedimento.**

Los sedimentos tienen un papel fundamental en los ciclos biogeoquímicos de muchos elementos por tener la gran capacidad de almacenar materia orgánica, nutrientes y contaminantes tóxicos (Middelburg y Van der Nat, 1993; Jahnke, 2005). En áreas sujetas a contaminación, como las afectadas por la acuicultura marina, la calidad del sedimento es perturbada por el ingreso de grandes cantidades de materia orgánica, parte de la cual es remineralizada y retorna a la columna de agua bajo formas disponibles para los productores primarios (Hall et al., 1992). El sedimento se transforma en una fuente interna de nutrientes, favoreciendo la eutrofización. En aguas poco profundas, la interfase agua-sedimento tiene una importancia esencial en los procesos de remineralización y flujos de nutrientes a través de la misma

(Niencheski y Jahnke, 2002). La degradación de materia orgánica en los primeros centímetros del sedimento marino, es un proceso principalmente microbiológico que involucra la mineralización propiamente dicha y la producción de biomasa bacteriana (Talin et al., 2003). Requiere de oxígeno disuelto (o de otros oxidantes cuando éste ya ha sido consumido), que es aportado por la columna de agua; se produce una importante carga de nutrientes que enriquece el agua intersticial y difunde hacia la columna de agua por gradientes de concentración. Dichos intercambios en la interfase agua-sedimento se denominan “flujos bentónicos”, y su magnitud es influenciada, además de por la cantidad de materia orgánica, por el tipo de sedimento, la turbulencia del agua, la bioturbación (movimiento del sedimento realizado por invertebrados bentónicos) y el aporte externo desde aguas subterráneas (Wassmann y Olli, 2004.). Bajo determinadas condiciones (coexistencia de microsítios óxicos y anóxicos en el sedimento), la eutrofización puede regularse a sí misma por exportación hacia la atmósfera de formas gaseosas de nitrógeno ( $N_2$  y  $N_2O$ ), principal regulador del ciclo de la materia orgánica en la mayoría de los sistemas marinos costeros (desnitrificación) (Christensen et al., 2000).

Los flujos bentónicos se pueden estudiar *ex situ*, es decir fuera de su ambiente natural, mediante la incubación de muestras de sedimento bajo condiciones controladas de laboratorio (Christensen et al., 2000; Berelson et al., 2003; Webb y Eyre, 2004) e *in situ*, es decir en el propio lugar, mediante cámaras bentónicas (Jahnke, 1990; Huettel y Rusch, 2000; Niencheski y Jahnke, 2002). Las cámaras bentónicas encierran un área conocida de la superficie del sedimento con un volumen determinado de agua de fondo que está en contacto con el mismo. Mediante la variación en la concentración de solutos en el agua, que está en contacto con el sedimento, en función del tiempo se estiman los flujos de solutos desde o hacia el sedimento (Viollier et al., 2003). Estos cálculos se basan en 4 asunciones:

1. condiciones estacionarias son válidas durante el periodo de incubación *in situ* con respecto al intercambio de soluto (ej. con respecto a gradientes de concentración dentro del sedimento y entre el sedimento y la capa de agua del fondo)
2. las tasas de los procesos biogeoquímicos en la columna de agua del fondo son despreciables comparadas con aquellas dentro del sedimento a menos que se midan de forma separada y se tomen en cuenta en el cálculo del flujo final (una forma de descontar los procesos en la columna de agua es poniendo una cámara con un plástico en el fondo)
3. el régimen hidrodinámico dentro de la cámara no altera el intercambio de solutos a través de la interfase agua-sedimento cuando se compara a condiciones naturales
4. el tamaño del muestreo/ medidas de áreas es representativo de un área mayor del fondo del mar.

Hay muy pocos estudios a nivel mundial sobre flujos bentónicos bajo piscifactorías tanto *in situ* (Hall et al., 1990, 1992; Holby y Hall, 1991, 1994; Freitas et al., 2008; Ferrón et al., 2009) como *ex situ* (Heilskov y Holmer, 2001; Nizzoli et al., 2007), existiendo en el Mediterráneo Occidental sólo estudios *ex situ* (Belias et al., 2007).

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# Capítulo 2: Objetivos

### 2.1.- Objetivo general y específicos

El objetivo general de esta tesis es cuantificar los flujos de oxígeno y nutrientes que se producen en la interfase agua-sedimento, mediante experimentos “*in situ*” de incubación con cámaras bentónicas, en una instalación de jaulas flotantes en mar abierto para el engorde de dorada (*Sparus aurata*) durante y tras el cese de su funcionamiento. Pero además se plantean una serie objetivos específicos:

- Analizar los efectos causados por la producción de dorada sobre la calidad del agua, a lo largo de un ciclo anual, mediante el análisis de parámetros físico-químicos y biológicos con una alta resolución vertical.
- Analizar los efectos causados por la producción de dorada sobre los sedimentos, a lo largo de un ciclo anual, mediante parámetros físico-químicos y analizando la estructura de la comunidad bentónica en términos de riqueza y densidad de especies.
- Estudiar la recuperación de la zona que fue afectada por una piscifactoría de dorada tras el cese definitivo de su actividad.

# Capítulo 3: Plan experimental y organización de la memoria

### 3.1.- Plan experimental

La zona de estudio se localizó en la costa occidental del Mediterráneo, España, concretamente en Burriana, donde se ubicaba una piscifactoría de dorada (*Sparus aurata*) (figura 3.1). La instalación se encontraba en mar abierto a unos 2 km de la costa y a una profundidad de 19 m. Constaba de 15 jaulas de engorde de 25 metros de diámetro (figura 3.2). La empresa empezó su actividad en el año 1999 y cesó la producción en junio 2009. En los últimos años de funcionamiento de la piscifactoría la producción fue de unas 500 t de dorada al año.

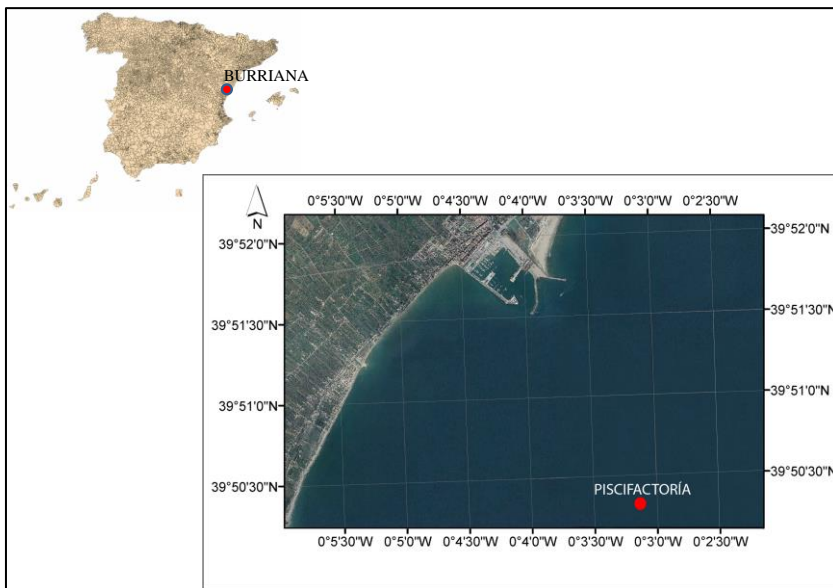


Figura 3.1: Situación de la zona de estudio



Figura 3.2: Jaulas de engorde en mar abierto

Este estudio se dividió en dos fases: fase 1 o de funcionamiento de la piscifactoría, la cual abarcó un ciclo anual y fase 2 o tras el cierre definitivo de la actividad acuícola. En dos lugares se realizaron las medidas en ambas fases: uno situado bajo una jaula ubicada en el centro de la instalación (I) ( $0^{\circ} 3' 11.101''$  W;  $39^{\circ} 50' 19.6243''$  N), y otro de referencia o control (C) ( $0^{\circ} 3' 6.1871''$  W;  $39^{\circ} 50' 21.4126''$  N), localizado a 130 m al nordeste de la piscifactoría, en dirección contraria a la velocidad de corriente dominante de la zona.

En la fase de funcionamiento se realizaron 4 campañas de muestreo, la primera se realizó en otoño (25/11/2008 en I y 01/12/2008 en C), la segunda en invierno (23/02/2009 en I y 28/02/2009 en C), la tercera en primavera (28/04/2009 en I y 02/05/2009 en C) y la cuarta campaña se realizó a principios de verano (17/06/2009 en I y 19/06/2009 en C).

En la fase tras el cese de la actividad acuícola se realizaron 4 campañas de muestreo más: la primera 1 mes después del cese (28/07/2009 en I y 30/07/2009 en C), la segunda 3 meses después del cese (22/09/2009 en I y 24/09/2009 en C), la tercera 9 meses después del cese (07/04/2010 en I y 09/04/2010 en C) y la última 2 años después del cese (28/07/2011 en I y C).

Se midieron flujos *in situ*, con cámaras bentónica (figura 3.3), de oxígeno disuelto y nutrientes (amonio, nitritos, nitritos más nitratos, fósforo soluble reactivo y ácido ortosilícico) en todas las campañas de muestreo menos en la última tras el cese de la actividad. Se utilizaron dos tipos de cámaras bentónicas, transparentes y opacas, para analizar el posible efecto de la producción primaria en el bentos. En cada muestreo se instalaron 6 cámaras; tres transparentes y tres opacas para intentar minimizar los efectos de heterogeneidad espacial. Se utilizaron cámaras bentónicas similares a las utilizadas por Freitas et al. (2008). Las cámaras consistieron en una semi-esfera de metacrilato de 40 cm de diámetro, con un volumen aproximado de 16.7 l, sobre un área de sedimento de 0.125 m<sup>2</sup>. Las cámaras disponían de un agitador manual para minimizar los gradientes de concentración (Niencheski y Jahnke, 2002). El proceso de homogenización fue adecuado para asegurar que la velocidad de mezcla (~ 60 rpm) fuera suficiente para homogeneizar el agua sin alterar la superficie del sedimento. Las cámaras se colocaron en el sedimento manualmente en inmersión con escafandra autónoma, siendo el tiempo de incubación total de 6 h, generalmente entre las 9:00 y las 15:00 h. La toma de muestras de agua del interior de las cámaras se hizo cada 2 h utilizando jeringuillas de plástico de 60 ml a través de un tubo de silicona que contenía una válvula que controlaba la abertura.





Figura 3.3: Cámaras bentónicas

También se tomaron en cada campaña y muestreo (instalación y control) 6 muestras de sedimentos inalterados utilizando corers (figura 3.4) (tubos de metacrilato de 30 cm de alto por 6.5 cm de diámetro interno): 3 para el estudio de parámetros físico-químicos (potencial redox, materia orgánica, porosidad, granulometría, fósforo total) y tres para la identificación y recuento de macroinvertebrados bentónicos. Inmediatamente tras la emersión de los corers, se midió el potencial redox del sedimento a 0.5 cm de profundidad y a tres de ellos se les cortó el centímetro más superficial para analizar los distintos parámetros físico-químicos. La porosidad fue calculada según Dell'Anno et al. (2002). Para la determinación del fósforo total del sedimento se realizó una digestión previa según Arocena y Conde (1999). La materia orgánica se analizó según el método de calcinación (Dell'Anno et al. 2002). Para determinar la granulometría del sedimento se utilizó la escala de Wentworth, (Shepard 1954). Los otros 3 corers destinados para el estudio de la macrofauna se tamizaron a través de una malla de 0.5 mm de luz y los organismos retenidos en el tamiz se anestesiaron con cloruro de magnesio al 7% durante 30 min (Castelli et al., 2004). Posteriormente se fijaron en una

solución de formol al 7% tamponado (Castelli et al., 2004), y no fueron apartadas de su conservación excepto para realizar las determinaciones correspondientes.



Figura 3.4: muestra inalterada de sedimento en un corer

En la primera fase, la de funcionamiento, también se tomaron muestras de agua cada 2 metros en la columna, más agua superficial y de fondo (un metro sobre el fondo marino) utilizando para ello una botella oceanográfica tipo Niskin. Los diferentes parámetros medidos y analizados fueron: transparencia, salinidad, pH, temperatura, sólidos suspendidos, clorofila *a*, amonio, nitritos, nitratos más nitritos, fósforo soluble reactivo, fósforo total, ácido ortosilícico y oxígeno disuelto. El nitrógeno inorgánico disuelto fue calculado como la suma de amonio más nitrato más nitrito. Se midió la transparencia de la columna de agua con un disco Secchi y la salinidad, pH y temperatura utilizando una Sonda multiparamétrica WTW Multi 340i. Las muestras de oxígeno disuelto (cámaras bentónicas y columna de agua) fueron fijadas inmediatamente y analizadas por el método yodométrico de Winkler (Baumgarten et al. 1996). Los nutrientes (cámaras bentónicas y columna de agua) fueron analizados mediante métodos descritos por Aminot y Chaussepied (1983) y adaptados por Baumgarten et al. (1996).

La clorofila *a* y los sólidos en suspensión fueron determinados usando las metodologías descritas en APHA, AWWA y WEF (2005).

Además en cada muestreo se obtuvo la velocidad media y dirección dominante de la corriente de la columna de agua usando un perfilador de corrientes multicelda “Doppler- Argonaut-XR” (figura 3.5).



Figura 3.5: Doppler- Argonaut-XR.

### 3.2.- Organización de la memoria

El trabajo realizado dio como resultado tres aportaciones en revistas científicas, cuyos contenidos se presentan en los diferentes capítulos de esta tesis doctoral:

El **capítulo 4 (fase de funcionamiento)**: describe los efectos causados por el engorde de dorada, en jaulas flotantes en mar abierto, en el Mediterráneo occidental sobre el metabolismo bentónico.

El **capítulo 5 (fase de funcionamiento)**: describe los efectos causados por el engorde de dorada, en jaulas flotantes en mar abierto, en el Mediterráneo occidental sobre la calidad del agua, los sedimentos y la estructura de la comunidad bentónica.

El **capítulo 6 (fase después del cese)**: describe la recuperación de una zona que fue afectada por el engorde de dorada en el mar Mediterráneo

tras el cese definitivo de la actividad. Se describen parámetros físico-químicos de los sedimentos, los flujos bentónicos *in situ* de oxígeno y nutrientes y la comunidad bentónica.

Los capítulos 4-6 se corresponden con los tres artículos que han constituido la base de la presente Tesis Doctoral:

**Capítulo 4:** T. Morata, • J. Sospedra • S. Falco • M. Rodilla (2012) Exchange of nutrients and oxygen across the sediment-water interface below a marine fish farm of *Sparus aurata* in the North Western Mediterranean Sea. Journal of Soils and Sediments, 12: 1623-1632. DOI: 10.1007/s11368-012-0581-2.

**Capítulo 5:** Tania Morata, Silvia Falco, Isabel Gadea, Javier Sospedra, Miguel Rodilla. (2013) Environmental effects of a marine fish farm of gilthead seabream (*Sparus aurata*) in the NW Mediterranean Sea on water column and sediment. Aquaculture Research. DOI:10.1111/are.12159.

**Capítulo 6:** Tania Morata, Silvia Falco, Miguel Rodilla. Benthic Recovery after the Cessation of a Gilthead Seabream Fish Farm in the Mediterranean. Enviado a Aquaculture.

*Nota: los capítulos 4-6 mantienen los requisitos de uniformidad de las revistas en las que se publicaron o se han enviado, aunque se han editado para facilitar su lectura y adaptarlos al formato de la presente Tesis Doctoral.*

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## Capítulo 4

Exchange of nutrients and oxygen across  
the sediment–water interface below a  
*Sparus aurata* marine fish farm in the  
north-western Mediterranean Sea

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*Journal of Soils and Sediments* (2012) 12:1623-1632  
DOI 10.1007/s11368-012-0581-2

### **Abstract**

*Purpose* This study analyzes the effects of aquaculture activities in open seawater in the north-western coastal waters of the Mediterranean Sea. It is the first of its kind to be based on benthic flux data gathered in situ below fish farms for this particular area.

*Materials and methods* Samples were collected on four sampling campaigns over a 1-year cycle under a *Sparus aurata* fish farm facility where benthic fluxes were measured in situ using light and dark benthic chambers. Bottom water and sediment samples were also collected. Data were compared to those for a nearby control station.

*Results and discussion* Significant differences were found (ANOVA,  $p < 0.05$ ) between concentrations of organic matter (OM), total phosphorus and redox potentials in sediments located under the cages and those of the control station. The consumption of dissolved oxygen (DO) by sediment and positive ammonium ( $\text{NH}_4^+$ ) fluxes was stimulated by OM content, with correlations of  $r = -0.60$  ( $p < 0.01$ ) and  $r = 0.70$  ( $p < 0.01$ ), respectively. The OM content of sediments was found to be consistently higher under the cages than at the control station, with the highest value ( $1.8 \pm 0.7\%$ ) under the cages observed during the early summer; values of DO and  $\text{NH}_4^+$  fluxes were  $-64 \pm 17$  and  $12.7 \pm 1.0 \text{ mmol m}^{-2} \text{ day}^{-1}$ , respectively.  $\text{PO}_4^{3-}$  fluxes were consistently higher in the fish farm sediments (between 0.58 and 0.98  $\text{mmol m}^{-2} \text{ day}^{-1}$ ) than those observed at the control station. Nitrate ( $\text{NO}_3^-$ ) fluxes were found to be consistently negative due to denitrification occurring in the sediments and were related to the concentration of  $\text{NO}_3^-$  in bottom waters ( $r = 0.92$ ,  $p < 0.01$ ). Si fluxes were shown to be associated with water temperature ( $r = 0.59$ ,  $p < 0.05$ ).

*Conclusions* The results imply that sediments located below cages accumulate organic matter originating from aquaculture activities, especially during summer months when this activity increases. Sediments undergo



biogeochemical changes that mainly affect fluxes of DO,  $\text{NH}_4^+$  and soluble reactive phosphorus, although these do not seem to have a significant impact on the quality of the water column due to the hydrodynamic characteristics of the area.

**Keywords** Aquaculture impacts·Benthic·Fish farm·Fluxes·Sediment

### 4.1.- Introduction

World aquaculture production increased dramatically from  $0.6 \times 10^6$  t in 1950 to  $68.4 \times 10^6$  t in 2008. Spain's aquaculture production is the highest of all EU member countries and was 249,070 t in 2008 (19.5% of total EU production); followed by France and Italy (APROMAR 2010). It is a growing economic activity which unfortunately brings with it environmental consequences (Belias et al. 2007; Borja et al. 2009).

There is growing concern in many countries, both among citizens and the scientific community, regarding the sustainability of this growing industry (Hargrave 2005). The magnitude of its ecological impact depends on the physical and oceanographic conditions of the fish farm site, water temperature and ecosystem absorption capacity. In addition to this, fish farm management and size, cultivation density, duration of cultivation operations, fish feed digestibility and fish health are all significant factors that need to be taken into account (Molina and Vergara 2005).

Studies of the environmental effects of aquaculture activities have focused on a number of aspects, including: changes occurring in the water itself (Maldonado et al. 2005) and in the sediment layers of fish farm facilities (Karakassis et al. 1998; Aksu and Kocatas 2007); disturbances in *Posidonia oceanica* meadows (Pergent-Martini et al. 2006); anoxic conditions of the sediment layer below cages (Hargrave et al. 1997); and the influence of current velocities (Black and McDougall 2002). Some studies have analysed metals and pigments found in sediments as well as bacteria

and multiple stable isotopes as indicators of environmental impact (Mazzola et al. 2000; Kaymakci et al. 2010). Others have focused on the environmental impact of aquaculture activities on nutrients and plankton (Neofitou and Klaoudatos 2008); on nutrients and benthic community structure (Mazzola et al. 2000; Yucel-Gier et al. 2007); and on the geochemistry of sediments and benthic organisms (Mazzola et al. 2000).

The enrichment in organic matter produced in the sediments below cages causes high rates of decomposition, which can modify the quality of the water around the facility. This can lead to a decrease in oxygen in the water column, promote eutrophication and turn the sediments anoxic, with the result that the organic matter decomposes anaerobically. It can also seriously affect the structure of the benthic community (Jackson et al. 2004). Sediment plays a crucial role in the cycling of nutrients and can operate as the main source of nutrients in the water column, especially in coastal ecosystems (Warnken et al. 2002). A greater understanding of the above processes would help to predict impacts and adopt practices in aquaculture activity geared towards a sustainable use of the marine environment. By measuring oxygen and nutrient fluxes, it is possible to identify the main mechanisms in the mineralisation process and quantitatively determine the importance of sediments in the global cycles of nutrients on fish farms (Riise and Roos 1997).

Thus, this study should be seen in the context of the widespread preoccupation which exists with the release of waste from aquaculture and its potential to cause great harm to the ecosystem through changes in the nutrient load. More specifically, the study focuses on the effects of gilthead sea bream (*Sparus aurata*) farming activities on benthic metabolism over a 1-year farming cycle. These activities involve the use of floating cages located in open seawater in the north-western waters of the Mediterranean Sea. Oxygen and nutrient fluxes at the sediment–water interface were determined via in

situ incubations using benthic chambers. All the data were compared with that collected at a control station.

It is worth pointing out that at the international level, there have been few in situ studies into benthic fluxes at fish farms (e.g. Holby and Hall 1991, 1994; Hall et al. 1992; Freitas et al. 2008; Ferrón et al. 2009), or ex situ studies (e.g. Heilskov and Holmer 2001; Nizzoli et al. 2007). Just one study of north-western Mediterranean waters has been completed to date and this involved ex situ experiments (Belias et al. 2007).

### **4.2.- Materials and methods**

#### **4.2.1.- Study area**

The study area is located in the north-western waters of the Mediterranean Sea, off the coastal town of Burriana (Spain), on the site of an intensive fish farm (UTM X: 752170; UTM Y: 4414096) located about 2 km from the coast at a depth of 19 m. The area is characterised by sandy sediments. The fish farm comprises 15 cages, and all measurements were taken from an area below a cage located at the centre of the fish farm. This farm produces 500 t annually and measurements were taken below a cage with a biomass of between 30 and 47 t. The sea breams were fed on commercial feed (conversion factor, 1.8 to 2), which was unequally distributed throughout the year as the feed rate varied according to temperature.

#### **4.2.2.- Sampling and sampling points**

Measurements were taken from two sampling points: the first was located below a fish farm cage (I) and the other, which was used as a control area (C), was located 100 m north of the fish farm, up-current from dominant sea currents. Samples were collected during four sampling campaigns: the

first in autumn (25 November 2008, I-1; 01 December 2008, C-1); the second in winter (23 February 2009, I-2; 28 February 2009, C-2); the third in spring (28 April 2009, I-3; 02 May 2009, C-3); and the fourth in early summer (17 June 2009, I-4; 19 June 2009, C-4). Control station sediments were similar to those found below the fish farm cages. The control station was not influenced by nutrients released by the fish farm activities and the 100-m distance was deemed sufficient as various authors (e.g. Karakassis et al. 1998; Yucel-Gier et al. 2007; Borja et al. 2009) have shown that the environmental effects of fish farm activities are limited to areas immediately beneath the cages.

### 4.2.3.- Benthic chamber experiments and analysis

To study nutrient and oxygen fluxes at the sediment–water interface, measurements were taken in situ using benthic chambers, similar to those used by Freitas et al. (2008). Light and dark chambers were used for each incubation, with three replicate samples being taken for each chamber type to minimise the effects of spatial heterogeneity. The measurements were taken using semi-spherical methacrylate chambers with a diameter of 40 cm and a volume of 16.7 l, covering a sediment surface area of 0.125 m<sup>2</sup>. The chambers contained a manual stirrer to minimise concentration gradients (Niencheski and Jahnke 2002).

The chambers were placed in the sediment manually by scuba divers, and the total incubation period was 6 h. Samples of water were taken from inside the chambers every 2 h using 60-ml plastic syringes inserted through openings controlled by a valve. Ammonium (NH<sub>4</sub><sup>+</sup>), nitrates (NO<sub>3</sub><sup>-</sup>), soluble reactive phosphorus (PO<sub>4</sub><sup>3-</sup>), silicate (Si) and dissolved oxygen (DO) concentrations were analysed. Benthic fluxes were estimated from the slope of a linear regression of the time series results and the chamber volume (Niencheski and Jahnke 2002) and Eq. (1), as used by Nizzoli et al. (2007):

$$(1) F = (C_t - C_o) \cdot (1/(A \cdot t)) \cdot V \cdot 24$$

where  $F$  (millimoles per square metre per day) is the estimated flow;  $C_t$  and  $C_0$  (millimoles) are the final and initial concentrations obtained in the linear fit;  $A$  (square metre) is the area of incubation;  $t$  (hours) is the total incubation time; and  $V$  (l) is volume of incubated water.

### 4.2.4.- **Bottom water and sediment**

At the start of each sampling day, the transparency of the water column was measured using a Secchi disk (Secchi depth), and salinity (Sal), pH and temperature (Temp) of bottom water were measured using a WTW Multi 340i multiparameter probe. Water samples were taken using a Niskin bottle at a depth of 0.5 m from the bottom to analyse DO and nutrients. Samples were also taken every 2 m from the water column to analyse for chlorophyll-*a* (chl-*a*).

Scuba divers visually inspected the sea bottom for signs of *Beggiatoa* spp. and phytobenthic assemblages. During each sampling session, three samples of unaltered sediment layers were taken from the fish farm and control area using corers with a length of 30 cm and an internal diameter of 6.5 cm in order to examine physical and chemical parameters such as redox potential (Eh), organic matter (OM), water content, porosity, granulometry and total phosphorus (TP). When the corers were brought up to the surface, their Eh was measured and 1 cm of sediment was removed from the uppermost layer of the sample for analysis of the various parameters.

### 4.2.5.- **Parameters considered and analytical methods**

The DO samples were fixed immediately and analysed in situ using the Winkler iodometric method (Baumgarten et al. 1996). For the analysis of dissolved nutrients, the samples were filtered using a cellulose acetate membrane filter with a pore size of 0.45  $\mu\text{m}$ . The  $\text{NH}_4^+$  was determined on the same day and the remaining samples were frozen for later analysis. The

nutrients were analysed using the methods described by Aminot and Chaussepied (1983) and adapted by Baumgarten et al. (1996). The chl-*a* was determined using the methodology described in APHA, AWWA and WEF (2005). The intensity of light at the bottom ( $I_z$ ) was calculated following Lorenti and De Falco (2004) using the Secchi depth and solar radiation data provided by the Valencian Institute of Agricultural Research. Sediment Eh was measured at a depth of 0.5 cm using a Crison PH25 potentiometer. Sediment water content was calculated as the difference between wet and dry weight and expressed as a percentage. Sediment porosity was determined according to Dell'Anno et al. (2002). To determine sediment TP, digestion was performed following Arocena and Conde (1999). OM was analysed using the combustion method (Dell'Anno et al. 2002). Granulometry was performed for the sediment samples using the Wentworth scale (Shepard 1954).

### 4.3.- Results

#### 4.3.1.- Bottom water and sediment

Table 1 lists the values for DO, nutrients, salinity, pH and temperature of bottom water, Secchi depths and the  $I_z$  values were calculated for the fish farm and the control station. The temperature varied between 12.9 °C in winter and 20.3 °C at the beginning of summer. Salinity ranged from 37.0 to 38.0 ‰. DO concentrations at the fish farm were found to be consistently lower than those observed at the control station. The highest concentration of  $\text{NH}_4^+$  was observed in autumn at the fish farm, with a value of 3.1  $\mu\text{M}$ , and the highest concentrations of  $\text{NO}_3^-$  were observed in autumn and winter.  $\text{PO}_4^{3-}$  concentrations were very low and there were no great differences among the four sampling campaigns: the values oscillated

between 0.10 and 0.26  $\mu\text{M}$ . The maximum Si value of 5.4  $\mu\text{M}$  was found in the bottom water of the control station in the early summer.

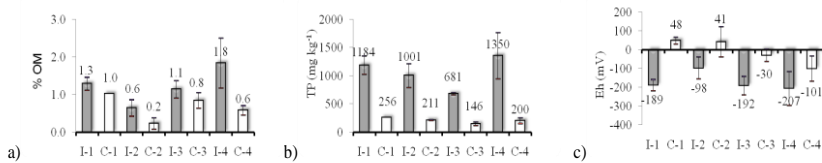
No differences in transparency were found between the fish farm and the control station, except for a control station sample taken in early summer, which had a higher value and resulted in a greater  $I_z$ . As can be seen from Table 1, the  $I_z$  value was quite low except for the early summer sample just mentioned, which was 12.3  $\text{W m}^{-2}$ . In fact, the  $I_z$  below the cages may have been overestimated as Secchi depths were taken next to the cage and the shade they generated over the sediment was not taken into account.

**Table 1** Parameters measured in bottom water of the fish farm (I) and the control station (C) in the four sampling campaigns

Parameters	Autumn		Winter		Spring		Early Summer	
	I	C	I	C	I	C	I	C
Temp ( $^{\circ}\text{C}$ )	16.0	15.0	12.9	12.9	16.0	16.1	20.3	18.6
Sal (‰)	37.8	37.9	37.9	38.0	37.0	37.4	37.4	37.4
pH	8.1	8.1	8.2	8.1	8.1	8.1	8.0	8.0
DO ( $\text{mg l}^{-1}$ )	4.4	7.1	8.4	8.7	7.9	9.4	7.8	9.0
$\text{NH}_4^+$ ( $\mu\text{M}$ )	3.1	0.5	0.4	<0.1	<0.1	<0.1	0.5	0.9
$\text{NO}_3^-$ ( $\mu\text{M}$ )	4.0	0.7	3.9	2.0	0.3	0.1	0.2	0.0
$\text{PO}_4^{3-}$ ( $\mu\text{M}$ )	0.23	0.13	0.12	0.11	0.22	0.26	0.10	0.10
Si ( $\mu\text{M}$ )	2.6	1.7	2.1	2.9	1.8	2.0	1.1	5.4
Secchi depth (m)	6.0	5.0	4.9	4.6	6.7	6.0	6.7	13.0
$I_z$ ( $\text{Wm}^{-2}$ )	0.2	0.2	0.2	0.1	1.0	0.6	2.4	12.3

Both the fish farm and the control station were characterised by sandy sediments with a grain size mode of between 0.125 and 0.063 mm and an average grain size corresponding to very fine sand. At both locations, water content was approximately 25% and porosity was 0.46. Differences were found only in the percentage of shell content ( $12.1 \pm 4.0\%$  under the fish farm and  $0.3 \pm 0.2\%$  at the control station). The value was 37 times higher under the cages due to anti-fouling activity (cleaning of cages).

Significant differences (ANOVA,  $p < 0.05$ ) were found between fish farm and control station sediments in OM content, TP concentrations and Eh measurements. In the four sampling campaigns, OM content and TP concentrations were found to be greater at the fish farm than at the control station (Fig. 1a, b). The highest OM content ( $1.8 \pm 0.7\%$ ) and TP ( $1,350 \pm 411 \text{ mg kg}^{-1}$ ) and greatest negative values for Eh ( $-207 \pm 90 \text{ mV}$ ) were found in the early summer samples taken from below cages. Reducing conditions were observed under the cages throughout the year; Eh values were found to be consistently negative and much lower than those observed at the control station (see Fig. 1c). Visual inspections did not reveal *Beggiatoa* spp.; however, microphytobenthic assemblages were observed in the control station sediments in the spring.



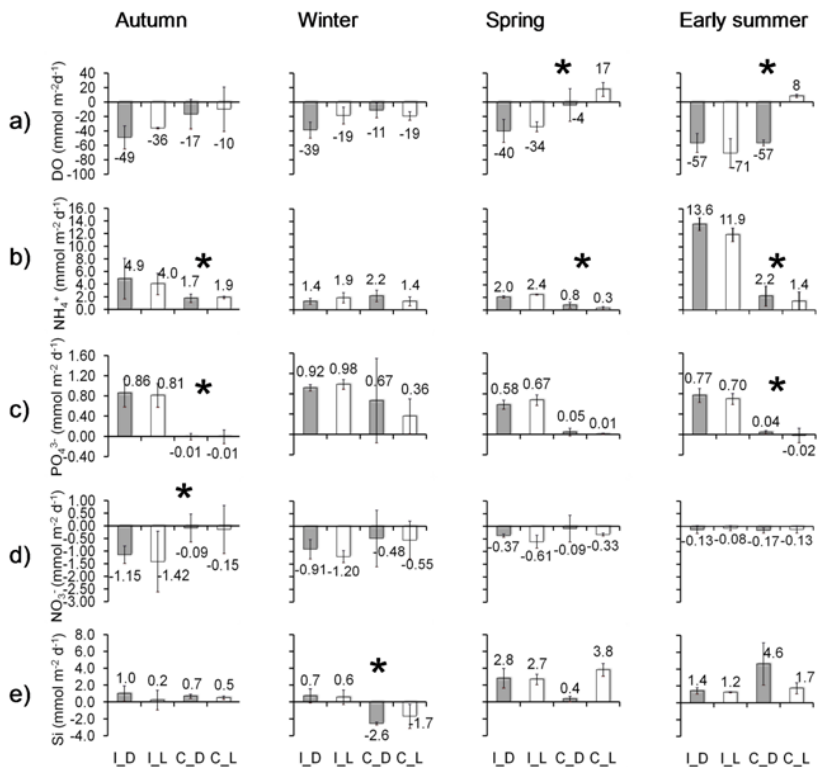
**Fig. 1 a–c** Values of organic matter (OM), total phosphorus (TP) and Eh in sediments under the fish farm (I) and the control station (C) in the four sampling campaigns.

#### 4.3.2.- Benthic fluxes

Analysis of all samples revealed no significant differences between the dark and light benthic chambers for the fluxes measured. In general, DO fluxes were negative (Fig. 2a), which indicates DO consumption by the sediment. These values were found to be more negative at the fish farm than at the control station, and the highest consumption value was found under the cages in the early summer (up to  $-71 \pm 20 \text{ mmol m}^{-2} \text{ day}^{-1}$ ). Significant differences (ANOVA,  $p < 0.05$ ) between the fish farm and the control station



were only observed in spring and early summer. It was during these seasons when a positive flux was also observed in the light benthic chambers at the control station (up to  $17 \pm 10 \text{ mmol m}^{-2} \text{ day}^{-1}$ , in spring). All the chambers showed positive fluxes in  $\text{NH}_4^+$  from the sediment to the water column (see Fig. 2b). Significant differences (ANOVA,  $p < 0.05$ ) were found between  $\text{NH}_4^+$  fluxes measured at the fish farm and at the control station in the autumn, spring and early summer. The latter sampling campaign showed the greatest differences, with the highest flux found under the fish farm ( $13.6 \pm 1.0 \text{ mmol m}^{-2} \text{ day}^{-1}$ ).



**Fig. 2** Benthic fluxes of dissolved oxygen ( $\text{DO}$ ), ammonium ( $\text{NH}_4^+$ ), soluble reactive phosphorus ( $\text{PO}_4^{3-}$ ), nitrate ( $\text{NO}_3^-$ ) and silicate ( $\text{Si}$ ) in dark ( $D$ ) and light ( $L$ ) chambers under the fish farm cage ( $I$ ) and at the control station ( $C$ )

in the four sampling campaigns. *Asterisk*: significant differences (ANOVA,  $p < 0.05$ ) between fish farm and control station.

$\text{PO}_4^{3-}$  fluxes were generally positive, i.e. phosphorus from the sediment was released to the water column, and were consistently greater below the fish farm than at the control station (see Fig. 2c) and significant differences were only found (ANOVA,  $p < 0.05$ ) in autumn, spring and early summer.  $\text{NO}_3^-$  fluxes were negative, i.e.  $\text{NO}_3^-$  from the water column was consumed by the sediment. These fluxes were found to be more negative at the fish farm in autumn, winter and spring than those observed at the control station (see Fig. 2d), although significant differences (ANOVA,  $p < 0.05$ ) were only found in autumn. Si fluxes were positive (see Fig. 2e), except for the samples taken in winter at the control station, which showed negative fluxes in both the light and dark benthic chambers. Significant differences (ANOVA,  $p < 0.05$ ) between Si fluxes at the fish farm and control station were only observed in the winter. The highest and most positive Si fluxes were found in spring and early summer.

### 4.4.- Discussion

#### 4.4.1.- Bottom water and sediment

Water temperature varied according to season. The lower concentrations of DO observed at the fish farm compared to those at the control station may be attributable to the consumption of oxygen by fish respiration and the aerobic decomposition of OM. The higher concentrations of  $\text{NH}_4^+$  observed at the fish farm in autumn may have been caused by fish excretion (Dosdat 2001; Aksu and Kocatas 2007) and possible decomposition of OM. The differences in  $\text{NO}_3^-$ , which were found to be greater at the fish farm, were most likely due to oxidation of  $\text{NH}_4^+$  to form  $\text{NO}_3^-$  (Dosdat 2001). The higher concentrations of  $\text{NO}_3^-$  in the Mediterranean in winter were also

found by Maldonado et al. (2005). As P is a limiting nutrient in the Mediterranean (Siokou-Frangou et al. 2010), this may be the reason why we found lower concentrations of P; these values are similar to those observed by Kaymakci et al. (2010). The highest concentration of Si at the sea bottom in summer was also observed by Kaymakci et al. (2010) and may be due to mineralization of biogenic Si accumulated at the sea bottom.

OM content was consistently higher under the cages than at the control station, a finding which is known to occur under fish farm cages located in open seawater (Mantzavarakos et al. 2007; Borja et al. 2009). OM in sediment mostly originates from non-ingested fish feed, either due to over-feeding or poorly managed diet or feeding regime. Accumulation of fish faeces, farmed fish mortality and cage cleaning may also increase OM in the sediment (Molina and Vergara 2005). The highest OM content was found in early summer under the cages owing to the higher temperatures, which increased the metabolism of the fish and, consequently, their rate of feeding. This led to greater losses in fish feed and greater rates of excretion. OM values observed in this study were similar to those seen at other fish farms (Sakamaki et al. 2006; Nizzoli et al. 2007) although Mantzavarakos et al. (2007) observed higher values in OM in the Mediterranean Sea.

In this study, TP in the sediment below the cages was consistently higher than at the control station, which is in line with the findings of Karakassis et al. (1998). The highest concentrations were found in summer, as was also observed by Mantzavarakos et al. (2007). All of the above values, along with those observed for OM, were due to the increase in solid waste originating from increased production, typical of the season. TP concentrations in sediments under Mediterranean Sea fish farms observed in other studies (e.g. Karakassis et al. 1998) were of a similar order of magnitude to our results; however, Mantzavarakos et al. (2007) found TP concentrations to be lower.

On the basis of the Eh measurements, we found sediments under the cages to be consistently more negative, with the greatest negative value observed in early summer possibly due to the higher OM content, as this measurement is highly correlated to OM content ( $r = -0.70$ ,  $p < 0.01$ ) and TP ( $r = -0.79$ ,  $p < 0.01$ ; Table 2). Reducing conditions in fish farm sediments were found in other studies (e.g. Karakassis et al. 1998; Ferrón et al. 2009).

The study illustrates the effects that aquaculture activity has on the sediment in the proximity of fish farms. The sediment experiences an increase in OM and TP content, which at the same time become more reductive. It is important for companies and the competent environmental authorities to take into account the changes in aquaculture activity throughout the year resulting from changes in the prevailing water temperature. In addition to the general effect on the sediments and hence the environment, there are also seasonal changes which cause an increase in aquaculture activity and thus have a greater impact. The amount of activity also affects the parameters measured in the bottom water, but since the farm facility is close to the open sea, the water is constantly being refreshed, and thus, the differences are not as pronounced as the parameters measured in the sediments.

### 4.4.2.- Benthic fluxes

No significant differences were found between dark and light benthic chambers for any of the fluxes measured, which can be explained by the fact that  $I_z$  values were close to zero for nearly all the samples taken, except for an early summer sample at the control station which was  $12.3 \text{ W m}^{-2}$ . The low rate of primary production seen at a depth of 19 m, therefore, did not significantly influence flux estimation. The negative fluxes of DO indicate that the sediments consumed oxygen from the water column in the case of the dark benthic chambers. This can be explained by the aerobic

mineralization of OM in which heterotrophic microorganisms use oxygen as the terminal electron acceptor. In the light benthic chambers, the majority of fluxes were found to be negative, which indicates that the consumption of oxygen by mineralization was higher than that which could have been released as a result of primary production.

Positive values for DO fluxes were measured only in spring and at the beginning of summer at the control station. Microphytobenthic assemblages were observed in the spring in this area as well as in the early summer, and  $I_z$  was greater (see Table 1). DO consumed by the sediments was generally more negative under the fish farm cages than at the control station due to the higher OM content of the fish farm ( $r = 0.60$ ,  $p < 0.01$ ) (see Table 2). The oxygen consumption of the sediment below the cages showed an increase over the control station of between  $13 \text{ mmol m}^{-2} \text{ day}^{-1}$  in the winter and approximately  $42 \text{ mmol m}^{-2} \text{ day}^{-1}$  in both the spring and early summer. The maximum value for sediment consumption of DO was observed in the early summer under the cages, co-occurring with higher bottom water temperatures ( $20 \text{ }^\circ\text{C}$ ), higher rates of feeding and higher OM content found in the sediment. These conditions were similar to those observed by Nizzoli et al. (2007) and Ferrón et al. (2009). Other studies that examined DO fluxes on aquaculture facilities obtained results similar to those seen in this study (e.g. Ferrón et al. 2009) or lower fluxes (e.g. Nizzoli et al. 2007; Freitas et al. 2008). We consider that sediment oxygen demand is an index which can be used to estimate the benthic regeneration rate, as well as being a good parameter for assessing how well this industrial activity is being managed. DO fluxes were also found to be inversely proportional to  $\text{NH}_4^+$  fluxes ( $r = -0.83$ ,  $p < 0.01$ ), which indicates that the greater the consumption of oxygen during mineralization of OM, the greater the release of  $\text{NH}_4^+$  to the water column (Berelson et al. 2003).

Below the cages, the flux of ammonium from the sediment to the water column was greater than at the control station, increasing by 11 mmol m<sup>-2</sup> day<sup>-1</sup> at the start of summer. NH<sub>4</sub><sup>+</sup> fluxes at the fish farm were greater in early summer (12.7±1.0 mmol m<sup>-2</sup> day<sup>-1</sup>), followed by autumn and then spring (see Fig. 2b). This correlated with the OM content in the sediment ( $r = 0.70, p < 0.01$ ). Ferrón et al. (2009) observed NH<sub>4</sub><sup>+</sup> fluxes of 5 to 20 mmol m<sup>-2</sup> day<sup>-1</sup>, and Nizzoli et al. (2007) found values ranging from 10 to 57 mmol m<sup>-2</sup> day<sup>-1</sup>. Both studies also found that the greatest fluxes occurred in the summer, when temperatures and aquaculture activity were higher. Especially noteworthy was the fact that for three of the four sampling campaigns, significant differences in NH<sub>4</sub><sup>+</sup> fluxes were observed between the fish farm and control station.

**Table 2** Pearson's correlation coefficients for relationships between variables measured in bottom water and sediment. Values in bold are significant at  $p < 0.05$ ; values in italics are significant at  $p < 0.01$ . The number of observations ranged from 8 to 16. *Temp* temperature, *bot* bottom, *sed* sediment.

	DO flux	PO <sub>4</sub> <sup>3-</sup> flux	Si flux	NH <sub>4</sub> <sup>+</sup> flux	NO <sub>3</sub> <sup>-</sup> flux	Temp bot	OM sed	Eh sed	TP sed	NO <sub>3</sub> <sup>-</sup> bot
DO flux	1.00									
PO <sub>4</sub> <sup>3-</sup> flux	<b>-0.56</b>	1.00								
Si flux	-0.17	-0.27	1.00							
NH <sub>4</sub> <sup>+</sup> flux	<b>-0.83</b>	<b>0.56</b>	-0.11	1.00						
NO <sub>3</sub> <sup>-</sup> flux	0.13	<b>-0.71</b>	0.24	-0.08	1.00					
Temp bottom	-0.32	-0.25	<b>0.59</b>	0.39	<b>0.51</b>	1.00				
OM sediment	<b>-0.60</b>	0.24	0.34	<b>0.70</b>	0.10	<b>0.71</b>	1.00			
Eh sediment	<b>0.67</b>	<b>-0.59</b>	-0.44	<b>-0.61</b>	0.30	<b>-0.53</b>	<b>-0.70</b>	1.00		
TP sediment	<b>-0.72</b>	<b>0.83</b>	0.00	<b>0.77</b>	-0.49	0.21	<b>0.69</b>	<b>-0.79</b>	1.00	
NO <sub>3</sub> <sup>-</sup> bottom	-0.13	<b>0.67</b>	-0.46	0.10	<b>-0.92</b>	<b>-0.63</b>	-0.19	-0.09	0.46	1.00

PO<sub>4</sub><sup>3-</sup> fluxes were higher under the cages than at the control station, rising from 0.44 mmol m<sup>-2</sup> day<sup>-1</sup> in winter to 0.84 mmol m<sup>-2</sup> day<sup>-1</sup> in autumn,

due to the higher content of TP in the sediment ( $r = 0.83$ ,  $p < 0.01$ ).  $\text{PO}_4^{3-}$  fluxes were correlated (see Table 2) to  $\text{NH}_4^+$  ( $r = 0.57$ ,  $p < 0.05$ ) and DO fluxes ( $r = -0.56$ ,  $p < 0.05$ ). This can be explained by the decomposition of OM, which involves an uptake of DO to produce  $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$ . Nevertheless, there was no direct relationship between this and OM content, probably due to the fact that the uppermost layer of the sediment, which was probably well oxygenated, retained  $\text{PO}_4^{3-}$ . We also obtained an interesting correlation between  $\text{PO}_4^{3-}$  flux and Eh ( $r = -0.59$ ,  $p < 0.05$ ), which was attributable to the fact that P may be released under anaerobic conditions (Rodríguez 1999), such as those observed in below the superficial sediment layer. Ferrón et al. (2009) observed flux data for  $\text{PO}_4^{3-}$  similar to those seen in this study.

Nitrate fluxes were consistently negative;  $\text{NO}_3^-$  in the water column was consumed by the sediment due to benthic denitrification occurring in the suboxic and anoxic sediment layers, where  $\text{NO}_3^-$  acts as a terminal acceptor of electrons (Herbert 1999). In samples taken in the autumn, winter and spring,  $\text{NO}_3^-$  fluxes were found to be more negative at the fish farm than at the control station;  $\text{NO}_3^-$  consumption is as much as  $1.2 \text{ mmol m}^{-2} \text{ day}^{-1}$  higher on the fish farm compared to the control, despite the fact that significant differences were found in autumn only. These differences may be explained by the higher concentrations of OM under the cages where suboxic/anoxic zones of the sediments are closer to the surface, favouring increased benthic denitrification. Greater negative fluxes were observed in autumn and winter under the cages, co-occurring with the higher concentrations of  $\text{NO}_3^-$  in the bottom water (see Table 1). The correlation between  $\text{NO}_3^-$  fluxes and initial concentrations of  $\text{NO}_3^-$  in the bottom waters was  $r = -0.92$  ( $p < 0.01$ ). Previous studies have shown a clear negative relationship between  $\text{NO}_3^-$  flux and  $\text{NO}_3^-$  concentration in bottom water (Sakamaki et al. 2006). The high concentrations of  $\text{NO}_3^-$  in the bottom waters

increase its flux into sediments, increasing  $\text{NO}_3^-$  concentrations in interstitial water, which in turn favours bacterial nitrate respiration. As the concentration of  $\text{NO}_3^-$  increases,  $\text{NO}_3^-$  is reduced to  $\text{N}_2/\text{N}_2\text{O}$  and N is lost from the system due to denitrification (Herbert 1999).

The highest and most positive Si fluxes were observed in spring and early summer due to the increase in Si dissolution rates caused by the higher temperatures (Cermelj et al. 2001). The correlation between Si fluxes and temperature (see Table 2) was 0.59 ( $p < 0.05$ ). Fish farm activity does not seem to affect Si fluxes as OM from the fish farm had a low Si content. Other studies (e.g. Freitas et al. 2008) also indicate that Si fluxes are not significantly influenced by aquaculture activities and they also find positive flows, similar to those seen in Ferrón et al. (2009).

To determine the implications that these fluxes might have for the water column, it is first necessary to quantify their importance to primary production. Morán and Estrada (2005) studied the north-western basin of the Mediterranean Sea and observed that total phytoplankton biomass was closely correlated with primary production and proposed a linear regression model which integrated daily rates of primary production ( $\text{PP}_{\text{int}}$ ) with integrated values of chl-*a* (chl- $a_{\text{int}}$ ). Since we had data from all our sampling campaigns for chl-*a* taken every 2 m depth in the water column, we were able to calculate the average chl- $a_{\text{int}}$  for both the control and fish farm. In both areas, the average chl- $a_{\text{int}}$  was similar, at around  $7.2 \text{ mg m}^{-2}$ . Subsequently, we used the relation postulated by Morán and Estrada (2005) to estimate the integrated daily production, which was  $139 \text{ mg C m}^{-2} \text{ day}^{-1}$  (ca.  $12 \text{ mmol C m}^{-2} \text{ day}^{-1}$ ). Assuming nutrient utilization ratios approaching Redfield and no nitrogen fixation (Redfield et al. 1963; Niencheski and Jahnke 2002), phytoplankton would require ca.  $2 \text{ mmol N m}^{-2} \text{ day}^{-1}$ ,  $0.1 \text{ mmol P m}^{-2} \text{ day}^{-1}$  and  $2 \text{ mmol Si m}^{-2} \text{ day}^{-1}$ . We calculated average N benthic flux from the sum of the fluxes of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and  $\text{NO}_2^-$  (data not shown) for both the control



and fish farm, giving 1.3 and 4.4 mmol N m<sup>-2</sup> day<sup>-1</sup>, respectively. At the control station, nearly three quarters of phytoplankton N requirements can be met by benthic N fluxes, whereas at the fish farm, the benthic fluxes are 2.5 times higher than these requirements. Average P benthic fluxes were 0.1 mmol P m<sup>-2</sup> day<sup>-1</sup> for the control and 0.8 mmol P m<sup>-2</sup> day<sup>-1</sup> for the fish farm, which indicates that phytoplankton P requirements are met by benthic fluxes in both cases, even though on the fish farm, they are eight times higher. The average benthic Si fluxes at the control and fish farm were similar at approximately 1 mmol Si m<sup>-2</sup> day<sup>-1</sup>, which is half of the diatom Si requirements. The above results show that the fish farm has a far higher availability of P and N than the control, while the contribution of Si by the sediment is the same in both locations. On the fish farm, these differences might lead to a modification in the structure of the phytoplankton community and promote the growth of species which do not use Si as a nutrient. However, the greater availability of N and P on the fish farm was not reflected in differences in PP<sub>int</sub>, which was similar in both zones, at around 7.2 mg m<sup>-2</sup>. This indicates that there is a high amount of dilution in the zone as a consequence of the high hydrodynamic behaviour of open seawater, making it impossible to detect differences in the phytoplankton biomass. This corroborates the observations made for similar fish farms in the Mediterranean by Pitta et al. (1999) and Sarà et al. (2006).

### 4.5.- Conclusions

Floating cage aquaculture in the marine environment generates an accumulation of OM in sediments, which reaches peak values in summer. The variations in the accumulation of OM occurring on fish farms are very closely linked to the metabolic activity of the fish bred on farms, and this depends on the water temperature. As a result, sediments undergo biochemical changes which primarily affect oxygen consumption, release of

$\text{NH}_4^+$  and  $\text{PO}_4^{3-}$  and  $\text{NO}_3^-$  consumption by the sediment. However, Si fluxes seem unaffected by fish farm activity since the contribution of Si by fish farms is currently negligible. In the future, if the use of fish feed of vegetable origin instead of fish meal becomes more widespread, it will become necessary to reassess the Si contributed by fish farm activity.

Our results for benthic flux in situ are the first obtained for caged fish farms in the Mediterranean and they show that sediments can play an important role in nutrient cycling. Furthermore, benthic fluxes can have an important influence the concentrations of oxygen and nutrients in the water column; oxygen consumption is as much as  $42 \text{ mmol m}^{-2} \text{ day}^{-1}$  higher on the fish farm compared to the control site. Although no important consequences are detected in the water column, the accumulation of organic waste and oxygen concentrations in the water near the bottom will need constant monitoring owing to the hydrodynamic features of such locations. The flux which has the greatest importance relative to the requirements of the primary producers is  $\text{PO}_4^{3-}$ . However, the system does not seem to be affected by the presence of such high fluxes, possibly because of the dilution due to the hydrodynamic behaviour of open seawater. In the future, it will be necessary to consider the broader (in time and space) repercussions that the input of nutrients has on the ecosystem as a whole.

### **4.6.- Acknowledgments**

We would like to thank the Caja del Mediterráneo for a predoctoral fellowship fund for this research and Antonio Asunción Acuigroup Maremar manager for the facilities and support in conducting the study. The translation of this paper was funded by the Universidad Politécnica de Valencia, Spain. We are grateful for the valuable comments of the anonymous reviewers on previous versions of the manuscript.

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# Capítulo 5

Environmental effects of a marine fish farm of gilthead seabream (*Sparus aurata*) in the NW Mediterranean Sea on water column and sediment

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*Aquaculture Research* (2013)  
DOI 10.1111/are.12159

### **Abstract**

This study examined the effects of organic enrichment on water column, sediments and macrofauna caused by a fish farm in the Mediterranean Sea. Samples were collected on four sampling campaigns over a one-year cycle. Significant differences were found in the water column in dissolved oxygen, dissolved inorganic nitrogen, phosphate and total phosphorus concentrations between the fish farm and the control. The increase in the dissolved inorganic nitrogen and phosphate concentrations at the fish farm modified the stoichiometric ratios between nutrients, with silicate acting as limiting nutrient at the fish farm 11% more than at the control. Nevertheless, chlorophyll *a* concentration in the water column was higher at the control station, probably due to the fouling of the underwater fish farm structures. Significant differences were found in sediment concentrations of organic matter, total phosphorus and redox potential between the fish farm and the control. The Canonical Correlation Analysis indicated that organic matter, total phosphorus, redox potential and % of gravels accounted for 68.9% of the total variance in the species data. Changes were observed in macrofauna, with a decrease in number of species and up to a nine-fold increase in abundance with respect to the control.

**Keywords:** aquaculture, nutrients, organic matter, macrofauna

### **5.1.- Introduction**

The rapid growth of aquaculture, in particular the intensive open seawater fish farming installations in the Mediterranean Sea, has generated a series of conflicts with traditional users of coastal waters such as fishermen and tourists (Porrello, Tomassetti, Manzuetto, Finoia, Persia, Mercatali & Stipa 2005), whilst becoming the focus and subject of a multitude of studies due to the environmental effects of such facilities (Mantzavrakos, Kornaros, Lyberatos & Kaspiris 2007).



The most common effects of farming fish in cages which are of greatest concern are mainly the issue of local eutrophication. The largest source of waste in aquaculture is organic matter coming from the fish feed (Sanz-Lázaro & Marín 2011). Generally speaking, approximately 1/4 of the nutrients added via fish feed are incorporated into the fish meat itself, while 3/4 remain in the water (Holmer, Wildfish & Hargrave 2005). This organic matter is relatively rich in organic carbon and nutrients such as nitrogen and phosphorus, released in particulate and dissolved form. The release of dissolved nutrients can provoke an enrichment of surrounding waters, giving rise to an increase in primary production in the affected areas (FAO, 1992), which in turn alters the composition of the algae species found in this area. The increase in algae biomass can lead to greater turbidity and lower concentrations of dissolved oxygen in the water column owing to the decomposition of algae biomass (La Rosa, Mirto, Favaloro, Savona, Sarà, Danavaro & Mazzola 2002; Garren, Smriga & Azam 2008). Nevertheless, the impact of fish farming tends to be more noticeable in the benthos than in the water column, as the waste from the cages tends to accumulate around and under the fish cages (Yucel-Gier, Kucuksezgin & Kocak 2007; Vita & Marin 2007). This accumulation of organic matter at the sediment surface increases the metabolism of the sediments which leads to increased oxygen consumption (Holmer & Kristensen 1992; Morata, Sospedra, Falco & Rodilla 2012), as oxygen is used by aerobic bacteria as an electron acceptor in respiration.

Although organic matter is considered to be the greatest source of pollution from fish farming, there are other contaminants that can have an adverse effect on benthic communities, such as the metals Cu, Zn and Cd (Dean, Shimmield & Black 2007), as well as chemotherapeutic agents (Davies, Mchenery & Rae 1997). These contaminants may cause interactive effects (Murray, Bulling, Mayor, Sanz-Lázaro, Paton, Killham & Sosal 2008)

and may also alter the benthic community structure and diversity. Macrofauna plays an important role in ecosystem functions such as the mineralisation of organic matter and nutrient recycling (Braeckman, Provoost, Gribsholt, Van Gansbeke, Middelburg, Soetaert, Vincx & Vanaverbeke 2010). Although many of these processes are carried out by bacteria living at the bottom of the sea, macrofauna also has an effect on these processes via bioturbation and bioirrigation.

The environmental assessment of aquaculture activities is a key component in decisions made by planners regarding the number and size of fish farms that can be installed at a given site. Moreover, a greater understanding and assessment of this medium is of vital importance to fish farm managers as it is related to fish health and ultimately to the profitability of the farm itself (Chou, Haya, Paon, Burrige & Moffat 2002).

Spain reports the largest production in aquaculture among European Member States. It is also one of Europe's main producers of gilthead seabream, at 20,360 tonnes in 2010 (14.6%) (APROMAR, 2011). It is important that this economic activity should be carried out whilst respecting the environment and ensuring the highest levels of protection for the site's natural setting. Yet few studies have been conducted in Spain on the impact of intensive fish farming in the sea (Delgado, Ruiz, Perez, Romero & Ballesteros 1999; Aguado & García 2004; Maldonado, Carmona, Echeverría & Riesgo 2005; Ferrón, Ortega & Forja 2009).

The aim of this study was to analyse the effects on water quality, sediment and benthic community structure that are produced by the open-sea cultivation of gilthead seabream (*Sparus aurata*, Linnaeus, 1758) in cages located in the Western Mediterranean Sea.

The potential impact of the waste generated by aquaculture on water column ecosystems has not been as widely studied as the effects of waste on sediments and benthic ecosystems, probably owing to the fact that it is more

difficult to identify and quantify such impacts (Olsen, Holmer & Olsen 2008). This study is notable for the high spatial resolution of the parameters measured in the water column, which were sampled every 2 metres. Most studies published in this field thus far have measured parameters of water at the surface and the bottom (La Rosa et al., 2002; Maldonado et al., 2005; Mantzavrakos et al., 2007; Kaymakci, Aksu & Egemen 2010) or at three depths at the most (Matijević, Kušpilić, Morović, Grbec, Bogner, Skejić & Veža 2009; Huang, Hsieh, Huang, Meng, Chen, Keshavmurthy, Nozawa & Chen 2011).

### 5.2.- Materials and Methods

The gilthead seabream fish farm where this study was conducted is located in the North Western waters of the Mediterranean Sea, off the Spanish coast. The fish farm installation is located in the open sea, about 2 km from the coast at a depth of 19 m. The facilities are composed of 15 fattening cages, which, together with the remaining structures, comprise a considerable submerged surface area which represents a considerable surface area for fouling growth and development. Fouling is caused by macroalgae and mainly suspensivores such as *Mytilus galloprovincialis* (Lamarck, 1819) and *Sagartia elegans* (Dalyell, 1848). Fish production for this farm is 500 t per year. The gilthead seabream are fed on commercial feed, dispensed manually by a pneumatic feeding system on a small boat. During the period of this study, the feed conversion ratio (FCR) of the fish farm was approximately 1.8 and 2. The amount of feed dispensed was approximately 1000 t, which was unequally distributed throughout the year as the feed rate varied according to temperature, with maximum feed rate occurring during the summer months and the minimum during the winter months.

This study covers a one-year cycle, with measurements taken from two sampling points, one located among the fish farm cages (0° 3' 11.10'' W;

39° 50' 19.62''N) and the control station (0° 3' 6.19''W; 39°50'21.41''N), located 130 m northeast of the fish farm. Samples were collected during four sampling campaigns, the first in autumn (25/11/2008 (I=Fish Farm Installation) "I-1" and 01/12/2008 (C= Control Station) "C-1"), the second in winter (23/02/2009 (I) "I-2" and 28/02/2009 (C) "C-2"), the third in spring (28/04/2009 (I) "I-3" and 02/05/2009 (C) "C-3") and the fourth in early summer (17/06/2009 (I) "I-4" and 19/06/2009 (C) "C-4").

Current velocity and direction was measured during sampling by way of a multi-cell current profiler (Acoustic Doppler current- Argonaut-XR, Wissenschaftlich-Technische Werkstätten (WTW), Weilheim, Germany).

Samples from the water column were taken every two metres. Surface and bottom water (one metre over the sea bed) was also sampled using a Niskin-type water sampler. Transparency (Secchi depth), salinity (Sal), pH, temperature (Temp), suspended solids (SS), chlorophyll *a* (Chl-*a*), dissolved inorganic nitrogen (DIN = ammonium (NH<sub>4</sub><sup>+</sup>) + nitrates (NO<sub>3</sub><sup>-</sup>) + nitrites (NO<sub>2</sub><sup>-</sup>)), soluble reactive phosphorus (PO<sub>4</sub><sup>3-</sup>), total phosphorus (TP), silicate (Si) and dissolved oxygen (DO) were measured in water column. Transparency was measured using a Secchi dish, and the salinity, pH and temperature with Multi-Parameter Instruments WTW Multi 340i (Sontek, San Diego, CA, USA). The DO samples were fixed immediately and analysed using the Winkler iodometric method (Baumgarten, Rocha & Niencheski 1996). For the analysis of dissolved nutrients, the samples were filtered using a cellulose acetate membrane filter with a pore size of 0.45 µm. The NH<sub>4</sub><sup>+</sup> concentration was determined on the same day and the remaining samples were frozen for later analysis. The nutrients were analysed using the methods described by Aminot & Chaussepied (1983) and adapted by Baumgarten et al., (1996). The Chl-*a* and SS were determined using the methodology described in APHA, AWWA & WEF (2005).

Scuba divers visually inspected the sea bottom for signs of *Beggiatoa* spp. and phytobenthic assemblages. During each sampling session, 3 samples were taken of unaltered sediment layers for both the fish farm and control station, using corers with a length of 30 cm and an internal diameter of 6.5 cm. When the corers were brought up to the surface, redox potential (Eh) was measured at a depth of 0.5 cm using a Crison PH25 potentiometer. The uppermost layer (1 cm) was removed to analyse granulometry, porosity, organic matter (OM) and total phosphorus (TP). Sediment porosity was calculated following Dell'Anno, Mei, Pusceddu & Danovaro (2002). To determine sediment TP, digestion was performed following Arocena & Conde (1999). OM was analysed using the combustion method (Dell'Anno et al., 2002). Granulometry was performed for the sediment samples using the Wentworth scale (Shepard, 1954). In addition, 3 additional cores were taken for subsequent identification and count of benthic macroinvertebrates. These cores were sieved using a 0.5 mm mesh and 7% magnesium chloride was used as anaesthetic. Organisms were later fixed in 7% formaldehyde solution. Simpson's diversity index was calculated following Cardona (2007).

Two-way ANOVA was used to determine the existence of significant differences ( $P < 0.05$ ) among the various parameters analysed in the water column and sediment. The factors chosen were “location” factor (fish farm facilities and control station) and “seasonal nature” factor (different sampling campaigns: fall, winter, spring and early summer). When data did not meet the assumptions for the ANOVA, we applied appropriate transformations. This task was carried out using the software Statgraphics centurion.

The effects of benthic environmental variables on the abundances of species in the macrofauna and their spatial variation were analysed by Canonical Correlation Analysis (CCA) using PC-ORD software.

### 5.3.- Results

#### 5.3.1.- Water column

Table 1 shows maximum and average values of water velocity and most frequent current direction for both the fish farm and control site. Maximum velocity ( $43 \text{ cm s}^{-1}$ ) was measured at the beginning of summer at the fish farm installation. Average velocity ranged between  $3$  and  $10 \text{ cm s}^{-1}$  at the fish farm and  $4$  to  $8 \text{ cm s}^{-1}$  at the control station. No significant differences were found between average velocities at the fish farm and control site nor among the different sampling sessions that took place throughout the year. The dominant current direction was found to be Northeast.

**Table 1:** Number of measurements, maximum and average speed and more frequent current direction of the fish farm installation (I) and the control station (C).

		Direction	Total measurements	Percent measurements	Maximum speed ( $\text{cm s}^{-1}$ )	Average speed ( $\text{cm s}^{-1}$ ) $\pm$ sd
Fall	I	NE	1095	13.42	18.15	$4.91 \pm 2.75$
	C	W	930	11.40	28.90	$8.04 \pm 6.59$
Winter	I	E	1691	20.72	19.70	$3.32 \pm 2.51$
	C	S	930	11.40	16.25	$5.34 \pm 2.50$
Spring	I	NE	1210	14.83	31.67	$5.65 \pm 4.09$
	C	SE	1127	13.81	14.44	$4.27 \pm 2.71$
Early Summer	I	N	638	7.82	43.29	$9.87 \pm 9.03$
	C	NE	539	6.61	36.68	$5.99 \pm 4.10$

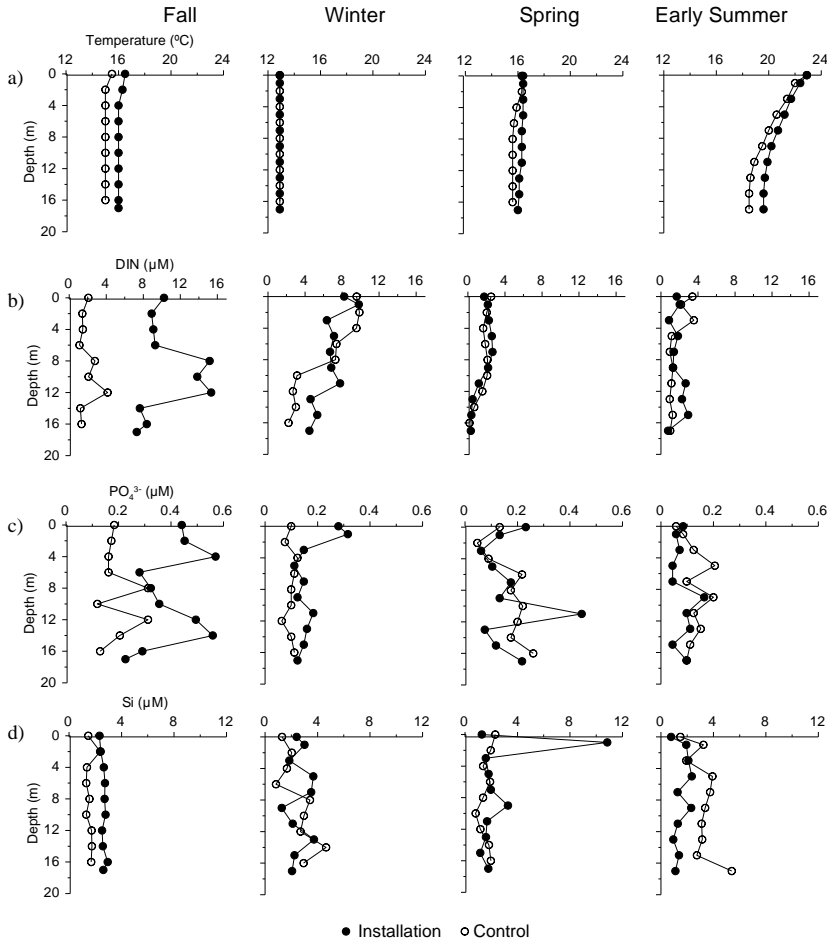
Table 2 shows maximum, minimum and average values of the parameters measured in the water column for every sampling campaign, for both the fish farm and control site.

**Table 2:** Ranges and averages (in parenthesis) of environmental parameters of the water column for the 4 sampling campaigns in the fish farm installation (I) and control station (C).

Environmental Parameter	Fall		Winter		Spring		Early Summer	
	I	C	I	C	I	C	I	C
Temperature (°C)	16.0-16.5 (16.1)	15.0-15.5 (15.1)	12.7-12.9 (12.9)	12.7-12.9 (12.9)	16.0-16.4 (16.3)	15.6-16.3 (15.8)	22.9-19.6 (20.7)	22.9-18.5 (20.0)
pH	7.86-8.12 (8.06)	8.00-8.14 (8.09)	7.92-8.12 (8.00)	7.95-8.06 (8.02)	8.05-8.13 (8.09)	7.99-8.09 (8.07)	7.95-8.17 (8.08)	7.98-8.06 (8.01)
Salinity (g L <sup>-1</sup> )	37.7-37.9 (37.8)	37.4-38.0 (37.9)	37.5-38.0 (37.8)	37.6-38.0 (37.8)	37.0-37.5 (37.4)	37.1-37.4 (37.3)	37.4-37.5 (37.4)	36.7-37.5 (37.3)
DO (mg L <sup>-1</sup> )	3.82-5.51 (4.94)	5.16-7.29 (6.31)	8.00-8.53 (8.22)	8.71-8.89 (8.77)	7.00-7.90 (7.57)	8.60-9.40 (9.01)	7.60-8.60 (7.84)	6.00-9.00 (7.73)
NH <sub>4</sub> <sup>+</sup> (μM)	3.13-11.44 (6.60)	0.50-1.75 (0.96)	0.36-4.04 (1.43)	0.01-1.31 (0.70)	0.01-1.27 (0.39)	0.01-0.76 (0.38)	0.10-1.35 (0.72)	0.35-3.00 (1.05)
NO <sub>3</sub> <sup>-</sup> + NO <sub>2</sub> <sup>-</sup> (μM)	2.59-5.96 (3.91)	0.28-3.25 (0.99)	4.11-6.10 (5.29)	2.20-8.58 (5.38)	0.30-2.25 (1.20)	0.16-2.25 (1.25)	0.16-2.57 (1.06)	0.15-2.58 (0.64)
DIN (μM)	7.29-15.33 (10.51)	1.11-4.15 (1.96)	4.47-9.81 (6.72)	2.21-9.87 (6.08)	0.31-2.66 (1.59)	0.17-2.50 (1.63)	0.77-2.94 (1.78)	0.95-3.55 (1.69)
TP (μM)	0.37-0.65 (0.50)	0.20-0.39 (0.29)	0.19-0.41 (0.26)	0.14-0.23 (0.20)	0.28-0.51 (0.36)	0.26-0.40 (0.35)	0.17-0.67 (0.30)	0.20-0.54 (0.31)
Si (μM)	2.32-2.94 (2.62)	1.31-2.37 (1.61)	1.27-3.72 (2.58)	0.83-4.65 (2.50)	1.16-10.84 (2.70)	0.81-2.31 (1.63)	0.94-2.35 (1.54)	1.48-5.40 (3.21)
Chl- <i>a</i> (μg L <sup>-1</sup> )	0.10-0.29 (0.25)	0.10-0.53 (0.28)	0.49-0.58 (0.52)	0.40-0.73 (0.60)	0.55-1.13 (0.74)	0.79-1.03 (0.91)	0.10-0.27 (0.12)	<0.2 (<0.2)
SS (mg L <sup>-1</sup> )	5-8 (6)	5-9 (8)	8-11 (9)	9-11 (10)	7-12 (10)	9-13 (11)	6-9 (7)	4-9 (8)
Secchi depth (m)	6.0	5.0	4.9	4.6	6.7	6.0	6.7	13

No significant differences in water temperature were found between the fish farm and control site but there were seasonal variations (Fig. 1a): the lowest values were in winter and the highest at the beginning of summer, while no significant differences were observed between autumn and spring. Only during the sampling campaign at the beginning of summer, at both the fish farm and the control site, was the surface water temperature 3 to 4 °C higher than the bottom water temperature. No significant differences in pH were found between the fish farm and control site. pH values remained between 7.9 and 8.2. No significant differences in salinity were found between the fish farm and control site and it remained uniform throughout the water column in all the samples. DO values in the water column were lower and significantly different at the fish farm in comparison with the control site. DO concentrations also showed seasonal differences, with the highest levels being observed in winter and spring and the lowest in autumn. No defined pattern was observed in the vertical distribution for DO. The levels of DIN,  $\text{PO}_4^{3-}$  and TP at the fish farm installation were significantly higher than those of the control station, with seasonal differences being found during some of the sampling campaigns. The DIN presented much lower concentrations, both at the fish farm installation and at the control site, during the sampling campaigns of spring and the beginning of summer. The seasonal variations in phosphorus were not high; the only notable increase in concentration was in the autumn campaign, in which the mean concentration in  $\text{PO}_4^{3-}$  at the fish farm was double that of the control station. In the vertical profiles of DIN (Fig. 1b) at the fish farm installation we observed peaks of concentration at intermediate depths in the samples for autumn, winter and the beginning of summer.





**Figure 1:** Temperature, DIN,  $PO_4^{3-}$  and DO in the water column in the fish farm installation and the control station in the 4 sampling campaigns.

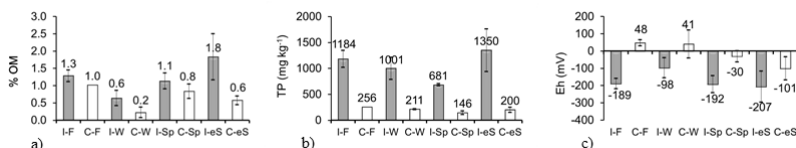
The vertical distribution profiles for  $PO_4^{3-}$  (Fig.1c) were quite similar to those of the TP: at the fish farm we observed clearly defined peaks at intermediate depths in the autumn and spring campaigns. No significant differences in Si concentrations were found at the two stations sampled or during the different seasons of the year. In the vertical distribution profiles of

Si, a subsurface peak was observed only at the fish farm installation in the spring campaign, but with no clear general pattern emerging (Fig. 1d). There were significant differences in Chl-*a* concentration between the fish farm installation and the control station, with the control site being the higher of the two. There were also differences among the different sampling campaigns: the highest Chl-*a* concentrations were in spring, with an average of 0.74 and 0.91  $\mu\text{g L}^{-1}$  at the fish farm and control respectively; while the lowest were recorded at the beginning of summer (Table 2). No clear pattern emerged in the vertical distribution of Chl-*a* in the water column. SS values ranged from 4 to 13  $\text{mg L}^{-1}$ , and were highest in spring at both the fish farm and the control site, coinciding with the highest observed values for Chl-*a*. There were significant differences between SS at the fish farm and that measured at the control station, with the highest concentrations being observed at the control station, however, there were no significant differences in transparency values between sites. No clear trend was observed in the vertical distribution of the SS.

### 5.3.2.- Sediment

Both the fish farm facility and the control station were characterised by sandy sediments with a grain size mode of between 0.125 mm and 0.063 mm and an average size corresponding to very fine sand. Differences were only observed in the percentage of gravels,  $6.3\pm 2.8$  at the fish farm versus  $0.1\pm 0.1$  at the control station. This sediment fraction, which represents particle sizes greater than 2 mm, is mainly composed of mussel valves, which in the case of the fish farm were found to be 40 times higher than that observed in the control station. At both locations the porosity of sediments was 0.46. Significant differences were found between fish farm and control site sediments in OM content, TP concentrations and Eh measurements. In the four sampling campaigns, OM content and TP concentrations were found

to be greater at the fish farm facility than at the control station (Fig. 2a and 2b). OM content was found to be three times and TP seven times higher at the fish farm than at the control site at the beginning of summer. The highest concentrations of OM ( $1.8\% \pm 0.7$ ) and TP ( $1,350 \pm 411 \text{ mg kg}^{-1}$ ) and the greatest negative values for Eh ( $-207 \pm 90 \text{ mV}$ ) were found in the early summer in samples taken from below cages. Reducing conditions were observed under the cages throughout the year; Eh values were found to be consistently negative and much lower than those observed at the control station (Fig. 2c).



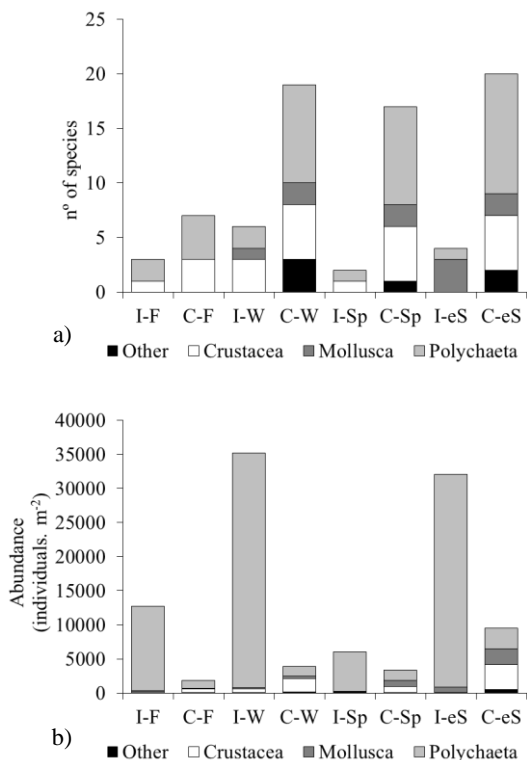
**Figure 2:** % OM, TP and Eh in sediments under the installation of the marine fish farming (I) and the control station (C) in the sampling campaigns in the Fall (F), Winter (W), Spring (Sp) and early Summer (eS).

### 5.3.3.- Benthic organisms

Visual inspections did not reveal *Beggiatoa* spp. (Trevisan, 1842); however, microphytobenthic assemblages were observed in the control station sediments in the spring.

Various organisms belonging to *Crustacea*, *Mollusca* and *Polychaeta* groups were found at the fish farm installation. These groups were found at the control station as well others belonging to the *Nematomorpha*, *Equinodermata*, *Sipuncula* and *Cnidaria* groups, in some of the sampling campaigns. Fig.3 shows the specific richness and abundance of benthic macrofauna, which clearly indicates the consistently lower specific richness and greater abundance of the fish farm when compared to the control

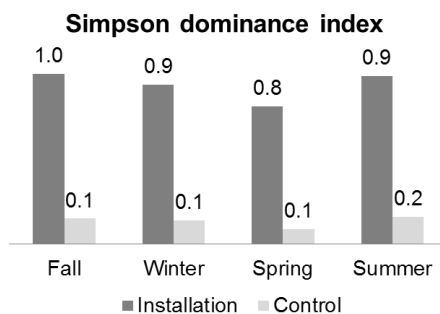
station. The number of species found at the fish farm installation was between 2 and 6, while at the control station, it was between 7 and 20.



**Figure 3:** a) number of species and b) density of individuals in sediments under the of the fish farm installation (I) and the control station (C) in the sampling campaigns in the Fall (F), Winter (W), Spring (Sp) and early Summer (eS).

However, the average abundance at the fish farm installation was  $21,419 \pm 14,339$  individuals  $m^{-2}$  whereas at the control station, it was  $4,584 \pm 3,440$  individuals  $m^{-2}$ . This trend was in line with the results of Simpson's diversity index (Fig. 4), which always showed higher values at the fish farm installation than at the control station. Simpson's diversity index

registered values of between 0.89 and 0.95 at the fish farm, and between 0.08 and 0.15 at the control site. In all sampling campaigns, polychaeta showed lower specific richness at the fish farm than at the control station. *Capitella capitata* (Fabricius, 1780) was consistently present at the fish farm although some *Owenia fusiformis* (Delle Chiaje, 1844) individuals were found in a few samples, as were *Diopatra neapolitana* (Delle Chiaje, 1841). All samples taken at the control station contained species such as *Nephtys hombergi* (Savigny, 1818) (Fig. 5a). *Hyalinoecia bilineata* (Baird, 1870) (Fig. 5b), *Goniada maculate* (Örsted, 1843), *Pectinaria koreni* (Malmgren, 1866), *Glycera* sp (Grube, 1850), in addition to species from the *Sabellidae* (Malmgren, 1866), *Spionidae* (Grube, 1850) and *Maldanidae* (Malmgren, 1867) families, which were found in at least two of the sampling campaigns taken at the control station. In contrast, the abundance of polychaeta was found to be consistently greater at the fish farm than at the control station, owing to the dominance of *Capitella capitata* under the fish cages. This

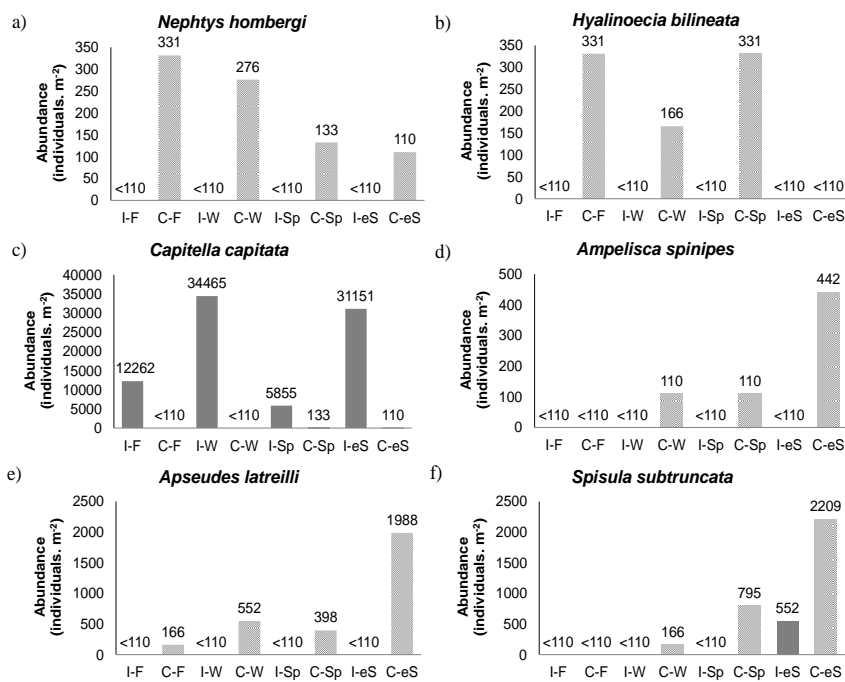


**Figure 4:** Simpson's diversity index at installation of the marine fish farming and at the control station in the 4 sampling campaigns.

species showed an abundance of between 5,855 and 34,465 individuals  $m^{-2}$  with the samples for winter and beginning of summer showing the maximum abundances (Fig. 5c). While the Crustacea exhibited the same general pattern

in terms of specific richness, that is, fewer species at the fish farm than at the control station, lower abundance at the fish farm was observed. Species such as *Ampelisca spinipes* (Boeck, 1861) and *Apseudes latreilli* (Milne-Edwards, 1820) which were detected at the control station were not observed at the fish farm (Fig. 5d and 5e). The sampling conducted at the beginning of the summer did not contain any crustacean species, while a maximum abundance was observed in winter, with 530 individuals  $m^{-2}$ . However the abundance of crustaceans at the control station ranged between 497 and 3,646 individuals  $m^{-2}$ , with a maximum observed in the sampling campaign carried out at the beginning of the summer. The number of species from the mollusc group was found to be low at both sites. The autumn sampling did not contain any mollusca species underneath the cages or at the control station. *Spisula subtruncata* (da Costa, 1778) was found at the fish farm in the sampling taken at the beginning of summer, with an abundance of 552 individuals  $m^{-2}$ . Yet this species was also found in samples taken at the control station in winter, spring and beginning of summer; the latter sampling exhibiting a maximum abundance for this species, which was found to be 2,209 individuals  $m^{-2}$  (Fig. 5f).

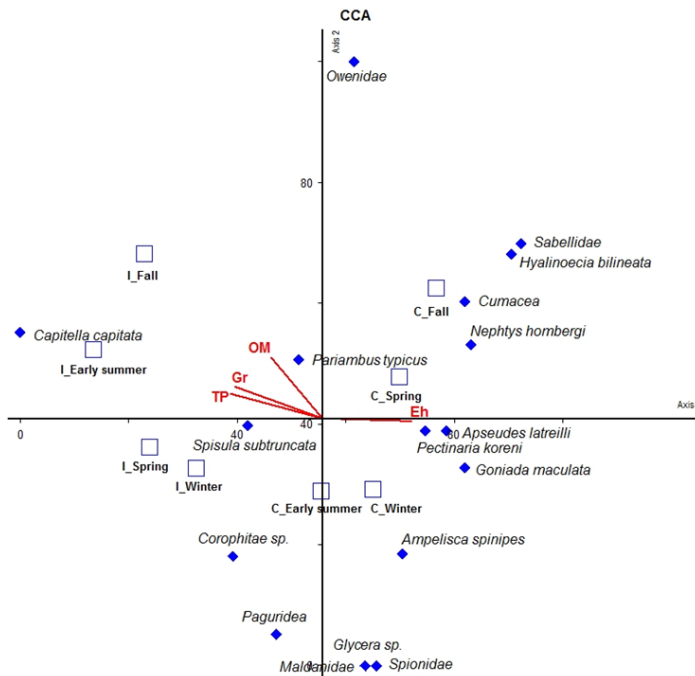
In the CCA, since rare taxa can distort the coordination points, the taxa that were only observed during a sampling campaign at either the fish farm installation or control station were excluded. The abundance values were converted into  $\log(\text{abundance}+1)$ . We considered a total of four benthic environmental variables (% OM, TP, Eh, % gravels). Analysis showed that the first three axes accounted for 68.9% of the total variance contained in the data for the species in the benthic community. The first axis accounted for 34.6%, the second, 22.6%, and the third, 11.6%. All the variables correlated with axis 1; with the correlation being positive for Eh ( $r = 0.89$ ), and negative



**Figure 5:** density of individuals of some species of macrofauna in sediments under of the fish farm installation (I) and the control station (C) in the sampling campaigns in the Fall (F), Winter (W), Spring (Sp) and early Summer (eS).

for % OM ( $r = -0.53$ ), TP ( $r = -0.92$ ) and % gravel ( $r = -0.88$ ). The only predictor variable with strong loading on axis 2 was % OM which had a positive correlation ( $r = 0.72$ ). The Pearson correlation between the species and the environmental variables was 0.96 and 0.98 for the first and second axes, respectively. The factors diagram (Fig. 6) respecting axis 1, showed a clear differentiation in the two sampled zones and a smaller differentiation among the different sampling campaigns in both zones. The four sampling campaigns at the fish farm installation appeared on the left or negative

whereas the sampling campaigns at the control station, with the exception of the beginning of summer, which was in the middle, appeared on the right or positive. As regards axis 2, differences were observed among the different sampling campaigns in the 2 zones. Among the species found, *Capitella capitata* stood out as being the only one located top left. Most of the species found only at the control station appeared to the right, and of these, species such as *Ampelisca spinipes*, *Aapseudes latreilli*, *Pectinaria Koreni*, *Goniada maculata*, *Glycera sp* and the families *Maldanidae* and *Spionidae* appeared bottom right.



**Figure 6:** CCA ordination diagram showing the study sites positions: installation (I) and Control (C) in the 4 sampling campaign (□) and distribution of species (◆) in relation to predictor variables: percentage of organic matter (OM), total phosphorus (TP), redox potential (Eh) and percentage of gravels (Gr).



### 5.4.- Discussion

#### 5.4.1.- Water column

The average velocity values ranged between 3 and 10 cm s<sup>-1</sup>, similar to those observed by Aguado & García (2004) in the Western Mediterranean Sea. The dominant current direction among all measurements made at both sites was found to be northeast. This minimizes the possibility that the control station could have been influenced by fish farm waste, as the control station had been situated up-current from dominant sea currents.

At both the fish farm installation and the control station, the water temperature during each sampling campaign varied according to the season. At the beginning of summer, no thermocline was observed, although there was a gradual decrease in temperature from the surface to the bottom due to the increase in incident solar radiation (Fig. 1a). The lower DO concentrations in the water column observed at the fish farm with respect to those seen at the control station are due to oxygen consumption produced by fish respiration, consumption of organic matter through aerobic decomposition and the nitrification of the reduced forms of nitrogen. In every case, values were found to be higher than the “farm’s critical value” (3.7 mg l<sup>-1</sup>), as per the recommended criteria established by Abo & Yokoyama (2007) for sustainable aquaculture production. The highest DO values were observed in winter and spring, due probably to the lower temperature and greater primary production respectively. The highest concentrations of DIN in the water column registered at the fish farm installation were due to supplies of both NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>+NO<sub>2</sub><sup>-</sup>. Ammonium nitrogen (Table 2) is the principal form of nitrogen that is excreted by the fish (Dosdat, 2000; La Rosa et al., 2002; Aksu & Kocatas, 2007), as well as the first component released by decomposition of organic matter in the water column and sediment. The particulate portion of the nitrogen from the fish

farm which is deposited in the sediment is rapidly decomposed biochemically and reincorporated into the water column (Christensen, Rysgaard, Sloth, Dalsgaard & Schwaerter 2000; Aguado, 2001; Cromey, Nickell & Black 2002). The highest value of  $\text{NH}_4^+$  was found to be  $11.4 \mu\text{M}$ , which means that, given the temperature, salinity and pH of the water, 2.65% of this concentration was in the form of ammonia (Johansson & Wedborg 1980), that is,  $5.2 \mu\text{g NH}_3 \cdot \text{L}^{-1}$ . This value was substantially lower than the maximum  $\text{NH}_3$  value recommended by the Environmental Protection Agency (EPA) ( $<100 \mu\text{g NH}_3 \cdot \text{L}^{-1}$ ) to avoid negative effects on fish growth. It was also lower than the levels recommended by Wajsbrot, Gasith, Krom & Popper (1991) for gilthead seabream fish farms to avoid adverse effects on growth and survival ( $<64 \mu\text{g NH}_3 \cdot \text{L}^{-1}$ ). The differences observed in  $\text{NO}_3^- + \text{NO}_2^-$  (Table 2), found in greater concentrations at the fish farm, probably due to the  $\text{NH}_4^+$  is quickly oxidized to the less toxic  $\text{NO}_3^-$  and  $\text{NO}_2^-$  (Dosdat, 2000), as well as from faecal nitrogen and non-ingested feed. We found generally lower concentrations of DIN during the sampling campaigns of spring and the beginning of summer compared to those of autumn and winter. This reflects the typical dynamics known for the western Mediterranean, caused by summer stratification of the water column due to shallow pycnoclines and maximum phytoplankton growth and nutrient uptake in the upper water layer due to increased temperature and irradiance (Maldonado et al., 2005). We also observed differences at the fish farm installation compared to the control site, especially during the autumn sampling campaign for concentrations of  $\text{PO}_4^{3-}$  and TP in the water column. This can be attributed to the excretion of phosphorus by fish in the form of dissolved orthophosphate, organic phosphorus compounds or non-ingested feed, which also contains phosphorus (Jover, 2000). The peaks found at intermediate depths for both DIN and de  $\text{PO}_4^{3-}$  at the fish farm installation were probably due to fish excretion and the location of the fish in the cages when the

samples were taken. In floating sea cages, the density of the fish can be affected by environmental gradients such as temperature, currents and variations in light (Juell & Fosseidengen, 2004). Fish are attracted to the optimum areas and avoid unfavorable areas. This causes high densities of fish in favorable areas with less competitive fish confined to the other areas and in lower densities (Johansson, Juell, Oppedal, Stiansen & Ruohonen 2007; Oppedal, Juell & Johansson 2007). This behaviour can affect different environmental parameters since high local concentrations of fish can lead to reduced water flow (Martins, Galhardo, Noble, Damsgard, Spedicato, Zupa, Beauchaud, Kulczykowska, Massabuau, Carter, Rey Planellas & Kristiansen 2012).

It should be noted that significant differences were seen in the majority of nutrients analysed in the water column, with respect to other studies. The high spatial resolution of the water column parameters may have contributed to these results. There are studies such as that conducted by Kaymakci et al., (2010) in which significant differences were not observed for any of the parameters measured in the water column (oxygen or nutrients) at eight fish farms around Salih Island and at control station at each fish farm. However, there are other studies which have shown significant differences in some of the water column parameters measured at sea fish farms with respect to control sites. For instance, La Rosa et al., (2002) also found significant differences in  $\text{PO}_4^{3-}$  concentrations; they did not, however, find differences in DIN. Yucel-Gier et al., (2007) found significant differences in  $\text{NO}_3^-$  concentrations, although they did not find differences in  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and  $\text{PO}_4^{3-}$ . Aksu & Kocatas (2007) also found significant differences in  $\text{NH}_4^+$  y  $\text{PO}_4^{3-}$  concentrations, but not in DO, y  $\text{NO}_3^- + \text{NO}_2$ .

The higher levels of DIN and  $\text{PO}_4^{3-}$  found in the water column of the fish farm installation compared to those of the control station, combined with the oligotrophic character of the Mediterranean Sea (Siokou-Frangou,

Christaki, Mazzocchi, Montresor, Ribera d'Alcalá, Vaqué& Zingone 2010) could cause over-fertilization and undesirable consequences for the ecosystem and fish farming. Although it is not present in the waste produced by aquaculture (Maldonado et al., 2005), silicate was also measured in order to calculate the stoichiometric ratios of nutrients, as another effect of increased nitrogen and phosphorus in the water column is the alteration of the stoichiometric ratio DIN:PO<sub>4</sub><sup>3-</sup>:Si. The criteria applied by Justic, Rabalais, Turner & Dortch (1995) were used in this study to identify the limiting nutrients at the fish farm and control station. Table 3 shows the percentages of cases in which each nutrient acted as a potential limiting nutrient, only taking into account nitrogen and phosphorus, as these nutrients are the ones that are added into the system by the aquaculture activity. It was observed that phosphorus was the limiting nutrient at the fish farm in three out of four sampling campaign (autumn, winter, beginning of summer), while phosphorus only acted as limiting nutrient at the control station in winter.

**Table 3:** Percentage when DIN or PO<sub>4</sub><sup>3-</sup> act as potential limiting nutrient.

	Sampling	%DIN limiting	%PO <sub>4</sub> <sup>3-</sup> limiting	% without limitation
<b>INSTALLATION</b>	Fall	0	70	30
	Winter	0	80	20
	Spring	50	20	30
	EarlySummer	20	50	30
<b>CONTROL</b>	Fall	56	0	44
	Winter	0	89	11
	Spring	56	11	33
	EarlySummer	50	30	20
<b>INSTALLATION</b>	4 campaigns	17	55	28
<b>CONTROL</b>	4 campaigns	40	33	27

This is likely attributable to the fish farm activity which releases just as much nitrogen into the system as it does phosphorus; however nitrogen is released in far greater quantities. Moreover, much of the nitrogen is released in dissolved form whereas phosphorus is mostly in particulate form (Yucel-Gier et al., 2007; Olsen et al., 2008). On the other hand, when DIN,  $\text{PO}_4^{3-}$  and Si are taken into consideration in the stoichiometric ratios (Table 4), it is observed that Si acts as the limiting nutrient at the fish farm facilities 11% more than at the control station. This may be explained by the fact that aquaculture releases are limited mainly DIN and  $\text{PO}_4^{3-}$ . Moreover, the limiting nutrient at the fish farm was mostly found to be  $\text{PO}_4^{3-}$  (43%), followed by Si (33%), whereas the limiting nutrients at the control station were found to be  $\text{PO}_4^{3-}$  and DIN, showing similar percentages (around 30%). It is also important to point out the higher levels of nutrients in the areas surrounding the fish farm, as these changes in the nutrients ratios generated by this activity could bring the increase of toxic phytoplankton species such as dinoflagellates in certain times of the year. Olivos, Masó & Camp (2002) and Vila, Garcés, Masó & Camp (2001) observed a relationship between the nutrient runoff along the continental water and/or the changes in the nutrients ratios induced by anthropogenic activities seasonal incidence and an increase in the presence of harmful dinoflagellates along the Catalan Coast and the North-western Mediterranean respectively.

However, despite the greater availability of nitrogen and phosphorus in the areas surrounding the fish farm, we found higher concentrations of Chl-*a* at the control station. This runs contrary to the general prediction that a greater availability of nutrients should lead to an increase in Chl-*a* concentrations. This could be due to many factors: the hydrodynamics of the study area might have contributed to the dilution and dispersion; also, the potential effects of the various substances used in aquaculture (to control diseases and antifouling substances) on primary production and/or the role of

macroalgae and suspensivores associated to the submerged structures of the fish farm. The hydrodynamics of the study area (current velocity was found to range between 3 a 10 cm s-1) could have added to dilution and dispersion, but this would also have affected nutrients such as phytoplankton biomass. We were also aware that this facility did not use antifouling substances as part of its management practices; thus, there was considerable biofouling. In addition, this study measured Chl-*a* concentrations in the water, which is not equivalent to the total primary production that may be taking place in the area; the role of macroalgae and suspensivores associated to the submerged structures of the fish farm and their direct consumption of nutrients and phytoplankton respectively was not taken into account. There are studies

**Table 4:** Percentage when DIN, PO<sub>4</sub><sup>3-</sup> or Si act as potential limiting nutrient.

	Sampling	%DIN limiting	%PO <sub>4</sub> <sup>3-</sup> limiting	%Si limiting	% with outlimitation
<b>Installation</b>	Fall	0	20	80	0
	Winter	0	80	20	0
	Spring	40	20	10	30
	EarlySummer	20	50	20	10
<b>Control</b>	Fall	33	0	44	22
	Winter	0	78	11	11
	Spring	33	11	33	22
	EarlySummer	50	30	0	20
<b>Installation</b>	4 campaigns	15	43	33	10
<b>Control</b>	4 campaigns	29	30	22	19

which attribute increased mussel growth (Cook & Black, 2003) and macroalgae (Chung, Kang, Yarish, Kraemer & Lee 2002) to the nutrient enrichment of the water column in fish farms. Cook, Black, Sayer, Cromey, Angel, Spanier, Tsemel, Katz & Eden (2006) observed higher fouling

biomass and different community compositions in fish farm installations as opposed to those of control sites. Although we did not measure biofouling biomass associated to the submerged fish farm structures directly, we were able to observe, albeit indirectly, significant growth at the fish farm installation. The dry weight of the valves found in the sediment under the cage was between 775 and 1,247 g m<sup>-2</sup>. This was the result of the cleaning operations in place at the fish farm, where removal of biomass is not adequately handled and therefore much of said biomass ends up in the sediment under the cages. Cugier, Struski, Blanchard, Mazurié, Pouvreau, Olivier, Trigui & Thiébaud (2010) claim that the wild suspensivores associated to shellfish farming are key elements in the control of primary production and concentrations of chlorophyll *a*.

Our study highlights the importance of researching vertical profiles in the water column as a means of evaluating the impact of fish farming. We found that at the fish farm installation there were lower concentrations of DO and higher concentrations of DIN, PO<sub>4</sub><sup>3-</sup> and PT than in the reference zone due to the aquaculture activity. The increase in the DIN and PO<sub>4</sub><sup>3-</sup> concentrations at the fish farm modified the stoichiometric ratios between nutrients, with Si acting 11% more as a limiting nutrient at the fish farm than at the control. Nevertheless, Chl-*a* concentration was higher at the control station. On the other hand, the seasonal changes observed in the majority of the variables studied in the water column were mainly due to the typical seasonal weather patterns of the Mediterranean (La Rosa et al., 2002; Maldonado et al., 2005), since they occurred both at the fish farm and the control station.

### 5.4.2.- Sediment

The differences between the fish farm and the control station in the percentage found in the sediment fraction (> 2 mm) were due to the shells

coming from the fouling removal performed on the submerged structures of the fish farm.

The OM content was consistently higher under the cages than at the control station, a finding which is known to occur under fish farm cages located in open seawater (Karakassis, Tsapakis, Hatziyanni, Papadopoulou&Plaiti 2000; Mantzavarakos et al., 2007, Borja, Rodríguez, Black, Bodoy, Emblow, Fernandes, Forte, Karakassis, Muxika, Nickell, Papageorgiou, Pranovi, Sevastou, Tomassetti & Angel 2009). OM in sediment mostly originates from non-ingested fish feed, either due to over-feeding or poorly managed diet. Accumulation of fish faeces, cultivated fish mortality and cage cleaning may also increase OM in the sediment (Molina & Vergara, 2005). The highest OM content was found under the cages in summer, due to the higher rates of organic matter deposition from the seabream production, which varies seasonally. As temperatures increase, fish metabolism increases and more fish feed is administered. This leads to greater excretion rates and fish feed wastage. This accumulation of organic matter at the sediment surface increases the metabolism of the sediments which leads to increased oxygen consumption (Morata et. al., 2012), as oxygen is used by aerobic bacteria as an electron acceptor in respiration. The OM values observed in this study were similar to those found in another fish farm studies (Sakamaki, Nishimura & Sudo 2006; Nizzoli, Bartoli & Viaroli 2007).

In this study, TP under the fish cages was always higher than at the control site; a result also seen by Karakassis, Tsapakis & Hatziyanni 1998, with the greatest concentrations in the summer months, as observed by Mantzavarakos et al., (2007). This, as in the case of OM, can be explained by the increase in solid waste that is generated as fish farm activity increases in summer. The TP concentrations in sediments under fish cages in the Mediterranean Sea observed by authors such as Karakassis et al., (1998) and



Porrello et al., (2005), were of an order of magnitude that was similar to the concentrations found in this study. Eh in the sediments is a key factor in determining the biochemical transformation of organic matter as well as distribution, type and physiological activity of bacteria and other microorganisms found in the sediment (Teasdale, Minett, Dixon, Lewis & Batley 1998). Eh was measured in the uppermost sediment layer. We found sediments under the cages to be consistently more negative, with the greatest negative value observed in early summer possibly due to the higher OM content. Other studies also found reducing sediments under fish farms such as those conducted by Karakassis et al. (1998), Karakassis et al. (2000) and Ferrón et al. (2009).

### 5.4.3.- **Benthic organisms**

The area under fish cages showed fewer animal groups as well as a lower specific richness in crustaceans and polychaetes. This is in keeping with Bellan-Santini, Lacaze & Poizat (1994), who state that, under normal conditions, oligotrophic systems such as the Mediterranean Sea, show a low abundance and high diversity of species, a situation that is not unlike the conditions observed at our control station.

As the CCA indicated, the environmental variables measured in the sediment were largely responsible for the differences found in the macrofauna at the fish farm installation and the control station with the TP and Eh showing the best correlation with the distribution and abundance of the species. The OM also partly explained the distribution and abundance of the species. There are greater quantities of OM under the cages (Fig. 2a) and this produces a decrease in oxygen concentrations in the sediment, which affects species that show high sensitivity to oxygen depletion (Diaz & Rosenberg, 1995).-In natural conditions, hypoxia is often associated with increased ammonia and hydrogen sulphide (Wu, 2002), substances which are

toxic to most organisms. Although the CCA explained most of the variability in the two study zones as well as giving high correlations between the environmental and biological variables, other environmental variables not measured in this study were also a probable cause of the low diversity found below the cages. For example, other contaminants from fish farming activities, namely metals and chemotherapeutic agents may also adversely affect benthic fauna (Dean et al., 2007; Davies et al., 1997), which may also lead to the disappearance of some species. Pinedo & Jordana (2008) classified the macrofauna of the Western Mediterranean area, according to each species sensibility/tolerance to organic enrichment in particular. Species were grouped into four categories: 1 – sensitive; 2 – indifferent; 3 – tolerant; 4 – opportunist species. According to this classification system, species such as *Ampelisca spinipes* belong to group 1 (species which are very sensitive to organic enrichment and present under unpolluted conditions) and species such as *Apseudes latreilli*, *Nephtys hombergi*, *Goniada maculata*, *Pectinaria koreni* and *Glycera sp* belong to group 2 were only observed at the control station. At the CCA, these species appeared to the right on the factors diagram. However, *Capitella capitata*, the dominant species in the fish farm installation (Fig. 5c) and responsible for the high dominance of the macrofauna under the cages (Fig. 4), was classified as a member of group 4, that is, as an opportunistic species (in pronounced unbalanced situations). These are deposit feeders, which proliferate in reduced sediments. The CCA showed that this species is associated with high values of OM, TP and gravel and highly reducing conditions in sediments. Karakassis et al., (2000) also found *Capitella capitata* to be the dominant species among macrofauna under two marine fish farms in the Mediterranean Sea. *Capitella sp.* is thought to be an indicator par excellence of anoxic conditions (Rosenberg, 2001; Wu, 2002). On the other hand, the increased abundance of this organism may, to a certain extent, limit accumulation of organic matter in sediments, as there is

an increased consumption of organic matter by this macrofauna type. According to Banta, Holmer, Jensen & Kristensen (1999), this phenomenon may account for up to 15% of the total respiration of sediments.

The results of this study demonstrate the general effect that organic enrichment has on marine sediments: namely, lower diversity and greater abundance of individuals. The benthic variables which showed the best correlation with the distribution and abundance of species were the concentrations of TP and Eh. The polychaete worm *Capitella capitata* was the dominant species under the cages, and thus can be considered a good indicator of organic contamination. Taxas such as *Ampelisca spinipes*, *Apeudes latreilli*, *Nephtys hombergi*, *Pectinaria koreni* and *Hyalinoecia bilineata* can be regarded as good indicators of non-disturbed areas.

### 5.5.- Conclusion

This study showed that fish farming activities located in marine ecosystems can give rise to certain environmental effects in the water column as these activities decrease the concentration of dissolved oxygen and increase the concentration DIN and  $\text{PO}_4^{3-}$ . These conditions occasionally altered the stoichiometric ratios between nutrients and the limiting nutrient in primary production. Some future lines of research that may prove useful may be the characterisation and quantification of phytoplankton species, as the alterations which may favour the development of species that do not require silicate, such as dinoflagellates. In addition, the fact that we found nutrient concentrations with lower levels of Chl-*a* at the fish farm facility suggests that it would be worth studying the role of marine biofouling on submerged fish farm structures in the uptake of nutrients, particulates and phytoplankton.

The sediment under the cages was found to have greater concentrations of organic matter and total phosphorus, as well as a redox potential that was more negative with respect to the sediments located in the

control station. This was attributed to the organic waste from the fish farm. Since the fish feeding rates were a function of water temperature, the sediments quality consequently also showed seasonal changes. The fish farming activity also generated a significant change to the structure of the benthic community under the fish cages, with a notable decrease in specific richness and nearly a nine-fold increase in abundance. The values of OM, TP, Eh and % gravels in sediments influence the distribution and abundance of species of the macrofauna, which limits the presence of sensitive species and favours population growth of opportunists such as *Capitella capitata*.

Although these impacts are quite localised and depend on a variety of factors arising from the fish farming activity itself and its location, this type of research may be of assistance in legislative processes, management practices and the adoption of measures by this industry to reduce the negative impact of these farming activities on the environment.

### **5.6.- Acknowledgements**

We would like to thank the Caja del Mediterráneo (CAM) for a pre-doctoral fellowship fund for this research and Antonio Asunción Acuigroup Maremar manager, for the facilities and support in conducting the study. The translation of this paper was funded by the Universidad Politécnica de Valencia, Spain.

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# Capítulo 6

## Benthic Recovery after the Cessation of a Gilthead Seabream Fish Farm in the Mediterranean

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*Enviado a Aquaculture (13-03-2013)*

### **Abstract**

After the cessation of a gilthead seabream (*Sparus aurata*) fish farm in the Mediterranean off the coast of Spain, a study was made of the recovery of the environment. Measurements made in the area that was affected by the fish farm and a control station included: the physico-chemical parameters of sediments (granulometry, porosity, organic matter, total phosphorus, and redox potential); nutrient and oxygen fluxes at the sediment-water interface; and the benthic macrofauna. Five sampling campaigns were made, one before the fish farm closed, and others at 1, 3, 9 and 24 months after cessation. The benthic flux of ammonium was the first parameter to recover, followed by benthic fluxes of phosphate and dissolved oxygen and % organic matter in the sediments, which 3 months after the cessation of the fish farm already showed levels similar to those in the control station. At 9 months after the cessation the other abiotic parameters of the sediments disturbed by the activity had recovered, such as the % coarse fraction, total phosphorus concentrations, and redox potential measurements. Fluxes of phosphate revealed a variable percentage, in function of the time that had passed since the cessation of the fish farm – the losses of total phosphorus in the sediment being from 30.2% to 1.2%. The recovery of the macrofauna was slower than the abiotic parameters. 3 months after the cessation, the abundance of *Capitella capitata* had descended drastically in the area that was affected by the fish farm, but similar levels of specific richness were not observed between the two sampled zones until 2 years after the cessation of the fish farm.

**Keywords:** Benthic fluxes; Nutrient fluxes; Oxygen fluxes; Interface sediment-water; Sediment geochemistry; Macrofauna

### **6.1.- Introduction**

More than half of all the sea products consumed by the world population are now produced in fish farms. This milestone has been reached

after four decades of continuous and intense development (APROMAR, 2012). The FAO (2010) estimates that by 2030, 65% of aquatic food will come from aquaculture. Ensuring high food standards is an essential goal in the European Union, and ensuring the food security, environmental protection, and social welfare of employees are inalienable principles.

For a correct environmental management, it is important to have a good knowledge about the processes that regulate the effects of the aquaculture residues on the ecosystem (Sanz-Lázaro and Marín, 2011) and understand the processes of ecosystem recovery when activity ceases or during a fallow (Aguado et al., 2012).

There is a considerable literature regarding the environmental effects of fish farming and following the expansion of fish farms various researchers have studied the impacts on the water column, sediments, fauna and flora in the vicinity of fish farm facilities. These studies – some of which were made in the early days of fish farming – include: Gowen and Bradbury (1987); Hall et al. (1990); Holby and Hall (1991); Holmer and Kristensen (1992); Diaz and Rosenberg (1995); Karakassis et al. (1998); and these studies have been supplemented over the years by Mazzola et al. (2000); La Rosa et al. (2002); Cancemi et al. (2003); Porrello et al. (2005); Pitta et al. (2005); Maldonado et al. (2005); Pergent-Martini et al. (2006). The most recent studies include: Yucel-Gier et al. (2007); Freitas et al. (2008); Ferrón et al. (2009); Olsen et al. (2008); Matijević et al. (2009); Siokou-Frangou et al. (2010), Sanz-Lázaro and Marín (2011); Huang et al. (2011); Morata et al. (2012). However, few studies have examined the evolution of the environment after production ceases – despite the fact that this information is needed for making future impact predictions (Aguado et al., 2012). The biogeochemical processes of recovery are poorly studied and the time needed to restore an ecosystem has not been determined (McGhie et al., 2000; Pereira et al., 2004; Gray and Elliott, 2009). Despite this, recovery has been studied under various settings, including situations of temporary cessation (fallowing between two

production periods) (McGhie et al., 2000; Brooks et al., 2003; Macleod et al., 2006, 2007; Vita and Marín, 2006; Lin and Bailey-Brock, 2008), and after the complete cessation of productive activity (Karakassis et al., 1999; Mazzola et al., 2000; Kraufvelin et al., 2001; Brooks et al., 2004; Macleod et al., 2004; Pereira et al., 2004; Sanz-Lázaro and Marín, 2006; Aguado et al., 2012). In the case of fallowing, sediment recovery is understood from a sustainability point of view: recovery to the extent of preventing a progressive deterioration of the sediments may be sufficient to support long-term farming operations (Macleod et al., 2006, 2007). A complete recovery is not then required or expected after fallowing. Nevertheless, in the context of definitive cessation, sediment recovery is understood as a return to conditions similar to those in adjacent undisturbed sediments (Thrush and Whitlatch, 2001; Sanz-Lázaro and Marín, 2006). Brooks et al. (2002, 2003, 2004) observed that a chemical remediation should first occur to allow a subsequent biological remediation. The degree of recovery is quite variable, depending mainly on the hydrological characteristics of the area, the sediment type, and in the case of fallowing, the duration of fallowing (Brooks et al., 2004; Pereira et al., 2004; Macleod et al., 2006, 2007; Lin and Bailey-Brock, 2008). Moreover, benthic response and thus recovery processes are scale-dependent and may vary in terms of the extent of the impact (Whitlatch et al., 1998; Villnäs et al., 2011). Additionally, biotic factors, such as community composition and peculiarities (dispersal, recruitment, life stage, etc.), and their relationships (competence, predation, etc.) influence recovery processes (Norkko et al., 2006).

The aim of this study is to analyse the recovery of an area in the Mediterranean affected by a gilthead seabream (*Sparus aurata*) fish farm after cessation of the fish farm. Analysis included the physicochemical parameters of the sediments, the development of benthic fluxes of oxygen and nutrients *in situ* – this being the first study to make these measures *in situ*

after the cessation of a farm. We also analysed the development of the macrobenthic sediment community.

### 6.2.- Materials and methods

The study area is located in the north-west Mediterranean, in the Gulf of Valencia (Spain), at the previous site of a fish farm producing gilthead seabream (*Sparus aurata*). The fish farm installation was located in the open sea, about 2 km from the coast at a depth of 19 m. The fish farm installation began in 1999 and closed in June 2009. Fish production for this farm was 500 t per year in the final years. The facility was composed of 15 fattening cages each 25 m in diameter. The parameters analysed in the sediments and the *in situ* measurements of fluxes were studied at two sampling points: one that was affected by the fish farm activity and located under one of the central cages in the installation (0° 3' 11.101'' W; 39° 50' 19.6243''N), and the control station (0° 3' 6.1871''W; 39° 50' 21.4126''N), located 130 m northeast of the fish farm and up-current from dominant sea currents (Morata et al., 2013). Samples were collected during five sampling campaigns: the first in early summer, before cessation of the fish farm (17/06/2009 (I = Fish Farm Installation) 'I-B' and 19/06/2009 (C = Control Station) 'C-B'); the second sampling campaign was collected 1 month after cessation of the fish farm (28/07/2009 'I-1MA' and 30/07/2009 'C-1MA'); the third sampling campaign was collected 3 months after cessation of the fish farm (22/09/2009 'I-3MA' and 24/09/2009 'C-3MA'); the fourth sampling campaign was collected 9 months after the cessation of the fish farm (07/04/2010 'I-9MA' and 09/04/2010 'C-9MA'); and the fifth sampling campaign at 24 months after cessation of the fish farm (28/07/2011 'I-24MA' and 'C-24MA'). In the final sampling campaign collection, benthic fluxes were not measured.

During each sampling campaign, three samples were taken of unaltered sediment layers from both the fish farm and control station, using

corers with a length of 30 cm and an internal diameter of 6.5 cm (33.18 cm<sup>2</sup>). The uppermost layer (1 cm) was removed to analyse granulometry, porosity, organic matter (OM), and total phosphorus (TP). Granulometry was performed for the sediment samples using the Wentworth scale (Shepard, 1954). Sediment porosity was calculated following Dell'Anno et al. (2002). OM was analysed using the combustion method (Dell'Anno et al., 2002). To determine sediment TP, digestion was performed following Arocena and Conde (1999). When the corers were brought to the surface, redox potential (Eh) was measured at a depth of 0.5 cm using a Crison PH25 potentiometer.

To measure nutrient and oxygen benthic fluxes in situ benthic chambers similar to those used by Freitas et al. (2008) and Morata et al. (2012) were used. Light and dark chambers were used for each incubation, with three replicate samples being taken for each chamber type to minimise the effects of spatial heterogeneity. The chambers were made of semi-spherical methacrylate with a diameter of 40 cm and a volume of 16.7 l, and covering a sediment surface area of 0.125 m<sup>2</sup>. The chambers contained a manual stirrer to minimise concentration gradients (Niencheski and Jahnke, 2002). The chambers were placed in the sediment manually by scuba divers, and the total incubation period was six hours. Samples of water were taken from inside the chambers every 2 hours using 60 ml plastic syringes inserted through openings controlled by a valve. The parameters analysed were: dissolved oxygen (DO), ammonium (NH<sub>4</sub><sup>+</sup>), nitrates (NO<sub>3</sub><sup>-</sup>), nitrites (NO<sub>2</sub><sup>-</sup>), phosphate (PO<sub>4</sub><sup>3-</sup>) and silicate (Si). Benthic fluxes were estimated from the slope of a linear regression of the time series results and the chamber volume (Niencheski and Jahnke, 2002), and Equation 1, as used by Nizzoli et al.

(2007): (1) 
$$F = (C_t - C_o) \cdot (1/(A \cdot t)) \cdot V \cdot 24$$
. Where F is the estimated flux in mmol m<sup>-2</sup> d<sup>-1</sup>; C<sub>t</sub> and C<sub>o</sub> are the final and initial concentrations (mmol) obtained in the linear fit; A is the area of incubation in m<sup>2</sup>; t is the total incubation time in hours; and V is volume of incubated water in l.



The DO samples were fixed immediately and analysed using the Winkler iodometric method (Baumgarten et al., 1996). For the analysis of dissolved nutrients, the samples were filtered using a cellulose acetate membrane filter with a pore size of 0.45  $\mu\text{m}$ . The  $\text{NH}_4^+$  concentration was determined on the same day and the remaining samples were frozen for later analysis. The nutrients were analysed using the methods described by Aminot and Chaussepied (1983) and adapted by Baumgarten et al. (1996).

Scuba divers visually inspected the sea bottom for signs of *Beggiatoa* spp. and phytobenthic assemblages.

To identify and count benthic macroinvertebrates three additional corers were taken in each area during all the sampling campaigns. These corers were sieved using a 0.5 mm mesh and 7% magnesium chloride was used as an anaesthetic. Organisms were later fixed in 7% formaldehyde solution. Simpson's diversity index was calculated following Cardona (2007).

A one-way ANOVA was used to determine the existence of significant differences ( $p < 0.05$ ) among parameters measured in sediment and benthic fluxes in each sampling campaign between the area affected by the fish farm and the control station. When data did not meet the assumptions for the ANOVA, we applied appropriate transformations. This task was carried out using Statgraphics Centurion software.

The effects of benthic environmental variables on the abundances of species in the macrofauna and their spatial variation were analysed using Canonical Correlation Analysis (CCA) and PC-ORD software. Since rare taxa can distort the coordination points, the taxa that were only observed during a sampling campaign at either the fish farm installation or the control station were excluded. The abundance values were converted into  $\log(\text{abundance} + 1)$ . After a preliminary analysis of the CCA, it was found the most important environmental variables. They were: benthic fluxes of  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$  and DO, % OM, concentration TP, Eh and % coarse fraction. The

analysis was made with the samplings campaign data before cessation and 1, 3, and 9 months after cessation, without including data from sampling campaign made 2 years after cessation due to benthic fluxes were not measured.

We consulted the website of Puertos del Estado (<http://www.puertos.es/>) for information on synoptic meteorology in the period June 2009 - July 2011. Specifically, we obtained data from the numerical modelling of node WANA 2084118, located less than six km from the study area.

### **6.3.- Results**

#### **6.3.1.- Sediment physico-chemical variables**

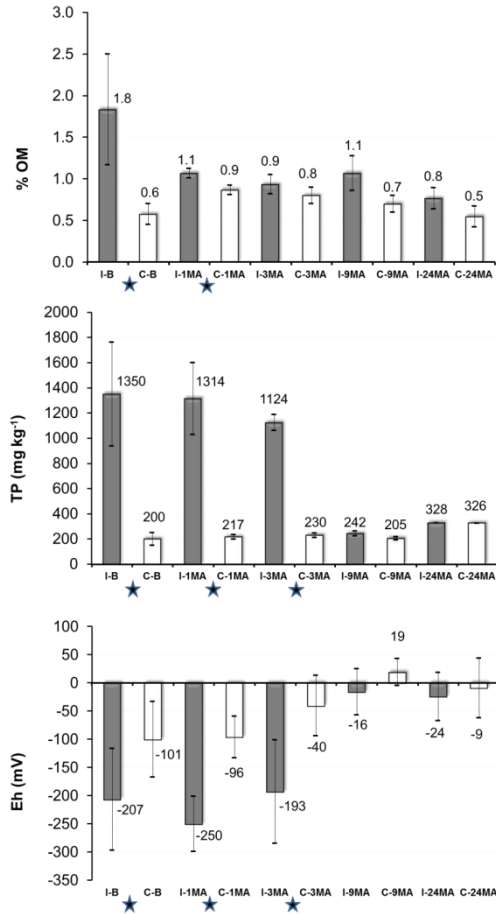
Table 1 shows the values corresponding to the granulometry and porosity of the sediments in the area that was affected by the fish farm and the control station. At both locations, the sediments were sandy with a grain size mode of between 0.125 and 0.063 mm and an average grain size corresponding to very fine. Porosity was very similar and in the area that was affected by the fish farm it was between 0.44-0.50 and in the control station between 0.43-0.49. In the sampling campaign before the cessation of the fish farm and in the first two samplings after the cessation (1 and 3 months), significant differences could be observed between the area that was affected by the fish farm and the control station in the % of the coarse fraction of the sediment (% Cf). This material represents particle sizes greater than 2 mm and is mainly composed of shells. These shells were mostly mussel valves, which in the area that was affected by the fish farm came from the cleaning of the fouling on the submerged structures of the fish farm when it was operating.

Figure 1 shows % OM, TP concentrations, and Eh measurements in the sediments of the area that was affected by the fish farm and the control

station in the five sampling campaigns. In the sampling campaigns before and 1 month after cessation, significant differences (ANOVA,  $p < 0.05$ ) were observed in the sediments of the area that was affected by the fish farm and the control station in % OM, TP concentrations, and Eh measurements. In the sampling campaign 3 months after cessation significant differences in the TP concentrations and Eh measurements were observed, although the % OM no longer showed significant differences. In the other sampling campaigns there were no significant differences between the sampled zones in any of the measured parameters.

**Table 1:** % coarse fraction, % sand, % silt+clay, % shells and porosity in sediments in the area that was affected by the fish farm (I), and the control station (C), in the five sampling campaigns: before cessation of the fish farm (B) and 1, 3, 9 and 24 months after cessation of the fish farm (1MA, 3MA, 9MA and 24MA respectively).

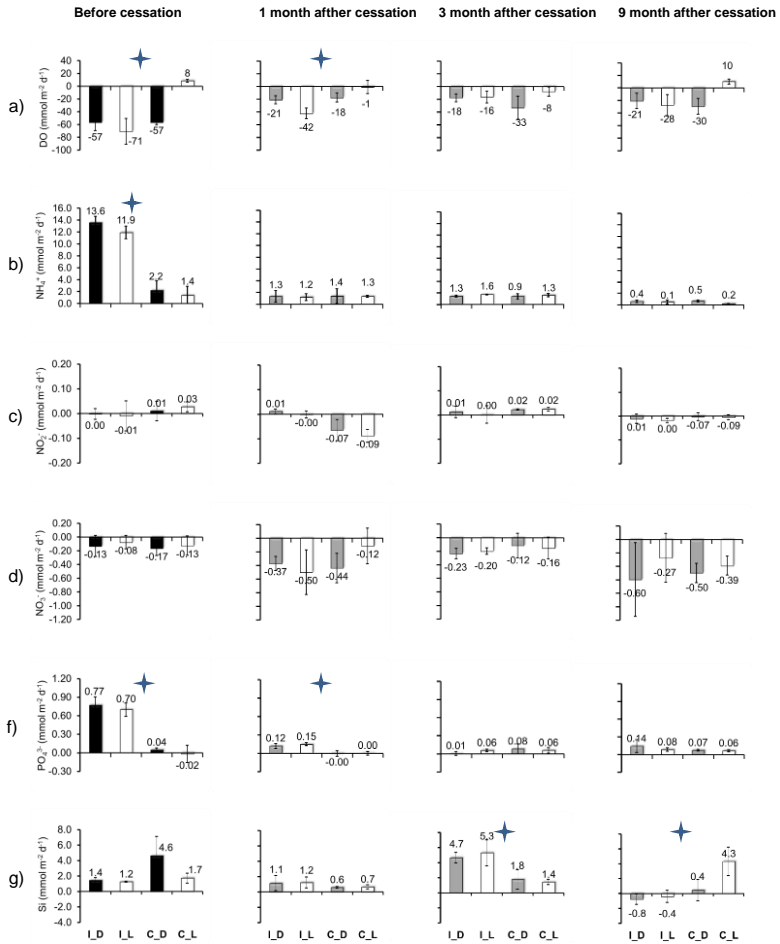
	%coarse fraction	%sand	%silt+clay	%shells	porosity
<b>I-B</b>	5.5±2.8	86.8±0.3	7.7±3.0	16.0±4.7	0.47±0.03
<b>C-B</b>	0.1±0.1	88.6±2.3	11.2±2.4	0.1±0.2	0.46±0.01
<b>I-1MA</b>	8.5±3.8	84.9±2.7	6.6±1.3	13.7±5.8	0.44±0.02
<b>C-1MA</b>	0.1±0.1	88.6±2.3	11.2±2.4	0.1±0.2	0.45±0.01
<b>I-3MA</b>	2.0±3.0	89.3±2.1	8.8±0.9	2.7±3.2	0.46±0.02
<b>C-3MA</b>	0.3±0.2	88.7±1.2	10.9±1.2	0.9±0.1	0.43±0.01
<b>I-9MA</b>	0.1±0.0	89.3±1.0	10.6±1.0	0.1±0.1	0.50±0.01
<b>C-9MA</b>	0.4±0.5	92.3±2.9	7.3±2.5	0.4±0.5	0.49±0.01
<b>I-24MA</b>	0.1±0.2	93.9±0.3	6.0±0.4	0.1±0.2	0.45±0.02
<b>C-24MA</b>	0.2±0.2	94.4±0.5	5.3±0.5	0.1±0.2	0.45±0.02



**Figure 1:** % OM, TP and Eh in sediments in the area that was affected by the fish farm (I) and the control station (C) in the five sampling campaigns: before cessation of the fish farm (B), and 1, 3, 9 and 24 months after cessation of the fish farm (1MA, 3MA, 9MA and 24MA respectively).

★ Differences between installation and control significant (ANOVA,  $p < 0.05$ )

6.3.2.- Benthic fluxes



**Figure 2:** Benthic fluxes of DO, NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup> and Si in dark (D) and light (L) chambers in the area that was affected by the fish farm (I), and the control station (C), before the cessation of the fish farm and 1, 3 and 9 months after cessation of the fish farm.

★ Differences between installation and control significant (ANOVA, p < 0.05).

Benthic fluxes were only measured before and 1, 3, and 9 months after cessation.

In general, DO fluxes were negative (Fig. 2a), which indicates DO consumption by the sediment, except at the control station in the light chambers, in the sampling campaign before the cessation and in the campaign 9 months after cessation, in which the fluxes were positive. Significant differences (ANOVA,  $p < 0.05$ ) were only observed between the area that was affected by the fish farm and the control station in the sampling campaigns before the cessation and during the campaign 1 month after cessation. In the area that was affected by the fish farm, the fluxes were more negative before cessation of the fish farm with an average of  $-64 \text{ mmol m}^{-2} \text{ d}^{-1}$ .

All the chambers showed positive fluxes in  $\text{NH}_4^+$  from the sediment to the water column (Fig. 2b). Significant differences (ANOVA,  $p < 0.05$ ) were only found between the area that was affected by the fish farm and the control station before the cessation of the fish farm, and the largest flux was found under the cages in the dark chambers ( $13.6 \pm 1.0 \text{ mmol m}^{-2} \text{ d}^{-1}$ ). In the remaining sampling campaigns, the fluxes were similar in both zones and were no greater than  $2 \text{ mmol m}^{-2} \text{ d}^{-1}$ .

$\text{NO}_2^-$  fluxes did not reveal a clear trend and were very low in comparison with the other measured fluxes in the sampling campaigns at both sites (Fig. 2c).  $\text{NO}_3^-$  fluxes were negative, meaning that  $\text{NO}_3^-$  was consumed by the sediment from the water column (Fig. 2d). No sampling campaigns showed significant differences in  $\text{NO}_2^-$  and  $\text{NO}_3^-$  fluxes between the area that was affected by the fish farm and the control station.

$\text{PO}_4^{3-}$  fluxes were generally positive (Fig. 2e), meaning there was an input of phosphorus from the sediment to the water column. Significant differences (ANOVA,  $p < 0.05$ ) were observed between the area that was affected by the fish farm and the control station in the sampling campaign before the cessation and in the campaign 1 month after the cessation. The greatest differences were observed before cessation with an average

difference of  $0.73 \text{ mmol m}^{-2} \text{ d}^{-1}$  and fluxes were higher under the cages. In the remaining sampling campaigns, the  $\text{PO}_4^{3-}$  fluxes in the area that was affected by the fish farm and the control station were very low in comparison with the sampling campaign measurements made before cessation in the area that was affected by the fish farm.

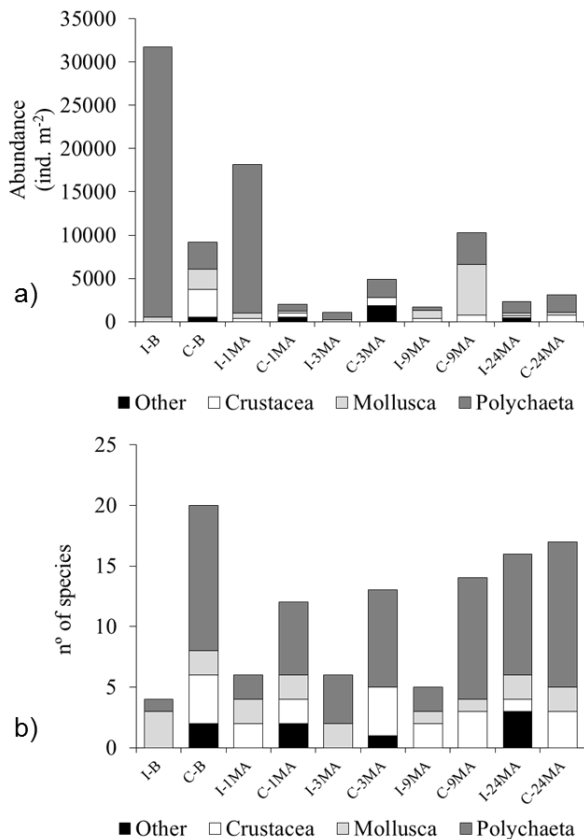
Si fluxes were positive (Fig. 2f) except for the sampling campaign 9 months after cessation in the area that was affected by the fish farm, which showed negative fluxes in both the light and dark benthic chambers. In the sampling campaigns 3 and 9 months after cessation there were significant differences (ANOVA,  $p < 0.05$ ) in the Si fluxes between the area that was affected by the fish farm and the control station.

### 6.3.3.- Benthic organisms

*Beggiatoa* spp. was not observed in any sampling campaign in the area that was affected by the fish farm nor in the control station.

Figure 3 shows the results for abundance and specific richness of benthic macrofauna. It can be clearly seen in the sampling campaigns before and one month after cessation that the area that was affected by the fish farm showed greater abundance (mostly accounted for by *Polychaeta*) when compared with the control station (Fig. 3a). In the remaining sampling campaigns the control station showed greater total abundance than the area that was affected by the fish farm, although in the sampling campaign 2 years after cessation the differences were minimal (only 1.3 times greater total abundance in the control station than in the area that was affected by the fish farm). For specific richness it can be seen that area affected by the fish farm was always considerably less rich than the control station, except for the sampling campaign 2 years after cessation of the fish farm when levels were similar (Fig. 3b). The area that was affected by the fish farm, as well as the control station, contained very few species of *Mollusca* and *Crustacea* in all the sampling campaigns. Among the *Crustacea*, *Apseudes latreilli* was

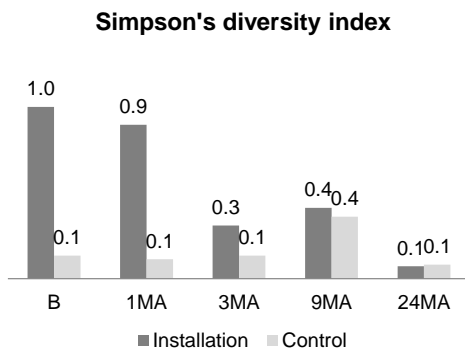
always present at the control station, nevertheless, in the area that was affected by the fish farm, this species was only found in the sampling campaigns conducted 9 months and 2 years after cessation. The number of species of *Polychaeta* found at the control station was between 6 and 12.



**Figure 3:** a) Density of individuals, and b) number of species in the area that was affected by the fish farm (I), and the control station (C) in the five sampling campaigns: before cessation of the fish farm (B) and 1, 3, 9 and 24 months after cessation of the fish farm (1MA, 3MA, 9MA and 24MA respectively).



These species belonged to the *Eunicidae*, *Glyceridae*, *Lumbrineridae*, *Nephtyidae*, *Pectinariidae*, *Phyllodoceidae*, *Spionidae*, *Maldanidae*, *Paraonidae*, and *Sabellidae* families. In the area that was affected by the fish farm the number of species ranged from a single species (*Capitella capitata*) in the sampling campaign before cessation to 10 species of *Polychaeta* in the sampling campaign 2 years after cessation, in which representatives of the *Eunicidae*, *Glyceridae*, *Lumbrineridae*, *Spionidae*, *Maldanidae*, *Sabellidae* and *Acoetidae* families were found.



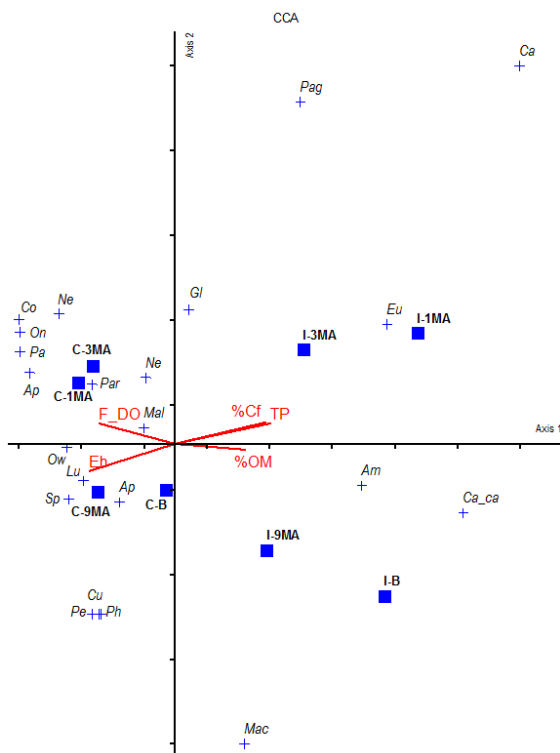
**Figure 4:** Simpson's diversity index in the area that was affected by the fish farm or installation (I) and the control station in the five sampling campaigns: before cessation of the fish farm (B), and 1, 3, 9 and 24 months after cessation of the fish farm (1MA, 3MA, 9MA and 24 MA respectively).

Simpson's diversity index (Fig. 4) in the control station was stable during all the sampling campaigns with a value of 0.1. Nevertheless, in the area that was affected by the fish farm Simpson's index was 1 and 0.9, before the cessation and 1 month after cessation, until reaching a value of 0.1 in the sampling campaign taken 2 years after cessation. It is worth noting that in the final sampling in the area that was affected by the fish farm organisms belonging to the *Nematomorpha*, *Equinodermata* and *Sipuncula* groups were

found for the first time, some of which had been found at the control station in some of the previous sampling campaigns.

### 6.3.4.- **Relationship between the environmental variables and the distribution of benthic organisms**

Environmental factors included in the CCA were those that showed differences between the area that was affected by the fish farm and the control station, namely: benthic fluxes of  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$  and DO, % OM, TP concentrations, Eh and % Cf, with the aim of detecting those that may be associated with the distribution of the benthic macrofauna. Analysis showed that the first three axes accounted for 70% of the total variance contained in the data for the species in the benthic community. The first axis accounted for 37.8%, the second 18.7%, and the third 13.5%. The correlations of the variables with the axes are shown in Table 2, with TP, % Cf, Eh, OD fluxes and % OM being the variables that best correlate with Axis 1. The Pearson correlation between the species and the environmental variables was 1.00 and 1.00 for the first and second axes, respectively, and the Monte Carlo permutation test ( $p < 0.05$ ) gave  $p = 0.004$  for the correlation between the environmental variables and the macrofauna – meaning that the observed correlations are significant. The factors diagram (Fig. 5) for Axis 1, showed a clear differentiation in the two sampled zones, given that in the four sampling campaigns (before and 1, 3, and 9 months after cessation) there was negative correlation for the control station, while the area that was affected by the fish farm was positively correlated.



**Figure 5:** CCA ordination diagram showing the study sites positions: area that was affected by the fish farm (I), and control station (C), in four sampling campaign (■), before cessation of the fish farm (B), and 1, 3 and 9 after cessation of the fish farm (1MA, 3MA and 9MA respectively); and distribution of species (+) in relation to predictor variables: flux of dissolved oxygen (F\_DO), % organic matter (%OM), total phosphorus (TP), redox potential (Eh) and % coarse fraction (%Cf).

Cu (*Cumacea*), Par (*Pariambidae*), Ap (*Apseudes latreilli*), Co (*Corophiidae*), Am (*Ampeliscidae*), Pag (*Paguridae*), Ca (*Cardiidae*), Mac (*Mactridae*), Ap (*Apistobranchidae*), Eu (*Eunicidae*), Gl (*Glyceridae*), Lu (*Lumbrineridae*), Ca\_ca (*Capitella capitata*), Mal (*Maldanidae*), Ne (*Nephtyidae*), On (*Onuphidae*), Ow (*Oweniidae*), Pa (*Paraoniodae*), Pe (*Pectinariidae*), Ph (*Phyllodocidae*), Sp (*Spionidae*), Ne (*Nematomorpha*).

Variable	Axis 1	Axis 2	Axis 3
NH <sub>4</sub> <sup>+</sup> fluxes	0.421	-0.334	0.288
PO <sub>4</sub> <sup>3-</sup> fluxes	0.507	-0.377	0.215
DO fluxes	<b>-0.705</b>	0.273	0.098
%OM	<b>0.648</b>	-0.081	0.440
TP	<b>0.885</b>	0.265	0.177
Eh	<b>-0.790</b>	-0.355	-0.089
%Cf	<b>0.834</b>	0.280	-0.144

**Table 2:** Correlations of the seven abiotic variables included in the CCA with three axes

#### 6.4.- Discussion

The disturbances observed at the fish farm before cessation were consistent with other studies on the effects of fish farms on the physicochemical properties of sediments, benthic fluxes, and macrofauna (Karakassis et al., 1998; Karakassis et al., 2000; Aguado et al., 2004; Maldonado et al., 2005; Nizzoli et al., 2007; Freitas et al., 2008; Ferrón et al., 2009; Morata et al., 2012). Seasonal variations in ‘natural’ organic material input, sedimentation, temperature, and larval availability will have an additional effect on the environmental and biological parameters measured; however, the observed gradual improvement after cessation in the abiotic and biotic variable measurements suggests that the effects are mainly related to the removal of fish farm waste residues (Pereira et al., 2004).

In this study, we examined environmental parameters that showed no disturbance due to fish farming, such as the Si fluxes. Fish farm activity does not seem to affect Si fluxes as OM from the fish farm had a low Si content (Tovar et al., 2000; Maldonado et al., 2005). Other studies (Freitas et al., 2008; Ferrón et al., 2009) also indicate that Si fluxes are not significantly influenced by fish farm activities. Differences found in the Si fluxes between

the area that was affected by the fish farm and the control station in the sampling campaign 3 months after cessation were due to the greater positive flux in the area that was affected by the fish farm. Nevertheless, in the sampling campaign made 9 months after cessation in the area that was affected by the fish farm the Si fluxes were negative, while in the control station they were positive. The positive fluxes of Si were mainly due to the dissolution of biogenic silica in the sediments and the negative flux of Si could be due to  $\text{Si(OH)}_4$  in the water precipitating to form suspended mineral silica; or the  $\text{Si(OH)}_4$  being absorbed by the diatom phytoplankton (De Vittor et al., 2012). Differences found in the Si fluxes could be due to the physicochemical conditions of the environment; the physical alteration of surface sediments; and/or spatial heterogeneity of sediments in terms of the presence of biogenic silica (dead or alive).

The  $\text{NO}_2^-$  fluxes were very low in comparison with other measured fluxes and there were no differences between the area that was affected by the fish farm and the control station, probably because this nitrogen compound is a transition molecule between  $\text{NH}_4^+$  and  $\text{NO}_3^-$  during nitrification (Herbert, 1999). Nitrate fluxes did not differ between the area that was affected by the farm and the control station and were negative in all cases.  $\text{NO}_3^-$  in the water column was consumed by the sediment due to benthic denitrification occurring in the suboxic and anoxic sediment layers where  $\text{NO}_3^-$  acts as a terminal acceptor of electrons (Herbert 1999). Other studies such as Christensen et al. (2000) and Morata et al. (2012) measured  $\text{NO}_3^-$  negative fluxes in sediments under the cages when farms were in operation and only obtained significant negative fluxes of  $\text{NO}_3^-$  when the concentration of  $\text{NO}_3^-$  in the water column was high. Some previous studies showed a clear negative relationship between  $\text{NO}_3^-$  flux and  $\text{NO}_3^-$  concentration in overlying water (Magalhaes et al., 2002; Sakamaki et al., 2006; Morata et al., 2012).

Of the environmental parameters measured that were affected by fish farming in the sampling before cessation, the  $\text{NH}_4^+$  flux was the first to recover by showing levels similar to those measured in the control station in sampling campaign 1 month after cessation. This result suggests that most of the nitrogenous organic matter provided by the farm, which mainly came from uneaten feed (the feed contained 42% crude protein) was rapidly degraded biochemically and reincorporated into the water column (Christensen et al., 2000; Aguado, 2001; Cromey et al., 2002).

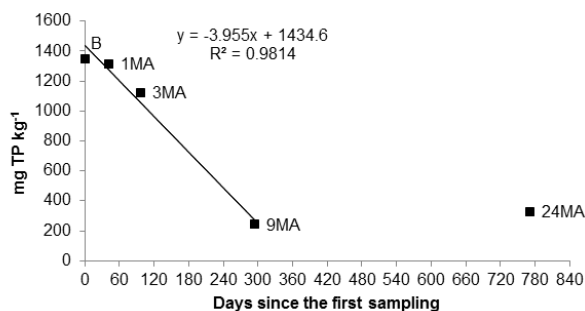
The next parameters to recover were % MO,  $\text{PO}_4^{3-}$  flux and OD flux, as there were no differences between the affected zone and the control in the sampling campaign taken 3 months after cessation. The % OM measurements showed that 1 month after cessation the decrease was one-third, yet there were still significant differences between the area that was affected by the fish farm and the control station. 3 months after cessation, the % OM was similar in both areas and the levels remained more or less constant in the subsequent sampling campaigns. OM in sediment mostly originates from non-ingested fish feed, either due to over-feeding or a poorly managed diet or feeding regime. Accumulation of fish faeces, farmed fish mortality, and cage cleaning may also increase OM in the sediment (Tovar et al., 2000; Molina and Vergara, 2005). This result shows that when the supply of OM from the farm stopped, the OM accumulated in the sediment was quickly mineralised. This rapid mineralisation of organic matter may have been accelerated by the high temperatures (Zweifel, 1999; Kirschbaum, 1995; Pereira et al., 2004) reached during the summer when the water temperature at the bottom was around 24°C. Moreover, the great abundance of *Capitella capitata* in this area before and 1 month after cessation (31,151 and 17,122 individuals  $\text{m}^{-2}$  respectively) may have contributed to the consumption of organic material. According to Banta et al. (1999), this phenomenon may account for up to 15% of the total respiration of sediments. The  $\text{PO}_4^{3-}$  flux, in the same way as % OM, also showed a large decrease in the area that was affected by the fish

farm 1 month after cessation (around five times less) and no differences between the affected zone and the control station were noticeable in the sampling campaign taken 3 months after cessation. It must be remembered that the production of dissolved phosphate in the first centimeters of sediment is directly related to the diagenesis of organic matter (Schuffert et al., 1994; Vink et al., 1997). While fluxes of  $\text{NH}_4^+$  y  $\text{PO}_4^{3-}$  before cessation were comparable with those of other studies such as that of Ferron et al. (2009), measurements made after cessation cannot be compared as no studies have been found measuring fluxes after a fish farm cessation. DO consumption by the sediment was more negative in the area affected by the farm in the sampling campaigns before and 1 month after cessation than at the control station and this is due to higher OM content (Fig. 2). The highest consumption of DO by sediment was found under the cages before the cessation of the farm (the average between the light and dark chambers was  $-64 \text{ mmol m}^{-2} \text{ d}^{-1}$ ). This maximum oxygen consumption is probably due to high residual levels of highly degradable organic carbon from the fish farm (Pereira et al., 2004) as it coincided with the highest OM content in the sediment (1.8%). Other authors who studied DO fluxes in fish farms found similar fluxes to those we found before cessation (Ferron et al., 2009), or even lower (Nizzoli et al., 2007; Freitas et al., 2008). At 3 months after cessation we found no significant differences between the area that was affected by the fish farm and the control station, as also occurred with % OM and the fluxes of  $\text{PO}_4^{3-}$ . Pereira et al. (2004) also measured the flux of DO, but *ex situ* after the cessation of a fish farm, and saw it as an early parameter of benthic recovery.

In the sampling campaign 9 months after cessation, the other variables in the disturbed sediment such as % Cf, TP and Eh also showed values similar to those measured in the control station. The recovery in the Eh measurements could be interpreted as a decrease in geochemical anaerobic processes, which is another symptom of chemical recovery. Other

authors such as Aguado et al. (2012), also observed an improvement in Eh measurements in the affected area eight months after cessation of a fish farm, but the levels were still significantly different from those measured at the controls and McGuie et al. (2000) used redox potential measurements to show that normal oxic conditions had returned after 12 months of fallow in a fish farm in Australia.

The % coarse fraction of the sediment in the area that was affected by the fish farm was gradually diminishing after the cessation due to burial and/or the hydrodynamics of the area.



**Figure 6:** Temporal variation of the concentration of TP in the sediments in the area that was affected by the fish farm in the five sampling campaigns: before cessation of the fish farm (B) and 1, 3, 9 and 24 months after cessation of the fish farm (1MA, 3MA, 9MA and 24MA respectively).

The decrease in the TP of the sediment in the area that was affected by the farm in the first 9 months sampled was nearly constant at about 3.955 mg TP kg<sup>-1</sup> d<sup>-1</sup> (Fig. 6). However, the measured benthic fluxes of PO<sub>4</sub><sup>3-</sup> did not signal this tendency (Fig. 2f). It must be taken into account that the TP measured in 1 cm of sediment includes both organic and inorganic phosphorus, as well as phosphorus adsorbed on the surfaces of sediment, and phosphorus that was chemically united to minerals as well as biologically assimilated by cells, in addition to as the content of organic detritus caused



by wildlife sediment and water column (House and Denison, 2002). In the area that was affected by the farm we compared the TP losses in the sediment with the measured benthic phosphate fluxes to estimate how much sediment TP loss was due to  $\text{PO}_4^{3-}$  fluxes. To achieve this, we transformed the  $\text{mg TP kg}^{-1} \text{ d}^{-1}$  in  $\text{mmol TP m}^{-2} \text{ d}^{-1}$  (flux units). To make this transformation we considered that the outer layer (1 cm) of  $1 \text{ m}^2$  of sediment had an average dry weight of 19 kg (data estimated from the weight of the first cm of the 6.5 cm diameter corers with an average humidity of 25%). The TP losses were  $2.42 \text{ mmol m}^{-2} \text{ d}^{-1}$ . We also assumed that the phosphate benthic fluxes came from the first centimetre of sediment and the  $\text{PO}_4^{3-}$  flux between two sampling campaigns was taken as that of the first sampling campaign. For example, between the first sampling campaign (June 2009) and the second (July 2009), it was assumed that benthic fluxes during the elapsed period were constant and equal to those measured in the June sampling campaign (mean flux between dark and light chambers of  $0.73 \text{ mmol m}^{-2} \text{ d}^{-1}$ ). We see that between the first and second sampling campaign (June 2009 and July 2009) fluxes of  $\text{PO}_4^{3-}$  could represent a maximum of 30.2% of TP losses (calculated from the levels found in the sediment, which were constant at  $2.42 \text{ mmol TP m}^{-2} \text{ d}^{-1}$  in the first 9 months). However, between the second and third sampling campaign (September 2009) fluxes explained 5.4%; and between the third and fourth sampling campaign (April 2010) only 1.2% of TP losses were due to phosphate benthic fluxes. The remaining TP losses could be partially related with the decrease of the opportunistic polychaete *Capitella capitata* observed in the affected area; as well as the burial of sediment and/or dispersal of waste due to the hydrodynamics of the area and passing storms. Between the third (September 2009) and the fourth sampling (April 2010), covering the months of autumn and winter, there were many storms in the study area. Specifically, between December 2009 and March 2010 there were a large number of adverse developments which caused winds and waves of great intensity in the study area. In December 2009 waves reached a

maximum height of 3.9 meters with peak period of 10 seconds; in January 2010 a maximum height of 3.2 meters with a peak period of 8.8 seconds was reached, in February a maximum height of 2.0 meters with peak period of 6.4 seconds was reached; and in March a maximum height of 2.0 meters with peak period of 11.1 seconds was reached. This indicates that it was a stormy period and storms are defined in the Mediterranean as periods when sea wave height exceeds 1.5 meters, or twice the annual average significant height (Julià, 2003). Unlike this study, in Guo et al. (2009) after 11 months of fallow in a fish farm in China, the levels of TP in sediments did not diminish and were around  $500 \text{ mg kg}^{-1}$ , more than twice the level of an area located 100 meters from the farm. Other studies, such as Aguado et al. 2012, also measured TP in sediment after the cessation of a fish farm and observed increases in the concentration of TP in the sediments of the area that had been affected. Karakassis et al. (1999) also observed a similar phenomenon and attributed it to secondary factors such as benthic algal production stimulated by the release of phosphate from the sediment under reducing conditions, which enriched the sediment with surges of organic matter.

The differences for macrofauna found between the area that was affected by the fish farm and the station control before cessation, were largely the consequence of organic enrichment that occurred in sediments under the cages (Morata et al., 2013). The organic enrichment affected the macrofauna and changed the relative contribution of trophic groups (Gallagher, 2010). The accumulation of organic matter produced a decrease in oxygen concentrations in the sediment, which affects species that show high sensitivity to oxygen depletion (Diaz and Rosenberg, 1995). In natural conditions, hipoxia is often associated with increases in ammonia and hydrogen sulphide (Wu, 2002), substances which are toxic to most organisms. *Capitella* sp. was found under the cages before the cessation in densities of  $31,151 \text{ ind m}^{-2}$  and it is thought to be an indicator par excellence of anoxic conditions (Rosenberg, 2001; Wu, 2002) and is classified as an

opportunistic species (pronounced in unbalanced situations) (Pinedo and Jordana 2008).

Unlike the other variables measured in this study, the macrofauna showed a slow recovery. In the sampling campaigns taken 3 and 9 months after cessation, the situation in the area that was affected by the farm showed a significant improvement, although differences could still be observed with the control station. The abundance of *Capitella capitata* decreased significantly at 3 months and 9 months (with values of 552 and 221 ind m<sup>-2</sup> respectively). This decline in *Capitella* was accompanied by an increase in the *Glyceridae* and *Nephtyidae* families which are carnivorous and mobile (Fauchald and Jumars 1979) and potential predators of other invertebrates. At the same time, examples of *Maldanidae*, sessile burrowers, were found and these species are very sensitive to organic enrichment (Borja et al., 2000). In contrast *Capitella* sp., is surface deposit-feeding and is typically found in areas with high organic carbon and reduced sediment (Borja et al., 2000). 9 months and more after cessation, individuals of *Apseudes latreilli* could be found in the area where there were none previously. This is probably explained by the physico-chemical properties of the sediment after recovery since the impact of fish farms in open water is limited to a relatively small perimeter around the cages (Mantzavrakos et al., 2007, Borja et al., 2009), and so the nearby areas that are unaffected are recolonised. The results of the CCA indicate that benthic environmental variables (mainly the TP, the % coarse fraction, the Eh, the OD flux and the % OM) were responsible for 70.0% of the total variance in the data of the species. These results showed that the development of the whole structure of the macrofauna is associated with improved abiotic conditions, which were potentially less aggressive for the growth of biota. However, it was not until 2 years after cessation that we found an absence of *Capitella capitata* in the area that was affected by fish farming. There was a specific richness that was very similar to that of the station control; and the Simpson index value was equal in both areas. Our

results are consistent with what several authors have suggested (Brooks et al., 2002, 2003, 2004) on the recovery of the soft bottom affected by organic discharge from fish farms, which suggest that the chemical recovery of the sediments is the first to occur and is necessary for a subsequent biological recovery. The recovery rate of an impacted system is difficult to compare with other locations, because it depends, among other things, on the characteristics of the area (Kraufvelin et al., 2001) and the ecological processes taking place (Dernie et al., 2003). However, we have reviewed other studies that have analysed the macrobenthic recovery after a fallow period or a fish farm cessation (Johannessen et al., 1994; Pohle et al., 2001; Pereira et al., 2004; Villnäs et al., 2011; Aguado et al., 2012). None of these studies finally observe a full recovery of all the macrofauna. A slow recovery of the macrobenthic community was reported after the cessation of a salmon farm in Norway and the community was still very much dominated by *Capitella capitata* a year after cessation (Johannessen et al., 1994). In Canadá, Pohle et al. (2001) found no recovery of the benthic macrofauna community 1 year after cessation of a farm. Pereira et al. (2004) found an evident macrobenthic community recovery 15 months after fish production stopped in a lake in Scotland, but it was still heavily dominated by opportunistic species. Villnäs et al. (2011) found only a partial recovery in the benthic macrofauna 2 years after the cessation of two fish farms in Finland. Aguado et al. (2012) found a significant trend toward recovery eight months after the cessation of a farm in the Mediterranean and that the abundance of polychaete families associated with organically enriched sediments had decreased, while the abundance of some sensitive and omnivorous families increased. However, there were still significant differences when compared to the control sites.

### 6.5.- Conclusions

Before the cessation of the fish farm, the abiotic and biotic conditions of the sediment under the cages showed differences when compared with the control station, mainly due to the continuous discharge of organic matter generated by the fish farm that accumulated in sediments under the cages. Although it is difficult to establish when there is a complete recovery of a benthic environment that has been heavily stressed, this study observed symptoms at various time scales that can be considered as partial recovery. These changes were attributed mainly to the cessation of the fish farm. The  $\text{NH}_4^+$  benthic flux was the first parameter to recover, just 1 month after cessation. This was followed by fluxes of  $\text{PO}_4^{3-}$  and OD and % OM in the sediments, which showed levels similar to the control station just 3 month after cessation. 9 month after cessation the remaining abiotic parameters of sediments (% coarse fraction, TP concentrations and Eh measurements) disturbed by the activity had recovered.

3 months after cessation the abundance of *Capitella capitata* had fallen drastically in the area that was affected by the fish farm, but the recolonisation of species tolerant of lower levels of contamination in unaffected nearby areas was slower, and similar levels of specific richness in the two sampling areas were not observed until 2 years after cessation.

In our study, the role of environmental fluxes in recovery after the cessation of the fish farm is limited to a maximum period of 3 months, as these are associated with the diagenesis of organic matter. In our case, a complete recovery was only observed after 2 years.

### 6.6.- Acknowledgments

We would like to thank the Caja del Mediterráneo (CAM) for a predoctoral fellowship fund for this research and Antonio Asunción Acuigroup Maremar manager for the facilities and support offered in conducting the study.

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# Capítulo 7: Discusión general

**7.1.- Fase 1. Efectos causados por la producción de dorada, en jaulas flotantes en mar abierto, en el Mediterráneo occidental sobre los sedimentos, la estructura de la comunidad bentónica, el metabolismo bentónico y la calidad del agua.**

**7.1.1.- Efectos sobre parámetros físico-químicos de los sedimentos**

El enriquecimiento orgánico es un impacto bien conocido y localizado bajo las jaulas de engorde en mar abierto (Hall et al., 1990; Mantzavrakos et al., 2007; Borja et al., 2009). Los restos orgánicos que llegan a los sedimentos provienen en gran parte del alimento suministrado que no es ingerido, bien por sobrealimentación o por una gestión inadecuada de la dieta o de su administración, además de las excreciones, mortalidad de los organismos cultivados y de la limpieza de las jaulas (Molina y Vergara, 2005).

La granulometría de los sedimentos, tanto en instalación como en control, fue arenosa con una moda de tamaño de grano de entre 0.125 mm y 0.063 mm y un tamaño medio que corresponde a arena muy fina. Se encontraron diferencias significativas en el porcentaje de la fracción mayor a 2 mm (% gravas) entre instalación y control. Esto se debió principalmente a las conchas que provienen de la limpieza del fouling de las estructuras sumergidas de la instalación. Esta biomasa en ocasiones no es gestionada adecuadamente y va a parar al sedimento bajo las jaulas. El mayor porcentaje de gravas se encontró en el muestreo de otoño en instalación con un 10.4% frente a un 0.2% que se encontró en el control (tabla 7.1). En ambos lugares la porosidad del sedimento fue muy similar con valores en torno al 0.46.

Se observaron diferencias significativas en el contenido de materia orgánica entre la instalación y la estación control y ésta siempre fue mayor en instalación (figura 7.1a Fase 1). El mayor contenido de materia orgánica se encontró en el muestreo de principios del verano bajo las jaulas debido a que en esta época del año la temperatura es mayor, por lo que el metabolismo de los peces aumenta al igual que la tasa de alimentación, teniendo como consecuencia mayores pérdidas de pienso y mayores tasas de excreción.

En la figura 7.1b Fase 1 se observa que las mayores concentraciones de fósforo total se dieron en los sedimentos bajo las jaulas frente a los controles. Al igual que en el caso de la materia orgánica, las mayores concentraciones se encontraron en el muestreo de principios de verano, y esto se debió al aumento de los residuos sólidos procedentes de la mayor actividad piscícola en esta época del año.

En los sedimentos, el potencial redox es el factor más importante que determina la transformación bioquímica de la materia orgánica, así como la distribución, tipo y actividad fisiológica de las bacterias y otros microorganismos que se encuentran en los mismos (Teasdale et al., 1998). Los valores del potencial redox siempre fueron más negativos bajo las jaulas (figura 7.1c Fase 1). El valor más bajo se dio en el muestreo de principios del verano debido posiblemente al mayor contenido en materia orgánica, ya que esta medida se correlaciona muy bien con el contenido de materia orgánica (correlación de Pearson:  $r = -0.70$ ,  $p < 0.01$ ) y también con el fósforo total del sedimento ( $r = -0.79$ ,  $p < 0.01$ ).

Este estudio ha mostrado que en los sedimentos bajo las jaulas se encontró: mayor porcentaje de la fracción mayor a 2 mm, mayor porcentaje de materia orgánica, mayor concentración de fósforo total y valores más negativos del potencial redox en comparación a la zona de referencia. En instalación, a lo largo de un ciclo anual, los valores más extremos de materia orgánica, fósforo total y potencial redox se apreciaron en el muestreo de

principios de verano debido a la mayor actividad acuícola en esta época del año.

### 7.1.2.- Efectos sobre la macrofauna

Otro efecto que pueden causar los desechos de la acuicultura es la modificación en las comunidades macrobentónicas (Yucel-Gier et al., 2007).

Se encontraron, en instalación, organismos pertenecientes a los grupos faunísticos *Crustacea*, *Mollusca* y *Polychaeta*, pero en la estación control además de estos también se identificaron organismos pertenecientes a los grupos *Nematomorpha*, *Equinodermata*, *Sipuncula* y *Cnidaria* en algunos de los muestreos. En la figura 7.2 Fase 1, se muestran los resultados de riqueza específica y abundancia de la macrofauna bentónica, observándose claramente que siempre en la instalación hubo una menor riqueza específica y mayor abundancia que en la estación control. En promedio, la zona control contó con  $16 \pm 6$  familias y  $4584 \pm 3440$  individuos.m<sup>-2</sup>, mientras que la instalación con  $4 \pm 2$  familias y  $21\ 419 \pm 14\ 339$  individuos.m<sup>-2</sup>. Esta tendencia también se reflejó en el índice de Simpson, que es un índice de dominancia, inverso al concepto de uniformidad o equidad de la comunidad, el cual manifiesta la probabilidad de que dos individuos tomados al azar de una muestra sean de la misma especie. Este índice tuvo constantemente valores más altos en la instalación que en la estación control (figura 7.3 Fase 1). En promedio, el índice de Simpson en la instalación fue de  $0.89 \pm 0.08$  y en la estación control de  $0.13 \pm 0.03$ . De acuerdo con Bellan-Santini et al. (1994) en sistemas oligotróficos como el mar Mediterráneo en condiciones normales se observa baja abundancia y alta diversidad, situación que se asemeja más a la que presentó la estación control.

Los poliquetos presentaron en todos los muestreos menor riqueza específica en la instalación que en la estación control. En la instalación la especie que siempre estuvo presente fue *Capitella capitata* aunque también se encontraron en algunos muestreos *Owenia fusiformis* y *Diopatra*



*neapolitana*. En la estación control estuvieron presentes especies como *Nephtys hombergi*, que se encontró en todos los muestreos. *Hyalinoecia bilineata*, *Goniada maculata*, *Pectinaria koreni*, *Glycera* sp. y especies de las familias *Sabellidae*, *Spionidae* y *Maldanidae* se observaron, en al menos dos de los muestreos en la estación control. Por el contrario la abundancia de poliquetos fue siempre mayor en instalación que en la estación control, debido a que *Capitella capitata*, que fue la especie que dominó bajo las jaulas, mostró una abundancia entre 5855 y 34 399 individuos.m<sup>-2</sup>, siendo en los muestreos de invierno y principios de verano cuando se observaron las máximas abundancias. En el caso de los crustáceos si bien la riqueza específica siguió la misma pauta general, es decir menor número de especies en instalación que en control, la abundancia fue menor en la instalación. En instalación no se observaron especies como *Ampelisca spinipes* y *Apsudes latreilli* las cuales si fueron detectadas en la estación control. En la instalación, en el muestreo de principios de verano, no se detectó ninguna especie de crustáceo, y en el muestreo de invierno fue cuando se encontró la máxima abundancia de crustáceos con 530 individuos.m<sup>-2</sup>. En la estación control, sin embargo, la abundancia de crustáceos varió entre 497 y 3646 individuos.m<sup>-2</sup>, siendo máxima en el muestreo de principios de verano. El número de especies de moluscos fue bajo en ambos sitios. En el muestreo de otoño no se encontraron moluscos ni bajo las jaulas ni en la estación control. *Spisula subtruncata* sólo se encontró en instalación en el muestreo de principios de verano con una abundancia de 552 individuos.m<sup>-2</sup>, sin embargo en la estación control esta especie se encontró en los muestreos de invierno, primavera y principios de verano, con el máximo de abundancia en este último (2209 individuos.m<sup>-2</sup>).

Pinedo y Jordana (2008) clasificaron la macrofauna del Mediterráneo occidental basándose en la sensibilidad / tolerancia de las especies, principalmente al enriquecimiento orgánico. Agruparon a las especies en cuatro categorías: 1-sensible, 2-indiferente, 3-tolerante y

4-especies oportunistas. Según esta clasificación las especies, encontradas sólo en la estación control, como *Ampelisca spinipes* está catalogada en el grupo 1 (especies muy sensibles al enriquecimiento orgánico y presente en condiciones no contaminadas) y especies como *Apeudes latreilli*, *Nephtys hombergi*, *Goniada maculata*, *Pectinaria koreni* y *Glycera sp* pertenecen a la categoría 2. Sin embargo *Capitella capitata*, muy abundante en la instalación y responsable de la alta dominancia que presenta la macrofauna bajo las jaulas, está catalogada como grupo 4, es decir, especie oportunista. *Capitella capitata* se alimenta de depósitos subsuperficiales (Borja et al., 2000) y prolifera en sedimentos reducidos. *Capitella* se considera como el indicador por excelencia de la falta de oxígeno (Rosenberg, 2001; Wu, 2002).

En este estudio se observó que la macrofauna bajo las jaulas presentó, en todas las campañas de muestreo, un menor número de especies y un aumento de la abundancia respecto del punto control. El poliqueto *Capitella capitata* fue la especie dominante bajo las jaulas, por lo que se puede considerar una buena especie indicadora de contaminación orgánica y taxones como *Ampelisca spinipes*, *Apeudes latreilli*, *Nephtys hombergi*, *Pectinaria koreni* y *Hyalinoecia bilineata* podrían ser buenos candidatos como indicadores de zonas no perturbadas para estudios posteriores en esta zona.

### 7.1.3.- Efectos sobre los flujos bentónicos

Para comprender mejor la biogeoquímica de los sedimentos y ver cómo se modifican los intercambios de oxígeno y nutrientes en la interfase agua-sedimento se calcularon los flujos bentónicos. Si el flujo es positivo quiere decir que hay aporte desde el sedimento a la columna de agua y si por el contrario el flujo obtenido es negativo quiere decir que se produce un consumo desde la columna de agua hacia el sedimento.

En general los flujos de oxígeno disuelto fueron negativos (figura 7.4a Fase 1), indicando un consumo de oxígeno por el sedimento. Éste

siempre fue más negativo bajo las jaulas que en la estación control. En primavera y principios de verano se observaron diferencias significativas entre instalación y control y en estas épocas también se observaron flujos positivos en las cámaras transparentes en la estación control (hasta  $17 \pm 10$   $\text{mmol.m}^{-2}.\text{d}^{-1}$ , en la campaña de primavera). En las cámaras transparentes en la instalación no se observó un flujo positivo de oxígeno en ningún muestreo, debido seguramente a la mayor mineralización aeróbica de materia orgánica y a la baja producción primaria que se pudo dar bajo las jaulas. La correlación de Pearson entre el consumo de oxígeno por el sedimento y el contenido en materia orgánica fue de  $r = 0.60$  ( $p < 0.01$ ). En la mineralización aeróbica de la materia orgánica los microorganismos heterótrofos usan el oxígeno como el receptor final de electrones. Este consumo de oxígeno en el sedimento bajo las jaulas se incrementó respecto a la zona de referencia entre  $13$   $\text{mmol.m}^{-2}.\text{d}^{-1}$  en invierno y alrededor de  $42$   $\text{mmol.m}^{-2}.\text{d}^{-1}$  tanto en primavera como a principios de verano. El valor máximo de consumo de oxígeno disuelto por el sedimento se encontró a principios de verano bajo las jaulas ( $-71 \pm 20$   $\text{mmol.m}^{-2}.\text{d}^{-1}$ ) coincidiendo con la mayor temperatura del agua de fondo ( $20^\circ\text{C}$ ), mayores tasas de alimentación y mayor contenido en materia orgánica en el sedimento.

El flujo de oxígeno disuelto también tuvo una relación inversa con el flujo de amonio ( $r = -0.83$ ,  $p < 0.01$ ), lo que indica que a mayor consumo de oxígeno en la mineralización de la materia orgánica mayor liberación de amonio a la columna de agua (Berelson et al., 2003). Bajo las jaulas se produjo un incremento del flujo de amonio del sedimento a la columna de agua respecto a la zona de referencia de hasta  $11$   $\text{mmol.m}^{-2}.\text{d}^{-1}$  a principios de verano. Los flujos de amonio en instalación fueron mayores a principios del verano ( $12.7 \pm 1.0$   $\text{mmol.m}^{-2}.\text{d}^{-1}$ ), cuando mayor temperatura y actividad piscícola hubo, seguido de otoño y primavera (figura 7.4b Fase 1), correlacionándose con el contenido en materia orgánica en el sedimento ( $r = 0.70$ ,  $p < 0.01$ ).

Los flujos de fosfato fueron mayores bajo las jaulas que en la estación control, llegándose a incrementar entre  $0.44 \text{ mmol.m}^{-2}.\text{d}^{-1}$  en invierno a  $0.84 \text{ mmol.m}^{-2}.\text{d}^{-1}$  en otoño (figura 7.4c Fase 1), debido a que en esta zona se encontraron mayores contenidos en fósforo total en el sedimento ( $r = 0.83$ ,  $p < 0.01$ ). Se observaron diferencias significativas en otoño, primavera y principios de verano. Los flujos de fosfato se correlacionaron con los del amonio ( $r = 0.57$ ,  $p < 0.05$ ) y los del oxígeno disuelto ( $r = -0.56$ ,  $p < 0.05$ ). Esto se debió a la degradación de la materia orgánica que consumió oxígeno disuelto y produjo amonio y fosfato. Por otra parte obtuvimos una correlación interesante entre el flujo de fosfato y el potencial redox ( $r = -0.59$ ,  $p < 0.05$ ), debido a que bajo condiciones anóxicas, como las encontradas en este estudio en la capa subsuperficial del sedimento, el fósforo se puede liberar (Rodríguez, 1999).

Los flujos del nitrato fueron siempre negativos (figura 7.5a Fase 1), es decir que existió un consumo de nitrato por el sedimento desde la columna de agua a causa de la desnitrificación bentónica que se produce en las regiones subóxicas y anóxicas de los sedimentos donde el nitrato actúa como un aceptor terminal de electrones (Herbert, 1999). En las campañas de otoño, invierno y primavera los flujos de nitrato fueron más negativos en instalación que en la estación control, hasta  $1.2 \text{ mmol.m}^{-2}.\text{d}^{-1}$ , a pesar de que sólo se dieron diferencias significativas en otoño. El mayor consumo de nitrato bajo las jaulas posiblemente sea debido a que, al haber mayor concentración de materia orgánica, las zonas subóxicas/anóxicas del sedimento están más próximas a la superficie, por lo que se produce una mayor desnitrificación bentónica. Los flujos más importantes se dieron en otoño e invierno bajo las jaulas de peces, coincidiendo con las mayores concentraciones de nitrato en la columna de agua. La correlación entre los flujos de nitrato y la concentración de nitrato en el agua de fondo fue de  $r = -0.92$ ,  $p < 0.01$ , coincidiendo con estudios previos como los de Magalhaes et al. (2002) y Sakamaki et al. (2006). Las altas concentraciones de nitrato en el agua de

fondo estimulan la velocidad del transporte de éste hacia los sedimentos, donde aumenta la concentración de nitrato en el agua intersticial, favoreciendo la respiración bacteriana de nitratos. Al aumentar la concentración de nitrato, se acelera la reducción de éste a  $N_2/N_2O$  y se produce una pérdida de nitrógeno del sistema por desnitrificación (Herbert, 1999).

Los flujos de nitrito no siguen una tendencia clara (figura 7.5b Fase 1). Esto probablemente se deba a que el nitrito es un compuesto de transición entre el amonio y nitrato en el proceso de nitrificación y entre el nitrato y  $N_2/N_2O$  en el proceso de desnitrificación béntica (Herbert 1999). Se observaron diferencias significativas entre instalación y control en las épocas de otoño, invierno y primavera. Los flujos más negativos bajo las jaulas se dieron en las campañas de muestreo de otoño e invierno, indicando con esto, que el proceso que más afecta a este compuesto en estas épocas es el de la desnitrificación béntica, coincidiendo también con los flujos más negativos de nitrato bajo las jaulas. La correlación de Pearson entre los flujos de nitrito y nitrato fue de  $r = 0.68$  ( $p < 0.05$ ).

Los flujos de nitrógeno inorgánico disuelto dieron siempre positivos y con una tendencia similar a los flujos de amonio, ya que este último fue el compuesto de nitrógeno que mayor representación tuvo dentro de los mismos. Los flujos en instalación también fueron mayores a principios del verano con  $12.62 \text{ mmol.m}^{-2}.\text{d}^{-1}$  frente a  $1.66 \text{ mmol.m}^{-2}.\text{d}^{-1}$  en la estación control.

Los flujos de sílice fueron positivos (figura 7.5c Fase 1), excepto en el muestreo de invierno en la estación control, donde obtuvimos flujos negativos tanto en las cámaras transparentes como en las opacas. Sólo se observaron diferencias significativas entre las medidas bajo las jaulas y el control en el muestreo realizado en invierno. Se observaron flujos más altos y positivos en primavera y principios de verano probablemente debido a que la tasa de disolución de la sílice aumenta con la temperatura (Cermelj et al.,

2001). La correlación entre los flujos de sílice y la temperatura fue de 0.59 ( $p < 0.05$ ), y además en esta época probablemente fue cuando más sílice biogénica pudo haber en el sedimento procedente de la muerte y sedimentación de diatomeas. La actividad acuícola no parece afectar al flujo de sílice (Freitas et al., 2008; Ferrón et al., 2009) ya que la materia orgánica que proviene de la piscifactoría tiene un bajo o nulo contenido en sílice (Tovar, 2000).

Los sedimentos bajo las jaulas sufrieron cambios biogeoquímicos que afectaron principalmente al consumo de oxígeno disuelto y a la liberación de amonio y fosfato por los sedimentos. El consumo de nitrato por el sedimento también se vio afectado por la actividad acuícola aunque depende en gran medida de la disponibilidad de nitrato en la columna de agua. El flujo de nitrito parece estar relacionado con el de nitrato. Sin embargo, los flujos de sílice no parecieron verse afectados por la actividad de la piscifactoría. No existieron diferencias, para ninguno de los flujos medidos, entre las cámaras transparentes y opacas durante la fase de producción debajo de las jaulas.

#### 7.1.4.- Efectos sobre la columna de agua

La actividad acuícola también pueden generar efectos sobre algunos parámetros ambientales de la columna de agua (La Rosa et al., 2002; Maldonado et al., 2005).

Los valores medios de velocidad oscilaron entre 3 y 10  $\text{cm}\cdot\text{s}^{-1}$  y la dirección que dominó entre las medidas realizadas en ambas zonas fue de Noreste. Esto minimizó la posibilidad de que la estación control pudiera haber estado influida por la dispersión de los desechos acuícolas, ya que se situó en dirección opuesta a la de las corrientes dominantes de la zona.

La temperatura no mostró diferencias significativas entre instalación y la estación control. No se observó estratificación térmica en ninguna campaña de muestreo debido probablemente a la poca profundidad que

presentó la zona de estudio (19 m). Sólo en la campaña de muestreo de principios de verano, tanto en instalación como en la estación control, la temperatura superficial fue de 3 a 4°C superior en comparación a la temperatura del agua de fondo.

En cuanto a las concentraciones de oxígeno disuelto, siempre fueron más bajas y significativamente diferentes en instalación en comparación a la zona control. Los valores de oxígeno disuelto oscilaron desde 3.82 hasta 8.60 mg.l<sup>-1</sup> en instalación y de 5.16 a 9.40 mg.l<sup>-1</sup> en la zona de referencia. Las menores concentraciones de oxígeno disuelto en la columna de agua en la instalación respecto al control, fueron consecuencia tanto del mayor consumo de oxígeno por el sedimento (de hasta 42 mmol.m<sup>-2</sup>.d<sup>-1</sup> más en instalación que en el control) como del consumo por parte de los peces, de la oxidación de las formas reducidas del nitrógeno y de la degradación aerobia de la materia orgánica de la columna de agua.

Se encontraron diferencias significativas en las concentraciones de nitrógeno inorgánico disuelto entre la instalación y la estación control. Fueron mayores las concentraciones de nitrógeno inorgánico disuelto en la columna de agua en la instalación debido principalmente a la mayor concentración de amonio, ya que ésta es la forma predominante de nitrógeno excretado por los peces (La Rosa et al., 2002; Aksu y Kocatas, 2007) además del primer compuesto de la degradación de la materia orgánica. Los aportes de amonio desde el sedimento a la columna de agua bajo las jaulas (de hasta 11 mmol.m<sup>-2</sup>.d<sup>-1</sup> más) sustentan parte de los niveles de amonio observados en el agua, siendo imposible cuantificar su proporción debido a los múltiples procesos en los que participa. Las diferencias de nitrato + nitrito, mayores en la instalación, principalmente se debieron a que el amonio pudo ser oxidado rápidamente por medio de la nitrificación.

También se observaron diferencias significativas en la concentración de fosfatos y de fósforo total entre la instalación y la estación control, donde se encontraron las mayores concentraciones en las proximidades de las jaulas.

Estos mayores niveles pudieron estar causados tanto por el mayor aporte de fósforo desde el sedimento (de hasta  $0.84 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  más) como por el fósforo excretado por los peces y al pienso no ingerido.

En la instalación se observaron variaciones espaciales a lo largo de la columna de agua. Éstas consistieron en picos de concentración a profundidades medias tanto de nitrógeno inorgánico disuelto como de fosfatos, que seguramente fueron debidas a la excreción de los peces y a la disposición de estos en las jaulas en el momento del muestreo. Especialmente en jaulas flotantes en mar, puede haber gradientes ambientales con temperatura, oxígeno, corrientes y variaciones de luz variable que condicionen la disposición de los peces. Los peces son atraídos a las áreas más óptimas y evitan las desfavorables. Esto provoca que densidades altas de peces se concentren en las zonas favorables y en las zonas subóptimas permanezcan los menos competitivos donde las densidades son menores (Johansson et al., 2007; Oppedal et al., 2007).

Se observaron cambios temporales en algunas de variables estudiadas en la columna de agua (temperatura, oxígeno disuelto, nitrógeno inorgánico disuelto y fosfatos) pero fueron debidos fundamentalmente a los patrones típicos de estacionalidad en el Mediterráneo (La Rosa et al., 2002; Maldonado et al., 2005), ya que se dieron tanto en la piscifactoría como en el sitio control.

Los mayores niveles de nitrógeno inorgánico disuelto y fósforo observados en instalación con respecto a la estación control, pueden ser un factor de gran relevancia si se tiene en cuenta el carácter oligotrófico del Mar Mediterráneo (Siokou-Frangou, et al., 2010). Aunque la sílice no está presente en los desechos de la acuicultura (Maldonado et al. 2005), ésta fue medida para poder realizar las relaciones estequiométricas de los nutrientes, ya que otro efecto del aumento de nitrógeno y fósforo en la columna de agua es la modificación de las relaciones estequiométricas; nitrógeno inorgánico disuelto/fosfatos/sílice. Se aplicaron los criterios utilizados por Justic et al.



(1995) para identificar la limitación de nutrientes en instalación y control. Los resultados mostraron que efectivamente el aumento en la concentración de nitrógeno inorgánico disuelto y fosfatos en instalación, modificó las relaciones estequiométricas de los nutrientes y la sílice actuó como nutriente limitante un 11% de veces más en instalación que en control. Esto pudo deberse a que los aportes de la actividad acuicultora fueron sólo de nitrógeno y fósforo. La concentración de sílice no estuvo relacionada con la actividad acuícola, por lo que ésta estuvo en función de procesos naturales (Maldonado et al., 2005). Hay que destacar las posibles implicaciones del incremento de nutrientes en las zonas cercanas a las piscifactorías, ya que producen cambios en las relaciones estequiométricas pudiendo propiciar, en algunas épocas del año, las condiciones nutricionales que soportan el desarrollo de especies fitoplanctónicas nocivas como dinoflagelados (Vila et al., 2001; Olivos et al., 2002).

Por otra parte, a pesar de encontrar mayores concentraciones de nutrientes en la instalación, la concentración de clorofila *a* fue mayor en la estación control. Este patrón va en contra de la predicción general de que una mayor disponibilidad de nutrientes debe producir un aumento de las concentraciones de clorofila *a*. La dinámica del agua en la zona de estudio (velocidad de la corriente fue de 3 a 10 cm.s<sup>-1</sup>) podría haber contribuido a la dilución y dispersión, así como las diversas sustancias químicas activas usadas en acuicultura podrían haber limitado la producción primaria en la zona. Además, hay que tener en cuenta el papel de las macroalgas y suspensívoros, asociados a las estructuras sumergidas de la piscifactoría, que podrían haber contribuido al consumo directo de nutrientes y de fitoplancton respectivamente, por lo que se podría haber estado subestimando la producción primaria total. Aunque no se midió directamente el biofouling asociado a las estructuras sumergidas de la piscifactoría, se observó indirectamente, un importante crecimiento en la instalación. El peso seco de las valvas que se encontraron en el sedimento bajo las jaulas estuvo entre 775

y 1247 g.m<sup>-2</sup>. Esto fue el resultado de las operaciones de limpieza, donde la eliminación del biofouling asociado a las estructuras de la piscifactoría no es manejado adecuadamente y por lo tanto, gran parte de esta biomasa termina en el sedimento bajo las jaulas.

En este estudio se apreciaron diferencias significativas en la mayoría de los nutrientes analizados en la columna de agua en contraste con otros estudios realizados en otras instalaciones (La Rosa et al., 2002; Yucel-Gier et al., 2007; Aksu y Kocatas, 2007; Kaymakci et al., 2010). Se encontraron diferencias significativas en la concentración de oxígeno disuelto, nitrógeno inorgánico disuelto, fosfatos y fósforo total entre la instalación y la estación control. La alta resolución vertical de muestreo de los parámetros de la columna de agua pudo haber contribuido a tales resultados. El aumento de nitrógeno inorgánico disuelto y fosfato modificó, en ocasiones, la relación estequiométrica de los nutrientes y el nutriente limitante para la producción primaria. Por otra parte, a pesar de encontrar mayores concentraciones de nutrientes en la instalación, la concentración de clorofila *a* fue mayor en la estación control.

#### **7.1.5.- Implicaciones de los flujos bentónicos en la columna de agua.**

Es imposible cuantificar, para oxígeno y nutrientes, la importancia del consumo/aportes de los sedimentos en la columna de agua debido a que estos están fuertemente influenciados por: la hidrodinámica, el intercambio con la atmósfera (en el caso de oxígeno y nitrógeno), además de los fuertes cambios temporales consecuencia de la estacionalidad. Sin embargo, se ha intentado relacionar los flujos en la interfase agua-sedimento de los nutrientes con los requerimientos por parte de la producción primaria planctónica. Morán y Estrada (2005), trabajando en el Mar Mediterráneo noroccidental, observaron que la biomasa total del fitoplancton estuvo fuertemente correlacionada con la producción primaria total y propusieron un modelo de

regresión lineal que relacionaba las tasas integradas diarias de la producción primaria con los valores integrados de clorofila *a* en la columna de agua. En este estudio al disponer, para todas las campañas, de datos de clorofila *a* tomados cada 2 m en la columna de agua, se pudo calcular el promedio integrado de clorofila *a* para los puntos control e instalación. Estos últimos fueron de 7.6 mg.m<sup>-2</sup> para la estación control y de 6.8 mg.m<sup>-2</sup> para la instalación. Debido a que en instalación se pudo haber estado subestimando la producción primaria total consecuencia del fouling asociado a las estructuras sumergidas, se tomó como valor integrado de clorofila *a* en la columna de agua, la media de ambas zonas (7.2 mg.m<sup>-2</sup>). Luego a partir de la relación planteada por Morán y Estrada (2005) se estimó la producción diaria integrada que fue de 139 mg C.m<sup>-2</sup>.d<sup>-1</sup> (aproximadamente unos 12 mmol C.m<sup>-2</sup>.d<sup>-1</sup>). Suponiendo que las tasas de utilización de nutrientes se aproximan a las de Redfield (Redfield et al., 1963) y que no se produce fijación de nitrógeno (Niencheski y Jahnke, 2002), el fitoplancton requeriría unos 2 mmol N.m<sup>-2</sup>.d<sup>-1</sup>, 0.1 mmol P.m<sup>-2</sup>.d<sup>-1</sup> y 2 mmol Si.m<sup>-2</sup>.d<sup>-1</sup>. Se calculó la media del flujo bentónico de nitrógeno inorgánico disuelto tanto para el punto control como para la instalación que fue de 1.3 y 4.4 mmol N.m<sup>-2</sup>.d<sup>-1</sup> respectivamente. En el punto control, casi las tres cuartas partes de los requerimientos de nitrógeno por parte del fitoplancton pudieron ser satisfechas por los aportes desde los sedimentos, mientras que los flujos bentónicos en instalación excedieron en 2.5 veces los mismos. La media de los flujos bentónicos de fosfato fueron de 0.1 mmol P.m<sup>-2</sup>.d<sup>-1</sup> para el control y de 0.8 mmol P.m<sup>-2</sup>.d<sup>-1</sup> para instalación indicando que los requerimientos de fosfato por parte del fitoplancton fueron proporcionados por los flujos bentónicos en ambos sitios, si bien en instalación estos fueron 8 veces superiores. Tanto en control como en instalación se observaron medias de flujos bentónicos de sílice en torno a 1 mmol Si.m<sup>-2</sup>.d<sup>-1</sup> lo que representó la mitad de los requerimientos de sílice para las diatomeas. Según estas estimaciones, en la instalación existió una disponibilidad mucho más elevada

de fósforo y nitrógeno que en el control, mientras que el aporte de sílice desde el sedimento es el mismo para ambas zonas. Estas diferencias podrían conllevar, en la instalación, una modificación en la estructura de la comunidad fitoplanctónica favoreciendo el desarrollo de las especies que no utilizan sílice como nutriente.

Tabla 7.1: Granulometrías, porcentaje de conchas y porosidad en los sedimentos de la instalación (I) y la estación control (C) en las 8 campañas de muestreo; 4 campañas en la fase 1 o de producción (1-otoño 2008, 2-invierno 2009, 3-primavera 2009, 4-principios de verano 2009) y 4 campañas más en la fase 2 o después del cese de la actividad (5- un mes después del cese, 6- tres meses después del cese, 7-nueve meses después del cese, 8- dos años después del cese).

		%Grava	%Arena	%Limo+Arcilla	%Conchas	Porosidad
FASE 1 PRODUCCIÓN	I-1	10.4±3.9	76.5±1.3	13.2±2.6	14.9±5.9	0.45±0.02
	C-1	0.2±0.1	89.8±2.2	10.0±2.1	0.4±0.2	0.45±0.02
	I-2	5.0±4.4	84.8±3.0	10.3±2.8	9.4±6.0	0.44±0.02
	C-2	0.1±0.1	91.2±2.7	9.2±2.4	0.5±0.2	0.45±0.01
	I-3	4.3±1.3	89.2±3.2	6.4±2.8	8.2±1.6	0.45±0.01
	C-3	0	91.5±1.6	8.4±1.6	0.1±0.1	0.47±0.02
	I-4	5.5±2.8	86.8±0.3	7.7±3.0	16.0±4.7	0.47±0.03
	C-4	0.1±0.1	88.6±2.3	11.2±2.4	0.1±0.2	0.46±0.01
FASE 2 DESPUÉS DEL CESE	I-5	8.5±3.8	84.9±2.7	6.6±1.3	13.7±5.8	0.44±0.02
	C-5	0.1±0.1	88.6±2.3	11.2±2.4	0.1±0.2	0.45±0.01
	I-6	2.0±3.0	89.3±2.1	8.8±0.9	2.7±3.2	0.46±0.02
	C-6	0.3±0.2	88.7±1.2	10.9±1.2	0.9±0.1	0.43±0.01
	I-7	0.1±0.0	89.3±1.0	10.6±1.0	0.1±0.1	0.50±0.01
	C-7	0.4±0.5	92.3±2.9	7.3±2.5	0.4±0.5	0.49±0.01
	I-8	0.1±0.2	93.9±0.3	6.0±0.4	0.1±0.2	0.45±0.02
	C-8	0.2±0.2	94.4±0.5	5.3±0.5	0.1±0.2	0.45±0.02

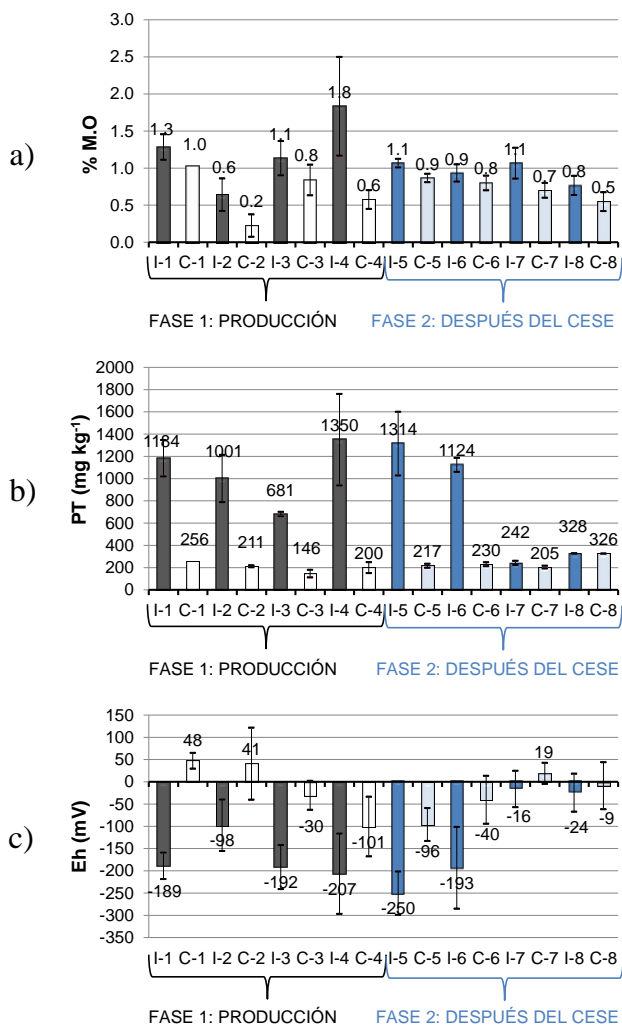


Figura 7.1: a) porcentaje de materia orgánica (%MO), b) fósforo total (PT) y c) potencial redox (Eh) en los sedimentos de la instalación (I) y la estación control (C) en las 8 campañas de muestreo; 4 campañas en la fase 1 o de producción (1-otoño 2008, 2-invierno 2009, 3-primavera 2009, 4-principios de verano 2009) y 4 campañas más en la fase 2 o después del cese de la actividad (5- un mes después del cese, 6- tres meses después del cese, 7-nueve meses después del cese, 8- dos años después del cese).

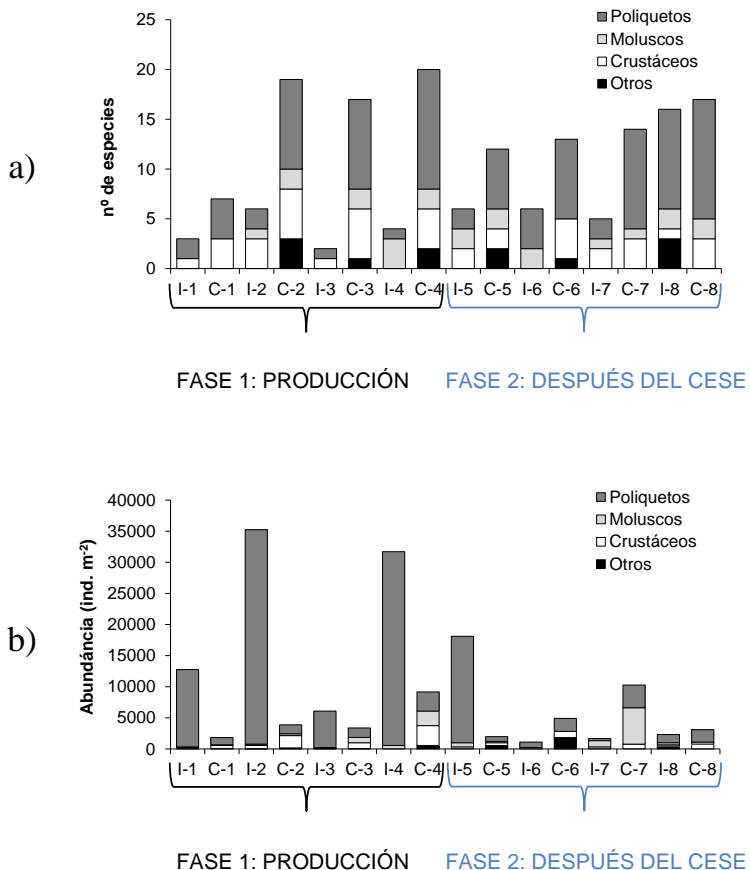


Figura 7.2: Macrofauna bentónica; a) número de especies y b) densidad de individuos en los sedimentos de la instalación (I) y la estación control (C) en las 8 campañas de muestreo; 4 campañas en la fase 1 o de producción (1-otoño 2008, 2-invierno 2009, 3-primavera 2009, 4-principios de verano 2009) y 4 campañas más en la fase 2 o después del cese de la actividad (5-un mes después del cese, 6- tres meses después del cese, 7-nueve meses después del cese, 8- dos años después del cese).

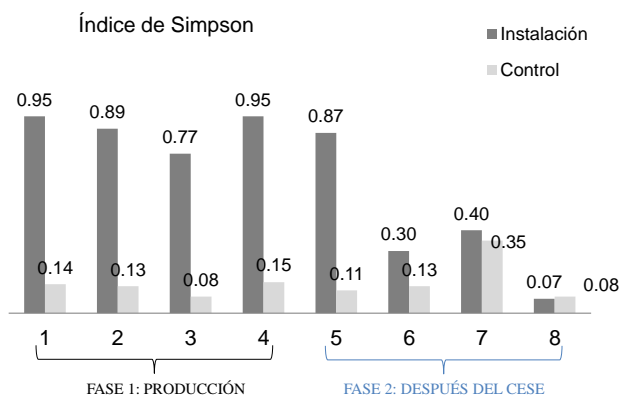


Figura 7.3: Índice de diversidad de Simpson en la instalación (I) y en la estación control (C) en las 8 campañas de muestreo; 4 campañas en la fase 1 o de producción (1-otoño 2008, 2-invierno 2009, 3-primavera 2009, 4-principios de verano 2009) y 4 campañas más en la fase 2 o después del cese de la actividad (5- un mes después del cese, 6- tres meses después del cese, 7-nueve meses después del cese, 8- dos años después del cese).



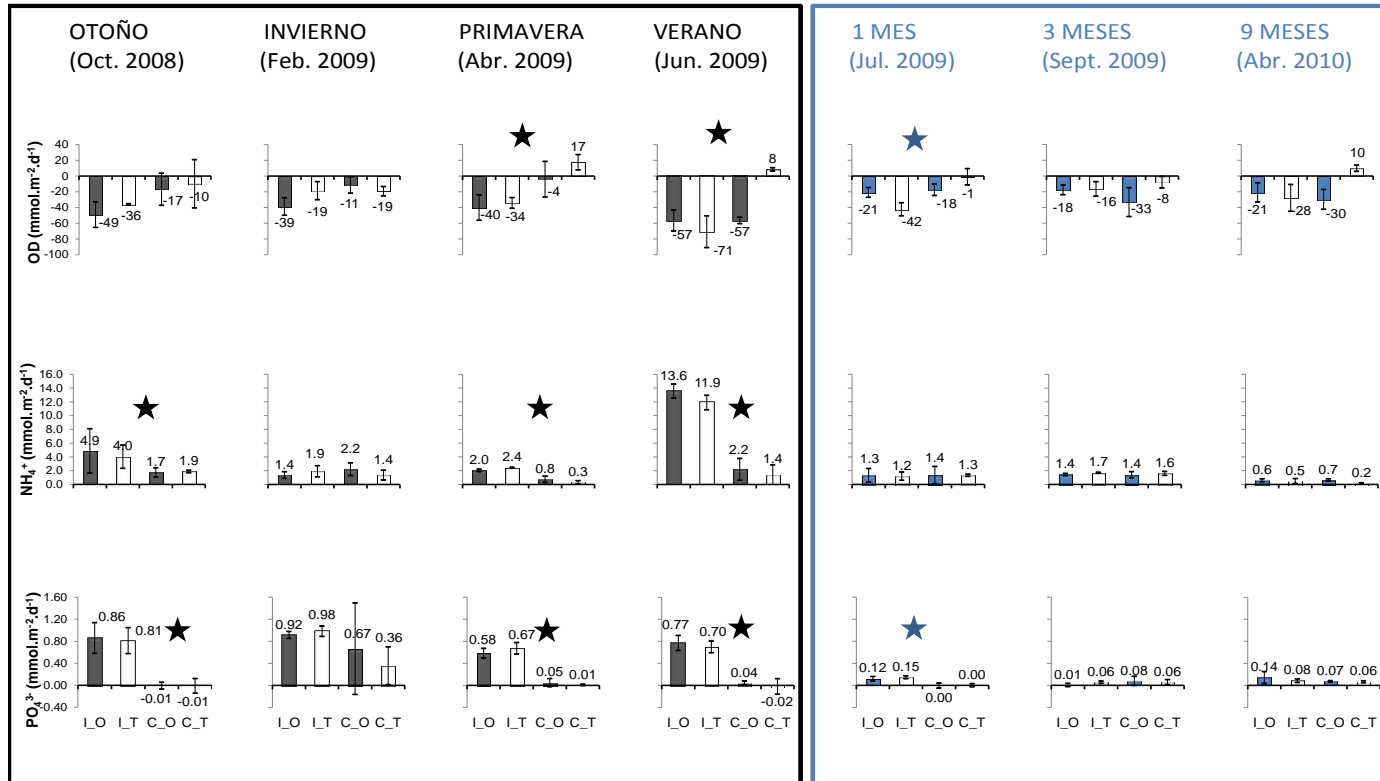


Figura 7.4: Flujos bentónicos de oxígeno disuelto (OD), amonio ( $\text{NH}_4^+$ ) y fosfato ( $\text{PO}_4^{3-}$ ) en cámaras opacas (O) y transparentes (T) bajo la instalación (I) y en la estación control (C) en 7 campañas de muestreo; Fase 1 (1-otoño 2008, 2-invierno 2009, 3-primavera 2009, 4-principios de verano 2009) y Fase 2 (1-un mes después del cese, 3-tres meses después del cese, 9-nueve meses después del cese). Estrella: diferencias significativas (ANOVA,  $p < 0.05$ ) entre instalación y control.

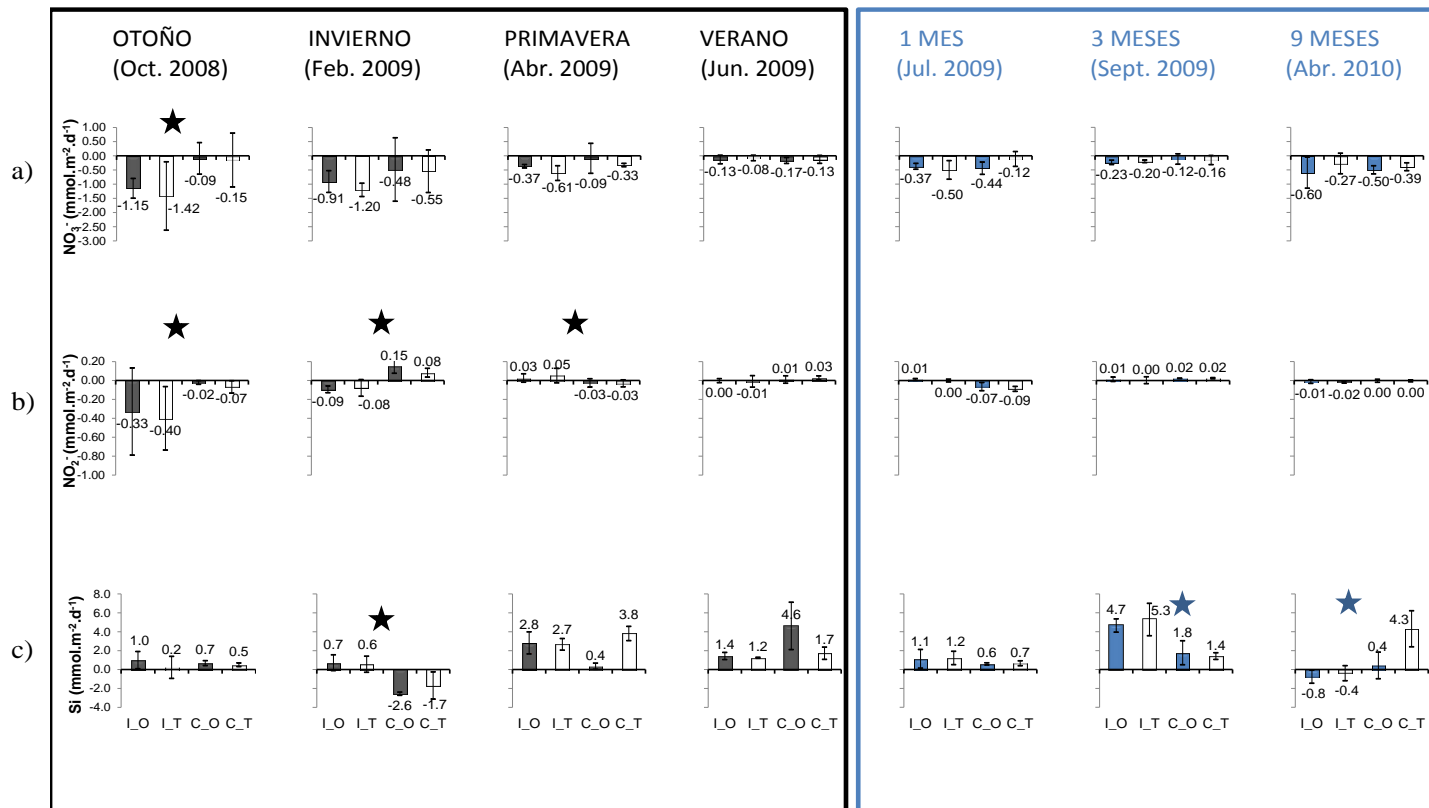


Figura 7.5: Flujos bentónicos de nitrato ( $\text{NO}_3^-$ ), nitrito ( $\text{NO}_2^-$ ) y sílice (Si) en cámaras opacas (O) y transparentes (T) bajo la instalación (I) y en la estación control (C) en 7 campañas de muestreo; Fase 1 (1-otoño 2008, 2-invierno 2009, 3-primavera 2009, 4-principios de verano 2009) y Fase 2 ( un mes después del cese, tres meses después del cese, nueve meses después del cese). Estrella: diferencias significativas (ANOVA,  $p < 0.05$ ) entre instalación y control.

## **7.2.- Fase 2. Recuperación de una zona que fue afectada por la producción de dorada en el mar Mediterráneo tras el cese definitivo de la actividad.**

### **7.2.1.- Recuperación de los parámetros físico-químicos de los sedimentos**

La tasa de recuperación de una zona afectada por la actividad acuícola y la forma en que se lleva a cabo sólo puede entenderse teniendo en cuenta la gran variabilidad de parámetros ambientales (Dernie et al., 2003). En este estudio, se midieron parámetros físico-químicos de los sedimentos para ver cómo se iban recuperando a medida que pasaba el tiempo desde el cese de la actividad.

El porcentaje de materia orgánica, fue el parámetro medido en los sedimentos que mostró una recuperación más rápida, ya que después de 1 mes del cese de la actividad había disminuido más de una tercera parte, y después de 3 meses los porcentajes de materia orgánica ya fueron similares a los encontrados en la estación control y se mantuvieron más o menos constantes en el resto de muestreos (figura 7.1a Fase 2). Este resultado mostró que al cesar el aporte de materia orgánica de la piscifactoría, los compuestos orgánicos acumulados en el sedimento fueron rápidamente mineralizados. Esta rápida mineralización se pudo ver favorecida por las altas temperaturas (Zweifel, 1999; Kirschbaum, 1995; Pereira et al., 2004) alcanzadas durante el verano, ya que la temperatura del agua del fondo estuvo alrededor de los 24°C en el mes de julio.

Otro parámetro perturbado por la actividad acuícola fue el porcentaje de la fracción gruesa del sedimento, que corresponde a tamaños mayores de 2 mm (% gravas). Este parámetro después de 1 y 3 meses del cese de la actividad acuícola, todavía mostró diferencias significativas entre

la zona que fue afectada por la piscifactoría y la estación control (tabla 7.1). Las diferencias encontradas se debieron principalmente al porcentaje de conchas dentro de esta fracción de sedimento. Estas conchas fueron principalmente valvas de mejillones, que en la zona afectada por la piscifactoría procedían de la limpieza del fouling de las estructuras sumergidas cuando la instalación estaba en funcionamiento. A medida que pasó el tiempo, este porcentaje de gravas fue disminuyendo en la instalación y en los muestreos después de 9 meses y 2 años ya no se encontraron diferencias entre los dos sitios, debido seguramente al enterramiento y/o hidrodinamismo de la zona.

Otro síntoma de recuperación química se observó a los 9 meses después del cese de la actividad, ya que dejaron de observarse diferencias significativas en la medida del potencial redox entre la instalación y el control (figura 7.1c Fase 2). Esta modificación podría ser interpretada como una disminución de los procesos geoquímicos anaerobios en la capa subsuperficial de los sedimentos.

Finalmente, el fósforo total del sedimento también disminuyó a medida que pasó el tiempo después del cese de la actividad. Curiosamente esta reducción fue casi constante los 9 primeros meses después del cese, de unos  $3.96 \text{ mg.kg}^{-1}.\text{d}^{-1}$ . Este parámetro dejó de dar diferencias significativas entre instalación y control en el muestreo después de 9 meses del cese de la actividad (figura 7.1b Fase 2). El enterramiento del sedimento y/o dispersión de los desechos debidos al hidrodinamismo de la zona y a los temporales habidos, podrían ser factores muy importantes a tener en cuenta en la disminución del fósforo total en el primer centímetro del sedimento. Casualmente entre el muestreo después de 3 meses del cierre (septiembre 2009) y el siguiente (abril 2010) se abarcaron los meses de otoño e invierno, épocas del año con desarrollo preferente de temporales en el litoral mediterráneo español (Olcina y Torres, 1997). Durante los meses de diciembre de 2009 a marzo de 2010 se produjeron en la zona de estudio un

elevado número de situaciones adversas que originaron varios temporales (Puertos del Estado, 2012).

Este estudio mostró que el porcentaje de materia orgánica se recuperó a los 3 meses después del cese, ya que no se observaron diferencias significativas entre la instalación y el control. A los 9 meses después del cese también se vieron recuperados el resto de parámetros abióticos de los sedimentos perturbados por la actividad, tales como el porcentaje de la fracción gruesa, el potencial redox y la concentración de fósforo total.

### 7.2.2.- Recuperación de la macrofauna

La recuperación potencial de un área después del enriquecimiento orgánico depende de los factores abióticos y bióticos (Díaz y Rosenberg, 1995). La recuperación química de los sedimentos es lo primero que se produce y es un requisito necesario para que la recuperación biológica tenga lugar (Brooks et al., 2002, 2003, 2004).

En la figura 7.2 Fase 2, se muestran los resultados de abundancia y riqueza específica de la macrofauna bentónica. En la campaña después de 1 mes del cese de la actividad, la zona que fue afectada por la piscifactoría todavía presentaba una mayor abundancia que la estación control, fundamentalmente debido a la de los poliquetos (figura 7.2b Fase 2). Ya en el resto de campañas de muestreo, la estación control mostró mayor abundancia que la zona que fue afectada por la piscifactoría, aunque en la campaña de muestreo después de 2 años tras el cese de la actividad las diferencias fueron mínimas. En cuanto a la riqueza específica, se observó que en la zona que fue afectada por la piscifactoría siempre fue bastante menor que en la estación control, excepto en la campaña de muestreo después de 2 años donde éstas fueron similares (figura 7.2a Fase 2).

Tanto en la zona que fue afectada por la piscifactoría como en la estación control se encontraron muy pocos moluscos y crustáceos en todas las campañas de muestreo. Entre los crustáceos, destacó *Apseudes latreilli*

que siempre estuvo presente en la zona control, sin embargo, en la zona que fue afectada por la piscifactoría, esta especie recién apareció en las campañas de muestreos pasados 9 meses y 2 años del cierre. Esto probablemente fue una consecuencia de que las condiciones físico-químicas de los sedimentos ya se habían recuperado, y dado que el impacto de las piscifactorías en aguas abiertas se limita a un perímetro relativamente pequeño alrededor de las jaulas (Mantzavrakos et al., 2007, Borja et al., 2009), se pudo producir una recolonización desde las áreas no afectadas.

Por otra parte, en la zona que fue afectada por la piscifactoría, se observó que la abundancia de *Capitella capitata* disminuyó considerablemente a los 3 y 9 meses (con valores de 552 y 221 individuos.m<sup>-2</sup> respectivamente). Esta disminución fue acompañada de un aumento de especies de las familias *Glyceridae* y *Nephtyidae* que son carnívoros móviles (Fauchald y Jumars 1979) y potenciales depredadores de otros invertebrados. Al mismo tiempo también se encontraron ejemplares de *Maldanidae*, excavadores sésiles, que son especies muy sensibles al enriquecimiento orgánico (Borja et al., 2000). Por el contrario *Capitella* sp., se alimenta de depósitos subsuperficiales, típico de áreas con elevado aporte de carbono orgánico e incluso en sedimentos reducidos (Borja et al., 2000).

Los valores del índice de Simpson (figura 7.3) en la zona control fueron bastante estables durante todas las campañas de muestreos con valores alrededor de 0.1. Sin embargo en la zona que fue afectada por la piscifactoría el índice de Simpson fue de 0.9 después de 1 mes del cese, hasta alcanzar el valor de 0.1 en el muestreo pasados 2 años del cese.

A diferencia de lo ocurrió con las variables físico-químicas medidas, la macrofauna mostró una recuperación más lenta. Hubo que esperar hasta 2 años desde el cese de la actividad acuícola, para que desapareciese *Capitella capitata* y la riqueza específica y el valor del índice de Simpson fuesen similares entre la instalación y la estación control. Se debe señalar también que en este último muestreo, en la zona que fue afectada por la piscifactoría,

por primera vez, se observaron organismos pertenecientes a los grupos *Nematomorpha*, *Equinodermata* y *Sipuncula*, los cuales se habían encontrado anteriormente en la estación control.

La tasa de recuperación de un sistema afectado es única y difícilmente comparable con otro, ya que depende, entre otras cosas, de las características básicas de la zona (Kraufvelin et al., 2001). Además, la forma en que se lleva a cabo en cada lugar, sólo puede entenderse teniendo en cuenta la gran variabilidad en los parámetros físico-químico ambientales (Dernie et al., 2003). A pesar de esto, se han revisado otros estudios que han analizado la recuperación macrobentónica tras un periodo de barbecho o cierre definitivo de una actividad acuícola (Johannessen et al., 1994; Pohle et al., 2001; Pereira et al., 2004; Villnäs et al., 2011; Aguado et al., 2012). Ninguno termina observando, en sus periodos de estudio, la recuperación total de la macrofauna.

### 7.2.3.- Recuperación de los flujos bentónicos

También se determinaron los flujos bentónicos para ver cuánto tiempo tardaron en recuperarse tras el cierre de la actividad acuícola, sin existir estudios previos que hayan medido ni *in situ* ni *ex situ* los flujos bentónicos de oxígeno y nutrientes tras el cese de una piscifactoría. La única excepción fue el estudio de Pereira et al. (2004) donde midieron sólo flujos de oxígeno *ex situ*.

Los flujos bentónicos sólo se midieron después de 1, 3 y 9 meses del cese de la actividad acuícola. No se midieron en la campaña realizada después de 2 años del cese, ya que en las campañas después de 3 y 9 meses del cese, ya no se obtuvieron diferencias significativas en ninguno de los flujos que las mostraban durante la fase de funcionamiento entre instalación y control.

Generalmente los flujos de oxígeno disuelto fueron negativos (figura 7.4a Fase 2) indicando un consumo de oxígeno por el sedimento, excepto en

el punto control en las cámaras transparentes, en la campaña de muestreo después de 9 meses del cierre, donde los flujos fueron positivos. Sólo se observaron diferencias significativas entre la zona que fue afectada por la piscifactoría y la estación control en la campaña después de 1 mes del cierre, por lo se puede decir que a los 3 meses después del cese, este flujo ya se había recuperado.

En todos los muestreos se observó un flujo positivo de amonio desde el sedimento a la columna de agua (figura 7.4b Fase 2). No se observaron diferencias significativas entre la zona que fue afectada por la piscifactoría y la zona control en ninguna de las campañas de muestreo después del cese de la actividad acuícola. Los flujos fueron similares en ambas zonas y no fueron superiores a  $2 \text{ mmol.m}^{-2}.\text{d}^{-1}$ . Este resultado sugiere que la mayor parte de la materia orgánica nitrogenada, aportada por la piscifactoría, fue rápidamente degradada bioquímicamente y reincorporada a la columna de agua (Christensen et al., 2000; Aguado, 2001; Cromey et al., 2002).

En general, los flujos de fosfato fueron positivos (figura 7.4c Fase 2), es decir, hubo aporte de fósforo desde el sedimento a la columna de agua. El flujo de fosfato siguió una tendencia similar al flujo de oxígeno disuelto y la materia orgánica tras el cese. Sólo en el muestreo después de 1 mes del cierre de la actividad se observaron diferencias significativas entre la piscifactoría y la estación control, si bien este flujo en instalación se vio fuertemente reducido con respecto al observado en la etapa de funcionamiento.

Los flujos del nitrato no mostraron diferencias entre la zona que fue afectada por la piscifactoría y la estación control y en todos los casos fueron negativos (figura 7.5a Fase 2), es decir que existió un consumo de nitrato por el sedimento a causa de la desnitrificación bentónica (Herbert 1999).

Los flujos de nitrito no mostraron diferencias significativas entre la zona que fue afectada por la piscifactoría y la estación control en ninguna campaña de muestreo después del cese (figura 7.5b Fase 2). Los flujos de



nitrito fueron muy bajos en comparación a los otros flujos medidos y no mostraron una tendencia clara. Esto probablemente se deba a que el nitrito es un compuesto de transición entre el amonio y nitrato en el proceso de nitrificación y entre el nitrato y  $N_2/N_2O$  en el proceso de desnitrificación bentónica (Herbert 1999).

Los flujos de sílice fueron positivos, excepto en la campaña de muestreo después de 9 meses del cierre, en la zona que fue afectada por la piscifactoría, donde fueron negativos, tanto en las cámaras transparentes como en las opacas (figura 7.5c Fase 2). Si bien en las campañas de muestreo después de 3 y 9 meses del cese de la actividad, se observaron diferencias significativas en los flujos de sílice entre la zona que fue afectada por la piscifactoría y la estación control, éstas no fueron una consecuencia de la actividad acuícola. La actividad acuícola no parece afectar al flujo de sílice ya que la materia orgánica que proviene de la piscifactoría tiene un bajo o nulo contenido en sílice (Tovar et al., 2000; Maldonado et al., 2005). Los flujos positivos de sílice pudieron deberse fundamentalmente a la disolución de la sílice biogénica presente en los sedimentos, mientras que los negativos pudieron ser consecuencia de que el  $Si(OH)_4$  presente en el agua pudo precipitar formando sílice mineral suspendida o a que el  $Si(OH)_4$  pudo ser absorbido por las diatomeas del fitobentos (De Vittor et al., 2012). Por lo que las diferencias encontradas en los flujos de sílice se pudieron deber a las condiciones físico-químicas del medio y/o a la heterogeneidad espacial de los sedimentos en cuanto a presencia de sílice biogénico, vivo o muerto.

De los flujos bentónicos medidos que más se vieron perturbados por la instalación acuícola, el flujo de amonio fue el primer parámetro en recuperarse tras 1 mes del cese, seguidos de los flujos de fosfato y oxígeno disuelto, los cuales a los 3 meses del cese de la piscifactoría ya mostraron niveles semejantes a los medidos en la estación control.

### **7.3.- Relación entre las variables ambientales de los sedimentos y la macrofauna incluyendo fase de funcionamiento y recuperación**

Teniendo en cuenta todas las campañas de muestreo menos la última, la de después de 2 años del cese de la actividad acuícola (donde no hubo datos de flujos bentónicos), se realizó un análisis de correlación canónica (CCA) para analizar los efectos de las variables ambientales bentónicas sobre las abundancias de las especies de la macrofauna y su variación espacial. Se incluyeron en el CCA siete variables ambientales que se vieron significativamente perturbadas por la actividad acuícola: los flujos bentónicos de amonio, de fosfato y de oxígeno disuelto, el porcentaje de materia orgánica, la concentración de fósforo total, la medida del potencial redox y el porcentaje de la fracción mayor a 2 mm o porcentaje de gravas. Debido a que los taxones raros pueden producir distorsión de las puntuaciones de coordinación, se excluyeron los taxones que sólo se observaron en una campaña de muestreo en instalación o control. El análisis señaló que los tres primeros ejes explicaron un 52.5% del total de la varianza contenida en los datos de la comunidad bentónica. El primer eje explicó el 41.2%, el segundo 7.6% y el tercero 3.7%.

Las correlaciones de las variables con los ejes se muestran en la tabla 7.2, siendo el fósforo total, el porcentaje de gravas, el potencial redox, el flujo de fosfato y el flujo de oxígeno disuelto, las variables que mejor se correlacionaron con el eje 1. La correlación de Pearson entre las especies y las variables ambientales fue de 0.998 para el primer eje y la prueba de permutación Monte Carlo ( $p < 0.05$ ) dio  $p = 0.002$  para la correlación entre las variables ambientales y la macrofauna, por lo que las correlaciones observadas fueron significativas. El diagrama de factores (figura 7.6) mostró una clara diferenciación entre las dos zonas muestreadas respecto del eje 1.

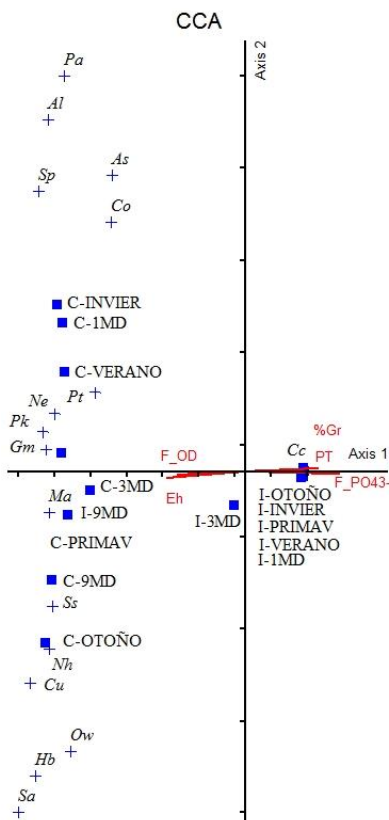


Figura 7.6: Diagrama de factores del CCA. Muestra la posición de las zonas de estudio (■): Instalación (I) y Control (C) en las diferentes campañas de muestreo: en fase de funcionamiento las campañas realizadas en otoño, invierno, primavera y verano y en la fase después del cese de la actividad, las campañas de muestreo de 1, 3 y 9 meses después del cese (1MD, 3MD y 9MD respectivamente) y la distribución de especies (+) en relación a las variables predictoras más significativas: flujo de fósforo ( $F_{PO_4^{3-}}$ ) y de oxígeno disuelto ( $F_{DO}$ ), la concentración de fósforo total (PT), la medida del potencial redox (Eh) y el porcentaje de la fracción mayor a 2 mm o porcentaje de gravas (%Gr). *Ampelisca spinipes* (As), *Apseudes latreilli* (Al), *Cumacea* (Cu), *Corophiidae* (Co), *Paguridae* (Pa), *Pariambus typicus* (Pt), *Spisula subtruncata* (Ss), *Hyalinoecia bilineata* (Hb), *Capitella capitata* (Cc), *Goniada maculata* (Gm), *Maldanidae* (Ma), *Nephtys hombergi* (Nh), *Owenidae* (Ow), *Pectinaria koreni* (Pk), *Sabellidae* (Sa), *Spionidae* (Sp), *Nematomorpha* (Ne).

Tabla 7.2: Correlación de las 7 variables abióticas incluidas en el CCA con los tres ejes.

Variable	Eje 1	Eje 2	Eje 3
Flujo amonio	0.377	-0.011	0.033
Flujo fosfato	<b>0.712</b>	0.062	0.085
Flujo oxígeno disuelto	<b>-0.647</b>	-0.073	-0.183
% materia orgánica	0.437	-0.217	-0.049
Fósforo total	<b>0.922</b>	-0.051	0.055
Potencial redox	<b>-0.753</b>	-0.145	0.104
% gravas	<b>0.831</b>	-0.047	0.017

La estación control siempre mostró correlación negativa, sin embargo la instalación mostró correlación positiva en todas las campañas de muestreo en la fase de funcionamiento y además en la campaña después de 1 mes del cese. En la campaña de muestreo después de 3 meses después del cese, la instalación ya obtuvo una correlación ligeramente negativa, reflejando en la campaña después de 9 meses del cese una correlación similar a la indicada en los muestreos en la estación control. En cuanto a la especie de macrofauna, se observa que *Capitella capitata* es la única especie que presenta correlación positiva con respecto al eje 1 y el resto de especies presenta correlación negativa.

El CCA, a pesar de explicar parte de la variabilidad dentro de las dos zonas de estudio y de presentar correlaciones altas entre las variables medioambientales y las biológicas, no explicó toda la variabilidad en la distribución de las especies. Otras variables ambientales no medidas en este estudio también pudieron influir en la distribución de las especies. Por ejemplo, otros contaminantes procedentes de la actividad piscicultura como metales y sustancias de quimioterapia también pueden causar efectos adversos sobre la fauna bentónica (Dean et al. 2007; Davies et al. 1997).

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# Capítulo 8: Conclusiones

### 8.1.- Fase de funcionamiento de la piscifactoría

La producción de dorada en jaulas flotantes afecta a calidad de los sedimentos y del agua, modificando los flujos de oxígeno y nutrientes en la interfase agua-sedimento.

- Mediante la caracterización físico-química y biológica de los sedimentos se apreció que:
  - Bajo las jaulas se generó una acumulación de materia orgánica y fósforo total y los sedimentos se volvieron más reductores, mostrando niveles máximos en verano. Las variaciones de los parámetros físico-químicos están íntimamente relacionadas con la actividad metabólica de la alimentación de los peces que a su vez depende de la temperatura del agua.
  - La alteración de los parámetros abióticos generó un cambio significativo en la estructura de la comunidad bentónica, ya que se produjo una notable disminución de la riqueza específica y un incremento de la abundancia de la macrofauna de hasta 9 veces. La presencia de especies sensibles al enriquecimiento orgánico fue muy limitada viéndose favorecida la oportunista *Capitella capitata* que mostró grandes incrementos poblacionales.
- En la columna de agua, la alta resolución vertical de muestreo permitió observar que:
  - En el entorno de las jaulas se produjo una disminución de la concentración de oxígeno disuelto y un aumento de nitrógeno inorgánico disuelto y fosfato, llegando a modificar la relación estequiométrica de los nutrientes y el nutriente limitante para la producción primaria.
  - A pesar de haber encontrado mayores concentraciones de nutrientes en las inmediaciones de las jaulas, no se apreció una correspondencia con los niveles de clorofila *a* probablemente

debido al papel que juega el biofouling marino de las estructuras sumergidas de las instalaciones piscícolas en el aprovechamiento de nutrientes, partículas y fitoplancton.

- Los flujos en la interfase agua-sedimento fueron los primeros obtenidos mediante ensayos *in situ* para piscifactorías en el Mediterráneo y señalaron que:
  - Debido a la mineralización aerobia de la materia orgánica, los sedimentos bajo las jaulas funcionaron como un sumidero de oxígeno disuelto y como una fuente de nitrógeno inorgánico disuelto y fosfato hacia la columna de agua. El amonio fue el principal compuesto de nitrógeno liberado desde los sedimentos, mientras que el nitrato, en menor proporción, fue consumido a causa de la desnitrificación que se produjo en capas subsuperficiales y que además se vio afectado por la disponibilidad de este compuesto en el agua de fondo. Los flujos de sílice no parecieron verse afectados por la actividad de la piscifactoría.
  - La media de los flujos bentónicos de nitrógeno, fósforo y sílice estimados en la instalación pudieron proporcionar: 2.5 veces los requerimientos de nitrógeno, 8 veces los requerimientos de fósforo y la mitad de los requerimientos de sílice por parte del fitoplancton en la columna de agua.

## 8.2.- Fase después del cese de la piscifactoría

La recuperación de una zona que fue afectada por una instalación acuícola se manifiesta, a distintas escalas temporales, mediante síntomas de recuperación parcial de los diversos parámetros abióticos y bióticos del medio. Esta recuperación está condicionada por las características

hidrológicas de la zona y el tipo de sedimento así como del grado y extensión del impacto. En este estudio se observó que:

- El primer parámetro en recuperarse fue el flujo de amonio, mostrando, en la zona que fue afectada por la actividad acuícola, niveles similares a los de la zona de referencia en el muestreo después de 1 mes del cese.
- Los siguientes parámetros que mostraron signos de recuperación fueron el porcentaje de materia orgánica en los sedimentos, y los flujos de fosfato y oxígeno disuelto, los cuales antes de 3 meses del cese de la piscifactoría ya mostraron niveles semejantes a los medidos en la estación control. También la abundancia de la macrofauna bentónica mostró señales claras de recuperación mediante un descenso significativo de *Capitella capitata*.
- A los 9 meses después del cese se vieron recuperados el resto de parámetros abióticos de los sedimentos perturbados por la actividad, tales como el porcentaje de fracción gruesa, concentración de fósforo total y el potencial redox.
- La recolonización llevada a cabo por las especies con tolerancia a niveles inferiores de contaminación, fue más lenta, no observándose, hasta el muestreo de 2 años del cese de la actividad, niveles de riqueza específica semejantes a los de la zona de referencia.
- El papel de los flujos en la recuperación ambiental tras el cese de la actividad acuícola estuvo limitado a un periodo máximo de tres meses, ya que estos estuvieron asociados a la diagénesis de la materia orgánica. Sin embargo, fue necesario que transcurriera más tiempo para la recuperación completa, que se observó tras dos años del cese de la actividad.