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Pigmentos indicadores: caracterización de la comunidad fitoplanctónica en ecosistemas marinos costeros

TESIS DOCTORAL

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A mi hija Mireia, a Jose y a nuestra familia

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RESUMEN

Las aguas costeras y estuarinas se caracterizan por una gran variabilidad, espacial y temporal, en sus características hidrológicas, físicas y químicas, que se refleja en la complejidad de la dinámica de sus comunidades fitoplanctónicas. Además, las zonas costeras están sometidas a una elevada presión antrópica, cuyo principal efecto es el aumento de los aportes de nutrientes de origen terrestre a las aguas receptoras, en las que puede llegar a provocar problemas de eutrofización.

El objetivo de esta tesis es caracterizar la variabilidad espacial de las comunidades fitoplanctónicas en aguas costeras y de transición caracterizadas por distintas fuentes de entrada de nutrientes. Obtener información sobre el funcionamiento de estos ecosistemas es necesario para orientar las medidas de gestión necesarias para mejorar la calidad de sus aguas.

En la presente tesis se realiza la clasificación taxonómica del fitoplancton con el programa CHEMTAX a partir de las concentraciones de pigmentos fotosintéticos halladas por cromatografía líquida de alta resolución en muestras de aguas costeras. Frente a las técnicas tradicionales de microscopía, esta metodología presenta una serie de ventajas entre las que cabe destacar el menor coste, la mayor rapidez y precisión. Además, se realiza una primera aproximación al uso de imágenes de satélite de alta resolución espacial, para estimar los niveles de clorofila *a*, parámetro indicador de la biomasa fitoplanctónica. La principal ventaja de esta técnica es que permite obtener una visión sinóptica de una determinada zona de estudio que no es posible obtener con las técnicas tradicionales de muestreo. Las características que presentan estas técnicas hacen de ellas

buenas candidatas para las exigencias de monitoreo de la Directiva Marco del Agua.

Los resultados de esta tesis indican que los principales problemas de calidad de aguas se dan en las áreas con un menor intercambio y dilución con el mar. En la presente tesis estas áreas están representadas por el Puerto de Gandía y la zona próxima a la desembocadura del río Serpis. Entre las recomendaciones para mejorar el estado de estos sistemas cabe destacar el control de las descargas ilegales de aguas residuales y perseguir el cumplimiento de las buenas prácticas agrícolas.

RESUM

Les aigües costaneres i els estuaris se caracteritzen per una gran variabilitat, espacial i temporal, en les seues característiques hidrològiques, físiques i químiques, que se reflecteix en la complexitat de la dinàmica de les seues comunitats fitoplanctòniques. A més, les zones costaneres estan sotmeses a una elevada pressió antròpica, el principal efecte de la qual és l'augment dels aportaments de nutrients d'origen terrestre a les aigües receptores, en les que pot arribar a provocar problemes d'eutrofització.

L'objectiu d'aquesta tesis és caracteritzar la variabilitat espacial de les comunitats fitoplanctòniques en aigües costaneres i de transició caracteritzades per distintes fonts d'entrada de nutrients. Obtenir informació sobre el funcionament d'aquests ecosistemes és necessari per a orientar les mesures de gestió necessàries per a millorar la qualitat de les seues aigües.

En la present tesis se realitza la classificació taxonòmica del fitoplàncton en el programa CHEMTAX a partir de les concentracions de pigments fotosintètics mesurats per cromatografia líquida d'alta resolució en mostres d'aigües costaneres. Front a les tècniques tradicionals de microscòpia, aquesta metodologia presenta una sèrie d'avantatges entre els que cal destacar el menor cost, la major rapidesa i precisió. A més, se realitza una primera aproximació a la utilització d'imatges de satèl·lit d'alta resolució espacial, per a estimar els nivells de clorofil·la *a*, paràmetre indicador de la biomassa fitoplanctònica. El principal avantatge d'aquesta tècnica és que permet obtenir una visió sinòptica d'una determinada zona d'estudi que no és possible obtenir en les tècniques tradicionals de mostreig. Les característiques que presenten aquestes tècniques fan d'elles bones

candidates per a les exigències de monitoratge de la Directiva Marc de l'Aigua.

Els resultats d'aquesta tesis indiquen que els principals problemes de qualitat d'aigües tenen lloc en les àrees en un menor intercanvi i dilució en la mar. En la present tesis aquestes àrees estan representades pel Port de Gandia i la zona pròxima a la desembocadura del riu Serpis. Entre les recomanacions per a millorar l'estat d'aquests sistemes cal destacar el control de les descàrregues il·legals

d'aigües residuals i perseguir el compliment de les bones pràctiques agrícoles.

ABSTRACT

Coastal and estuarine waters are notable for the high spatial and temporal variability in their hydrological, physical and chemical properties. This variability is reflected in the complex dynamics of their phytoplankton community.

In addition, coastal areas are subject to high anthropogenic pressure, the main effect of which is an increase in nutrient loads from terrestrial origin to receiving waters, where they can cause eutrophication problems.

The aim of this thesis is to characterize the spatial variability of the phytoplankton communities in coastal and transitional waters which receive nutrient inputs from various sources. Obtaining information about the functioning of these ecosystems is required so that the right management measures can be adopted to improve the water quality.

In this thesis, the concentrations of photosynthetic pigments in coastal water samples were measured using high-performance liquid chromatography. This data was then entered into the CHEMTAX software to obtain the taxonomic composition of the phytoplankton. Compared to microscopy, the main advantages of this technique are lower cost, more rapid analyses and greater precision. Furthermore, an initial attempt is made to estimate chlorophyll *a* levels, which are an indicator of phytoplankton biomass, by using high spatial resolution satellite images. The main advantage of this technique is that it provides a synoptic view not attainable with traditional monitoring. The characteristics of both these techniques make them suitable for the monitoring requirements of the Water Framework Directive.

The results of this thesis indicate that the main problems of water quality are found in areas with reduced exchange and dilution with the sea. In this thesis, these areas are represented by Gandia Harbour and an area near the Serpis river mouth. The need to control illegal waste water discharges and to ensure the proper implementation of the existing agricultural best management practices, are among the most important management recommendations to improve the state of these systems.

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Contenido

Capítulo 1: Introducción

1 Antecedentes

La eutrofización ha sido descrita como una de las amenazas actuales más importantes para la integridad de los ecosistemas costeros (Maier et al., 2009). Los principales efectos derivados de este proceso son los cambios en la composición de la comunidad fitoplanctónica y una mayor frecuencia de los episodios de floraciones de algas nocivas (Harmful Algal Blooms, HABs, en la literatura inglesa) (Heisler et al., 2008). Estas últimas incluyen tanto especies responsables de la síntesis de toxinas como productores de elevada biomasa, los cuales pueden causar hipoxia, anoxia y muertes indiscriminadas de vida marina al alcanzar concentraciones muy elevadas. El resultado es una disminución de la biodiversidad y la calidad del agua (Herrera-Silveira & Morales, 2009, Maier et al., 2009).

Existe un gran consenso en que el principal factor que promueve la eutrofización es el aumento en la disponibilidad de nutrientes (principalmente nitrógeno y fósforo) (Paerl, 2009). Los ecosistemas costeros son particularmente vulnerables a los aportes de nutrientes por su escasa profundidad y su relativamente débil intercambio con el mar abierto

(Maier et al., 2009). Además, estos sistemas están sometidos a una elevada presión antrópica, ya que el 75% de la población mundial se concentra en la franja litoral, y la tendencia va en aumento (Paerl, 2006).

Los ecosistemas costeros reciben y procesan gran cantidad de nutrientes procedentes de fuentes difusas (principalmente escorrentías superficiales, descargas subterráneas y deposición atmosférica) y de fuentes puntuales (principalmente ríos y descargas de efluentes de aguas residuales a través de emisarios submarinos) (Paerl, 2006). El uso del suelo en la cuenca vertiente determina en gran medida las características de las descargas de nutrientes. En zonas donde predomina la agricultura son características las descargas con un elevado contenido en nitratos (Maier et al., 2009). En zonas urbanas, las descargas de aguas residuales (urbanas e industriales) pueden producir un aumento de nutrientes, especialmente de fósforo, en las aguas receptoras (Artioli et al., 2008). El sílice, aunque su principal fuente es natural (disolución de los silicatos), también se ve influenciado por el uso del suelo. La construcción de presas para regular el caudal de los ríos y aumentar el aprovechamiento de los recursos hídricos superficiales, para riego y abastecimiento urbano, provoca una disminución en los aportes de sílice aguas abajo (Falco et al., 2010).

Además, los ecosistemas costeros se caracterizan por una elevada variabilidad en sus características físicas y químicas, por lo que se pueden encontrar variaciones en el nutriente potencialmente limitante (nitrógeno, fósforo y sílice), a lo largo del gradiente de salinidad y según la época del año (Paerl, 2009). Disponer de información sobre el funcionamiento de estos ecosistemas es fundamental para tomar medidas que obtengan los resultados esperados (Conley, 2009).

Con el objetivo de reducir la eutrofización en los últimos años se han adoptado diversas políticas para reducir las entradas de nutrientes de origen antrópico a las cuencas. Por ejemplo, en la legislación europea encontramos la Directica 91/271/CEE de Tratamiento de Aguas Residuales o la Directiva 91/676/CEE de Nitratos. Sin embargo, el éxito conseguido ha sido variable, y en general, se han obtenido mayores reducciones de los nutrientes provenientes de fuentes puntuales (aguas residuales) que vierten en localizaciones discretas y más fácilmente identificables (Artioli et al., 2008). Dado que los costes para conseguir reducciones en las emisiones de nutrientes son sustanciales, desarrollar una estrategia apropiada de gestión de los nutrientes es muy importante (Conley et al., 2009).

La importancia de las aguas costeras y de transición, y de los problemas de eutrofización que las afectan debido a la elevada presión antrópica, ha sido reconocida en Europa, por la Directiva Europea 2000/60/CEE, también conocida como la Directiva Marco del Agua, y en Estados Unidos, por el Programa Nacional de Evaluación de la Eutrofización Estuarina (National Estuarine Eutrophication Assessment (NEEA) Program) (Whitall, 2007). Estas normativas establecen sistemas de monitoreo para vigilar el cumplimiento de los objetivos de calidad del agua establecidos, de parámetros físicos, químicos y biológicos.

La Directiva Marco del Agua establece el seguimiento de distintos parámetros biológicos, entre los que cabe destacar la composición taxonómica del fitoplancton y la biomasa fitoplanctónica (cuyo indicador es la clorofila *a*), para caracterizar la calidad de las masas de agua. El requerimiento de un sistema de evaluación basado en la composición taxonómica del fitoplancton encierra cierta dificultad debido a las variaciones espaciales y temporales de la comunidad fitoplanctónica, a la diversidad de las diferentes masas de agua, y a que las técnicas de microscopía tradicionalmente utilizadas son laboriosas, costosas y pueden presentar resultados inconsistentes entre diferentes laboratorios (Duarte et al., 1990; Schlüter et al., 2000).

La clasificación taxonómica basada en la especificidad de los pigmentos fotosintéticos, analizados por cromatografía líquida de alta resolución (HPLC), se presenta como una técnica alternativa que permite el análisis de cientos de muestras de forma rápida y a un coste menor (Wright et al., 1996; Higgins et al., 2011). La aplicación estadística CHEMTAX (CHEMical TAXonomy software) permite diferenciar cualquier clase algal independientemente de si los pigmentos marcadores corresponden o no a una sola clase (Mackey et al., 1996; Latasa, 2007).

Por otra parte, la variabilidad espacial, la variabilidad temporal, los muestreos y los errores analíticos hacen que la caracterización de los elementos biológicos sea imprecisa y es necesaria la toma de suficientes muestras para poder caracterizar este error (Carstersen, 2007). Frente al elevado coste económico de las técnicas de muestreo y análisis tradicionales, el monitoreo mediante imágenes de satélite se plantea como una alternativa viable para el seguimiento de la concentración de clorofila *a*, como indicador de la biomasa fitoplanctónica, en los programas de monitoreo de la Directiva Marco del Agua (Chen et al., 2004). Las imágenes de satélite permiten obtener una visión sinóptica que es especialmente importante en ambientes con un alto grado de variabilidad en sus características físico-químicas y en las entradas de nutrientes, que se reflejan en la complejidad de las comunidades biológicas (Elliot and Quintino, 2007). Entre estos ambientes los ecosistemas estuarinos y costeros son uno de los ejemplos más representativos.

2 Objetivos

Los objetivos de la presente tesis son los siguientes:

1) Analizar la composición de pigmentos fotosintéticos en muestras de agua principalmente marinas mediante cromatografía líquida de alta resolución (HPLC)
2) Clasificar taxonómicamente las comunidades fitoplanctónicas presentes en estas muestras mediante el programa CHEMTAX (CHEMical TAXonomy)
3) Estudiar la variabilidad espacial de los grupos fitoplanctónicos en función de los aportes de nutrientes de origen terrestre en una zona costera que incluye los siguientes casos de estudio:

   Caso de estudio 1: instalación portuaria caracterizada por aportes de agua dulce y un reducido intercambio con el mar: Puerto de Gandía (Valencia, España)

Caso de estudio 2: área costera cerrada y de escasa profundidad caracterizada por los aportes de un sistema fluvial: desembocadura del río Serpis, playa de Venecia (Gandía, España)

Caso de estudio 3: área de influencia de un emisario submarino que vierte aguas residuales urbanas: emisario de la Estación Depuradora de Aguas Residuales de Gandía (Valencia, España)

Caso de estudio 4: área costera abierta, sin ningún aporte de los citados anteriormente: playa del Ahuir (Gandía, España)

Caso de estudio 5: área a 9 km de la línea de costa caracterizada por estar poco afectada por aportes terrígenos

4) Comparar la distribución espacial de las comunidades fitoplantónicas en diferentes épocas del año: primavera vs. verano y período húmedo vs. período seco
5) Ajustar y validar un modelo para estimar la clorofila *a* (indicador de biomasa fitoplanctónica) con el sensor de alta resolución espacial Quickbird en la franja costera

3 Referencias

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Capítulo 2: Metodología

1 Área de estudio

El área de estudio se encuentra situada en el sector más al sur del Golfo de Valencia (España), en el mar Mediterráneo occidental. Corresponde a la franja costera de la ciudad de Gandía, que comprende desde el sur de la desembocadura del río Xeraco (también llamado río Vaca) hasta el sur de la desembocadura del río Serpis (Fig. 1).

En esta zona el clima es típicamente mediterráneo y el régimen fluvial está caracterizado por la variabilidad de sus precipitaciones. El caudal de los ríos Xeraco y Serpis presenta una alternancia estacional entre el período seco (verano) y el período húmedo, con lluvias concentradas principalmente en otoño; aunque esta variación estacional se ve atenuada por la intensa regulación artificial. Mientras que el río Xeraco tiene una longitud de 16.6 km y desemboca en una zona abierta, el río Serpis tiene una longitud de 74.5 km y desemboca en una zona más cerrada, la playa de Venecia, que se encuentra situada al sur del Puerto de Gandía (Fig. 1) (Hermosilla, 2005).

La llanura aluvial de estos ríos se sitúa sobre el acuífero detrítico de la Plana de Gandía-Denia, el cual se caracteriza por tener altos niveles de nitratos, que exceden el límite de 50 mg L^{-1} fijado por la Directiva de Nitratos (Directiva 91/676/CEE). Por ello los municipios del área afectada han sido declarados zona vulnerable a la contaminación por nitratos. La principal causa del alto contenido en nitratos es la actividad agrícola que se viene desarrollando a lo largo de la historia en esta región.

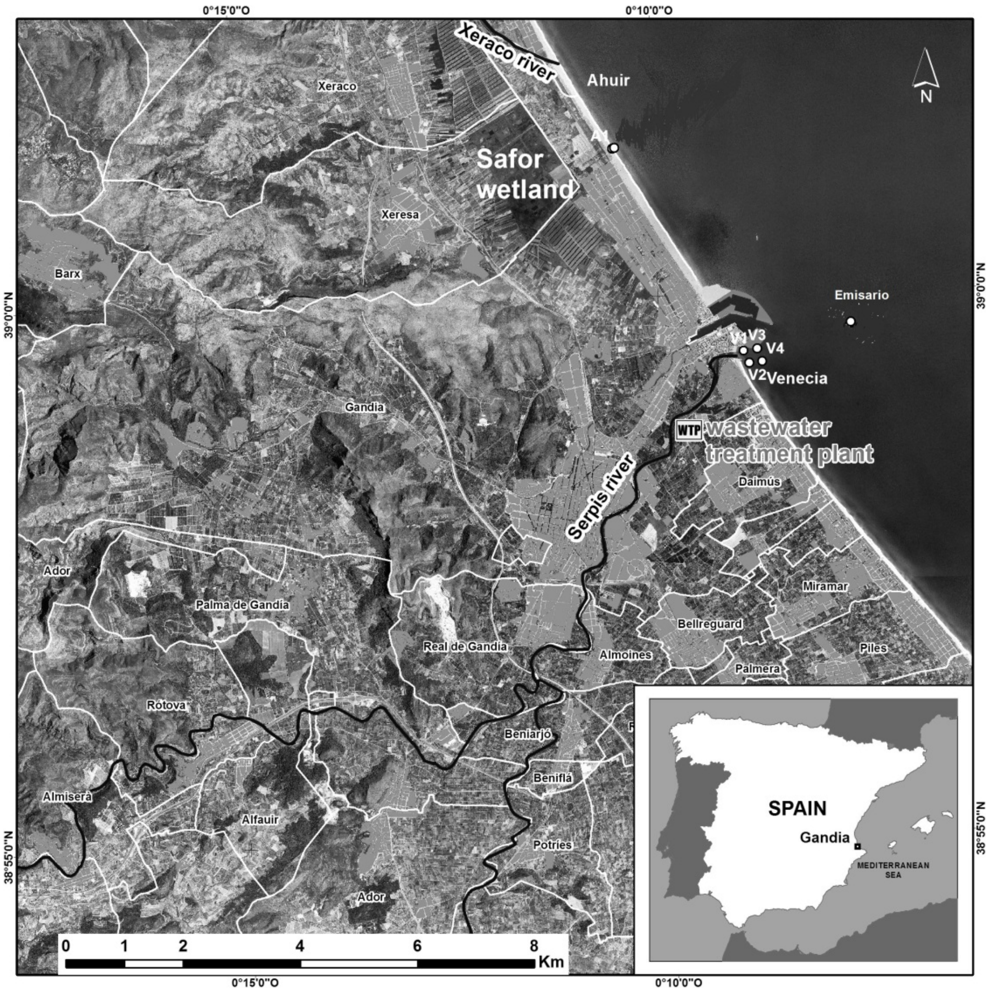

Figura 1 Área de estudio

En la llanura aluvial se encuentra una zona húmeda, La Marjal de La Safor (Fig. 1), que fue declarada lugar de interés comunitario (LIC) según la Directiva Hábitats (92/43/CEE). Los cultivos agrícolas que forman parte importante de este ecosistema han ido cambiando a lo largo del tiempo. En la actualidad los principales cultivos son cítricos y hortícolas. La extensión de tierras dedicadas a la agricultura también ha ido evolucionando, en concreto, ha experimentado una regresión debido a la expansión urbanística de los municipios costeros. El aporte hídrico más estable a La Marjal es el de agua subterránea del acuífero de la plana de Gandía-Denia, que a su vez es alimentado por descargas del acuífero cárstico de la Serra Grossa. El funcionamiento hídrico de La Marjal está regulado de forma artificial, por un complejo sistema de canales y bombeos, para satisfacer las necesidades de riego, así como para impedir problemas de inundación tanto de los cultivos como de las zonas urbanas construidas en las tierras ganadas a La Marjal. Las aguas drenadas de La Marjal por este sistema de bombeo se descargan al interior del Puerto de Gandía. Además, existe una zona de descarga subterránea situada al final de la zona urbanizada de la costa de Gandía, situada en la playa del Ahuir, donde se producen salidas difusas debidas a la presión que ejerce el sistema acuífero (Ballesteros-Navarro, 2003).

Como ya se ha avanzado, los municipios costeros han experimentado un crecimiento urbanístico significativo, que fue especialmente importante en las dos últimas décadas. Una de las principales causas de esta expansión es la actividad turística que, en los municipios del área de estudio, se basa fundamentalmente en el turismo residencial (Mantecón y Huete, 2007). La principal planta de tratamiento de las aguas residuales generadas en estos municipios es la planta de Gandía, la cual recoge el agua de 17 municipios de la comarca de La Safor: Almoines, Bellreguard, Beniarjó, Beniflà, Benirredrá, Daimús, Gandía, Guardamar de La Safor, La Font d'En Carròs, L'Alqueria de la Comtessa, Miramar, Palmera, Piles, Potries, Rafelcofer, Real de Gandía y Villalonga (Fig. 1). La planta vierte los efluentes tratados a través de un emisario submarino situado a aproximadamente 1900 m de distancia de la desembocadura del río Serpis y a una profundidad aproximada de 18 m (Fig. 1).

2 Estructura de la tesis

La variabilidad espacial y estacional de los nutrientes y la comunidad fitoplanctónica en el área de estudio descrita, se analiza en los capítulos del 3 al 6, ambos inclusive, de esta memoria de tesis, a través de los casos de estudio definidos en los objetivos.

Estos capítulos corresponden a cuatro artículos científicos, los cuales han sido enviados a revistas de reconocido prestigio. En la siguiente tabla se detalla el estado en que se encuentran en el momento de finalizar la presente memoria de tesis.

La estructura de la memoria de tesis en artículos científicos resulta más dispersa que la estructura tradicional. Con el fin de dotar a la memoria de un hilo conductor que ayude a seguir el grado de cumplimiento de los objetivos propuestos, se incluye en el capítulo 7 una discusión general de los resultados. En los capítulos 8 “Conclusiones” y 9 “Futuras líneas de investigación” se analizan los avances que se han obtenido en la tesis y se trata de generalizar el enfoque propuesto a otros lugares de similares características.

Tabla 1 Relación de capítulos de la presente memoria de tesis vs. artículos científicos

NºCAPÍTULO	TÍTULO ARTÍCULO	AUTORES	REVISTA	INDEXACIÓN	ESTADO
Capítulo 3	**Influence of nutrient inputs from a wetland dominated by agriculture on the phytoplankton community in a shallow harbour at the Spanish Mediterranean coast**	Sebastiá; M.T. Rodilla; M. Sanchis; J.A., Altur; V. Gadea; I., Falco, S.	Agriculture, Ecosystems and Environment ISSN 0167-8809	2010 JCR Science Edition FI 2.790	**Publicado en: Volumen 152 Páginas 10-20 DOI: 10.1016/j.agee.2012.02.006**
Capítulo 4	**Effects of freshwater inputs on the receiving waters of a Mediterranean coastal area: nutrients and phytoplankton analysis**	Sebastiá, MT, Rodilla, M.	Environmental Management ISSN 0364-152X	2010 JCR Science Edition FI 1.503	En revisión
Capítulo 5	**Nutrients and phytoplankton dynamics in the receiving waters of a Mediterranean river: the role of precipitation variability**	Sebastiá, M.T., Rodilla, M., Falco, S., Sanchis, J.A	Journal of Hydrology ISSN 0022-1694	2010 JCR Science Edition FI 2.514	En revisión
Capítulo 6	**Estimation of chlorophyll “a” on the Mediterranean coast using a Quickbird image**	M.T. Sebastiá, J. Estornell, M. Rodilla, J. Martí and S. Falco	Revista de teledetección ISSN 1133-0953	LATINDEX DICE	**Aceptado** Se publicará en el número de Junio de 2012

3 Metodología

La presente memoria de tesis incluye directamente artículos científicos en revistas reconocidas internacionalmente, tal y como se ha especificado en el epígrafe anterior. Por ello, cada uno de los capítulos correspondientes a dichos artículos (capítulos del 3 al 6) incluye un apartado de metodología el cual se halla explicado de forma sucinta, según el formato característico de las publicaciones científicas. En consecuencia, existe un solape entre capítulos en este apartado.

En el presente capítulo de esta memoria de tesis se trata de explicar la metodología con mayor detalle y en relación con los objetivos propuestos.

3.1 **Diseño campañas de muestreo: análisis de la variabilidad espacial y temporal**

El diseño de las campañas de toma de muestras es fundamental para el análisis de la variabilidad espacial de los grupos fitoplanctónicos en función de los aportes de nutrientes de origen terrestre en una zona costera.

Las campañas de muestreo se han diseñado teniendo en cuenta las particularidades de cada uno de los aportes de agua dulce así como de las aguas receptoras.

Caso de estudio 1: instalación portuaria caracterizada por aportes de agua dulce y un reducido intercambio con el mar, Puerto de Gandía (Valencia, España) (Fig. 1). Este caso de estudio se desarrolla en el capítulo 3 “Influence of nutrient inputs from a wetland dominated by agriculture on the phytoplankton community in a shallow harbour at the Spanish Mediterranean coast”.

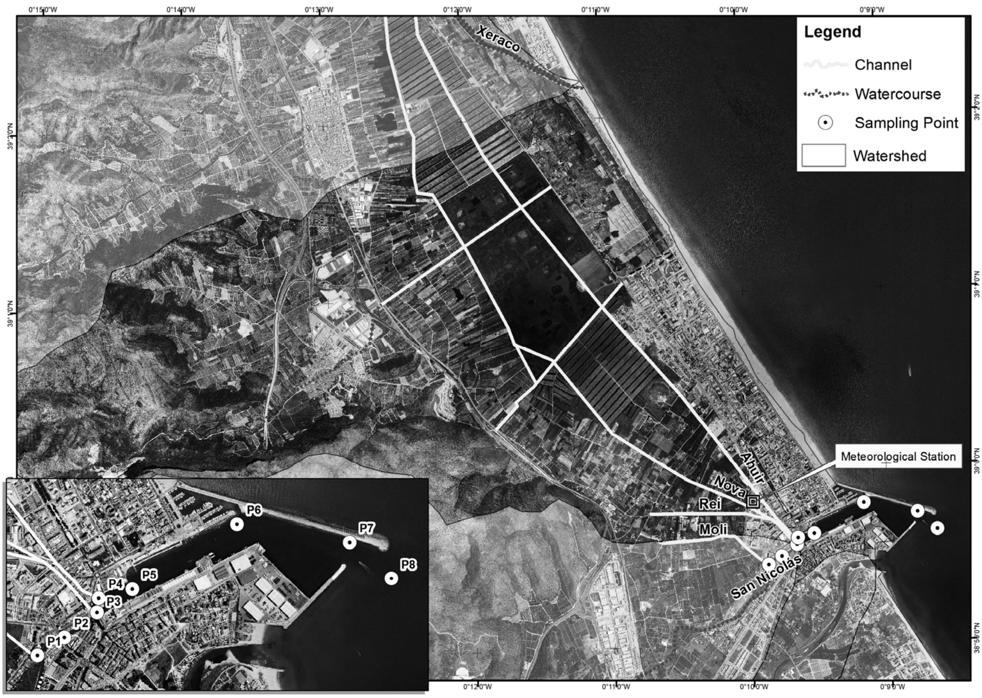

Figura 2 Localización punto campañas de muestreo en el Puerto de Gandía

El Puerto de Gandía recibe aportes de agua dulce a través de 3 acequias que drenan La Marjal de la Safor. Dada la escasa profundidad y sección de estas acequias se toma una única muestra de agua en cada una a 0.05 m de profundidad, la cual se considera representativa del caudal de agua dulce vertido (Fig. 2 puntos P1, P3 y P4). El caudal vertido por estas acequias genera una capa superficial de agua dulce sobre la cuña de agua de mar que hace que el puerto funcione como un estuario estratificado la mayor parte del año. La extensión y profundidad de esta capa superficial de agua dulce depende en gran medida del caudal vertido, así como de la dirección del viento. Los puntos de muestreo se distribuyen a lo largo de un gradiente longitudinal desde los puntos de descarga hasta el exterior de la zona portuaria, siendo P2 y P5 (Fig. 2) los puntos situados inmediatamente después de los puntos de vertido. En la tabla 1 se muestran las coordenadas de los puntos de muestreo, que se tomaron mediante GPS (modelo Garmin 60C, precisión 3-5 m), así como la distancia desde el primer punto de vertido (P1). Para tener en cuenta la variabilidad de la columna de agua y captar la zona de interfase se toman muestras a distintas profundidades (0, 0.05, 0.10, 0.30, 0.50, 0.75 y 1m) utilizando un muestreador de aguas superficiales (Superficial Water Sampler, SWAS, Mösso et al., 2008), así como una muestra extra sobre el fondo con una botella oceanográfica horizontal Van Dorn. El muestreo con el SWAS permite una elevada precisión en la zona más superficial donde se espera la mayor variabilidad en las características físico-químicas y biológicas de la columna de agua.

Tabla 2 Localización de los puntos de muestreo en la sección longitudinal del Puerto de Gandía

Punto	Coordenadas geográficas	XUTM	YUTM	Distancia a P1 (m)
P2	N38 59.586 W0 09.663	745886	4319919	176
P5	N38 59.688 W0 09.492	746127	4320114	566
P6	N38 59.832 W0 09.135	746634	4320398	1181
P7	N38 59.801 W0 08.828	747079	4320354	1774
P8	N38 59.664 W0 08.344	747785	4320123	2041

Se realizaron dos campañas de muestreo en dos épocas de estudio primavera (período húmedo) y verano (período seco), a priori distintas, en cuanto a aportes de agua dulce y cargas de nutrientes. El período húmedo en la zona de estudio abarca desde otoño hasta primavera, con las principales precipitaciones concentradas en otoño. No obstante, desde el punto de vista del funcionamiento hídrico de La Marjal cabe esperar mayores drenajes a través de los canales artificiales durante la primavera. Durante el verano, la ausencia de precipitaciones (o su escasez) y el uso de aguas subterráneas para riego y abastecimiento urbano, hace que descienda el nivel freático del acuífero, llegando a detectarse problemas de intrusión marina (Ballesteros-Navarro, 2003). Las lluvias de otoño sirven para recargar el acuífero después de este período. El nivel freático del acuífero es más elevado hacia el final del período húmedo, siendo entonces cuando son más necesarios los bombeos para evitar los problemas de asfixia radicular de los cultivos de La Marjal. Además, el período de abonado de los cítricos, cultivo predominante en el área, es a principios de marzo.

Caso de estudio 2: área costera cerrada y de escasa profundidad caracterizada por los aportes de un sistema fluvial, desembocadura del río Serpis, playa de Venecia (Valencia, España). Este caso de estudio se trata en dos de los capítulos posteriores.

En el capítulo 4 “Effects of freshwater inputs on the receiving waters of a Mediterranean coastal area: nutrients and phytoplankton analysis” se estudia la variabilidad espacial de los nutrientes y los grupos fitoplanctónicos en dos épocas de estudio (primavera, estación lluviosa y verano, estación seca). El aporte de agua desde los ríos al mar genera una pluma de agua dulce sobre el agua salina. Para analizar la variabilidad espacial se toman muestras en 3 puntos situados a lo largo de un gradiente longitudinal desde la desembocadura del río. La posición de estos puntos depende de la morfología de la pluma, la cual está condicionada por las corrientes marinas y el oleaje, que dependen en gran medida del viento. En estos 3 puntos de muestreo (V5, V6 y V7) se toman muestras a 0.05, 0.10 y 0.75 m con el SWAS. En el contorno de la pluma (Fig. 3, V1 a V4) se espera una menor variabilidad espacial de la columna de agua, por lo que se toman muestras únicamente en superficie y en profundidad (50 cm sobre el fondo) con una botella oceanográfica Van Dorn.

En el capítulo 5 “Nutrients and phytoplankton dynamics in the receiving waters of a Mediterranean river: analysis of the wet and the dry season” se estudia la variabilidad temporal en una estación, con muestreos desde mayo de 2008 hasta agosto de 2009, con una frecuencia media quincenal. El punto de muestreo se sitúa a 7.5 m de profundidad frente a la desembocadura del río Serpis. Dado que a esta profundidad la influencia de la pluma es mínima se muestrea únicamente en superficie, con botella oceanográfica Niskin, y en profundidad (50 cm sobre el fondo) con botella oceanográfica Van Dorn.

Caso de estudio 3: área de influencia de un emisario submarino que vierte aguas residuales urbanas, emisario de la Estación Depuradora de Aguas Residuales de Gandía (Valencia, España). En el capítulo 4 se estudia la variabilidad espacial de los nutrientes y los grupos fitoplanctónicos en dos épocas de estudio (primavera y verano). En este caso el mayor caudal de agua dulce vertido por el emisario se produce en verano, durante la temporada turística, debido al incremento de población. El emisario submarino descarga las aguas residuales tratadas a través de una tubería situada a 18 m de profundidad, a una distancia aproximada de 1900 m de la costa. El agua dulce vertida por el emisario asciende a la superficie donde forma una pluma cuya morfología depende principalmente de la dirección del viento. Para analizar la variabilidad espacial se toman muestras en 3 puntos situados a lo largo de un gradiente longitudinal desde el punto de afloramiento del efluente del emisario. La posición de estos 3 puntos (O5, O6 y O7) depende de la morfología de la pluma, se toman muestras a 0.05, 0.10 y 0.75 m con el SWAS. En el contorno de la pluma (Fig. 3, O1 a O4) se espera una menor variabilidad espacial de la columna de agua, por lo que teniendo en cuenta la profundidad de la columna de agua se toman muestras a 0.05, 5, 10 y 15 m con una botella oceanográfica Niskin.

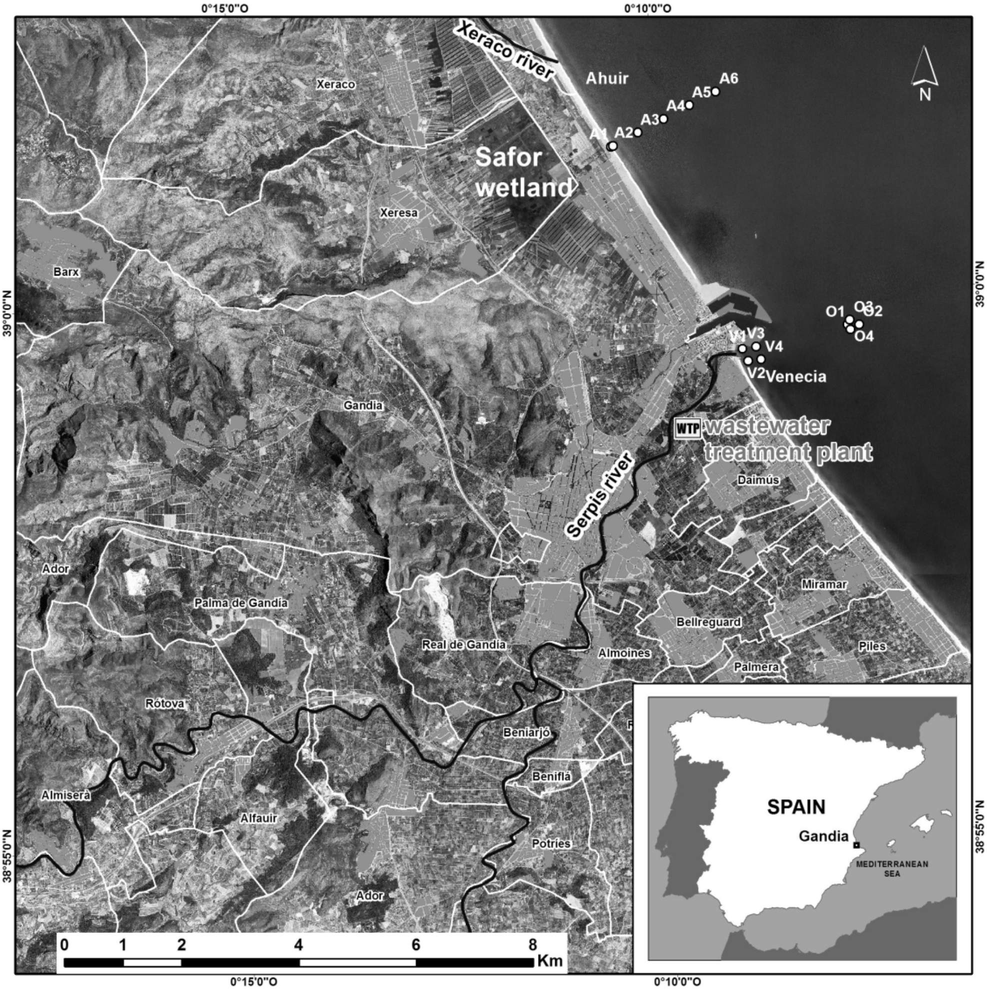

Figura 3 Localización puntos de muestreo casos de estudio 2, 3 y 4

Caso de estudio 4: área costera abierta, sin ningún aporte de los citados anteriormente, playa del Ahuir (Gandía). En esta zona de estudio no existe ninguna fuente puntual de aporte de agua dulce. Los aportes de aguas continentales son de origen difuso y provienen de la zona de contacto del acuífero de la Plana de Gandía-Denia (Ballesteros-Navarro, 2003). En el capítulo 4 se estudia la variabilidad espacial de los nutrientes y los grupos fitoplanctónicos en dos épocas de estudio (primavera, época lluviosa, y verano, época seca). En esta área, el muestreo se realiza a lo largo de un gradiente longitudinal desde la línea de costa, para tratar de captar el gradiente característico en las características físico-químicas y biológicas de las áreas costeras en mares micromareales como el Mediterráneo (Flo et al., 2011). El primer punto de muestreo, el punto A se localiza en la zona de surf, el segundo punto A1 (Fig. 3) se localiza inmediatamente después de la zona de surf, el tercer punto A2 (Fig. 3) se localiza a 50 m de la línea de costa, y los siguientes puntos (Fig. 3, desde A3 hasta A6) se sitúan cada 500 m. En la zona más somera (Fig. 3, A y A1) se toma una única muestra que se considera representativa de la columna de agua. En el resto de puntos se toma una muestra en superficie y una muestra cada 5 m de columna de agua con una botella oceanográfica Niskin, y una muestra cerca del fondo (50 cm) con una botella oceanográfica Van Dorn.

Caso de estudio 5: área a 9 km de la línea de costa caracterizada por estar poco afectada por aportes terrígenos. En el capítulo 5 se estudia la variabilidad temporal en una estación, con muestreos desde mayo de 2008 hasta agosto de 2009, con una frecuencia media quincenal. El punto de muestreo se sitúa en aguas marinas a 36.5 m de profundidad y 9000 m de distancia a la costa, fuera de la línea imaginaria que uniría lo cabos de Cullera y La Nao y que separa aguas costeras de aguas marinas. Dada la escasa influencia de los aportes terrestres y la estabilidad de la columna de agua se muestrea únicamente en superficie, con botella oceanográfica Niskin, y en profundidad (50 cm sobre el fondo) con botella oceanográfica Van Dorn.

Caso de estudio 6: la elevada variabilidad espacial de las zonas costeras hace que la caracterización físico-química y biológica de sus aguas con las técnicas tradicionales de toma de muestras sea muy compleja. En este sentido, el uso de técnicas alternativas como el uso de imágenes de satélite ofrece la posibilidad de obtener una versión sinóptica que no es posible por otros medios. En el capítulo 6 "Estimation of chlorophyll *a* on the Mediterranean coast using a QUICKBIRD image", se desarrolla un estudio preliminar implementado en un único escenario durante el período seco (verano), que evalúa la capacidad de un sensor de alta resolución espacial, Quickbird, para resolver la variabilidad espacial de la clorofila *a* en la franja costera. Para el desarrollo del modelo de estimación de la clorofila *a* se realizó un muestreo con 16 puntos distribuidos aproximadamente en 3 transectos paralelos a la línea de costa.

3.2 Obtención de parámetros ambientales

3.2.1 Caudales

El caudal de las acequias que vierten al Puerto de Gandía se midió in situ con un correntímetro.

La medida del caudal del río Serpis en la desembocadura in situ resulta técnicamente más compleja, por ello se utilizan los datos tomados por la Confederación Hidrográfica del Júcar (CHJ) en las estaciones de aforo de Font en Carrós (río Serpis) y de Vernissa (río Vernissa, tributario del Serpis), situadas a aproximadamente 10 km de la desembocadura. Estos datos se pueden consultar on-line gracias al Sistema Automático de Información Hidrológica (SAIH) del Ministerio de Agricultura, Alimentación y Medio Ambiente. La medida de caudal se proporciona en $m^3\ s^{-1}$ y la frecuencia consultada ha sido la diaria (aunque también existe información quinceminutal y horaria).

3.2.2 Datos meteorológicos

Los principales datos meteorológicos utilizados por su relevancia en esta memoria de tesis son pluviometría y viento.

Los datos de pluviometría se han obtenido de las estaciones pluviométricas de la CHJ Font en Carrós y Vernissa cuyos datos se pueden consultar on-line también gracias al Sistema Automático de Información Hidrológica (SAIH) del Ministerio de Agricultura, Alimentación y Medio Ambiente. La medida de precipitación se proporciona en mm y la frecuencia consultada ha sido la diaria (aunque también existe información quinceminutal y horaria).

Los datos de viento (velocidad y dirección) se han obtenido de la estación meteorológica situada en la Escuela Politécnica Superior de Gandía, y han sido facilitados por el profesor Josep Llinares Palacios del Departamento de Química de la Universidad Politécnica de Valencia. Esta estación está situada en el entorno de la zona de estudio.

3.3 Análisis de parámetros físico-químicos

La metodología de medida *in situ* (salinidad, temperatura) o análisis en el laboratorio de los distintos parámetros físico-químicos (salinidad, sólidos en suspensión, nutrientes) se halla descrita en el apartado de metodología de cada uno de los capítulos del 3 al 6 de la presente memoria de tesis.

3.4 Análisis de pigmentos fotosintéticos por HPLC

En este epígrafe se detalla la metodología utilizada para alcanzar el primer objetivo propuesto en esta memoria de tesis: analizar la composición de pigmentos fotosintéticos en muestras de agua, principalmente marinas, mediante cromatografía líquida de alta resolución (HPLC).

3.4.1 Obtención de muestras

En cada punto se tomó una alícuota, en botellas de plástico opacas de 2 L. Las muestras se conservan y transportan en neveras con hielo, a 4ºC, desde su recolección hasta su posterior procesado, protegiéndolas en todo momento de la exposición a la luz.

El diseño de cada muestreo y la obtención de muestras están explicados con mayor detalle en cada uno de los capítulos.

3.4.2 Filtración

La muestra se filtró en el menor tiempo posible desde su recolección (transcurrió un máximo de 5 horas hasta la filtración de la última muestra), a través de filtros GF/F Whatman de 25 mm de diámetro y 0.7 μm de tamaño de poro.

Se utilizó una bomba de aceite para vacío, Telstar S8, con manorreductor para poder controlar la presión de filtración, y que en todo momento se mantuviera por debajo de los 200 mbar (< 150 mmHg), para evitar la rotura de las células y la pérdida de su contenido durante el proceso de filtración.

Todo el procesado de la muestra se realizó bajo luz tenue y las probetas utilizadas fueron envueltas en papel de aluminio, todo esto para minimizar los procesos de fotodegradación.

Es importante en los análisis de HPLC filtrar el mayor volumen posible para poder medir precisamente la mayoría de los principales pigmentos. Una verificación cualitativa para determinar si se está filtrando un volumen suficiente consiste en contar el número de pigmentos accesorios (clorofilas *b*, *c*1, *c*2, *c*3 y carotenoides) cuantificados, excluyendo los productos de degradación de la clorofila (Trees et al., 2000). La mayoría de los grupos de algas contienen al menos 4 pigmentos accesorios medibles con HPLC (Jeffrey et al., 1997). Por lo tanto, las muestras que no cumplan con este número mínimo de pigmentos accesorios pueden tener problemas de límite de detección relacionados con bajos ratios señal/ruido y/o técnicas de concentración inadecuadas (vg, bajos volúmenes filtrados).

En nuestro estudio los volúmenes de filtración varían entre 0.5 y 2 L, el volumen filtrado varía dependiendo de la concentración de partículas que se observa en los filtros durante la filtración (Bidigare et al., 2003). Para aguas estuarinas y costeras Pinckney et al. (2011) recomiendan que el tiempo total de filtración no supere los 10 minutos, para evitar que la lisis de las células pueda resultar en una subestimación del contenido real de pigmentos.

Una vez filtrada la muestra, los filtros se doblan por la mitad y se secan con papel de filtro para minimizar el agua retenida por el filtro. Posteriormente se envuelven en papel de aluminio, se congelan en nitrógeno líquido y se almacenan a -20ºC.

En Jeffrey et al. (1997) se hallan publicados los resultados de un ensayo que identifica los métodos más eficientes para almacenar los filtros con la mínima degradación. El almacenamiento de los filtros bajo nitrógeno líquido (-196ºC) es el más recomendado para la preservación y recuperación del 96% de los pigmentos hasta aproximadamente 1 año. Sin embargo, para cortos períodos de almacenaje, el almacenamiento de los filtros en congeladores de -20ºC resulta en porcentajes de recuperación mayores al 95%. Dado que no se disponía de depósitos criogénicos, ni tampoco de ultracongelador (-80ºC), se optó por almacenar los filtros a -20ºC y realizar la extracción y análisis de las muestras en un período nunca superior a las tres semanas.

3.4.3 Extracción

Para la extracción de muestras se sigue el siguiente procedimiento. La extracción de los filtros se realiza dentro de jeringas de plástico con 3 mL acetona (calidad HPLC). La muestra se homogeneiza mediante ultrasonidos durante 40 s a una potencia aproximada de 200 W con un sonicador (homogeneizador de ultrasonidos), modelo SONOPULS HD200 (Bandelin), con una micropunta de 6 mm de diámetro. Se deja extraer durante 12 horas a 4ºC en la oscuridad (Lambert et al., 1999). Posteriormente, el extracto se filtra con un filtro de jeringa PTFE de 17 mm y 0.2 μm de poro. El extracto se vierte directamente a viales de vidrio ámbar, donde se conserva hasta su análisis. Los extractos son analizados en el HPLC antes de 24 horas. Inmediatamente antes del análisis, el extracto se diluye con agua milliQ, en una proporción 1:0.2, para evitar la distorsión de los picos debido a la precipitación de los pigmentos más hidrófobos (Latasa et al., 2001).

Se eligió como disolvente la acetona porque las pérdidas de pigmentos con el tiempo durante el proceso de extracción son menores que con el metanol y su toxicidad es menor (Latasa et al., 2001). La elección del volumen de extracción, está condicionada por la dilución de la muestra (si se utilizan volúmenes muy altos se corre el riesgo de que los pigmentos minoritarios se hallen por debajo del límite de detección), y por la necesidad de bañar completamente el filtro; además, el uso de volúmenes de extracción muy bajos puede resultar en errores en la estimación debido a la evaporación del disolvente.

3.4.4 Análisis cromatográfico

Para el análisis de los pigmentos mediante cromatografía líquida de alta resolución (HPLC), se utilizó el método C18 de Wright et al. (1991), ligeramente modificado según Hooker et al. (2000). El método de Wright et al. (1991) fue diseñado como método de referencia en programas internacionales como el JGOFS (Joint Global Ocean Flux Study) y recomendado por el grupo de trabajo de la UNESCO del SCOR (Scientific Comittee on Oceanic Research). Este método se sigue utilizando actualmente (Garrido et al., 2011).

Las separación de los pigmentos se realizó en una columna ODS-2 Spherisorb C18, 250 x 4.6 mm y 5 µm tamaño de partícula, para prolongar la vida útil de la columna se utilizó una precolumna ODS-2 Spherisorb C18, 50 x 4.6 mm y 5 µm tamaño de partícula.

El equipo utilizado fue un cromatógrafo líquido de Agilent HP Serie 1100, equipado con:

- Bomba cuaternaria
- Compartimento termostatado columna
- Loop de muestra, acero inoxidable, 200 µL
- Detector de diodos (DAD Diode Array Detector)

- Programa de adquisición e integración de datos ChemStation
- Módulo de programa espectral de la ChemStation LC

Dado que el sistema de inyección utilizado es manual, la jeringa utilizada (500 µL) se llena hasta el máximo volumen posible, llenando el loop de 200 µL al menos 2 veces, para asegurarse el llenado del loop, ya que para conseguir un llenado del loop superior al 95% se debe inyectar entre 2 y 3 veces su volumen y para llegar a un porcentaje superior al 99% es necesario entre 4 y 5 veces el volumen del loop (Latasa et al., 1996), cabe tener en cuenta que este es un paso clave ya que gran parte de la variabilidad de los resultados se puede originar aquí.

Los parámetros de detección seleccionados fueron los siguientes:

- El detector de diodos (DAD) recogía el espectro de absorción entre 350-750 nm, franja de absorción de clorofilas y carotenoides.
- Se obtiene el cromatograma para la longitud de onda de 436 ± 4 nm
- Rendija (slit) 4 nm. Se fija una rendija igual al ancho de banda
- Ancho de pico (tiempo de respuesta) 0.05 min (1 s): la elección de este parámetro es muy importante, de él depende en buena medida que seamos capaces de observar los picos en el cromatograma.

Este valor debe de ser del orden del pico más estrecho que se vaya a observar.

Inicialmente los picos se integraron automáticamente según las siguientes condiciones:

- Área de rechazo 1 mAU
- Altura de rechazo 0.05 mAU
- Ancho de pico 0.05 min

La elección de estos parámetros viene determinada por las características de los picos observados y su finalidad es distinguir entre picos y línea base.

Dado que en nuestras muestras es habitual encontrar pequeñas cantidades de los pigmentos minoritarios o de los pigmentos pertenecientes a grupos fitoplanctónicos con escasa presencia, encontraremos picos muy pequeños, tanto en altura como en área, por tanto, estos parámetros se fijan en valores muy reducidos, para evitar perder picos al confundirse con la línea base.

Posteriormente, todos los resultados se inspeccionaban visualmente, y, en aquellos cromatogramas en que las condiciones iniciales de integración no ajustaban correctamente la línea base, se volvió a integrar con la opción de integración del programa ChemStation que buscaba los parámetros óptimos de integración (Hooker et al., 2000).

La temperatura de la columna se mantuvo constante a 30ºC durante los análisis gracias al horno de la columna, esto nos permitió estabilizar los tiempos de retención, lo que facilita la identificación de los picos, ya que la temperatura afecta seriamente a la elución de los compuestos; un cambio diurno de la temperatura ambiente de 5ºC (típico de la mayoría de laboratorios) puede producir un cambio de hasta el 10 % de los tiempos de retención. La elección de la temperatura se realizó en base a que es altamente recomendable mantener la columna termostatada, al menos, entre 5 y 8ºC por encima de la temperatura ambiente.

Las fases móviles utilizadas fueron:

- Fase A: metanol: acetato de amonio (pH = 7.2) 80:20 (V: V)
- Fase B: acetonitrilo: agua milliQ 90:10 (V: V)
- Fase C: acetato de etilo 100 %

Todos los disolventes utilizados en la preparación de las fases móviles eran de grado HPLC (Baker), una vez preparadas las fases móviles se filtraban a través de filtros de membrana PTFE de 47 mm y 0.45 µm de poro.

El gradiente analítico, con un flujo constante de 1mL min^{-1}, era el siguiente:

Tabla 3 Gradiente analítico Hooker et al. (2000)

Tiempo (min)	% A	%B	%C
0.0	100	0	0
2.0	0	100	0
2.6	0	90	10
13.6	0	65	35
18.0	0	31	69
23.0	0	31	69
25.0	0	100	0
26.0	100	0	0
34.0	100	0	0

Al finalizar los análisis se realizaba un gradiente que constituía el protocolo de apagado (Jeffrey et al., 1997), de este modo la columna se conserva en acetato de etilo hasta su siguiente uso. A su vez, al inicio de los análisis se realizaba el gradiente de apagado invertido, lo que constituía el protocolo de inicio, en el cual se sustituye la fase móvil C (acetato de etilo), en la que se había conservado la columna, por la fase A (metanol: acetato de amonio). Este último paso es indispensable, dado que ambas fases móviles, A y C, son inmiscibles por su diferente polaridad.

En la siguiente tabla se presenta el protocolo de apagado, para un flujo constante de 1mL min^{-1}:

Tabla 4 Gradiente para el protocolo de apagado o conservación de la columna

Tiempo (min)	% A	% B	% C
0	100	0	0
3	0	100	0
6	0	0	100
16	0	0	100
17	0	0	0

En la siguiente tabla se presenta el protocolo de inicio, para un flujo constante de 1mL min$^{-1.}$

Tabla 5 Gradiente para el protocolo de inicio

Tiempo (min.)	% A	%B	%C
0	0	0	100
10	0	0	100
13	100	100	0
16	100	0	0
17	100	0	0

3.4.5 Identificación de los picos

Los picos del cromatograma se identificaron con la ayuda de una librería generada previamente con los espectros de absorción de 13 pigmentos patrón, los cuales fueron adquiridos en el DHI Water and Environment Institute (Hørsholm, Denmark). Además, se consultó la publicación Jeffrey et al (1997), la cual proporciona información sobre el orden y el tiempo de elución, así como del espectro de absorción de más de 50 pigmentos.

En la siguiente tabla se muestra el orden de elución de los pigmentos así como los tiempos de retención medios (n=7) calculados a partir del análisis de una mezcla patrón según Hooker et al. (2000):

Tabla 6 Tiempo de retención medio de los pigmentos

Nº pico	Pigmento	Tr (min.)	Desv. Est.
1	Peridinina	7.8	0.02
2	19'butanoyloxyfucoxantina	8.1	0.02
3	Fucoxantina	8.8	0.03
4	19'hexanoyloxyfucoxantina	9.3	0.03
5	Neoxantina	9.7	0.03
6	Prasinoxantina	10.8	0.03
7	Violoxantina	11.6	0.03
8	Diadinoxantina	13.0	0.03
9	Aloxantina	14.4	0.03
10	Luteína	16.0	0.03
11	Zeaxantina	16.5	0.20
12	Clorofila *b*	18.4	0.02
13	Clorofila *a*	19.6	0.01

En el ANEXO I se incluyen cromatogramas representativos de las muestras analizadas en cada uno de los capítulos de esta memoria de tesis.

3.4.6 Cálculo concentración de pigmentos

La concentración individual de cada pigmento se calcula como:

$$Cmuestrai=\frac{Amuestrai \cdot Vextracto}{Fi \cdot Vinyectado \cdot Vmuestrai}$$

Donde:

- $C_{muestrai}$: concentración del pigmento en µg L^{-1}
- $A_{muestrai}$: área del pico del pigmento para una inyección en mUA
- $V_{extracto:}$ volumen extraído en mL
- F_i: factor de respuesta
- $V_{inyectado}$: volumen inyectado en mL
- $V_{muestra}$: volumen de muestra filtrado en L

El factor de respuesta del sistema HPLC, para un pigmento i (F_i), se calcula como la pendiente de la recta de regresión de las áreas del pico del patrón frente a la masa de los patrones (µg) .Este factor se utiliza para cuantificar la concentración de cada pigmento en la muestra.

3.5 Clasificación taxonómica

En este epígrafe se detalla la metodología utilizada para alcanzar el segundo objetivo propuesto en esta memoria de tesis: clasificar taxonómicamente las comunidades fitoplanctónicas presentes en las muestras de agua mediante el programa CHEMTAX.

La composición de la comunidad fitoplanctónica se determinó a partir de la concentración de los principales pigmentos fotosintéticos mediante el programa CHEMTAX (CHEMical TAXonomy) (Mackey et al. 1996) versión 1.95 (S. Wright, comunicación personal).

Este programa utiliza como punto de partida una matriz inicial de ratios de pigmentos de cada uno de los grupos fitoplanctónicos presentes. Esto implica la necesidad de obtener previamente información de la comunidad fitoplanctónica a través de las técnicas tradicionales de microscopía, para determinar qué grupos incluir en la matriz inicial (Higgins et al., 2011). Esto hace que ambas técnicas se utilicen de forma complementaria (Cartaxana et al., 2009; Seoane et al., 2011).

Sin embargo, según señala Latasa (2007) es paradójico que para obtener información de la comunidad fitoplanctónica a través de los pigmentos fotosintéticos, sea necesario en primer lugar tener información de la comunidad fitoplanctónica. Latasa (2007) ofrece una aproximación metodológica al uso de CHEMTAX que permite superar este inconveniente.

En la presenta tesis se utiliza la metodología desarrollada por Latasa (2007), tal y como se aplica en Latasa et al. (2010), para el cálculo de la composición taxonómica de las muestras a partir de las concentraciones de pigmentos. En el Anexo II se presentan las matrices ratio finales utilizadas.

Aun así, la información microscópica es una fuente valiosa de información taxonómica que es crítica para una evaluación cualitativa de los resultados químico-taxonómicos. Dado que obtener recuentos microscópicos de todas las muestras analizadas por HPLC no es muy factible, se recomienda hacer un escrutinio de algunas muestras representativas (Higgins et al., 2011). En la presente tesis se analizaron por microscopía invertida (metodología descrita en el Capítulo 3 de esta memoria) muestras representativas del Puerto de Gandía (Capítulo 3 de esta memoria) y del área de influencia de la desembocadura del río Serpis (Capítulos 4 y 5 de esta memoria) para identificar los grupos presentes.

3.6 **Análisis estadístico**

El análisis estadístico de los resultados se ha realizado con los paquetes estadísticos Statgraphics 5.1 y SPSS 16.0, de los cuales dispone licencia la Universidad Politécnica de Valencia.

Los pasos seguidos para el análisis estadístico de los datos son los siguientes.

En primer lugar, se realiza un análisis univariado de cada una de las variables estudiadas. En este análisis se hallan los principales estadísticos descriptivos (media, mediana, desviación típica, máximo, mínimo, rango, desviación estándar) y se estudia la normalidad de la variable. La mayoría de las variables no siguen una distribución normal, lo que condiciona la elección de los métodos de análisis multivariado utilizados (test de Kruskal-Wallis y análisis de correlación de Spearman).

En segundo lugar, se efectúa un análisis de conglomerados para agrupar las muestras en función de los niveles de salinidad y nutrientes, con el objetivo de crear grupos de muestras lo más homogéneos posibles antes de aplicar el programa CHEMTAX para el análisis de la composición del fitoplancton. El análisis jerárquico de conglomerados es una herramienta exploratoria que está diseñada para revelar grupos naturales dentro de un conjunto de datos que de otro modo no son aparentes. Se utiliza cuando el número de objetos a agrupar es pequeño (menor de unos pocos cientos). El criterio básico para crear los conglomerados es la distancia entre objetos. Para un conjunto de datos determinado, la formación de los conglomerados depende de los siguientes parámetros: el método de conglomeración y el método de medida de la distancia (Norusis, 2004). En la presente memoria de tesis se ha utilizado el método de conglomeración de Ward, y como medidas de distancia, la distancia de bloque-ciudad y la distancia euclídea al cuadrado, las cuales producen idénticos resultados para el nivel de conglomeración deseado.

Para estudiar la variabilidad espacial (distintas zonas de estudio) y estacional (primavera vs. verano, época lluviosa vs. época seca) de los grupos fitoplanctónicos y nutrientes se utiliza la prueba no paramétrica de Kruskal-Wallis. Los test no paramétricos para varias muestras independientes se utilizan para determinar si los valores de la variable de interés son o no diferentes entre uno o más grupos. Estos test se aplican cuando las condiciones para la aplicación del ANOVA (ANalysis Of VAriance) no se cumplen (normalidad de la distribución de los datos y homocedastiticidad de la varianza) (Norusis, 2004). El test de Kruskal-Wallis no asume la normalidad de la variable. Este test indica que los grupos son diferentes, pero no dice qué grupos son diferentes (Norusis, 2004). Para averiguar qué grupos son diferentes entre sí se puede utilizar el test de Mann-Whitney test o la prueba gráfica de la muesca de la mediana en lo gráficos de caja-bigotes (esta última en Statgraphics 5.1).

Para analizar la correlación entre variables se utiliza el coeficiente rho de Spearman. El análisis de correlación bivariado calcula la asociación por pares de un grupo de variables y muestra los resultados en una matriz.

Este análisis es útil para determinar el grado y la dirección de la asociación entre dos variables ordinales. El análisis de correlación de Spearman se puede utilizar independientemente de la distribución de las variables y los valores extremos tienen escaso efecto (a diferencia de otros análisis como el de Pearson que se utilizan con variables que siguen una distribución normal) (Norusis, 2004).

Además, en el capítulo 4 “Effects of freshwater inputs on the receiving waters of a Mediterranean coastal area: nutrients and phytoplankton analysis”, se ha utilizado el programa CANOCO 4.5., el cual es muy utilizado para el análisis multivariado de datos ecológicos(Ter Braak y Smilauer, 2002).

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Capítulo 3: Influence of nutrient inputs from a wetland dominated by agriculture on the phytoplankton community in a shallow harbour at the Spanish Mediterranean coast

1 Introduction

It is well known that agriculture is the main source of nitrogen in many regions: less than a half of the total nitrogen input via fertilizers and animal manure in crop production is effectively used, while the remainder is dissipated into the wider environment, where it contributes to a range of ecological and human health effects (Galloway et al., 2008). One of the main ecological effects originates when nitrogen leaves fields in surface runoff and is discharged to coastal ecosystems. There, it induces enhanced primary phytoplankton production that can lead to severe eutrophication problems (Cloern, 2001; Glé et al., 2008).

However, the phenomenon of eutrophication does not only depend on nitrogen inputs, but also on the phosphorus and silica inputs and on the relative nutrient composition (Cloern, 2001; Ludwig et al., 2009). In addition to increasing primary production, the alteration of nutrient ratios, in particular the imbalance of nitrogen and phosphorus with respect to silica has inevitable effects on the taxonomic composition of phytoplankton communities, as it can provoke a shift in primary production from diatoms to non-siliceous algae, often harmful for the ecological equilibrium (Ludwig et al., 2009). Lastly, these changes in phytoplankton community often precede larger-scale, longer-term changes in ecosystem function, including shifts in nutrient cycles, food webs, and fisheries (Paerl et al., 2010).

The EU has already adopted several directives and policies intended to combat eutrophication with varying degrees of success. While phosphorus levels have been decreasing since the 1990s, a reduction in nitrogen emissions is more difficult to achieve. Phosphorus pollution is normally dominated by point sources which are easier to target, thanks mainly to the ban on phosphorus detergents and phosphate removal in sewage plants. But the relationship of nitrogen with farming and the diffuse nature of the sources makes nitrogen regulation more difficult (Artioli et al., 2008).

Eutrophication problems are especially relevant in wetlands. In recent decades, increased regulation of wetlands and more intensive farming have increased the nutrient loading to many coastal ecosystems world-wide. Proper functioning of wetlands depends on groundwater and surface water hydrology. However, the hydrology of these ecosystems has been traditionally manipulated to satisfy the different cultivation needs (Hook, 1993). The main environmental and socioeconomic impacts of water regulation in wetlands are discussed in López (1999).

The Safor Wetland (Western Mediterranean) is an example of wetland regulation. The main freshwater input to the Safor Wetland is groundwater discharge, and the second main input is due to precipitation and infiltration over the area. Climatically, autumn and spring are the rainy seasons while summer is the dry period in this Mediterranean area. Nowadays, the hydrology of the wetland is anthropogenically manipulated to satisfy cultivation needs (mainly citrus). To prevent crop root asphyxia, in the wet seasons, water is pumped through the irrigation channels to the sea to decrease the phreatic level. But, there are other factors that make this regulation necessary: the intense urbanization process experienced in recent decades also means pumping water in order to avoid flooding of urban areas, and the population increase in summer, as the Spanish coast is a popular tourist destination, makes it necessary to increase pumping to supply drinking water from the wells located in the detritic aquifer which nourishes the wetland.

Beklioglu et al. (2007) have highlighted the need for information on the role of hydrology and major nutrients (nitrogen and phosphorus) in the phytoplankton ecology of shallow Mediterranean lakes in order to develop criteria for water quality in this climatic zone. The phytoplanktonic community of the shallow water bodies of this area has been studied by Rodrigo et al. (2003) but no study has focused on the receiving waters. Studying the receiving waters is especially important given the oligotrophic character of Mediterranean waters, where discharges of freshwater and associated nutrients play a key role in marine productivity (Ludwig et al., 2009).

This study analyzes the role of nutrient and nutrient ratio variations in determining the phytoplankton community in Gandia Harbour in relation to freshwater inputs from the Safor Wetland. It analyzes these variations in terms of spatial and seasonal composition and abundance of phytoplankton groups, using diagnostic photopigment analysis.

2 Materials and methods

2.1 Study area

The Safor Wetland (on Spain’s Mediterranean coast) is a protected ecosystem declared a Site of Community Importance (SCI) under the Habitats Directive (92/43/EEC), as it is considered one of the best preserved wetlands in Spain. Agricultural practices have been part of this ecosystem with different crops throughout history (e.g. sugar cane in the 15th and 16th century; corn, wheat and the white mulberry tree in the 18th century; and rice in the 19th century). Nowadays, characteristic crops in this area are citrus and horticultural crops.

The Valencian Regional government (through Order 13/2000, DOGV nº 3677, 2000-01-31) declared the municipalities of the Safor Wetland a nitrate-vulnerable zone, in accordance with the Council Directive 91/676/EEC (hereafter referred to as the Nitrates Directive). The Good Agricultural Practices Code published by the regional government (through Order 7/2010, DOCV nº 6212/23.02.2010), also in accordance with the Nitrates Directive, establishes that the recommended nitrogen doses for citrus crops is 200-250 kg ha^{-1} year^{-1} for surface irrigation and 180-220 kg ha^{-1} year^{-1} for drip irrigation. For horticultural crops, doses are similar, depending on the crop. However, in this region agriculture has been more intensive than in other areas, due to the mild climate, and traditionally these doses have been exceeded outstandingly (MARM, 2010). In consequence, nitrogen excess has been lixiviated to the aquifers or poured into surface streams.

The area is drained by an orthogonal network of artificial channels (Fig. 1) and has several pumping stations. The Ahuir channel is the main collector and its flow can be pumped to two watercourses: northward to the Xeraco watercourse which outflows directly to the sea (the Mediterranean) or southward to the San Nicolas watercourse which outflows into the Gandia Harbour. San Nicolas is an ephemeral watercourse which drains an area of 50 km^2 and it is about 14 km long; it is generally inactive; it carries great quantities of water only when torrential rain falls. The flow is only continuous in the last 1.5 km due to the inputs of freshwater draining the Safor Wetland. The harbour has an average depth of 5 m, and a maximum depth of 10 m restricted to a small area where merchant ships tie up. Water residence time is above 30 days on average in the harbour, so eutrophication problems are more likely to be found here. The harbour can be considered a small stratified estuary with a shallow freshwater layer due to freshwater inputs from the wetland for most of the year. The last 1.5 km of the San Nicolas ephemeral watercourse and the Gandia Harbour were selected as the study area.

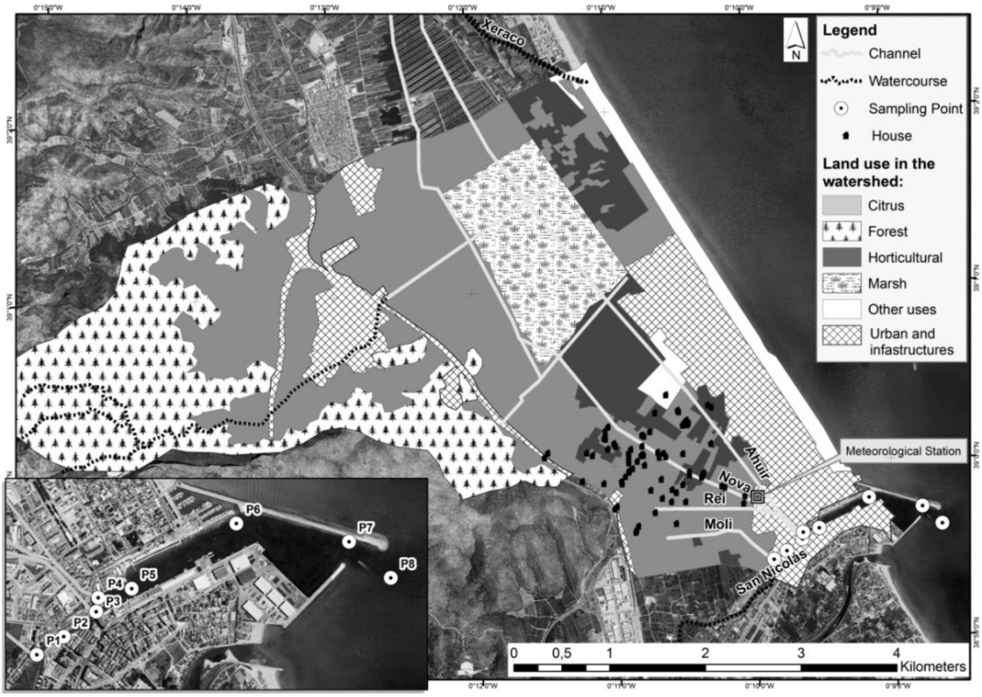

Figure 1. The Gandia Harbour and land uses in its drainage area. Location of sampling stations: Point 1 (P1), Point 2 (P2), Point 3 (P3), Point 4 (P4), Point 5 (P5), Point 6 (P6), Point 7 (P7) and Point 8 (P8) and meteorological station. P1, P3 and P4 are located respectively in the channels of Molí, Rei and Ahuir-Nova

Gandia Harbour is a commercial, fishing and recreational harbour located in the southernmost sector of the Valencian Gulf (South-Western Mediterranean). Apart from the Ahuir channel, the harbour receives freshwater inputs from the Molí, Rei, and Nova channels (Fig. 1). The final few metres of the Rei, Nova and Ahuir channels are buried underground and flow into the harbour through two outlets. The Rei irrigation channel outlet is Point 3 (P3) in Fig. 1 (see detailed photo), while the Nova and Ahuir channels meet and flow out at Point 4 (P4).

Present land uses (Fig. 1) in the drainage area described include citrus (990.7 ha), horticultural crops (215.8 ha), forest (588.1 ha), wetland (224.8 ha) and urban use (409.6 ha). Anthropogenic land uses, including agricultural (48%) and urban (16%) use, represent 66% of the watershed, while forest and marsh account for 32% of the drainage area.

This area is located over the Plana de Gandia-Denia detritic unconfined aquifer, which provides the necessary water resources for crop irrigation; however its shallow phreatic level causes problems of root asphyxia. To prevent this problem, freshwater from the aquifer is pumped into Gandia Harbour through the irrigation channels described above. Due to continued agricultural practices, nitrate levels in the aquifer have exceeded the limit of 50 mg L^{-1} established by the Nitrates Directive, so freshwater discharges are characterized by high nitrogen loads.

Concerning the main phosphorus input of anthropogenic origin, municipal wastewater is treated in the sewage treatment plant of Gandia and discharged into the sea through a submarine outfall at an approximate distance of 1900 m from the harbour. However, there are some second homes on the non-urban soil of the Safor wetland (Fig. 1), which are not connected to the wastewater collection system. Many of them discharge wastewater directly into the surface channels (Nova, Ahuir and Rei channels), others have septic tanks. Even in the second case, wastewater ends in the irrigation channels, because wastewater infiltrates from the septic tanks to the shallow aquifer and water is continuously pumped from the aquifer into the surface channel as described above.

Regarding phosphorus input of agricultural origin, the recommended phosphorus doses for citrus crops is 70 kg ha^{-1} year^{-1} for surface irrigation and 80 kg ha^{-1} year^{-1} for drip irrigation (MARM, 2010). One unique dose of phosphorus fertilizers is surface-applied generally in March (Legaz and Primo-Millo, 1988). He et al. (2006) analyzed the concentration and forms of phosphorus in the surface runoff from field-scale studies of an analogous study area: citrus and horticultural crops in a flat landscape with shallow water table, artificial drainage and similar phosphorus fertilization. They found that dissolved inorganic phosphorus (DIP) was the dominant form in the total dissolved phosphorus and its concentration varied widely from <0.01 to 9.85 mg L^{-1} in the runoff waters, but generally DIP concentration was above the 0.01 mg L^{-1} critical concentration for eutrophic shallow lakes recovery (Beklioglu et al., 2007).

2.2 Sampling strategy

Water samples were taken at the 8 sampling points shown in Fig.1. Sampling points were chosen to evaluate the nutrient input of the irrigation channels and its influence on Gandia Harbour phytoplankton structure. Sampling was designed with high spatial resolution as recommended in Zablotowicz et al. (2010) because phytoplankton can vary on a scale of meters.

Point 1 (P1) was situated in the first irrigation channel, the Molí channel, which flows into Gandia Harbour. Point 2 (P2) was located after the inflow of the Molí channel and before the inflow of the two other irrigation channels to the harbour. Point 3 (P3) and Point 4 (P4) were situated in the Rei and Nova-Ahuir irrigation channels respectively. Points 5 (P5) to 8 (P8) were located on a longitudinal seaward transect, starting with P5 after the contribution of the Rei and Nova-Ahuir channels and finishing with P8 outside the harbour but under its direct influence.

Samples were taken in two hydrological periods: the wet one in spring, on 15 April 2009, and the dry one in summer, on 06 August 2009. Only one water sample was collected at 0.05 m depth in each irrigation channel because of their scarce depth and flow. At the other points, water samples were collected at different depths in the water column (0, 0.05, 0.10, 0.30, 0.50, 0.75 and 1 m) using a Superficial Water Sampler (Mösso et al., 2008) and one extra sample just above the bottom with a horizontal Van Dorn bottle. Water samples were kept in a cool box (4ºC) and transported to the laboratory.

Flow measurements were made with a calibrated current meter in the irrigation channels. At each measurement point, flow discharge was gauged by taking velocity within subsections (at 60% of the subsection's depth and averaged for 90 s) along the stream's cross section. A computer program integrated flows for the point.

Wind speed and direction were measured in the weather station located approximately 500 m from the harbour (Fig. 1).

2.3 Laboratory analysis

The following parameters were analyzed in all the samples: salinity, suspended solids (SS), nitrate, nitrite, and ammonium, dissolved inorganic phosphorus (DIP) and dissolved silicate (DSi). Dissolved inorganic nitrogen (DIN) was calculated as the sum of nitrate, nitrite and ammonium. Salinity was determined by means of a conductivity meter Multi 340i/SET WTW, using the Practical Salinity Scale. Nutrients were analyzed colorimetrically using the method of Aminot and Chaussepied (1983).

Samples for phytoplankton pigment analysis were filtered on GF/F fiberglass filters (25 mm diameter). Pigments were extracted using acetone (100% HPLC grade) and were measured using reverse-phase high-performance liquid chromatography (HPLC). The HPLC method employed was that proposed by Wright et al. (1991) slightly modified as per Hooker et al. (2000). The system was calibrated with external standards obtained commercially from the DHI Water and Environment Institute (Hørsholm, Denmark). Once the concentration of important photosynthetic pigments was determined, the phytoplankton community was studied using the CHEMTAX program (Mackey et al., 1996). Diagnostic photopigment analyses are able to detect significant changes in phytoplankton community composition over a broad range of time scales and as such are well suited for monitoring programs designed to assess short- and long-term trends in water quality in response to nutrient enrichment (Niemi et al., 2004). Phytoplankton samples were fixed with formaldehyde, concentrated according to UNE EN 15204:2006, based on Utermohl (1958), and qualitatively examined under a LEICA DM IL inverted microscope. CHEMTAX was applied following the procedures described in Latasa (2007) using version 1.95 (S. Wright, pers. comm.) to obtain the contribution to chlorophyll *a* (Chl-*a*) of the phytoplankton groups identified.

2.4 **Statistical analysis**

Sampling points were grouped according to similar salinity, DIN, DIP and DSi properties as determined by cluster analysis. Clustering dendograms were generated using STATGRAPHICS 5.1. City-block distances were calculated and samples clustered according to Ward's method (Latasa et al., 2010). Pigment samples were separated into subsets following the results of the cluster analysis, and CHEMTAX was applied independently to each subset (Latasa et al., 2010) to obtain the contribution of 8 phytoplankton groups to the chlorophyll a stock: diatoms, dinoflagellates, euglenophytes, chlorophytes, cryptophytes, prymnesiophytes, prasinophytes and cyanobacteria.

A non-parametric one-way analysis of variance (Kruskal-Wallis) was performed to statistically assess variations in the median fraction of chl-*a* of each phytoplankton taxon within the identified clusters and sampling seasons. Variations in the nutrient concentration between clusters and seasons were also assessed.

Spearman rank correlation analyses were performed on environmental parameters (DIN, DIP, DSi, DIN/DIP, DSi/DIN, DSi/DIP, salinity and season) and phytoplankton groups in order to examine significant relationship.

3 Results

3.1 Physical and chemical parameters

Most of the physical and chemical parameters that were measured in this study showed a longitudinal gradient from the discharge points of the irrigation channels to the sea (Fig. 2). Salinity varied between 19.1 and 22.4 at the upper station (P2) surface and 37.0 and 37.5 at the lower station (P8) surface. The difference in temperature over the salinity gradient was rather small, generally less than 1ºC. In spring, temperatures ranged from 16.6ºC to 15.8ºC, surface and bottom respectively, at P2; and 15.8ºC and 14.9ºC at P8. In summer, temperatures ranged from 26ºC to 25.8ºC, surface and bottom respectively, and there were no significant changes along the longitudinal transect.

Predominant wind direction in the spring sampling was W-NW, while in summer it was E-SE. This caused greater marine water entry into the harbour in summer due to the orientation of its entrance channel (Fig. 1). Total flow measurements in the irrigation channels were 0.74 $m^3 s^{-1}$ in spring and 0.34 $m^3 s^{-1}$ in summer. More specifically, the Molí irrigation channel (sampling point P1) flow was 0.23 $m^3 s^{-1}$ in spring and 0.10 $m^3 s^{-1}$ in summer; Rei channel (P3) flow was 0.28 $m^3 s^{-1}$ and 0.04 $m^3 s^{-1}$ respectively; and the Nova-Ahuir channel (P4) was 0.23 $m^3 s^{-1}$ in spring and 0.20 $m^3 s^{-1}$ in summer. Nova-Ahuir flow was similar in both seasons, while Molí flow was reduced by half and Rei flow was practically non-existent in summer. Suspended solids were rather low, average 12 ± 5 mg L^{-1}, and so were not included in the statistical analysis.

The cluster analysis of salinity, DIN, DIP and DSi variables identified two major clusters, designated A and B. Samples included in cluster A were the samples from the irrigation channels (P1, P3 and P4) and the samples from P2 and P5 (from 0 to 0.75 m water column depth). All other samples were included in cluster B. Examination of the variables of the different clusters (Table 1) revealed that cluster A was characterized by significantly lower salinities and higher nutrient concentrations than cluster B. Nutrients showed an opposite longitudinal gradient to that of salinity and decreased from the landside to the seaside of the harbour. The spatial and seasonal variations in salinity and nutrients have been depicted in Fig. 2. No significant variation was observed for nutrient concentration between the two seasons, except for DIP concentration, which showed a significantly higher concentration in spring. Nitrate was the most dominant nitrogen form at all sampling points and the highest values were observed at the irrigation channel sampling points for both seasons with values around 200 μM. DIP concentrations were rather low and did not exceed 1 μM, for cluster A, and 0.2 μM, for cluster B. The highest DIP values were observed in the Rei (P3) and Nova-Ahuir (P4) irrigation channels and at sampling points P2 and P5 and were similar for both seasons, except in the Nova-Ahuir (P4) channel where DIP showed a summer increase (0.16 to 0.97 μM). Average DSi concentrations were 50.7 μM for Cluster A and 12.0 μM for Cluster B. In spring, the highest DSi values were found in the irrigation channels: all samples were around 100 μM DSi. In summer, DSi content in the irrigation channels decreased considerably, mainly in the Molí (P3) channel (9 μM).

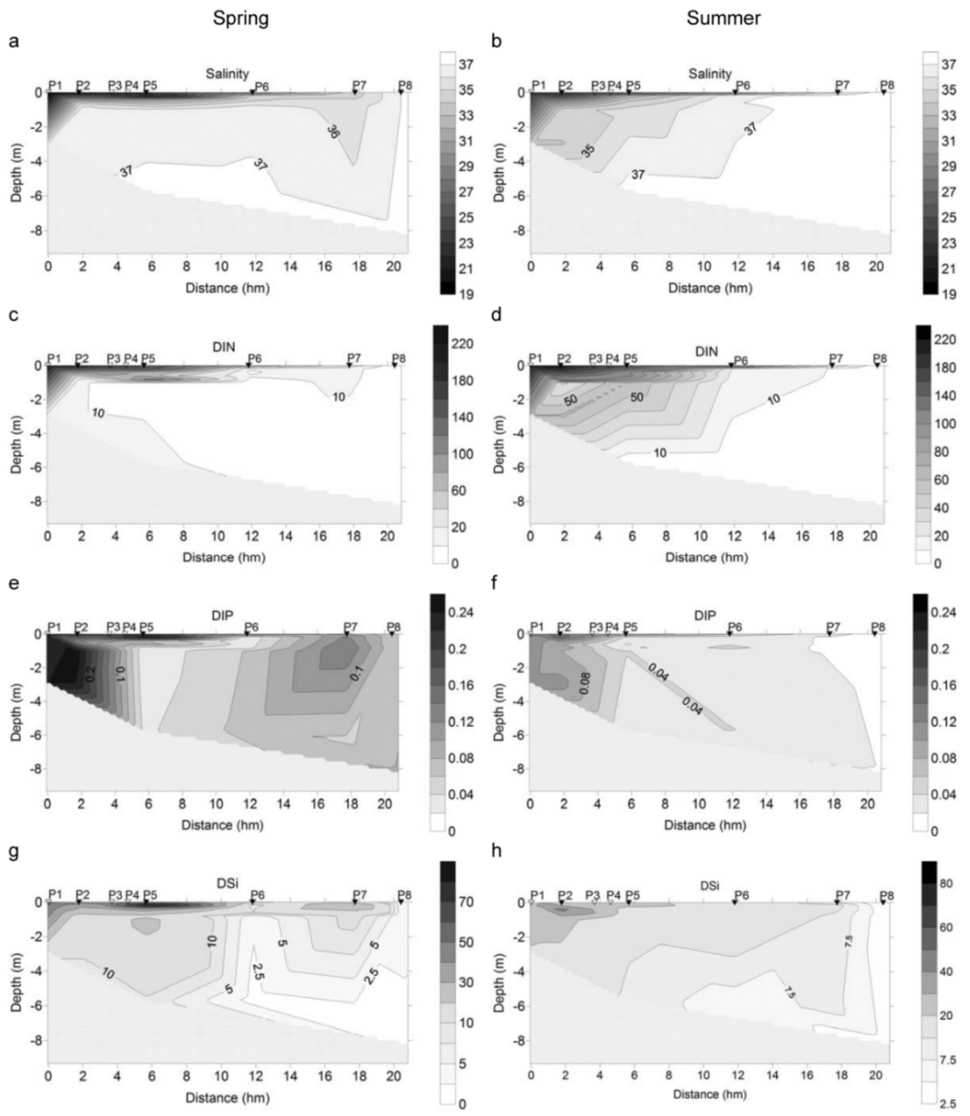

Figure 2 Vertical profiles of salinity (PSU). DIN (µM). DIP (µM) and DSi (µM) according to a gradient of distance towards the coast. Two different periods have been distinguished: spring (left column) and summer (right column). Distance on the x-axis is scaled in hectometres from the starting point of the section: Point 1 (P1). The end point is located at the most distant station: Point 8 (P8). The black inverted triangles

indicate the exact location of the sampling points and the white ones indicate the exact discharge point of irrigation channels

In order to better define potential nutrient control, we compared nutrient ratios between DIN, DSi and DIP concentrations with Redfield ratios (Si:N:P = 16:16:1). In the DIN:DIP and DSi:DIP ratios, phosphorus was always the limiting nutrient, except for an isolated instance of DIN limitation in spring at the P8 near-bottom sample. The average DIN:DIP and DSi:DIP ratios were 1968 and 476 respectively in Cluster A and 379 and 300 respectively in Cluster B (Table 1), showing a seaward decreasing gradient (Fig. 3 a, b, e, f). Regarding the DSi:DIN ratio, conditions were Si-limited in Cluster A, where this ratio remained under 1 in both seasons. In the irrigation channels, due to the constant DIN levels and the silica decrease, the DSi:DIN ratio decreased from 0.5 in spring to less than 0.2 in summer. In cluster B, Si-limited conditions and N-limited conditions alternated (Fig. 3 c, d).

Table 1 Descriptive statistics for salinity, nutrients (all expressed in µM units) and nutrient ratios for the identified clusters. Cluster A includes samples from the irrigation channels (P1, P3 and P4) and samples from P2 to P5 (from 0 to 0.75 m water column depth) Cluster B includes all other samples

	Salinity	NH_4	NO_2	NO_3	DIN	DIP	DSi	DIN/DIP	DSi/DIP	DSi/DIN
CLUSTER A										
Average	17.8	1.8	0.9	175.8	178.5	0.18	50.7	1968	476	0.3
Standard deviation	13.5	1.2	0.3	41.3	41.3	0.22	36.6	1435	359	0.2
Minimum	0.1	0.5	0.3	94.1	96.4	0.02	9.0	232	20	0.0
Maximum	34.8	4.6	1.4	243.2	245.5	0.97	106.0	5309	1367	0.6
CLUSTER B										
Average	36.3	0.8	0.2	12.0	13.0	0.06	12.0	399	316	1.0
Standard deviation	1.2	0.6	0.1	7.4	8.0	0.05	8.7	320	200	0.6
Minimum	34.2	0.1	0.0	2.9	3.4	0.01	2.8	53	46	0.4
Maximum	38.0	2.4	0.4	28.8	30.3	0.19	32.1	1143	622	2.5

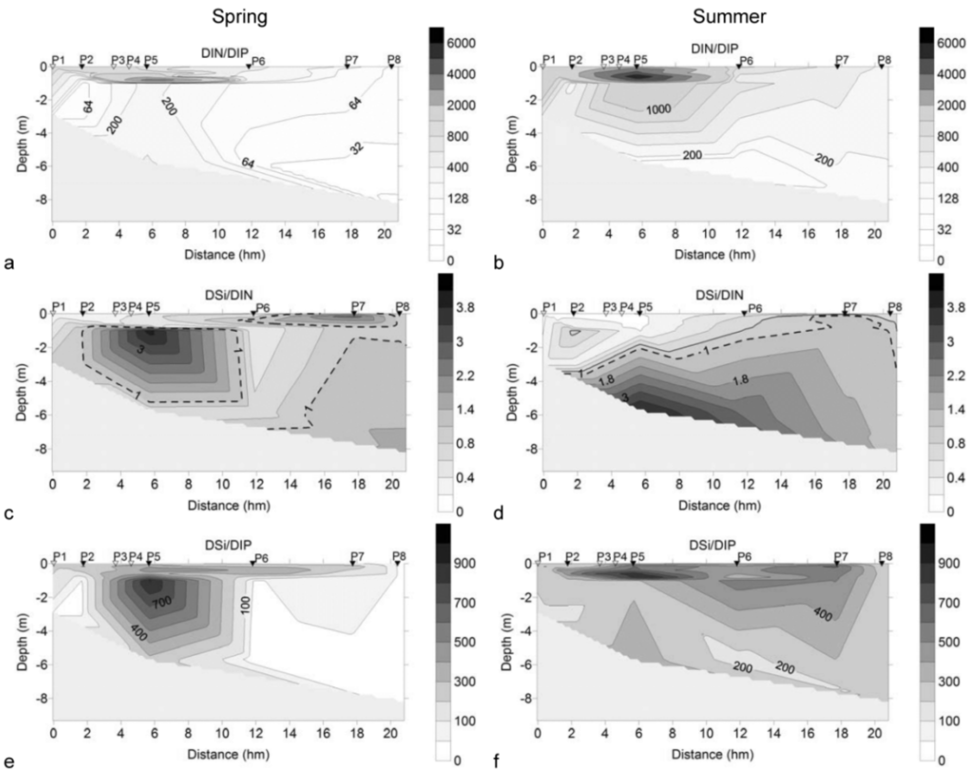

Figure 3 Vertical profiles of DIN:DIP. DSi:DIN and DSi:DIP molar ratios. The section details are as for Fig. 2. The dashed line in figures c and d represents a 1:1 DSi:DIN molar ratio

3.2 **Phytoplankton abundance and composition**

3.2.1 Total chlorophyll *a*

The spatial and seasonal variation in total chlorophyll *a* (chl-*a*) is shown in Fig. 4.

Chl-*a* concentration showed significant spatial variation with the highest values observed in the harbour after the freshwater inputs and a decreasing seaward gradient. In spring, the highest chl-*a* values were observed at P5, with a maximum of 8.8 μg L^{-1} at 1 m depth, and the lowest values were found at P8 with 1.4 μg L^{-1}. In summer, the highest values were measured at P2, with a maximum of 11.5 μg L^{-1} at 1 m depth, and the lowest values were found at P8 with 1.1 μg L^{-1} at surface. In the irrigation channels, chl-*a* concentration did not show a significant seasonal variation in the Rei (P3) channel, while in the other channels it increased in summer. The chl-*a* concentration varied from 2.4 to 10.2 μg L^{-1} in the Molí (P1) channel (spring and summer respectively); from 6.0 to 5.6 μg L^{-1} in the Rei (P3) channel; and from 1.9 to 4.5 μg L^{-1} in the Nova-Ahuir (P4) channel.

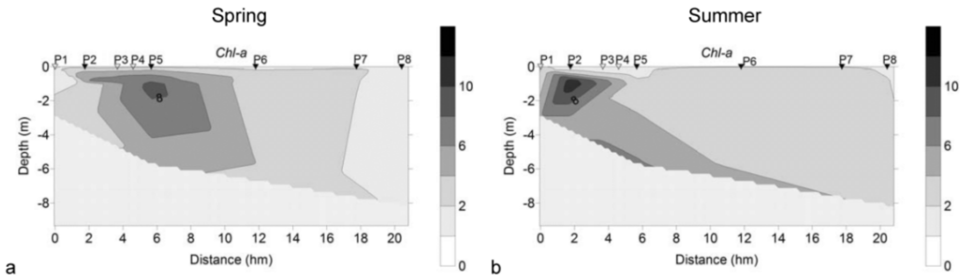

Figure 4 Vertical profile of total chl-*a* (µg/L). The section details are as for Fig. 2

3.2.2 Irrigation channels: P1, P3 and P4 sampling points

In terms of the contribution of the different groups of algae to total chlorophyll *a*, in the irrigation channels diatoms were the most important group in spring (Table 2). The contribution of this group diminished in summer (Table 3), and even disappeared from the Molí (P1) channel, being replaced by an increase in flagellate organisms - mainly euglenophytes in the Molí and Nova-Ahuir (P4) channels and chlorophytes in the Rei (P3) and Nova-Ahuir (P4) channels. Although all three channels were under silica limiting conditions in both seasons, diatoms only disappeared from the Molí channel, which had the lowest DSi:DIN ratio (0.04).

Table 2 Contribution (%) of different phytoplankton groups to total chlorophyll a calculated using CHEMTAX for spring sampling. P1; P3 and P4 are the sampling points located in the irrigation channels which were only sampled at 0.05 m depth

Sampling point	Depth (m)	Diatoms	Dinoflagellates	Euglenophytes	Chlorophytes	Cryptophytes	Prasinophytes	Prymnesiophytes	Cyanobacteria
P1	0.05	23.3	1.6	11.4	21.4	17.8	9.2	14.1	1.3
P2	0.05	25.2	31.9	37.2	5.7	0.0	0.0	0.0	0.0
	0.75	0.0	34.3	0.0	62.7	0.6	2.5	0.0	0.0
P3	0.05	40.3	11.6	9.5	17.1	2.0	17.8	0.0	1.7
P4	0.05	52.7	6.3	12.5	20.5	0.0	6.9	0.0	1.1
P5	0.05	15.0	20.5	49.1	4.3	5.5	0.1	5.0	0.6
	0.75	11.8	45.6	14.7	14.1	5.6	1.0	7.2	0.0
P6	0.05	3.7	11.1	44.5	16.2	1.5	2.4	20.5	0.0
	0.75	6.8	14.6	17.6	5.7	3.0	42.6	9.7	0.0
P7	0.05	3.5	11.0	28.2	0.0	2.1	35.4	19.7	0.0
	0.75	2.7	9.7	18.5	0.0	2.9	37.0	29.2	0.0
P8	0.05	0.0	1.4	5.5	0.0	3.6	16.0	72.8	0.7
	0.75	0.0	0.0	0.0	0.0	3.2	32.1	64.7	0.0

Table 3 Contribution (%) of different phytoplankton groups to total chlorophyll *a* calculated using CHEMTAX for summer sampling. P1; P3 and P4 are the sampling points located in the irrigation channels which were only sampled at 0.05 m depth

Sampling point	Depth (m)	Diatoms	Dinoflagellates	Euglenophytes	Chlorophytes	Cryptophytes	Prasinophytes	Prymnesiophytes	Cyanobacteria
P1	0.05	0.0	0.0	39.0	16.8	22.7	0.0	21.5	0.0
P2	0.05	13.6	0.0	49.4	16.6	4.2	0.0	0.0	16.2
	0.75	4.3	0.4	58.3	18.7	13.4	0.0	0.0	4.9
P3	0.05	16.5	1.5	2.5	60.6	10.7	0.0	0.0	8.1
P4	0.05	22.7	0.0	30.0	35.7	7.1	4.5	0.0	0.0
P5	0.05	26.3	1.1	35.5	5.1	9.0	0.0	0.0	22.9
	0.75	28.9	7.7	29.2	3.8	9.8	0.0	0.0	20.6
P6	0.05	18.0	6.6	59.5	6.4	3.7	0.0	0.0	5.8
	0.75	21.8	5.2	59.3	0.0	4.0	0.0	0.0	9.7
P7	0.05	10.2	7.3	66.2	0.0	2.2	1.2	0.0	12.7
	0.75	44.6	0.0	0.0	0.0	20.9	0.0	0.0	34.5
P8	0.05	1.3	6.8	19.3	4.9	2.8	0.0	33.7	31.3
	0.75	0.0	6.7	11.3	8.2	1.7	0.0	33.2	38.9

The contribution of euglenophytes in spring to total chl-*a* was small - around 10% in all three channels (Table 2) - while in summer they were the main group in the Molí channel (39%) and the second main group in the Nova-Ahuir (30%) (Table 3). Chlorophytes were the second main group in spring chl-*a* contribution (Table 2), while in summer they were the main group in the Rei (61%) and Nova-Ahuir (36%) channels (Table 3). Abundance of dinoflagellates and prasinophytes was significantly higher in spring, though their contribution to total chl-*a* was small, except for prasinophytes in the Rei channel (18%) (Table 2). Cryptophytes were an important group in the Molí channel in both seasons (18% spring and 23% summer) (Tables 2 and 3), while in the other two channels, they were more abundant in summer though their contribution to total chl-*a* was less important (11% Rei channel and 7% Nova-Ahuir).

Prymnesiophytes were only found in the Molí channel with a 14% contribution to chl-*a* in spring and 21% in summer (Tables 2 and 3). Cyanobacteria contribution to total chl-*a* was only relevant in the Rei channel in summer where they accounted for 8% (Table 3).

3.2.3 The estuary: P2, P5; P6 and P7 sampling points

In the harbour, flagellates were the main contributing group to total chl-*a* in both seasons, while the contribution of other groups such as diatoms and cyanobacteria was generally minor (Tables 2 and 3). In spring, euglenophytes were the most important group at P2, P5 and P6 down to a depth of 0.10 m, while in deeper waters (0.75 m), predominant groups were chlorophytes at P2, dinoflagellates at P5 and prasinophytes at P6 (Table 2). In summer, euglenophytes predominated in the whole water column at these same points (Table 3). At P7, the most important group in spring were prasinophytes (maximum of 68% at 0.10 m) and in summer, euglenophytes down to 0.10 m water depth (for reference see abundance at 0.05 m, Tables 2 and 3). The chlorophytes found were typical of freshwater, and as such they showed a negative correlation (Table 4) with salinity and their abundance was statistically higher in cluster A. They were also inversely correlated with DSi:DIN ratio and showed a positive correlation with DIN and DIP (Table 4). In the harbour, their maximum abundance was found at P2 in both seasons at 0.75 m depth (Tables 2 and 3). Dinoflagellate abundance decreased towards the coast and was only important at P2 and P5 just after the freshwater inputs. Freshwater prasinophytes were not found at P2 or P5 in spring or in summer (Tables 2 and 3). In spring, a prasinophytes population was found from P6 to P7, with its maximum abundance at 0.10 m depth accounting for 60% of total chl-*a* at P6 and 68% at P7. The statistical analysis indicated that this group was more abundant in Cluster B (Table 2). Other flagellate contributions to total chl-*a* were less important.

In spring, diatoms showed their maximum contribution to chl-*a* at P2 and P5, after the freshwater inputs, where they were the third main group (Table 2). On the other hand, in summer, diatoms were the second main group at P5 and P6 in the whole water column, and at P7 they were the main group at 0.75 m depth, with a contribution even greater than that of euglenophytes (Table 3).

Cyanobacteria were more abundant in summer (statistical analysis), while their spring contribution to total chl-*a* was reduced to less than 1% at all sampling points and depths (Tables 2 and 3). Two populations were detected: one population typical of freshwater and present in all irrigation channels in spring and only in the Rei channel in summer; and the other population, typical of marine waters. In the harbour, the freshwater population was observed in the surface samples from P2 and P5 (16.2% and 22.9% respectively in spring). The marine population showed an increase in abundance towards the sea; this population was directly correlated with salinity and inversely correlated with DIP concentration (Table 4).

3.2.4 Outside the estuary: P8 sampling point

Outside the harbour, at P8, prymnesiophytes were the most important group in spring, while an assemblage of prymnesiophytes (33.7%) and cyanobacteria (31.3%) predominated in summer (Tables 2 and 3). The prymnesiophyte marine population increased in abundance between P5 and P8 in spring and was present only at P8 in summer. Prymnesiophytes showed a negative correlation with DIN:DIP and DSi:DIP ratios (Table 4).

3.2.5 Blooming algal groups

It is important to highlight that in the microscope analysis, some potentially blooming species and genera (Moncheva et al., 2001) were detected: the dinoflagellates *Dinophysis caudata*, *Ceratium furca*, *Prorocentrum micans*, *Gymnodinium* spp., *Heterocapsa* sp., *Scrippsiella* spp.; diatoms of the genus *Amphora* spp. and *Pseudo-nitzschia* spp.; and euglenophytes of the genus *Eutreptiella* sp.

Table 4 Rank correlation matrix (Spearman's) between phytoplankton groups and environmental variables

	Diatoms	Dinoflagellates	Chlorophytes	Cryptophytes	Euglenophytes	Prasinophytes	Prymnesiophytes	Cyanobacteria	Marine cyanobacteria
Salinity	-0.31	0.02	-0.60[a]	-0.19	-0.05	0.03	0.18	0.18	0.55[b]
DIN	0.40[b]	-0.09	0.63[a]	0.20	0.20	-0.31	-0.40[b]	0.04	0.03
DIP	0.10	0.16	0.36[b]	0.01	-0.01	0.12	-0.05	-0.31	-0.61[b]
DSi	0.38[b]	0.20	0.40[b]	0.07	0.20	-0.15	-0.28	-0.01	-0.10
DIN/DIP	0.46[a]	-0.16	0.49[a]	0.24	0.30	-0.42[b]	-0.38[b]	0.21	0.40
DSi/DIN	0.32	0.16	-0.64[a]	-0.26	-0.14	0.35[b]	0.28	-0.12	-0.09
DSi/DIP	0.45[a]	0.09	0.04	0.08	0.23	-0.25	-0.35[b]	0.27	0.36

[a] $p < 0.01$
[b] $p < 0.05$
DIN - Dissolved Inorganic Nitrogen, DIP - Dissolved Inorganic Phosphorus, DSi - Dissolved Silicate

4 Discussion

Climatically, autumn and spring are the rainy seasons while summer is the dry period in this Mediterranean area. Thus, the phreatic level is higher in spring and to prevent crop root asphyxia, water is pumped through the irrigation channels to the sea. On the other hand, in summer, the population increase makes it necessary to increase pumping to supply drinking water from the wells located in the detritic aquifer. In addition, citrus crops need at least two waterings during summer. This causes the flow reduction found in the irrigation channels in summer, when irrigation return flows are the most important source of freshwater. Gandia harbour can be considered a small stratified estuary with a shallow freshwater layer for most of the year. However, in summer, due to the reduction in freshwater inputs and dominant wind direction, this freshwater layer nearly disappears.

In Gandia Harbour, the range of nitrate and silicate concentrations was of the order of those found in typical nutrient-enriched areas (Domingues et al., 2005). However, phosphorus concentrations were particularly low, in the same order of magnitude as those measured in non-polluted coastal areas (Aminot and Chaussepied, 1983; Glé et al., 2008).

It is broadly accepted that in marine systems, nitrogen is the limiting nutrient, whereas phosphorus limits freshwater systems. There is, however, evidence of seasonal and spatial variations of the limiting nutrient in estuarine systems (Domingues et al., 2005; García-Pintado et al., 2007). In the study area, the nutrient concentration in the irrigation channels and in the harbour is influenced by the soil use in their watershed. The clear predominance of agricultural practices (48% of watershed soil) and the discharge of treated urban wastewater through the submarine outfall, causes an excess of nitrogen and an imbalance in the DIN:DIP ratio. In addition, the entry of marine water into the harbour is also characterized by phosphorus limiting conditions, as shown by several studies in the Mediterranean, which identify phosphorus as the main limiting nutrient in phytoplankton productivity (Estrada, 1996; Olivos et al., 2002), in contrast with other marine systems.

Chemical weathering of silicates on land is the main process that supplies dissolved and particulate silicate to rivers. However, in this area, groundwater discharges from the Gandia-Denia detritic aquifer are also rich in silicates. The high silica levels found in both seasons also caused the imbalance in the DSi:DIP ratio. Thus, phosphorus was always the primary potentially limiting nutrient for phytoplankton growth, while DIN and DSi spatially alternated as secondary potentially limiting nutrients.

The reduction of DIP concentration to below 0.01 mg L^{-1} (approx. 0.32 µM) has been pointed out as the first necessary step in the recovery of eutrophic shallow lakes. However, for warm shallow lakes in the Mediterranean region there is experimental evidence that this threshold should be even lower (Villena and Romo, 2003). Despite the fact that the average DIP levels in the study area are generally below this limit, an isolated instance of DIP increase was observed in the Nova-Ahuir outflow in summer (0.97 µM). DIP concentrations in estuaries are often found to be highest during summer corresponding to a strong temperature-dependent release of phosphorus from sediments (García-Pintado et al., 2007). In Gandia Harbour, reduced water flow and the direction of dominant winds increase water residence time and this may provide longer contact with sediment that may also enhance internal release of phosphorus. However, DIP levels are significantly higher in spring, when phosphorus fertilizers are applied, which may point to a phosphorous origin from allochthonous sources (agricultural runoff) rather than from sediment release. DIP spring maximum was 0.30 µM, so the isolated DIP increase found in summer (see above) could not be explained by agricultural runoff. An alternative explanation for this increase can be found in the wastewater discharge from the second homes located in the study area (Fig. 1) which are only inhabited during summer.

Chlorophyll *a* concentration was of the order of that found in other eutrophic estuarine waters (Rodríguez et al., 2003; Seoane et al., 2005) and coastal lagoons (Coelho et al., 2007) of moderate biomass but much lower than that found in very eutrophic estuaries (Ansotegui et al., 2001) and coastal lagoons (Villena and Romo, 2003).

Diatoms are recognised as the most opportunistic species as far as taking advantage of nutrient availability is concerned (Fogg, 1991). In this study, diatoms showed a significant positive correlation with DIN and DSi concentration, and with DIN:DIP and DSi:DIP ratios (Table 4), confirming a preference for eutrophic conditions. In the irrigation channels diatoms were the most important group in spring and their contribution diminished in summer, being replaced by an increase in flagellate contribution to total chl-*a*. This numerical displacement of diatoms by flagellates, in geographically diverse regions experiencing decreased DSi:DIN and DSi:DIP ratios due to a decreased availability of silicate relative to nitrogen and phosphorus, has been well documented (Moncheva et al., 2001). In the harbour, high diatom contribution was also linked to high nutrient availability from freshwater discharges in spring (P2 and P5), while the highest contribution at P7 at 0.75 m depth in summer was attributed to the higher DSi:DIP ratios (Fig.3f) in this location due to lower phosphorus concentrations (Fig. 2f) in summer. DSi:DIN ratios, which showed a balanced value close to 1 at P7 (Fig. 3d), indicate diatom uptake (Glé et al., 2008). This high contribution of diatoms in summer has been reported and attributed to freshwater taxa that grow well in freshwaters as well as in brackish waters (Seoane et al., 2005). The diatom distribution can also be related with higher flushing rates as they are more abundant in the channels and in the sampling points located at their outflow (P2 and P5).

If we group the dinoflagellates, cryptophytes, prasinophytes, chlorophytes, and euglenophytes as flagellates, these dominated over diatoms at all the harbour sampling points and depths (P2, P5, P6 and P7), except for P7 at 0.75 m depth in summer. The dominance of euglenophytes has been observed in other eutrophic systems where it has been related with high nutrient levels and decreasing turbulence (Olli et al., 1996; Çelik and Ongun, 2007). The positive correlation of chlorophytes with DIN and DIP (Table 4) showed that this group was characteristic of the most eutrophic conditions. In contrast with diatoms, chlorophytes were inversely correlated with the DSi:DIN ratio (Table 4), so they outcompeted diatoms when this ratio value was lower. Their high contribution at P2 at 0.75 m depth in both seasons could be due to the sedimentation process of chlorophytes affected by the higher salinity. Dinoflagellates, cryptophytes and prasinophytes have been reported as eutrophic groups by Latasa et al. (2010) in the northwestern Mediterranean Sea. Higher abundance of prasinophytes at P6 and P7 could be attributed to euryhaline species (Seoane et al., 2005). The dominance of flagellates can explain the moderate chlorophyll *a* levels observed. It is assumed that small phytoplankton (which includes flagellates) cannot reach high biomasses, since biomass elevation actuates a feedback mechanism favouring predators and which may finally enhance the rate of primary production rather than biomass accumulation (Fogg, 1991; Bel Hassen et al., 2008).

Outside the harbour, the dominance of prymnesiophytes in spring (Table 2) and the dominance of the prymnesiophyte and cyanobacteria assemblage in summer (Table 3) coincide with the observations of Latasa et al. (2010). These two groups prefer more oligotrophic waters, and prymnesiophytes are outcompeted by cyanobacteria when nutrient content decreases. In this case, prymnesiophytes were significantly less abundant in summer, when DIP and DSi values were lower at P8 (Fig. 2 e, f, g, h), and the cyanobacteria contribution to total chl-*a* was higher. This preference for oligotrophic conditions is reflected in the prymnesiophytes' negative correlation with DIN:DIP and DSi:DIP ratios (Table 4). Higher contributions of smaller cells during summer are reported for several estuaries and it is well established that these organisms attain maximum growth rates at temperatures higher than those for diatoms and green algae (Domingues et al., 2005). The dominance of these groups outside the harbour indicates that the eutrophication problems are mainly restricted to the harbour area.

Among the potentially blooming species identified, the dinoflagellate *Dinophysis caudata* is responsible for the synthesis of the toxin DSP okadaic acid and the diatom *Pseudo-nitzschia* spp. synthesises the ASP toxin (domoic acid). In humans, these toxins can cause the poisoning syndromes known as diarrheic and amnesic shellfish poisoning (DSP and ASP respectively) (Anderson, 2009).

5 Conclusions

To develop appropriate management strategies, it is necessary to fully understand how ecosystems function by first of all establishing the relationships between phytoplankton composition, abundance and nutrient patterns. Nutrient concentrations in the aquifers depend on anthropogenic land uses and agricultural practices. Intensive farming and wetland regulation have increased groundwater discharge characterized by high nitrate levels to coastal ecosystems, such as shallow estuaries. High phosphorus levels in receiving waters usually coincide with phosphorus fertilization period, but other sources such as uncontrolled wastewater discharges also cause phosphorus increases. In ecosystems with phosphorus limiting conditions and high nitrate and silicate levels, a continued input of phosphorus could trigger the undesired effects of phytoplankton species responsible for the generation of harmful blooms. The correct implementation of the existing agricultural best management practices is needed to reduce nitrogen and phosphorus loading. But for effective control of the eutrophication effects strict control over wastewater point sources should be also exercised. This management strategy has been proposed for similar eutrophicated systems such as La Albufera (Valencia, Spain) (Villena and Romo, 2003) and El Mar Menor (Murcia, Spain) (García-Pintado et al., 2007). Reducing phosphorus inputs would make phosphorus more limiting and result in nitrogen from anthropogenic sources being less useful for ecosystem productivity.

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Capítulo 4: Effects of freshwater inputs on the receiving waters of a Mediterranean coastal area: nutrients and phytoplankton analysis

1 Introduction

Estuaries and coastal areas are ecosystems of high natural and socio-economic value that are at high risk of suffering the negative impact of human activities (Angelidis and Kamizoulis 2005). One of the main reasons for this risk is the high loads of nutrients that they receive from terrestrial origin, either from point (e.g. submarine wastewater outfalls) or diffuse sources (e.g. atmospheric, leaching from surrounding land) (Paerl 2006). Nutrients inputs in adequate proportions and quantities are essential for primary producers. However, excessive anthropogenic nutrient inputs can lead to undesirable effects associated with eutrophication, including harmful algal blooms (HABs), and alterations in the natural composition of the phytoplankton community, which may, in turn, change the ecosystem food webs and nutrient cycling dynamics (Paerl et al. 2010).

Nevertheless, regardless of the high anthropogenic nutrients loading into coastal systems, phytoplankton biomass may be limited by a specific nutrient due to an alteration in the N:P:Si nutrient ratios. The concept of nutrient limitation is a keystone of eutrophication research (Smith et al. 1999; Turner 2002) and as important as element loading rates and concentrations. Identifying and quantifying the key anthropogenic nutrient input sources is essential to adopting management measures that can target input for maximum effect.

Eutrophication has been described as one of the greatest contemporary threats to the integrity of coastal ecosystems (Vidal et al. 1999). Two key European policy instruments were introduced in the early 1990s to address the most significant pressures leading to eutrophication: the Nitrates Directive (ND, 91/676/EEC), which considers the agricultural contribution and the Urban Waste Water Treatment Directive (UWWTD 91/271/EEC), which focuses on urban sewage discharges. However, implementation of these directives has met with varying degree of success: point sources (UWWTD) are localized and are easier to monitor and control, while diffuse sources (ND) are much more difficult to regulate (Artioli et al. 2008; Maier et al. 2009).

Phytoplankton is a priority item in different national and international water quality and eutrophication assessment policies, such as the National

Estuarine Eutrophication Assessment (NEEA) Program (USA) (Whitall et al. 2007), the OSPAR convention (North-East Atlantic) (OSPAR 2003) and the Water Framework Directive (WFD 2000/60/EC). The WFD clearly addresses the status of coastal and transitional waters (such as estuaries, rias and coastal lagoons), integrating these within groundwater and inland surface water management. It bases its assessment on ecological quality status, requiring evidence of impact on biological quality elements as well as supporting physico-chemical data to confirm the likely cause of the impact (Maier et al. 2009). Biological parameters include composition, biomass and abundance of phytoplankton (WFD 2000/60/EC).

Although quality classification schemes for chlorophyll *a* concentrations as a measure of phytoplankton biomass have been developed, the requirement of the WFD for an evaluation system based on the taxonomic composition of the phytoplankton community is more difficult to satisfy (Cartaxana et al. 2009). Furthermore, taxonomic composition is usually assessed by standard microscopy, and accurate species enumeration is laborious, costly and the results can be inconsistent among different laboratories (Duarte et al. 1990; Schlüter et al. 2000). As an alternative approach, the phytoplankton community can be categorized into functional groups on the basis of taxonomic specificity of pigments determined with high performance liquid chromatography (HPLC) (Wright et al. 1996) coupled with the CHEMTAX program (Mackey et al. 1996). However, this technique has been mainly addressed as a complement to standard microscopy techniques (Cartaxana et al. 2009; Seoane et al. 2011). As Latasa (2007) observed "Pigment specialists are confronted with the paradox of requiring information about the composition of phytoplankton in order to learn about the pigment composition of phytoplankton groups and, ultimately, the composition of phytoplankton". In order to overcome this problem a procedure was suggested by Latasa (2007), and appraised by the authors involved in the development of CHEMTAX (H. Higgins and S. Wright pers. comm.).

The objective of this study is to characterize the nutrient levels and the phytoplankton community in a coastal Mediterranean area which can be defined as a pollution-sensitive area. That is, according to the UNEP/WHO (1999) definition "estuaries and coastal waters of natural or socio-economic value that are at higher risk to suffer negative impacts from human activities". In these areas is necessary to evaluate both the vulnerability of the system and the risk for environmental degradation (Angelidis and Kamizoulis 2005). Vulnerability is determined by natural characteristics (for instance, semienclosed waters are more sensitive to pollution impacts), while risk is determined by human activities (Angelidis and Kamizoulis 2005). In this study three systems characterized by different hydrodynamic characteristics and a distinctive main nutrient source, linked to a specific human activity, are compared. The aim of this comparison is to prioritize environmental protection actions. This research was triggered by the recent urban development of the area and the projected enlargement of a commercial harbour that may modify both system risk and vulnerability.

2 Materials and methods

2.1 Study area

The study area (Fig. 1) is located in the southernmost sector of the Valencian Gulf (Western-Mediterranean Sea) on the coast of the Safor County (Spain). Three important factors coincide here, which determine whether a coastal area is a sensitive area according to Angelidis and Kamizoulis (2005). Namely: 1) the presence of tourism as major income generation activity; 2) the presence of important nutrient sources in the drainage basin: a submarine wastewater outfall, harbour facilities and important agricultural runoff and 3) a river mouth area with low flushing rate, high water residence time, shallow mean depth and low mean slope of the sea floor.

Tourism is one of the main economic activities in this area. In 2009 the Valencia region received 5.1 million international tourists and 3.1 million national tourists from other Spanish regions (IET Spain's Tourism Statistics Centre 2010). Tourism development in the Safor County is mainly based on residential development (Mantecón and Huete 2007) and is based on a "leisure and holiday" product type labelled as a "sun and beach" product (Yepes and Medina 2005). The average density in the coastal municipalities of the Safor county (from Gandia to Piles) is 963 inhabitants km^{-2} in winter (1314 inhabitants km^{-2} in Gandia city) (INE 2009). However, that figure triples in summer. Statistical data of tourism accommodation from the Spanish INSTITUTO NACIONAL DE ESTADÍSTICA (INE 2009) did not explain the real summer increase. Published data for the coastal municipalities of the study area are the following: 28 hotels with accommodation for 5496 guests; 4 camp sites with 2822 pitches; 2053 regulated apartments, with 10590 beds. However, beds in non-regulated apartments far exceeded these numbers and explain the summer population increase more accurately. Property ownership increased from 91703 in 2004 to 106384 in 2008 according to the land registry (INE 2009) due to the coastal urbanization process and many of these apartments are devoted to mass tourism.

The impact of mass tourism on the Spanish Mediterranean coast has been addressed by other authors (Vera and Ivars 2003; Yepes and Medina 2005). It is important to highlight that one impact of the summer population increase is the seasonality of the wastewater flow, which is considerably higher during summer. The treatment plant of Gandia is designed to serve a 133300 inhabitants population and treat 46350 m^3 day^{-1} of average flow from 17 municipalities of the Safor County. These municipalities have a population of 115328 inhabitants in winter (INE 2009); however, this population triples in summer as described above. The treatment plant is located next to the Serpis River (Fig. 1), it discharges treated wastewater through a submarine wastewater outfall at an approximate distance of 1900 m from the Serpis river mouth. In addition, the plant has two overflow channels that discharge directly to the river. The first overflow channel discharges next to the plant, mainly during torrential rain episodes because wastewater and pluvial waters are not separated. The other overflow channel discharges near the river mouth. In summer, discharges have been detected through this channel as consequence of the population and wastewater flow in excess of designed capacity.

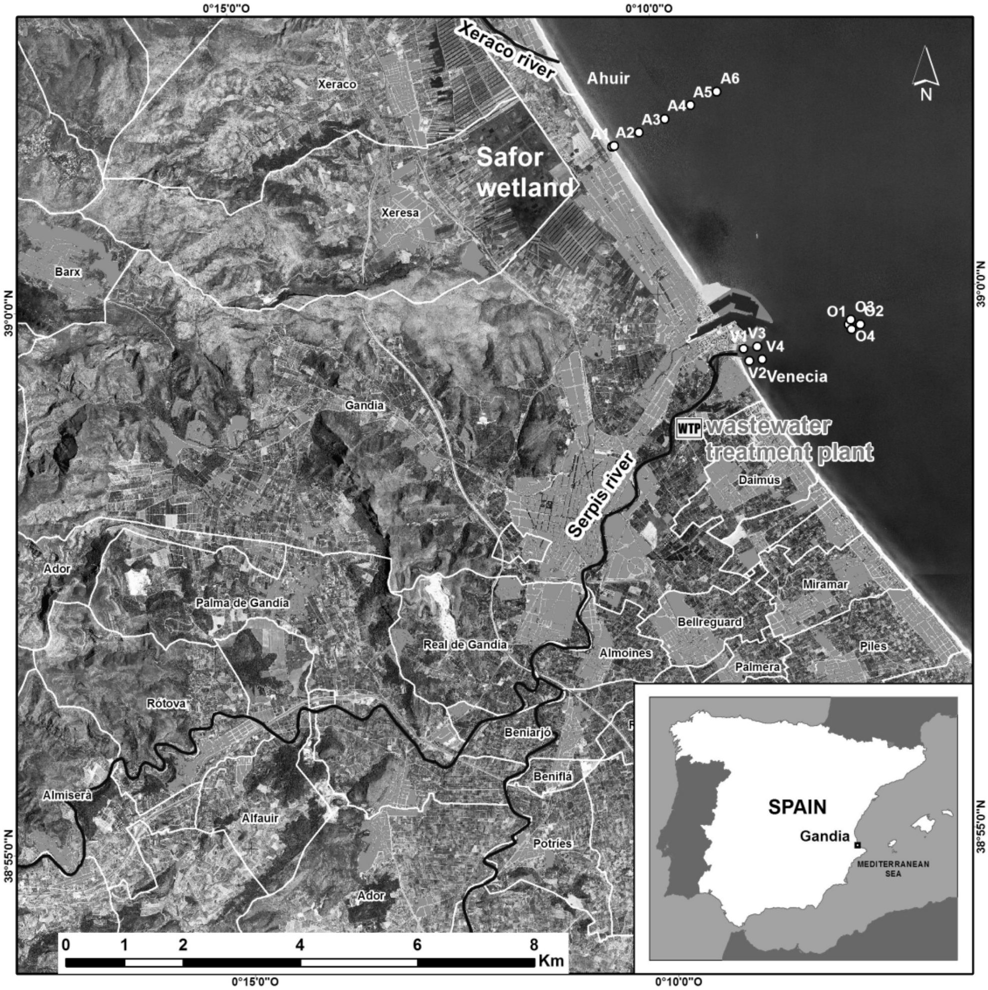

Figure 1 Study area and sampling points. Sampling point code: first character corresponds to the sampling site (A – Ahuir, V – Venecia (Serpis River outflow), O – submarine outfall)

Another source of economic activity in the Safor County is Gandia Harbour, which is a commercial, fishing and recreational harbour. The main traffic in the harbour is paper reels and wood packages; other merchandise is wood pulp, rice, and mainly citrus fruits. The commercial area is 260000 m^2 and it has an additional 70000 m^2 of storage area with 32000 m^2 of warehouses. It has a nautical club with a 21000 m^2 dock with capacity for 400 sports boats. In 2009, the total traffic in the harbour was 251000 tons (including fisheries and provisioning), mainly of imported products (APV 2009). There is currently a project for enlarging the harbour in order to increase the capacity for commercial and recreational activities, including the construction of a new commercial dock (APV 2009; Decision 28734 BOE nº 214, 6 September 2011) In Fig. 2 the harbour as it is now, and the projected enlargement can be observed.

Two rivers flow into the study area: at the northern end is the Xeraco river mouth, which is a small river, 16.6 km in length, and the Serpis River, which outflows southward and which has a basin of 752.8 km^2 and is 74.5 km in length. These rivers have a Mediterranean regime characterized by a high seasonality, with a dry period during summer, and a wet period with episodes of torrential rain, mainly in autumn (Garófano et al. 2009). While the Xeraco River outflows in a more open area, the Serpis River outflows on Venecia Beach, on the southern side of Gandia Harbour. According to the Angelidis and Kamizoulis (2005) definition of marine systems vulnerability; Venecia Beach is a system characterized by high vulnerability because it is a semi-enclosed bay (enclosed by the harbour and the river mouth) (Fig. 2), with shallow water (depth < 10 m) and mean slope < 1%. Northeast currents are dominant in the area, followed by southeast currents (CEDEX 1997). The new commercial dock will reduce the impact of northeast currents (Fig. 1) on the Serpis river mouth and the Venecia Beach causing an increase in the water residence time.

Figure 2 Gandia harbour as it is now (left side) and the projected enlargement (right side)

The alluvial plain was totally occupied by the Safor wetland and crops until the seventies. Nowadays, this area shares its agricultural activity, mainly citrus fruits and vegetables, with the tourism of the urban areas. The Safor Wetland is a protected ecosystem declared a Site of Community

Importance (SCI) under the Habitats Directive (92/43/EEC), as it is considered one of the best preserved wetlands in Spain. The wetland is drained by a network of artificial channels which outflows into Gandia Harbour. Agricultural land use represents 48% of the watershed. Due to continued agricultural practices, nitrate levels in the detritic aquifer have exceeded the limit of 50 mg L^{-1} established by the Nitrates Directive (Directive 91/676/EEC), and it has been declared a Nitrate Vulnerable Zone. It is likely that the largest groundwater inputs of nutrients to near shore waters will occur in regions of aquifer bearing strata outcropping on the coast and occurring within nitrate vulnerable zones (Maier et al. 2009). In the study area, these conditions are found on Ahuir beach which is located at the end of Gandia's urban area and which receives discharge from the Plana Gandia-Denia detritic aquifer, quantified at 66 Hm^3 $year^{-1}$ (2.1 m^3 s^{-1}) (Ballesteros-Navarro 2003).

2.2 **Sampling design**

Sampling was designed in order to characterise the systems receiving the major nutrient inputs along the coast. These systems were each characterized by a distinctive main nutrient source (groundwater discharge, river discharge or submarine wastewater outfall discharge) and by different hydrodynamics (water residence time).

The first study area was Ahuir beach which receives groundwater discharges from the Plana de Gandia-Denia aquifer. Samples were taken in an orthogonal transect (Fig. 1). The first sample was collected in the surf zone (A), the second sample right behind the surf zone (A1), the next sample was collected 50m from the coastline (A2) and subsequent samples were collected every 500 m along the transect (A3 to A6). Samples were collected with Niskin bottles each 5 m of water column depth and with a Van Dorn bottle 50 cm above the bottom. Maximum sampling depth was 18 m in A6.

The Serpis river mouth was selected as the second study area which we call Venecia area. Samples were collected as described below. Only one sample was collected in the Serpis River near its mouth. Four fixed sites were sampled drawing a 200 m square around the mouth (V1 to V4 plume contour stations, Fig. 1). Samples were collected with Niskin bottles near the surface at each fixed site, and an extra sample was collected with a Van Dorn bottle 50 cm above the bottom in V3 (2.8 m depth) and V4 (2.1 m depth). Three variable sites were sampled depending on wind and river plume directions. At these sites, water samples were collected at different depths in the water column (0.05, 0.10 and 0.75 m) using a Superficial Water Sampler (SWAS) (Mösso et al. 2008), because density driven vertical stratification was expected.

The third study area was located around the submarine wastewater outfall outlet. Four fixed sites (O1 to O4) (Fig. 1) were sampled around the outlet within 100 m approximate range. At these points, samples were collected with a Niskin bottle at 0.05, 5, 10 and 15 m. Three variable sites (O5 to O7) were sampled depending on wind and submarine wastewater outfall plume directions, water samples were collected at different depths in the water column (0.05, 0.10 and 0.75 m) using the SWAS, because a haline vertical stratification was expected.

Samples were taken in two hydrological periods: the wet one in spring, and the dry one in summer. Intensive samplings were performed in the study areas described above, as close together in time as possible (within a week maximum): in July 2008, in April 2009 and in July 2009. Samples were separated into three subsamples for nutrient, pigment and phytoplankton analysis. Water samples were kept in a cool box (4ºC) and transported to the laboratory.

2.3 Laboratory analysis

The following parameters were analyzed in all the samples: salinity, suspended solids (SS) nitrate, nitrite, and ammonium, dissolved inorganic phosphorus (DIP), dissolved silicate (DSi) and phytoplankton pigments. Representative samples were microscopically screened to avoid missing taxa following the recommendations of Higgins et al. (2011).

Dissolved inorganic nitrogen (DIN) was calculated as the sum of nitrate, nitrite and ammonium. Salinity was determined by means of an induction conductivity meter Multi 340i/SET WTW, using the Practical Salinity Scale. Nutrients were analyzed colorimetrically using the method of Aminot and Chaussepied (1983). Dissolved oxygen concentration was measured using the Winkler titration technique (Grasshoff 1983).

Samples for phytoplankton pigment analysis were filtered on GF/F fiberglass filters (25 mm diameter). Pigments were extracted using acetone (100%) and were measured using reverse-phase high-performance liquid chromatography (HPLC). The HPLC method employed was that proposed by Wright et al. (1991) slightly modified as per Hooker et al. (2000). The system was calibrated with external standards obtained commercially from the DHI Water and Environment Institute (Hørsholm, Denmark).

Phytoplankton samples were fixed with formaldehyde, concentrated according to UNE EN 15204:2006, based on Utermohl (1958), and qualitatively examined under a LEICA DM IL inverted microscope.

2.4 **Phytoplankton composition and abundance**

Once the concentration of important photosynthetic pigments was determined, the phytoplankton community was studied using the CHEMTAX program (Mackey et al. 1996) version 1.95 (S. Wright, pers. comm.) to obtain the contribution to total chlorophyll-*a* (chl-*a*) from the phytoplankton groups identified with microscopy.

To obtain reliable results, CHEMTAX should be applied to a data set where pigment ratios within the different groups do not change (Latasa et al. 2010). In order to identify groups of samples with similar characteristics, a cluster analysis was performed using STATGRAPHICS 5.1 to group samples according to salinity and nutrients (DIN, DIP and DSi). City-block distances were calculated and samples clustered according to Ward's method. Pigment samples were separated into two subsets following the results of the cluster analysis: the first subset included marine samples, and the second subset included freshwater samples with higher nutrients concentration. Freshwater samples include: Serpis River samples and samples from river plume stations (V5, V6 and V7) from 0.05 to 0.10 m water column depth (only April 2009 and July 2009). CHEMTAX was applied independently to each subset (Latasa et al. 2010) to obtain the contribution of 8 phytoplankton groups to the chl-*a* stock: diatoms, dinoflagellates, euglenophytes, chlorophytes, cryptophytes, prymnesiophytes, prasinophytes and cyanobacteria.

CHEMTAX was applied following the procedures described in Latasa et al. (2010) and using the same parameters (elements varied = 10). The average of the last 6 ratio estimations was incorporated into the final pigment ratio matrix when a clear convergence was observed. In the absence of a clear convergence, the average of each pigment ratio was incorporated. This final matrix was then used to estimate the contribution of the different groups to chl-*a* stock. The output values are presented in Table 2.

2.5 **Statistical analysis**

A non parametric one-way analysis of variance (Kruskal-Wallis) was used to test differences in physico-chemical variables and phytoplankton composition and abundance between sampling areas and seasons.

After studying the phytoplankton data distribution in relation to the physico-chemical variables, the Redundancy Analysis (RDA) was selected from among the different multivariate ordination methods available (Ter Braak and Smilauer 2002). Phytoplankton taxonomic groups were included in CANOCO 4.5 as dependent variables and physico-chemical variables were included as independent variables. The statistical significance of the relationships was evaluated using Monte Carlo permutation tests with manual forward selection procedure, under 499 permutations (Seoane et al. 2011).

3 Results

3.1 Environmental variables

Mean water temperature was 15.7ºC in spring conditions (April 2009) and 27ºC in summer conditions (July 2008 and July 2009). Water temperature showed a longitudinal gradient decreasing from landward to seaward in all the sampling campaigns in the Ahuir study area due to increasing water column depth. The difference in temperature over the gradient was rather small, generally around 1ºC. In the submarine wastewater outfall area, the difference between subsurface temperature and above bottom (approximately 20 m depth) temperature was 0.3ºC in spring 2009 and nearly 1ºC in both summers, so no thermal stratification was observed. In the Venecia area due to the shallow depth and the reduced size of the study area, water temperature differences were minimal.

In the Ahuir area, dissolved oxygen concentration ranged from 6.0 mg L^{-1} in July 2009 to 10.6 mg L^{-1} in April 2009, with higher values in spring near the shore (Table 1). Salinity showed a longitudinal gradient that increased seawards in July 2008 (range 36.7 to 37.6) and April 2009 (range 36.6 to 37.4) (Table 1). In July 2009 salinity was significantly higher than both July 2008 and April 2009 ($p < 0.01$) and no gradient was observed. Suspended solids concentration was not significantly different between sampling seasons ($p > 0.05$); average values were below 11 mg L^{-1} (Table 1), and its value decreased with increasing water column depth.

The Serpis flow, measured in the gauging stations Font En Carrós and Vernisa of the Júcar Hydrographic Confederation (CHJ), which are located approximately 10 km from the river mouth, was null during summer sampling, while in April 2009 it was 5.7 m^3 s^{-1}. Summer is the dry period in this Mediterranean area and total monthly precipitation was 31.8 mm in July 2008 and 41.2 mm in July 2009, while in April 2009 (spring) monthly precipitation was 156.6 mm. In spring, a precipitation event of 89.8 mm was registered 3 days before sampling in the Venecia area. Dissolved oxygen concentration was significantly higher in spring 2009 ($p < 0.01$): its values ranged between 6.9 and 9.6 mg L^{-1} (Table 1). The lowest dissolved oxygen values were observed in summer 2009 (ranging from 5.9 to 7.1 mg L^{-1}) when the Serpis river sample registered the minimum value of 3.1 mg L^{-1} (Table 1). Salinity in the receiving waters did not show statistically significant differences between sampling seasons ($p > 0.05$) given that salinity in the plume contour samples (V1 to V4) was always above 30. However, salinity in the plume sampling stations (V5 to V7) was lower in spring 2009 (under 5 in subsurface samples); while in July 2008 it was above 33, coinciding with a salinity measure in the river sample of 14.9. Suspended solids were significantly higher in July 2009 ($p < 0.01$) and ranged from 9 to 27 mg L^{-1}, with higher values in the plume points (V5 to V7).

In the submarine wastewater outfall area, average dissolved oxygen concentration showed was significantly higher ($p < 0.01$) in spring with an average value of 8.7 mg L^{-1} (Table 1) while in summer average values were 7.6 mg L^{-1} in 2008 and 7.4 mg L^{-1} in 2009. Salinity average values were above 37 in all the sampling seasons (Table 1). Only sampling point O5, located just above the outfall outlet, showed salinity values below 36 (35.2

in O5 at 0.05 m depth in April 2009 and 35.9 in July 2008). Significantly higher salinity values were observed in July 2009 ($p < 0.05$). Maximum suspended solids concentration was under 13 mg L^{-1} (Table 1).

Comparison of the three study areas revealed no significant difference ($p > 0.05$) in the salinity, dissolved oxygen concentration and suspended solids between the Ahuir and the submarine wastewater outfall area. On the other hand, the Venecia area had significantly lower salinity ($p < 0.01$) and dissolved oxygen concentration ($p < 0.01$) and higher suspended solids concentration ($p < 0.01$).

Table 1 **Descriptive statistics for suspended solids (SS), salinity, dissolved oxygen (DO), nutrients, nutrient ratios and chlorophyll-*a* for the three systems and sampled periods (all stations and depths are included in the calculation for each system). DIN: Dissolved Inorganic Nitrogen, DIP: Dissolved Inorganic Phosphorus, DSi: Dissolved Silicate. The standard deviation for nutrient ratios has been calculated by error propagation.**

		AHUIR				VENECIA				SERPIS	OUTFALL			
	jul-08	MEAN	S.D.	MIN	MAX	MEAN	S.D.	MIN	MAX	MEAN	MEAN	S.D.	MIN	MAX
JULY 08	**SS (mg L^{-1})**	7	2	4	10	8	2	5	11	10	6	2	2	9
	Salinity	37.2	0.3	36.7	37.6	36.1	1.3	34.0	37.5	14.9	37.1	0.4	35.9	37.6
	DO (mg L^{-1})	7.8	0.4	7.4	8.5	7.2	0.4	6.5	7.9	7.8	7.4	0.8	5.7	9.1
	DIN (µM)	0.60	0.62	0.15	2.10	13.91	10.17	2.90	30.40	46.31	4.32	3.44	1.00	11.50
	DIP (µM)	0.168	0.034	0.120	0.230	0.383	0.222	0.170	0.860	1.130	0.193	0.103	0.060	0.350
	PT (µM)	0.44	0.12	0.32	0.71	0.51	0.16	0.30	0.90	2.46	0.39	0.20	0.14	0.98
	DSi (µM)	1.08	0.51	0.70	2.30	11.10	6.99	4.20	25.40	44.10	3.36	1.91	1.30	7.20
	DIN/DIP	4	4	1	12	36	48	7	145	41	22	30	7	36
	DSI/DIP	6	4	4	19	29	35	7	93	39	17	19	5	55
	DSi/DIN	1.8	1.2	1	6	0.8	1.0	0	2	1	0.8	0.9	0	4
	Chl-*a* (mg m^{-3})	1.6	0.5	0.9	2.4	3.9	3.4	0.9	11.7	76.0	0.9	0.9	0.2	2.8
APRIL 09	**SS (mg L^{-1})**	10	3	6	19	12	4	7	23	8	5	2	1	9
	Salinity	37.2	0.2	36.6	37.4	24.5	15.4	1.0	37.2	0.2	37.1	0.6	35.2	37.7
	DO (mg L^{-1})	8.5	1.0	6.9	10.6	8.5	0.8	6.9	9.6	6.4	8.7	0.5	7.5	9.4
	DIN (µM)	5.91	1.79	3.24	8.36	27.29	20.28	4.50	55.16	52.10	7.08	3.04	1.41	12.68
	DIP (µM)	0.001	<0.001	0.001	0.001	0.165	0.157	0.020	0.463	0.350	0.330	0.466	0.049	1.959

		AHUIR				VENECIA				SERPIS	OUTFALL			
	jul-08	MEAN	S.D.	MIN	MAX	MEAN	S.D.	MIN	MAX	MEAN	MEAN	S.D.	MIN	MAX
	PT (µM)	0.18	0.06	0.07	0.33	0.74	0.39	0.34	1.42	0.40	0.43	0.48	0.09	1.91
	DSi (µM)	3.91	2.58	0.99	9.41	19.47	17.28	0.84	45.71	44.32	4.51	4.03	1.07	19.20
	DIN/DIP	11812	3584	6479	16726	165	279	65	407	149	21	39	6	104
	DSI/DIP	7823	5151	1985	18812	118	216	8	354	126	14	31	5	70
	DSi/DIN	0.7	0.4	0	3	0.7	1.3	0	1	1	0.6	1.5	0	3
	Chl-*a* (mg m^{-3})	2.4	2.3	0.8	9.2	3.7	1.6	1.8	6.4	71.5	0.8	0.2	0.5	1.2
JULY 09	**SS (mg L^{-1})**	11	12	5	60	17	5	9	27	12	10	2	5	12
	Salinity	37.4	0.2	37.1	37.6	33.4	5.5	20.2	37.4	1.3	37.4	0.2	37.0	37.7
	OD (mg L^{-1})	7.1	0.6	6.0	8.0	6.8	0.3	5.9	7.1	3.14	7.6	0.3	7.0	8.1
	DIN (µM)	1.10	0.92	0.22	3.59	20.18	15.99	3.88	47.36	53.87	1.73	1.24	0.11	4.07
	DIP (µM)	0.064	0.043	0.025	0.162	0.147	0.147	0.036	0.514	0.288	0.025	0.048	0.001	0.187
	PT (µM)	0.49	0.09	0.28	0.65	0.47	0.40	0.20	1.70	1.54	0.60	0.17	0.14	0.85
	DSi (µM)	2.96	0.95	1.15	5.33	23.29	22.58	3.16	64.06	142.22	2.97	1.38	1.25	7.01
	DIN/DIP	17	26	2	98	138	247	40	319	187	68	178	4	8150
	DSI/DIP	46	46	16	154	159	313	44	337	493	117	276	20	14022
	DSi/DIN	2.7	2.7	1	14	1.2	2.3	0	2	3	1.7	4.0	0	14
	Chl-*a* (mgm^{-3})	1.0	1.1	0.2	4.8	1.8	0.7	1.2	4.2	2.4	0.5	0.2	0.3	0.9

3.2 Nutrients concentration

In the Ahuir area, dissolved inorganic nitrogen (DIN) concentration ranged from 0.15 to 8.36 μM. The highest DIN values were observed in spring ($p < 0.01$), and ranged from 3.24 to 8.36 μM (Table 2). Nitrate was the most dominant nitrogen form in all sampling seasons. Dissolved inorganic phosphorus (DIP) concentration was significantly higher in summer ($p < 0.01$), with the highest values attained in July 2008 (ranging from 0.120 to 0.230 μM), while in spring, DIP values were below the analysis detection limit (Table 1). The lowest dissolved silicate (DSi) concentration was observed in July 2008 ($p < 0.01$), and ranged from 0.70 to 2.30 μM, while the maximum DSi values were attained in spring 2009 with 9.41 μM (Table 1).

In the Venecia area, nitrate was the most dominant nitrogen form in all sampling seasons, as was the case with the Ahuir area. Nitrate ranged from 2.17 to 50.83 μM, and DIN from 2.90 to 55.16 μM (Table 1). In this area no significant differences were observed in nitrate and DIN concentrations among sampling campaigns ($p > 0.05$). DIP concentration was significantly higher in July 2008 and ranged from 0.170 to 0.860 μM (Table 1). DSi showed no significant differences among sampling campaigns ($p > 0.05$).

In the submarine wastewater outfall area, nitrate was the most dominant nitrogen form in spring (average nitrate concentration was 6.88 µM and DIN was 7.08 µM) when DIN values were the highest ($p < 0.01$). Ammonium concentration was significantly higher in July 2008 ($p < 0.01$), when the DIN concentration was dominated by this ion (average ammonium concentration was 2.45 µM and average DIN was 4.32 µM). The lowest DIN and DIP ($p < 0.01$) values were observed in July 2009. DSi concentration did not show any statistical difference among the sampling campaigns ($p > 0.05$) and ranged from 1.07 to 19.20 µM (Table 1).

When the three study areas were compared, Venecia area showed significantly higher concentrations of nitrate, nitrite, DIN and DSi, than the Ahuir and the submarine wastewater outfall areas ($p < 0.01$ for all the variables). Venecia also showed the highest values for ammonium ($p < 0.01$) and DIP concentrations ($p < 0.01$); although in this case the submarine wastewater outfall area had higher values than the Ahuir area.

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3.3 Nutrient ratios

In order to better define potential nutrient control, nutrient ratios between DSi, DIN and DIP concentrations were compared with Redfield ratios (DSi:DIN:DIP = 16:16:1) in Figure 3.

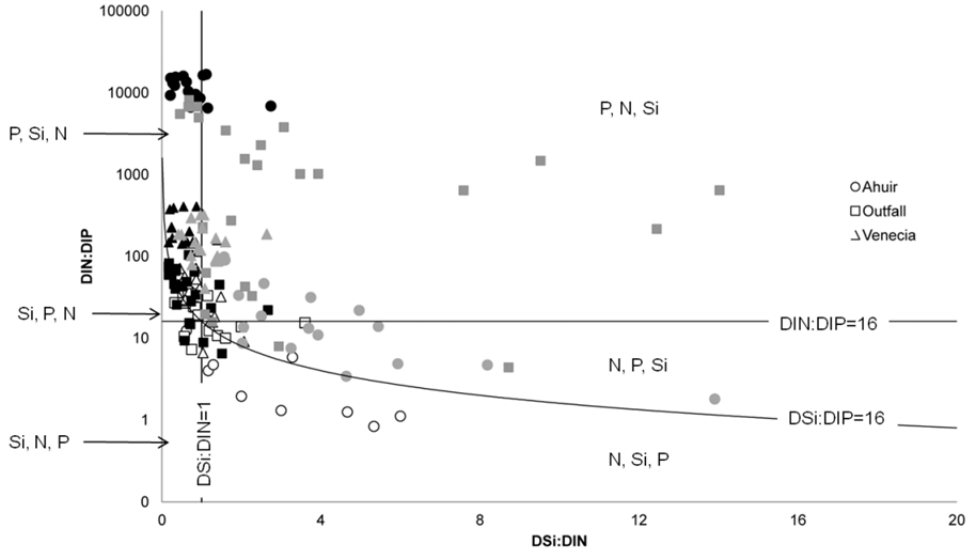

Figure 3 Synthetic graph of DSi:DIN:DIP molar ratios in the coastal waters of Gandia. In each area delimited Redfield ratios (DSi:DIN:DIP = 16:16:1), the potential limiting nutrients are reported in order of priority. Sample symbol correspond to the sampling site (circle –Ahuir, triangle – Venecia, square – Outfall). Sample symbol colours correspond to the season and year (white – summer 2008, black – spring 2009, grey summer – 2009)

In the Ahuir area, the highest DIN:DIP and DSi:DIP ratios were observed in spring 2009 ($p < 0.01$ for both variables), when average ratios were 11812 and 7823 respectively (Table 1) and clearly indicated a primary potential deficiency of phosphorus. DSi:DIN ratio ranged from 0.2 to 2.7 (Table 1), and in most of the sampling sites it was below the Redfield ratio (1:1) (Fig. 3). In summer, the lower DIN:DIP and DSi:DIP ratios, together with the higher DSi:DIN ratio, caused a change in the main potential limiting nutrient. In summer 2008, nitrogen was the first potential limiting nutrient at all the sampling sites, while in summer 2009 nitrogen was the first potential limiting nutrient only from A4 to A6 (all sampling depths), and phosphorus was the potential limiting nutrient from A1 to A3 (Fig. 3).

In the Venecia area the highest DIN:DIP and the DSi:DIP ratios were measured in the 2009 sampling campaigns ($p < 0.01$ for both variables). In July 2008, when these ratios were lower, DIN:DIP ranged from 7 to 145 (Table 1), and DSi:DIP ranged from 7 to 93 (Table 2). Phosphorus was the first potential limiting nutrient in all the sampling campaigns and sites, except for an isolated instance in July 2008 of a V3 subsurface sample, where the minimum of both ratios was attained (Fig. 3). The DSi:DIN ratio had significantly higher values in summer ($p < 0.01$) than in spring, however this ratio was always close to the Redfield one (Table 1).

In the submarine wastewater outfall area, the three nutrient ratios reached their highest values in July 2009 ($p < 0.01$ for the three ratios). While the DSi:DIP and DSi:DIN ratio showed no significant difference between July 2008 and April 2009, the DIN:DIP ratio was higher in April. Phosphorus was the first potential limiting nutrient at all sampling sites and depths in July 2009 (Fig. 3), while in the other sampling campaigns, the potential limiting nutrient alternated between nitrogen, silicate and phosphorus depending on the sampling site and water depth (Fig. 3).

Among areas, no significant difference was observed in the DIN:DIP ratio ($p > 0.05$). The DSi:DIN and the DSi:DIP ratios reached their highest values in the Ahuir area. While no difference was observed in the DSi:DIN ratio between the submarine wastewater outfall and the Venecia areas, significantly higher DSi:DIP ratio values were detected in the submarine wastewater outfall area.

3.4 **Phytoplankton abundance and composition**

The chlorophyll-*a* (chl-*a*) mean values and its main descriptive statistics are summarized in Table 1. Comparison of the three areas studied showed that the Venecia area had the highest significant chl-*a* values ($p < 0.01$), while the outfall area had the lowest values, which were mainly below 1 mg m^{-3}. In the three areas, the highest chl-*a* values were attained in spring (p <

0.01). The samples taken in the Serpis River reached the maximum chl-*a* values in July 2008 with 76.0 mg m^{-3} and in April 2009 with 71.6 mg m^{-3}, while in July 2009 the chl-*a* value was similar to that measured in the receiving waters (Table 1).

The pigment ratio values of the final matrix used to estimate the contribution of the different groups to chl-*a* stock are presented in Table 2. It is important to highlight that in the microscope screening of representative samples, two potentially blooming dinoflagellates were detected in both summers in the Venecia area: *Alexandrium* sp. and *Dinophysis caudata*. In Fig. 4 the spatial and seasonal average contribution of each phytoplankton group to total chl-*a* is represented as estimated with CHEMTAX. In this figure, dinoflagellates, cryptophytes, prasinophytes, chlorophytes, and euglenophytes were grouped as flagellates (prymnesiophytes, which also comprise non flagellate organisms, were represented separately).

Table 2 Output matrices of pigment to chl a ratios obtained from CHEMTAX for the samples of both clusters. Per: peridinin; 19'But: 19'-butanoyloxyfucoxanthin; Fuco: fucoxanthin; 19'Hex: 19'-hexanoyloxyfucoxanthin; Neo: neoxanthin; Pras: prasinoxanthin; Viol: violaxanthin; Allo: alloxanthin; Lut: lutein; Zea: zeaxanthin; Chl b: chlorophyll b. –: pigment not present in phytoplankton group. Cluster 2 includes freshwater samples: Serpis River samples and samples from river plume stations (V5, V6 and V7) from 0.05 to 0.10 m water column depth (only April 2009 and July 2009). Cluster 1 includes marine samples: all other samples

	Per	19'But	Fuc	19'Hex	Neo	Pras	Viol	Allo	Lut	Zea	Chl b
Diatoms											
Cluster1	-	-	0.169	-	0.001	-	-	-	-	-	-
Cluster2	-	-	0.246	-	0.000	-	-	-	-	-	-
Dinoflagellates											
Cluster1	0.524	-	-	0.169	-	-	-	-	-	-	-
Cluster2	0.231	-	-	0.008	-	-	-	-	-	-	-
Euglenophytes											
Cluster1	-	-	-	-	0.013	-	-	-	-	-	0.539
Cluster2	-	-	-	-	0.001	-	-	-	-	-	0.317
Chlorophytes											
Cluster1	-	-	-	-	0.071	-	0.030	-	0.130	0.057	0.091
Cluster2	-	-	-	-	0.001	-	0.090	-	0.176	0.002	0.198
Cryptophytes											
Cluster1	-	-	-	-	-	-	-	0.129	-	-	-
Cluster2	-	-	-	-	-	-	-	0.617	-	-	-
Prasinophytes											
Cluster1	-	-	-	-	0.068	0.090	0.114	-	0.027	0.073	0.147
Cluster2	-	-	-	-	0.086	0.094	0.045	-	0.020	0.012	0.298
Prymnesiophytes											

	Per	19'But	Fuc	19'Hex	Neo	Pras	Viol	Allo	Lut	Zea	Chl b
Cluster1	-	0.020	0.220	0.405	-	-	-	-	-	-	-
Cluster2	-	0.003	0.140	0.185	-	-	-	-	-	-	-
Cyanobacteria											
Cluster1	-	-	-	-	-	-	-	-	-	0.443	-
Cluster2	-	-	-	-	-	-	-	-	-	0.263	-

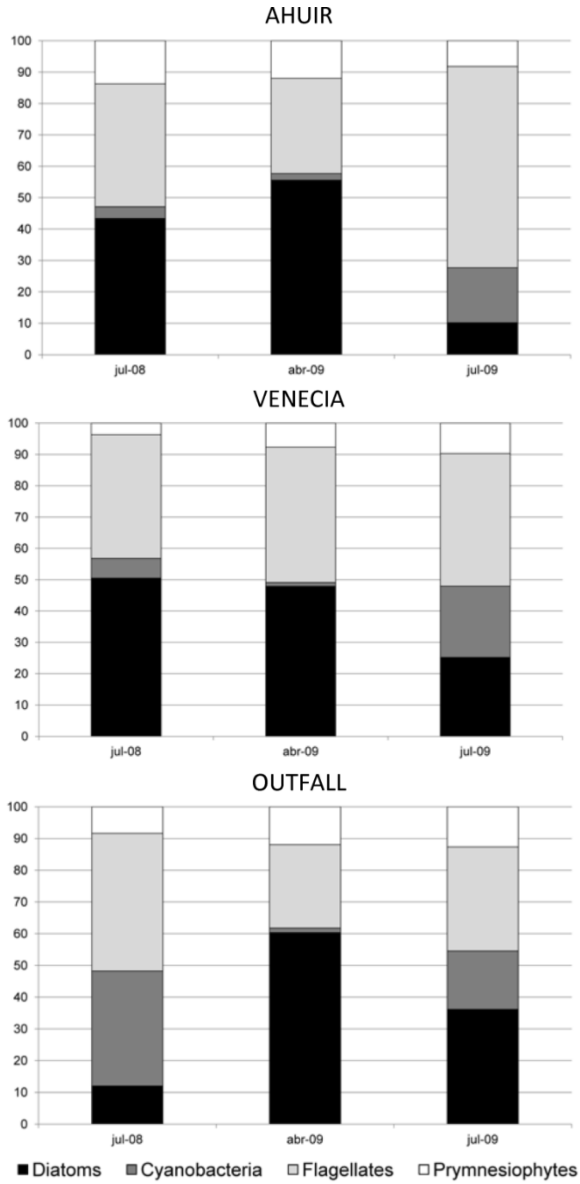

Figure 4 Relative contribution of each phytoplankton group as estimated with CHEMTAX. The average value for each study area and sampling campaign is represented. Dinoflagellates, cryptophytes, prasinophytes, chlorophytes, and euglenophytes were grouped as flagellates

In spring 2009, diatoms were the dominant group with a percentage of total chl-*a* higher than 50% in the three studied areas (Fig. 4); no significant abundance difference was observed among areas ($p > 0.05$). Flagellates were the second main group, and its contribution to total chl-*a* was nearly one third at the outfall and Ahuir areas, and nearly one half at Venecia (Fig. 4). Flagellate abundance showed no significant difference among areas ($p > 0.05$), but chlorophytes were more abundant in the Venecia area ($p < 0.01$). The contribution of prymnesiophytes to total chl-*a* was around 10% in the three areas (Fig. 4) (no significant abundance differences were detected, $p > 0.05$). The contribution of cyanobacteria was the lowest with approximately 2% of total chl-*a* (Fig. 4).

In the summer sampling campaigns cyanobacteria abundance significantly increased ($p < 0.01$) in the three areas (Fig. 4). In July 2008, the highest cyanobacteria contribution was found in the submarine wastewater outfall area ($p < 0.01$), where this group was the second main group (36% of total chl-*a*) after flagellates (44%) (Fig. 4). In July 2009, no significant cyanobacteria abundance difference was observed among areas ($p > 0.05$). Diatoms abundance was significantly lower than that observed in spring in the submarine wastewater outfall area in both summer campaigns ($p < 0.01$). In July 2008 the lowest diatom contribution was observed in this area ($p < 0.01$). In the Ahuir area, the summer diatom decrease was only significant in July 2009 ($p < 0.01$), while in the Venecia area the summer decrease, though observable, was not significant ($p > 0.05$) (Fig. 4). In the Ahuir area, a significant increase in flagellate contribution was observed (64% of total chl-*a*) in July 2009, mainly due to an increase in prasinophytes. Due to this increase, these groups' abundance was significantly higher than that observed at Venecia and the outfall areas ($p < 0.01$).

The results of the Redundancy Analysis (RDA) are displayed in Fig. 5. For a more detailed interpretation of the triplots, see Ter Braak (1994). The RDA analysis was performed with the HPLC-CHEMTAX data in terms of chl-*a* biomass. The RDA retained 6 variables: salinity, ammonium, nitrate, dissolved inorganic phosphorus (DIP), dissolved silicate (DSi) and DIN:DIP ratio. These variables together explained 31% of the variance in the phytoplankton composition.

The phytoplankton groups were classified according to their association with the environmental variables. The arrow length indicates the goodness of fit of each group's biomass to the displayed ordination, and acute angles between two vectors indicate a high positive correlation. In general, the phytoplankton groups can be divided into two main groups, depending on their response to a gradient related with nutrient concentration (Axis 1) or a gradient related with salinity (Axis 2) (Fig. 5). In the first group, diatom biomass was higher in waters with a high nitrate concentration and high DIN:DIP ratio, while cyanobacteria, prasinophyte and to a minor extent prymnesiophyte biomass was higher in low nitrate concentration and low DIN:DIP ratio. In the second group, euglenophyte and chlorophyte biomass was higher in low salinity, while cryptophyte and dinoflagellate biomass was higher at high salinity. In addition, the biomass of euglenophytes and chlorophytes was strongly correlated with a high ammonium concentration while cryptophyte and dinoflagellate biomass was strongly correlated with low silica concentration.

Samples taken from the Serpis River and river plume (Venecia area) reflected the salinity gradient in the spring and summer 2009 sampling campaigns; this gradient was more pronounced in spring (Fig. 5 black triangles). In general, the stations sampled in spring were clearly different from the stations samples in summer: summer samples (Fig. 5, white symbols year 2008 and grey symbols year 2009) were associated with lower nutrient concentration, and spring samples (Fig. 5, black symbols) with higher concentration.

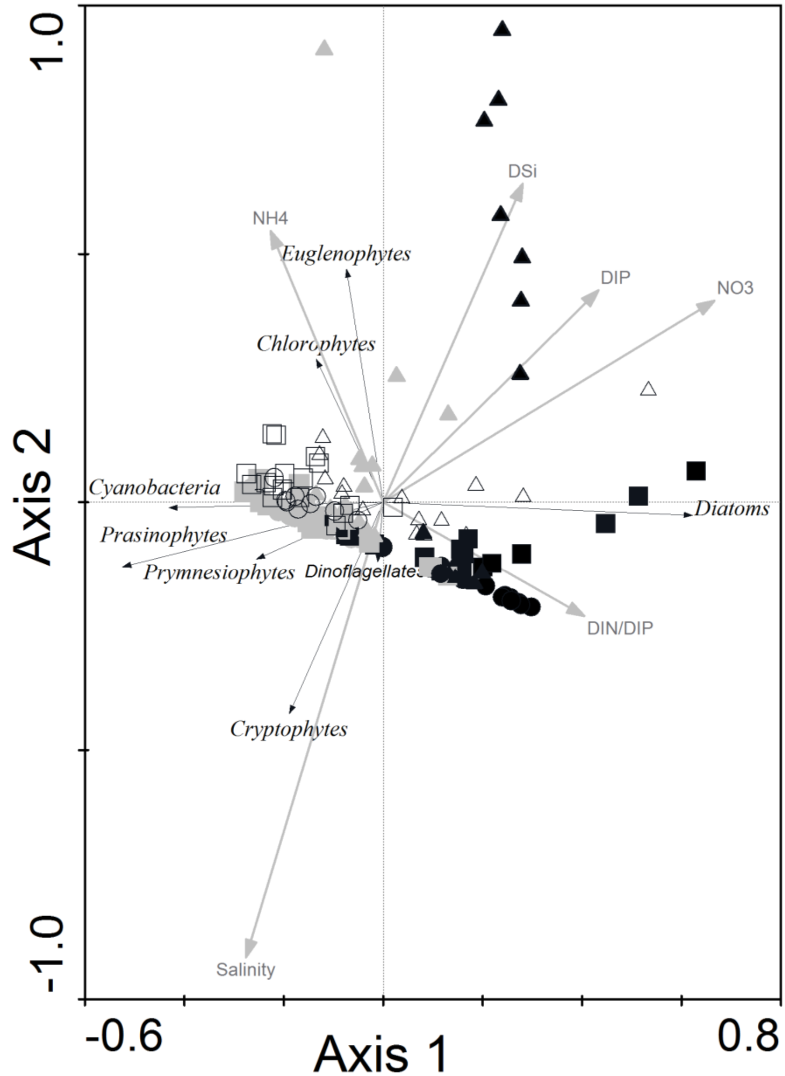

Figure 5 Correlation plots of the redundancy analysis (RDA), on the relationship between the environmental variables (grey arrows), the biomass of the phytoplankton groups (black arrows) and samples. Sample symbol correspond to the sampling site (circle –Ahuir, triangle – Venecia, square – Outfall). Sample symbol colours correspond to the season and year (white – summer 2008, black – spring 2009, grey summer – 2009)

4 Discussion

Climatically, autumn and spring are the rainy seasons while summer is the dry period in this Mediterranean area. So, higher salinity values were expected in the Ahuir and the Venecia areas in the summer sampling campaigns, due to reduced groundwater and river discharge respectively. In this area, summer groundwater discharge is also reduced because the summer population increase makes it necessary to increase pumping from the aquifer to supply drinking water from the aquifer wells. In addition, the aquifer provides freshwater for the citrus crops in the area which need at least two watering during summer. In the Ahuir, salinity was significantly higher in July 2009. Analysis of all the sampling points in the receiving waters revealed no significant salinity differences among seasons at Venecia but salinity at the Serpis river plume sampling points was lower in spring.

In contrast, the submarine wastewater outfall discharges are lower from autumn to spring and higher in summer, when the tourist population increases and thus the wastewater that needs treatment. In summer, the higher flow of wastewater makes it sometimes necessary to discharge partially through the overflow channel located near the river mouth. A wastewater discharge through the overflow channel could explain the low salinity value, 1.3, measured in the Serpis river sample in July 2009 (Table 1) and the plume samples classified into freshwater cluster when river flow was null at the CHJ gauging stations, which are located 10 km from the river mouth. The lower dissolved oxygen concentration and the higher suspended solids measured in the receiving waters reinforce this hypothesis. In addition, significantly higher salinity values were observed in the submarine wastewater outfall area in this sampling campaign, thus indicating a reduced discharge.

The dominant nitrogen forms followed the expected spatial and seasonal pattern. In the Ahuir and Venecia areas, nitrate was the most dominant form due to agriculture runoff (both groundwater and surface runoff). At the submarine wastewater outfall, a major ammonium contribution was expected in the summer campaigns when the flow was greater due to the population increase. However, this major ammonium contribution was observed only in July 2008. A wastewater release through the overflow channel directly into the Serpis River in July 2009, and then a lower flow in the outfall outlet, could explain the nitrate dominance in this sampling campaign. In fact, samples from the Serpis River and river plume followed the ammonium gradient in this campaign (Fig. 5, grey triangles).

When the three areas were compared, the lowest salinity values, the highest nutrient concentrations (ammonium, DIN, DSi and DIP) and the highest chl-a concentration were observed in the Venecia area (Kruskal-Wallis test). In this area, seasonal variations were minimal. Despite the reduced summer discharges of the Serpis River, no significant differences were observed in the DIN and DSi concentration between seasons, while the ammonium concentration was higher in July 2009. This was attributed partially to the sporadic releases of wastewater through the described overflow channel in the dry periods, which are an additional silica and nitrogen source. In addition, the high residence time of this semienclosed bay leads to higher nutrient accumulation and to greater exchanges with the sediment interface given the shallow water column depth (< 5 m). Silica dissolution at the sediment-water interface could be efficient during summer due to high temperatures and higher bacterial abundance, which has been pointed out as the cause of high silica levels in similar areas (Glé et al. 2008; Garmendia et al. 2010). In this area, phosphorus was always the potential limiting nutrient and phytoplankton composition and abundance has not shown any significant seasonal difference (except for the summer cyanobacteria increase and a minor chlorophyte contribution in July 2009).

In the Ahuir area, DIN and DSi concentrations were highest in April 2009 (Kruskal-Wallis test results) due to higher groundwater discharge, which is rich in both nitrates and silicates. A seasonal variation in the potential limiting nutrient was observed also: phosphorus was potentially limiting in spring, while a nitrate potential limitation was found in summer. This summer nitrogen deficiency, which is linked to the decrease of freshwater inputs, has been observed in other coastal areas and has been attributed also to autotroph consumption (Glé et al. 2008). The highest DSi:DIN and DSi:DIP ratios were found in this area, so despite the summer silica depletion, silica was not the potential limiting nutrient. In this area ammonium and DIP concentrations were lower than in the Venecia and the submarine wastewater outfall areas (Kruskal-Wallis test results); no direct wastewater discharge was found in the Ahuir, while the other areas received wastewater discharges, rich in both nutrients.

In the submarine wastewater outfall area (located 1.9 km from the coastline and 17.5 m water column depth), the dilution of treated wastewater with marine water results in dominant phosphorus limiting conditions, given that phosphorus has been identified as the most important limiting nutrient in the Mediterranean (Estrada 1996; Olivos et al. 2002).

Previous research has been developed further north in the Balearic sea coastal area (north-western Mediterranean) as reviewed in Olivos et al. (2002). This research focused on the spatial gradient of nutrients and chlorophyll-*a* in relation to their distance from the land (sample stations were grouped into coastal field, middle field and far field). Using the Olivos et al. (2002) sampling point classification, our three study areas fall into the coastal field (approximately within the first 2 km from the coast). Average chl-a concentration in the 3 areas falls into the range observed by Olivos et al. (2002) in the coastal field. Olivos et al. (2002) highlighted the silicate limitation in more than 50% of the cases and explained it in terms of the excess of N and P overshadowing the Si abundance. In contrast, in our study area, Si-limited conditions were scarce and that allowed the persistence of the diatom population at high levels even in summer. The Safor wetland location over the detritic aquifer can explain the high silica groundwater discharges in our study area. Wetland species of Gramineae are characterised by high silica content (typically 10-15% dry shoot weight); this biogenic silica, after decomposition of organic material, remains in the soil and it is lixiviated to the aquifer (Conley 2002). Even if the natural vegetation surface has decreased in the last decades, it still remains an important soil use. Thus, biogenic silica is an important element in the terrestrial biogeochemical cycle that must be considered in addition to the chemical weathering of land silicates.

The spatial gradient observed in phytoplankton biomass (total chl-*a*) both in spring and summer can be explained by the different factors controlling the dynamics of different phytoplankton groups, mainly nutrient availability and water exchange. In the Venecia area, the continued nutrient inputs and the reduced water exchange (high water residence time) explained the higher biomass, while in the submarine wastewater outfall area, despite the nutrient inputs, the dilution with low phosphorus marine waters determined the lowest chl-*a* values.

In the Ahuir and the submarine wastewater outfall areas, phytoplankton abundance and composition showed the typical seasonal cycle observed in other coastal areas. A shift from diatom-dominated communities and maximum phytoplankton biomass (total chl-*a*) in spring, to flagellate-dominated communities and minimum biomass in summer was observed (Garmendia et al. 2010). This can be observed in the RDA analysis where the stations sampled in spring (Fig. 5, black circles and squares) produced clearly different results from the stations sampled in summer (Fig. 5, white and grey circles and squares). Diatoms are recognised as the most opportunistic species as far as taking advantage of nutrient availability is concerned (Fogg 1991). In this study, diatoms showed a significant positive correlation with nitrate, phosphorus and silica concentration, and with DIN:DIP ratio and their abundance was higher when these conditions prevailed (RDA, Fig. 5). The cyanobacteria, prasinophyte and to a minor extent prymnesiophyte biomass correlation with low nitrate concentration and low DIN:DIP ratio (RDA, Fig. 5) has been linked to more oligotrophic conditions that mainly happen in summer (dry period) in this area (Sebastiá et al. 2012; Chapter 5 of this document).

In the Venecia area summer decrease in diatom abundance was not significant according to the Kruskal-Wallis test results. The key factor supporting the summer population seems to be the continuous availability of silica (even in the dry period), together with the optimal light and temperature conditions. This has also been observed in other estuarine areas, such as the Oka estuary (Bay of Biscay, Northern Spain) (Garmendia et al. 2010), which receive wastewater discharges from a treatment plant. A shorter time scale study (fortnightly) was developed from May 2008 to August 2009 in this area (Sebastia et al., Chapter 5 of this document), which confirmed that diatom relative abundance did not show the typical seasonal cycle of maximum abundances in spring and minimum abundances in summer. Diatom minimum abundance is observed after freshwater inputs as they are replaced for increases in flagellates (Chapter 5 of this document), as it happens in other estuarine areas where flow and salinity play a significant role in regulating phytoplankton communities (Chan and Hamilton 2001). In the RDA analysis (Fig. 5) two situations can be observed that reflect the freshwater inputs: 1) samples taken in April 2009 (black triangles) are linked to higher freshwater discharge after a rain episode with high silica concentration, and 2) samples taken in July 2009 (grey triangles) are linked to a wastewater release with high ammonium concentration. In these samples, both euglenophyte and chlorophyte biomass was higher. Chlorophytes are restricted to freshwater conditions (Chan and Hamilton 2001).

Dinoflagellates were a minor group in terms of relative abundance and total biomass in the three studied areas. However, their importance should not be disregarded since the dinoflagellates identified in the microscope analysis, *Alexandrium* sp. and *Dinophysis caudata*, are potentially Paralytic Shellfish Poisoning (PSP) and Diarrheic Shellfish Poisoning (DSP) producers respectively (Vila et al. 2001). In addition, potentially toxic diatoms of the genus *Pseudo-nitzschia*, which synthesises the ASP toxin, have been recorded inside the harbour (Sebastia et al. 2012). Blooms of this genus have also been observed northerly in the western-Mediterranean (Vila et al. 2001). In the Mediterranean Sea, Harmful Algal Blooms (HABs) are a widespread problem and the proliferation of these harmful dinoflagellates (*Alexandrium* sp. and *Dinophysis* sp.) has been related with conditions of low turbulence and flushing rates (Vila et al. 2001, CIESM 2010). Semi-enclosed gulfs and bays, such as the Venecia area, near important harbours and big cities are at higher risk of suffering HABs (EEA 1999, Vila et al. 2001). In these sense, the construction of new harbours or the enlargement of existing ones may affect coastal hydrodynamics and then enhance these conditions (CIESM 2010).

In this study, we found that at the Ahuir and the submarine wastewater outfall areas the effects of freshwater inputs (groundwater and wastewater discharge respectively) were reduced due to a greater water exchange with the oligotrophic Mediterranean waters. On the other hand, the highest nutrient and chl-*a* concentrations were observed in the Venecia area, which is a semi-enclosed area. This agrees with the observations of Flo et al. (2011) for the north-western Mediterranean Sea. They highlighted that the dominance of urban land and the presence of river inflows are the primary source of coastal inshore waters (CIW, 200 m from the shoreline) variability; but their impact on coastal nearshore waters (200-1500 m from the shoreline, such as the Ahuir area) or coastal offshore waters (> 1500 m, such as the submarine wastewater outfall area) is minimal. In CIW, nutrient enrichment and eutrophication risk are higher in enclosed or semi-enclosed areas (Flo et al. 2011), such as the Venecia area in this study.

Harbours can create semi-enclosed areas partially due to the alteration of coastal morphology and water currents. This alteration has been pointed out as one potential environmental impact of harbours (Gupta et al. 2005; Peris-Mora et al. 2005; Darbra et al. 2009). The alteration of sea floor has been selected as the only indicator for monitoring this impact in some environmental management plans (EMP) (Peris-Mora et al. 2005); while others include water quality parameters such as chlorophyll-a concentration, phytoplankton biomass and ratio of diatom/dinoflagellates (Xu et al 2004). The results of this study suggest that monitoring of the above mentioned water quality parameters should be included in harbour's EMP. The research of Vila et al. (2001) reinforces this statement as they observed that "the monitoring of confined waters could be used as an early warning system for detection of algal blooms". Moreover, according to Flo et al. (2011), it is important that monitoring programs (e.g. Water Framework Directive monitoring program), avoid overlooking environmental problems in localized areas.

5 Conclusions

In this study three Mediterranean coastal systems have been compared. The results showed that the highest nutrient concentration and phytoplankton biomass were measured in the Venecia area, which is a semi-enclosed area. In this area potentially toxic dinoflagellates were detected, even its abundance was minor. Two factors coincide in this area that may trigger dinoflagellates blooms: 1) the harbour enlargement will increase the vulnerability of this system and maybe provide the proper conditions of confinement, 2) the wastewater discharges through the overflow channel to the Serpis River will continue supplying phosphorus. Management measures should first target phosphorus inputs, as this is the most potentially limiting nutrient in the Venecia area and it comes from a point source that is easier to control.

Our recommendation is that for successful integrated management, local problems should be taken into account in monitoring programs. This is especially important in the Mediterranean Sea and in seas with similar characteristics (e.g., low tidal ranges) (EEA 1999; Flo et al. 2011). More specifically, harbour environmental management plans should include regular monitoring of water quality in adjacent waters to identify adverse phytoplankton community changes. The use of HPLC analysis and CHEMTAX could help to do so at a reasonable cost.

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Capítulo 5 Nutrients and phytoplankton dynamics in the receiving waters of a Mediterranean river: the wet and the dry season

1 Introduction

Estuaries and coastal areas receive high loads of nutrients of terrestrial origin, either from point sources, which flow out at discrete and identifiable locations (e.g. rivers, submarine wastewater outfalls) or non-point sources, which are rather diffuse and highly variable from year to year depending on climate and rainfall (e.g. surface and groundwater runoff) (Paerl, 2006). Among nutrients, nitrogen and phosphorus are both required to support marine productivity and are the key limiting nutrients in most aquatic and terrestrial ecosystems. However, the increase in these nutrients due to anthropogenic activities requires their inputs into coastal marine ecosystems to be reduced in order to minimise eutrophication problems (Paerl et al., 2010). The influence of these terrestrial inputs is especially important in the marine oligotrophic areas, such as the Mediterranean Sea (Romero et al., 2007).

Several policies have been adopted to reduce nutrient inputs with varying degrees of success depending on the source (Artioli et al., 2008). While improvements in wastewater treatment have achieved reductions in inputs of phosphorus and nitrogen from point sources, the largest nutrient contribution for coastal marine environments is from diffuse sources, especially those from agricultural land runoff (fertilizers), and reducing this is a difficult and slow process (Carstersen et al., 2006). Then, there is a tendency to consider nutrients from wastewater of minor importance for the environment compared with nutrients from agriculture (Torrecilla et al., 2005).

Rivers play a major role in the delivery of nutrients to coastal ecosystems, both from natural (e.g. silicates weathering) and anthropogenic (urban and industrial wastewater, agriculture runoff) sources. Wastewater treatment plants (WWTPs) can discharge treated effluent to rivers or to coastal areas through submarine wastewater outfalls. The objective of the 91/271/EEC Council Directive, concerning urban wastewater treatment, was to protect the environment from the adverse effects of wastewater discharges. Different studies have demonstrated that the submarine outfall discharges of treated effluent which comply with the Directive, have either no significant effects or only minor effects on the quality of receiving waters

(Juanes et al., 2005). However, wastewater discharge can cause water quality problems in rivers and this is especially relevant in Mediterranean rivers because of the hydrologic singularities of the Mediterranean climate and fluvial regime (Torrecilla et al., 2005).

In Mediterranean-type climate regions (areas surrounding the Mediterranean Sea, parts of western North America, parts of western and southern Australia, the south-west of South Africa and parts of central Chile), characteristic precipitation is scarce and torrential and has an extremely high spatial and temporal variability. Natural river discharge, driven by precipitation variability, has two distinct seasons: wet and dry (Gasith and Resh, 1999; González-Hidalgo et al., 2005). Thus, wastewater discharges present different problems depending on the season.

During the wet season combined sewers overflows (CSO) can reach receiving waters without treatment (Clark et al., 2007). The importance of storm overflows for water quality have been recognized by the 91/271/EEC Directive, Annex I: *the design, construction and maintenance of collecting systems shall be undertaken in accordance with the best technical knowledge not entailing excessive costs, notably regarding limitation of pollution of receiving waters due to storm water overflows*.

During the dry season, wastewater discharge can account for up to 90% of the total river flow (Molinos-Senante et al., 2011). In addition, fluctuating winter and summer populations in tourist areas, typical on Mediterranean coasts, provoke significantly variable wastewater loadings that can exceed the capacity of the treatment facilities (Aguilera et al., 2001; García-Pintado et al., 2007).

Several studies have addressed the quality problems of Mediterranean rivers receiving wastewater discharges, for instance, on the Mediterranean coast of the Iberian Peninsula, Torrecilla et al. (2005) studied the Ebro River (NE Spain) and Molinos-Senante et al. (2011) studied the Serpis River (Eastern Spain). On the other hand, studies on receiving coastal waters have mainly addressed the water quality in the submarine outfall areas (Aguilera et al., 2001) and not river mouths. Some studies of Californian recreational bathing beaches have addressed the effects of CSO discharges but have focused on fecal indicator bacteria (total coliforms, fecal coliforms and enterococci) and benthic invertebrates (Schiff et al., 2003; Clark et al., 2007).

Nutrient inputs into coastal ecosystems can induce eutrophication problems, among these, harmful algal blooms of species responsible for the synthesis of toxins and high-biomass producers that can cause hypoxia and anoxia and indiscriminant mortalities of marine life after reaching dense concentrations (Heisler et al., 2008). As regards marine recreational bathing beaches, these effects can cause beach closures that negatively impact the local economy of tourist areas. Thus, adequate management of nutrient sources is essential because clean water and healthy coastal habitats are clearly fundamental to successful coastal tourism which is characteristic of the Mediterranean climate regions (Hall, 2001). However, to develop appropriate management strategies, it is necessary to fully understand how ecosystems function by first of all establishing the relationships between phytoplankton and nutrient sources and patterns.

This study analyzes the seasonal variation of phytoplankton communities in the coastal receiving waters of the Serpis River input, which is a Mediterranean river basin in Spain. A comparison is established with marine waters to assess different patterns between high and low terrestrial influence areas. Two scenarios are analyzed: the wet and the dry period with distinctive characteristics. The aim is to provide information about ecosystem functioning that could help management decisions and could be extrapolated to other Mediterranean climate areas with similar characteristics.

2 Materials and methods

2.1 Study area

The study area (Fig. 1) is located in the East of the Iberian Peninsula, at the southernmost sector of the Valencian Gulf (Western-Mediterranean Sea) on the coast of the Safor County (Spain). The main annual rainfall is above 600-650 mm in the entire County, with values above 800 mm in the rainiest areas, with most precipitation concentrated into short-episodic storm events in autumn. For a 10-year return period daily rainfall is expected to be higher than 200 mm (Hermosilla, 2005).

The main river draining this area is the Serpis River which has a basin of 753 km^2 and is 74.5 km in length. The Serpis River receives freshwater from its tributary the Vernissa River (150 km^2). Both rivers have a Mediterranean regime characterized by a high seasonality, with a dry period during summer, and a wet period mainly in autumn (Hermosilla, 2005). These rivers are artificially regulated by a complex system of weirs and irrigation channels that provide freshwater for the irrigated crops of the Safor County. The Beniarrés reservoir (30 hm^3 maximum capacity) (Fig. 1) was built in the second half of the 20th century to increase the regularity of the Serpis River (Hermosilla, 2005).

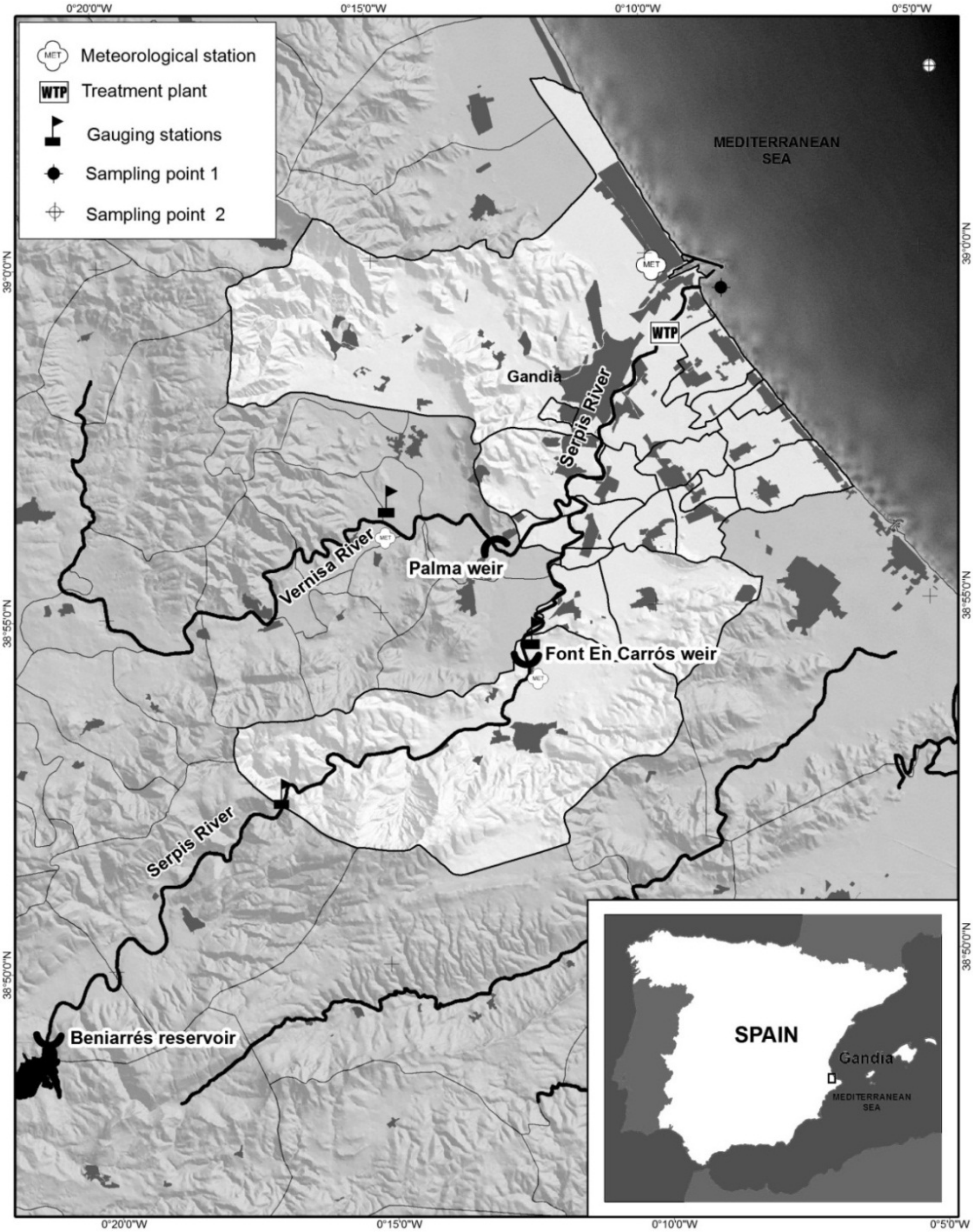

Figure 1 Study area and sampling points location

In the Serpis Basin there are 30 WWTPs that receive wastewater from the main urban and industrial areas, treating around 31 Mm^3 per year. In total, 24 of the 30 plants discharge treated water to the Serpis River, while the remaining six plants discharge treated water into the sea (Molinos-Senante et al., 2011). Downstream of the Beniarrés reservoir, the most important WWTP is the Gandia plant (Fig. 1) which serves a total of 133300 inhabitants from 17 municipalities of the Safor County. This plant discharges 17 million m^3 of treated wastewater per year to the sea through a submarine outfall (approximately 1900 m from the Serpis river mouth); however it also has two overflow channels that discharge directly to the river. One channel discharges besides the plant and the other near the river mouth.

The first overflow channel is used when the plant capacity is exceeded due to rain episodes. The plant treats an average flow of 46350 m^3 day^{-1} and its design flow is 60000 m^3 day^{-1}, which makes a difference of 13650 m^3 day^{-1}. But the urban area without wastewater and pluvial waters separation amounts to nearly 15 million square meters (see areas in Fig. 2) with almost zero infiltration. So, even small rain episodes cause combined sewer overflows because the plant capacity is exceeded. The wastewater collector, depicted in Fig. 2, is parallel to the Serpis River and combined sewer is also discharged into the Serpis directly from the collector when its capacity is exceeded.

The second overflow channel is generally used in summer, when the plant capacity is exceeded due to the population increase. The average density in the coastal municipalities of the Safor county is 963 inhabitants km^{-2} in winter (1314 inhabitants km^{-2} in Gandia city) (INE, 2009), but that figure triples in summer due to tourism activity which is mainly based on residential development (Mantecón and Huete 2007).

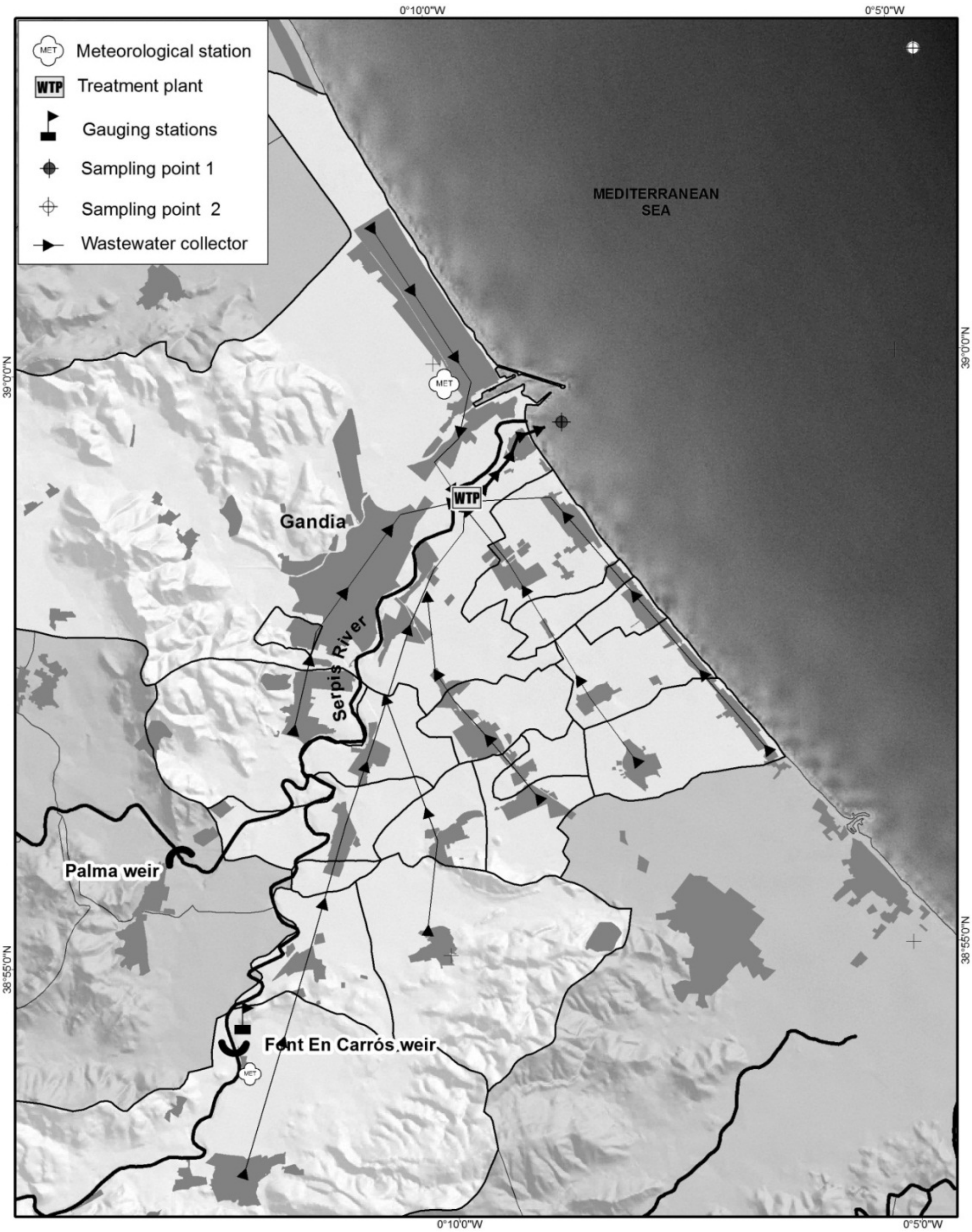

Figure 2 The combined sewer is depicted through the study area municipalities to the Gandia treatment plant

The Serpis River lower basin is characterised by dominant urban and agricultural uses. The alluvial plain was totally occupied by the Safor wetland and crops until the seventies. Nowadays, this area shares its agricultural activity, mainly citrus fruits and vegetables, with the tourism of the urban areas. The recommended nitrogen doses for citrus and horticultural crops have been traditionally exceeded in this area (MARM 2010). In consequence, both surface runoff and groundwater flow are characterized by high nitrate levels. In the detritic aquifer, nitrate levels have exceeded the limit of 50 mg L^{-1} established by the Nitrates Directive (Directive 91/676/EEC), and it has been declared a Nitrate Vulnerable Zone.

The Serpis River outflows on the southern side of Gandia Harbour creating an enclosed area due to the orientation of the harbour entrance channel (Fig. 1). The dominant currents from the southeast contribute to increased water residence time in this area. High nutrient inputs to ecosystems with low water renewal rates are especially important for water quality deterioration.

2.2 Sampling design

Field work was done from May 2008 to August 2009, with an average interval of 15 days. Sampling frequency was higher in the warm seasons, while in January and February 2009 there was no sampling due to bad weather conditions. Sampling took place at two stations along a gradient of distance from the landside (Fig. 1). Point 1 (CW, coastal waters) was located near the coastline (7.5 m depth), south of Gandia Harbour and facing the Serpis river mouth. Point 2 (MW, marine waters) was located at approximately 9 km from the coastline (36.5 m depth) outside the imaginary line that would link the Cullera Cape and La Nao Cape and which separates coastal waters from marine waters. The sampling points were chosen to compare the nutrient concentrations and seasonal variations of phytoplankton in two areas characterized by high (CW) and low (MW) influence of terrestrial inputs.

Temperature and salinity (expressed as practical salinity units, psu) were measured in the water column with a High Accuracy Conductivity, Temperature and Depth Meter (NXIC CTD). Samples were collected with Niskin bottles near the surface and with a Van Dorn bottle 50 cm above the bottom at each sampling point. Water samples were kept in a cool box (4ºC) and transported to the laboratory.

Rainfall and river flow data for the studied period were available from the Vernissa and Font En Carrós meteorological and gauging stations of the Júcar Hydrographic Confederation (CHJ). The Vernissa gauging station measures the Vernissa River flow, while the Font En Carrós gauging station measures the Serpis River flow before receiving the Vernissa input (Fig. 1). Rainfall in this study is presented as daily data (Fig. 3). The cumulative values of the 5 days (120 h) prior to sampling were determined and used in further analysis.

2.3 **Laboratory analysis**

The following parameters were analysed in all the samples: salinity, suspended solids (SS), nitrate, nitrite, and ammonium, dissolved inorganic phosphorus (DIP), total phosphorus (TP) and dissolved silicate (DSi). Dissolved inorganic nitrogen (DIN) was calculated as the sum of nitrate, nitrite and ammonium. Salinity was determined by means of an induction conductivity meter Multi 340i/SET WTW, using the Practical Salinity Scale. Nutrients were analysed colorimetrically using the method of Aminot and Chaussepied (1983). A persulphate digestion (Valderrama, 1981) was made for TP and the phosphate was subsequently analyzed. Dissolved oxygen concentration was measured using the Winkler titration technique (Grasshoff, 1983).

Samples for phytoplankton pigment analysis were filtered on GF/F fiberglass filters (25mm diameter). Pigments were extracted using acetone (100%, HPLC grade) and were measured using reverse-phase high-performance liquid chromatography (HPLC). The HPLC method employed was that proposed by Wright et al. (1991) slightly modified as per Hooker et al. (2000). The system was calibrated with external standards obtained commercially from the DHI Water and Environment Institute (Hørsholm, Denmark). Once the concentration of important photosynthetic pigments was determined, the phytoplankton community was studied using the CHEMTAX program (Mackey et al. 1996) version 1.95 (S. Wright, pers. comm.) to obtain the contribution to chlorophyll *a* (chl-*a*) of the 8 phytoplankton groups present (diatoms, dinoflagellates, euglenophytes, chlorophytes, cryptophytes, prymnesiophytes, prasinophytes and cyanobacteria). To obtain reliable results, CHEMTAX was applied following the procedures described in Latasa et al. (2010) and using the same parameters (elements varied = 10). The average of the last 6 ratio estimations was incorporated into the final pigment ratio matrix when a clear convergence was observed. In the absence of a clear convergence, the average of each pigment ratio was incorporated. This final matrix was then used to estimate the contribution of the different groups to chl-*a* stock.

2.4 **Statistical analysis**

In order to identify groups of samples with similar characteristics, a cluster analysis was performed using SPSS 16.0 to group samples according to salinity and nutrients (DIN, DIP and DSi). Squared Euclidean distances were calculated and samples clustered according to Ward's method. CHEMTAX was applied independently to each identified subset to obtain the contribution of each phytoplankton group to the chl-*a* stock (Latasa et al., 2010).

A non parametric one-way analysis of variance (Kruskal-Wallis) was used to test differences in physico-chemical variables and phytoplankton composition and abundance between sampling points and seasons (wet and dry seasons).

Spearman rank correlation analyses were performed on environmental variables (Serpis flow, rain, salinity and dissolved oxygen) and nutrient concentrations (NH_4^+, DIN, DSi, DIP, TP) in order to examine significant relationship between environmental variables and nutrient concentrations in coastal waters. Significant relationship between physical-chemical variables (temperature, salinity, DO, NH_4^+, DIN, DSi, DIP, DIN:DIP, DSi:DIP, DSi:DIN) and phytoplankton groups was evaluated in coastal and marine waters.

3 Results

3.1 Environmental variables

The rainy season started in September 2008 and lasted until June 2009 (Fig. 3). Maximum rainfall was registered in October, 376 mm at Vernissa and 562 mm at Font En Carrós, with a daily maximum of 111 mm at Vernissa and 139 mm at Font En Carrós, where the 100 mm threshold was exceeded three times in October. Summer was the dry season, with 14 mm of average rainfall.

The general pattern of the Vernissa River flow rates recorded during the study period (Fig. 3) followed a seasonal cycle characterised by a 6.3 $m^3 s^{-1}$ average flow in the rainy season and 0 $m^3 s^{-1}$ values during summer. The Serpis River flow, recorded at the Font En Carrós station, was 1.1 $m^3 s^{-1}$ on average in the rainy season. During summer a water flow higher than 30.0

$m^3\,s^{-1}$ (Fig. 3) was registered on 5 isolated days due to water releases from the Beniarrés reservoir to satisfy crop irrigation needs. Flow showed a significant correlation with rain which was weaker for the Font En Carrós station (r_s = 0.378, $p < 0.001$) while at Vernissa station, flow was r_s = 0.649, $p < 0.001$. No significant correlation was found between the flow measured at the Vernissa and the Font En Carrós gauging stations (Fig. 1) (r_s = 0.188, $p > 0.05$). At the coastal water station, NH_4^+, DIN and DSi concentrations were significantly correlated with rain and Serpis flow, calculated as the sum of the flow measured at the above mentioned gauging stations (Table 1). DIP concentrations did not show any significant correlation with environmental variables or nutrient concentrations (Table 1). TP was significantly correlated with rain but not with Serpis flow (Table 1).

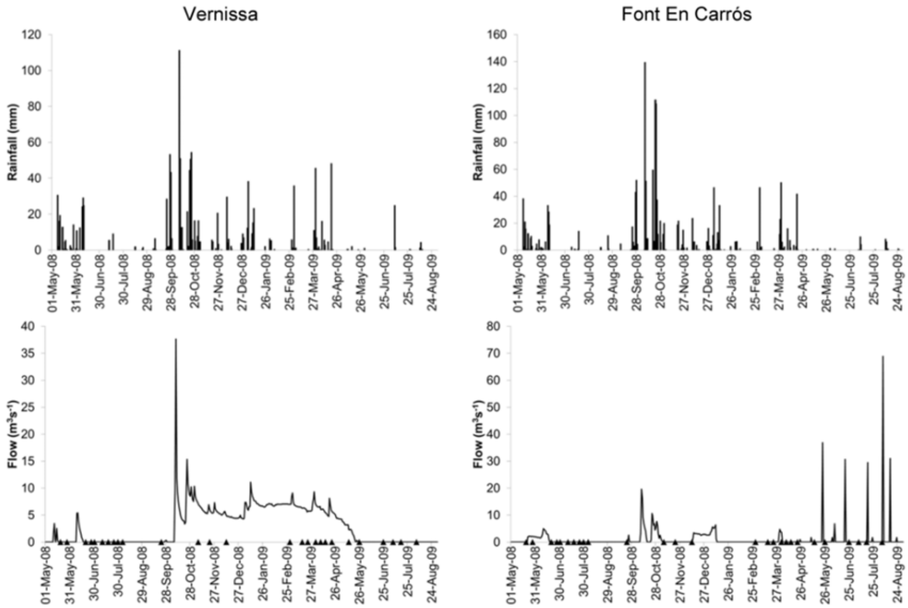

Figure 3 Daily rainfall and flow registered in Vernissa and Font En Carrós (CHJ) meteorological and gauging stations. The sampling dates are pointed out by triangles

Water temperatures during the study period followed a seasonal evolution characterised by the lowest values in winter and maximum values in summer. Surface temperature was similar in CW (P1) and MW (P2) with a minimum value of 12.3ºC in winter and maximum value of 27.9ºC in summer. Bottom temperature was generally lower in MW (7.5 m depth CW and 36.5 m depth MW), where the lowest temperature, 11.8ºC, was observed in March. By the end of the summer, the temperature gradient between surface and bottom waters was 1ºC in CW and 10ºC in MW. Dissolved oxygen concentration ranged from 6.4 to 9.9 mg L^{-1} at CW, and from 6.7 to 10.5 mg L^{-1} at MW, with higher values from December to April. While water column temperature at the two sampling points was statistically different ($p < 0.05$), no significant differences ($p > 0.05$) were observed in the oxygen concentration, although it was slightly higher in marine waters.

Table 1 Rank correlation matrix (Spearman's rs) of main physical and chemical parameters measured in Gandia coastal waters (n=54) from May 2008 to August 2009. ***p < 0.001, **p < 0.01, *p < 0.05

	Salinity	DO	NH_4^+	DIN	DSi	DIP	TP
Serpis flow	-0.04	0.53***	0.28*	0.35*	0.39**	0.00	-0.04
Rain	-0.32*	0.25	0.50***	0.41**	0.30*	0.09	0.30*
Salinity		0.04	-0.32*	-0.37**	-0.24	0.02	-0.39**
DO			0.12	0.27*	0.27*	-0.14	-0.12
NH_4^+				0.23	0.27*	0.14	0.52***
DIN					0.56***	-0.05	0.05
DSi						0.19	0.16
DIP							0.29*

Salinity values at CW were significantly lower than at MW ($p < 0.001$); mean values for the study period were 35.4 and 37.0 in CW and MW surface waters, and 36.8 and 37.4 respectively in bottom waters. At CW, salinity was significantly lower in surface waters ($p < 0.05$) and water column salinity was inversely correlated with rain (rain accumulated in the 5 days prior to sampling, Table 1); however, no correlation was observed with the Serpis flow (Table 1). At CW, salinity also showed a negative correlation with NH_4^+, DIN and TP concentrations (Table 1). At MW, no significant differences were observed between surface and bottom waters ($p > 0.05$). The seasonal variation of salinity can be observed in Fig. 4. Salinity differences between the rainy and the dry season were more pronounced at CW: average surface salinity was 35.7 in the rainy season and 36.7 in the dry season, while minimum values were 24.3 and 33.8 respectively (MW average surface salinity 37.2 in both seasons). However, seasonal differences were not significant at either station ($p > 0.05$).

Suspended solids ranged from 6 to 19 mg L^{-1} at CW and from 2 to 15 mg L^{-1} at MW, and were significantly higher at CW ($p < 0.001$).

3.2 **Nutrient concentrations and ratios**

Analysis of variance showed that DIN ($p < 0.001$), DSi ($p < 0.05$) and TP ($p < 0.01$) concentrations were significantly higher at CW, as well as the DIN:DIP ratio ($p < 0.05$), while the DSi:DIN ratio was significantly lower ($p < 0.05$). No significant differences between sampling points were found in NH_4^+ and DIP concentrations ($p > 0.05$) or in the DSi:DIP ratio ($p > 0.05$). At CW, DIN and DSi concentrations ($p < 0.001$) and the DIN:DIP and DSi:DIP ratios ($p < 0.05$) were significantly higher in surface water samples. At MW, water column characteristics were more homogenous: there were no statistical differences between nutrient concentrations (NH_4^+, DIN, DIP, TP and DSi) ($p > 0.05$) and nutrient ratios ($p > 0.05$) of surface and bottom waters.

The cluster analysis identified 4 clusters numbered according to decreasing salinity and increasing DIN, DSi and DIP concentrations. The descriptive statistics are summarized in Table 2. Cluster 1 included all samples from MW; cluster 2 included mainly bottom samples from CW; cluster 3 included mainly surface samples from CW; and cluster 4 included only 4 samples which were CW surface samples from 21 November 2008, 12 December 2008, 2 April 2009 and 22 April 2009. Bottom samples from CW which were included in cluster 3 where samples taken on 7 and 21 November 2008, 12 December 2008 and on April 2009 sampling campaigns (2, 8, 14 and 22 April). Bottom samples from CW included in cluster 3 and samples included in cluster 4 coincided with accumulated rain above 10 mm in the 5 days prior to sampling.

Dissolved inorganic nitrogen (DIN) concentrations during the study period ranged from 0.1 to 50.5 µM at CW and from 0.1 to 8.0 µM at MW (Fig. 4); the highest values were measured in the rainy season. DIN concentration was dominated by NO_3^- which registered maximum values of 49.4 µM at CW and 7.1 µM at MW. Though there were no significant differences in NH_4^+ concentrations at the sampling points ($p > 0.05$), concentrations below the method detection limit were more commonly found at MW than at CW. The seasonal tendency of NH_4^+ was similar to that of DIN (Fig. 4), with higher values after rain episodes, except for the maximum values attained on July 1 and 18 at CW bottom waters when no rain was registered.

Table 2 Descriptive statistics for salinity and nutrients (expressed in µM units) for the identified clusters

	Salinity	DIN	DSi	DIP
CLUSTER 1				
Average	37.2	1.47	1.90	0.04
Standard deviation	1.0	1.53	1.14	0.05
Maximum	38.5	8.00	4.50	0.23
Minimum	33.9	0.10	0.20	<0.01
CLUSTER 2				
Average	36.8	1.38	2.11	0.07
Standard deviation	1.2	1.02	1.14	0.09
Maximum	38.0	4.56	4.10	0.43
Minimum	34.0	0.12	0.70	<0.01
CLUSTER 3				
Average	36.2	6.56	3.96	0.05
Standard deviation	1.7	2.59	2.44	0.05
Maximum	37.5	12.34	10.00	0.21
Minimum	30.0	2.12	0.50	<0.01
CLUSTER 4				
Average	32.3	40.54	12.00	0.08
Standard deviation	5.5	9.43	3.75	0.11
Maximum	37.0	50.48	16.30	0.24
Minimum	24.3	30.87	7.50	0.01

Dissolved silicate (DSi) ranged from 0.5 to 16.3 μM at CW, and from 0.2 to 4.5 μM at MW (Fig. 4). The highest DSi concentrations were reached in the rainy season. Its maximum values were synchronous with rain events and with DIN maximum concentrations, except for April 2 (2009) when DSi did not peak. The lowest DSi concentrations were observed in June and early July.

Dissolved inorganic phosphorus (DIP) varied from < 0.01 μM to 0.43 μM at CW and from < 0.01 μM to 0.23 μM at MW (Fig. 5). The DIP seasonal pattern was not clear. Total phosphorus (TP) ranged from 0.04 to 1.14 at CW and from 0.01 to 1.20 at MW (Fig. 5) and showed a seasonal pattern similar to that of DIN and DSi (Fig. 4).

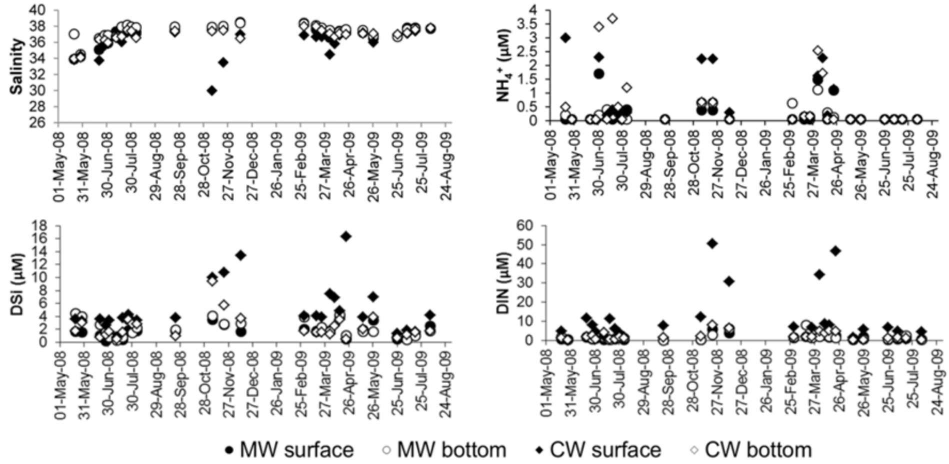

Figure 4 Temporal variation of salinity, dissolved inorganic nitrogen (DIN) (µM), ammonium (NH4$^+$) (µM) and dissolved silicate (DSi) (µM)

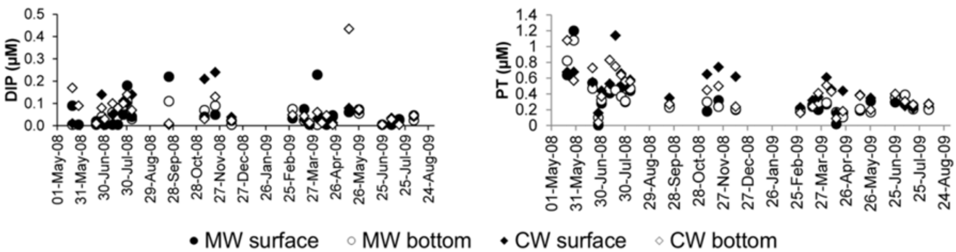

Figure 5 Temporal variation of dissolved inorganic phosphorus (DIP) (µM) and total phosphorus (TP) (µM)

In order to better define potential nutrient control, nutrient ratios between DSi, DIN and DIP concentrations were compared with Redfield ratios (DSi:DIN:DIP = 16:16:1) as shown in Figure 6. The DIN:DIP ratio was always above 16 at CW surface waters (except an isolated ratio of 14 on

August 5, 2008) (Fig. 6). The average ratio was 530, and the maximum ratio 3438, which was attained on April 22, 2009. At CW bottom waters and at MW (surface and bottom waters), DIN:DIP ratio values above 16 also prevailed. Values below the Redfield ratio were detected from mid-July to the beginning of November 2008 (only end of July at CW bottom waters) and in the second half of May 2009. The DSi:DIP ratios were high compared with Redfield ratios (average ratio was 179 at CW and 155 at MW), and only some points were below 16 with no clear seasonal tendency. As a consequence of these high DIN:DIP and DSi:DIP ratios, P was the potentially most deficient nutrient for phytoplankton growth during most of the study period, with DIN and DSi alternating as the secondary potentially deficient nutrient. In coastal waters, DSi secondary potentially limiting conditions were predominant all year round, except in May and from the second half of July to the beginning of September when DIN was the secondary potentially limiting nutrient. In contrast, in marine waters, DIN potentially limiting conditions prevailed. DSi was potentially limiting only in short periods: in April, from June to the beginning of July and from the second half of November to the end of December.

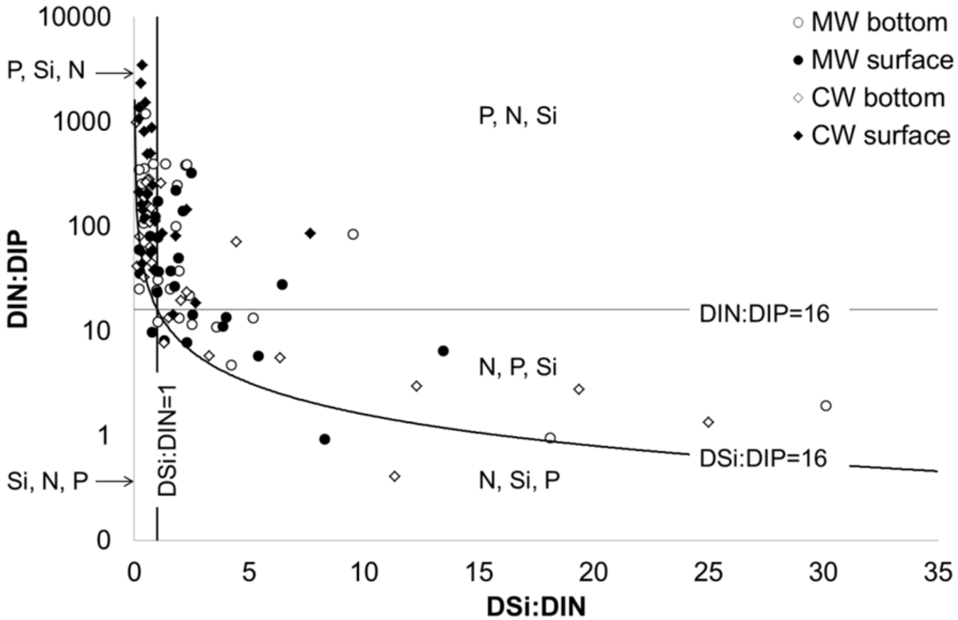

Figure 6 Synthetic graph of DSi:DIN:DIP molar ratios in coastal (CW) and marine waters (MW). In each area delimited Redfield ratios (DSi:DIN:DIP = 16:16:1), the potential limiting nutrients are reported in order of priority

3.3 Phytoplankton abundance and composition

Comparison of both sampling points showed that coastal waters (CW) had the highest significant chl-*a* values ($p < 0.01$) which ranged from 0.31 to 4.32 mg m^{-3} and had an average value of 1.43 mg m^{-3}. In marine waters (MW) chl-*a* values varied from 0.05 to 1.43 mg m^{-3} and the average value was 0.47 mg m^{-3}. The maximum chl-*a* concentration attained in marine waters corresponded with the average value observed in coastal waters.

In both areas, high chl-*a* values were observed in March (early spring), followed by a chl-*a* decrease in April and a recovery in May. At CW, the highest chl-*a* values were attained on March 16, 2009, while at MW, maximum values were measured on May 26, 2009. From June to December chl-*a* concentration was lower in both coastal and marine waters (average 1.1 mg m^{-3} at CW and 0.36 mg m^{-3} at MW; maximum 2.05 mg m^{-3} at CW and 0.68 mg m^{-3} at MW).

Absolute phytoplankton abundance was significantly higher at CW for all groups ($p < 0.05$), except for euglenophytes and chlorophytes that did not show statistically significant differences ($p > 0.05$) between the two sampling points. Relative abundance of dinoflagellates, prasinophytes and prymnesiophytes was higher at CW ($p < 0.05$), while diatom, euglenophyte and cyanobacteria contribution to total chl-*a* was higher at MW ($p < 0.05$). No significant differences were found in chlorophyte and cryptophyte relative abundance between the two sampling points ($p > 0.05$). In Fig. 7 the seasonal variation of each phytoplankton group is represented, as estimated with CHEMTAX, in terms of the contribution of the different groups of algae to total chlorophyll *a*.

In MW surface waters, diatom contribution to total chl-*a* was important all year round and ranged from 25 to 85% of chl-*a* (except for an isolated instance when diatoms were not detected on July 30, see Fig. 7). The diatom bloom started in early spring and lasted until the end of June. In this period the average diatom percentage of chl-*a* was 72%. No correlation was observed between diatoms and the variables included in the analysis (Table 3). Amongst flagellates the main group was generally euglenophytes (Fig. 7). The maximum flagellate abundance was observed from November to December, when euglenophytes accounted for 30% of total chl-*a* on average. The prymnesiophyte contribution to chl-*a* was 12% on average, and their maximum abundance was observed on July 30, when they accounted for 42% of chl-*a*. Cyanobacteria abundance was below 10% of chl-*a* from November to the end of May. An abundance increase was observed during the summer months with 34% of average contribution. Maximum cyanobacteria abundance reached 58% at the end of July 2008 and 47% at the beginning of August 2009 (Fig. 7). Cyanobacteria relative abundance was inversely correlated with DIN. The seasonal variation of phytoplankton groups in MW bottom waters followed the same tendency described for surface waters (Fig. 7). The main significant difference was a higher relative contribution of euglenophytes ($p < 0.01$) and a lower contribution of prymnesiophytes ($p < 0.05$).

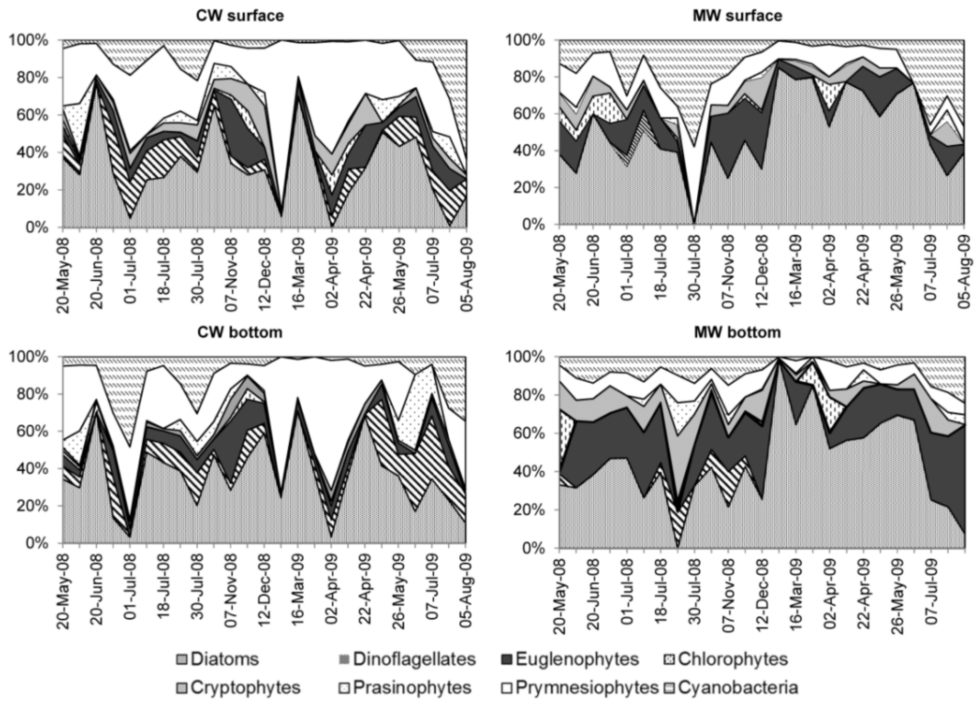

Figure 7 Temporal variation of relative abundance of phytoplankton groups as estimated with CHEMTAX

In CW, several diatom peaks were observed throughout the study period and not only in spring (Fig. 7), and a maximum chl-*a* percentage of 77% was attained in June 2008. Diatom relative abundance was not correlated with any of the variables included in the analysis (Table 3). Average flagellate abundance was 30% for the study period. Higher flagellate abundance was detected after the rain episodes due to an increase in euglenophyte, chlorophyte and cryptophyte abundance (Fig. 3 and 7). Chlorophyte abundance was inversely correlated with salinity (Table 3). During the dry season, high flagellate abundance was linked with an increase in dinoflagellates (Fig. 7). Average abundance of prymnesiophytes was 30% for the study period although several peaks were observed, generally after rain periods, with a high of 90% of total chl-*a* on 1 March 2009 (Fig. 7). Cyanobacteria abundance was below 5% of chl-*a* from the end September to mid-May. An abundance increase was observed during the summer months with 20% of average contribution. Cyanobacteria relative abundance was inversely correlated with several variables: dissolved oxygen, DIN, DSi, DIN:DIP ratio and DSi:DIP ratio (Table 3). The seasonal variation of phytoplankton groups in CW bottom waters was equal to that observed in surface waters. Only cryptophyte abundance, both absolute and relative, was significantly higher in surface waters ($p < 0.05$). This group was positively correlated with the following variables: NH_4^+, DIN, DSi, DIN:DIP ratio and DSi:DIP ratio (Table 3). A negative correlation with the DSi:DIN ratio was also observed (Table 3). None of the other phytoplankton groups produced any significant differences between surface and bottom waters.

Table 3 Rank correlation matrix (Spearman's rs) of main physical and chemical parameters with relative abundance of phytoplankton groups, in coastal waters (P1, n=52) and marine waters (P2, n=50) of Gandia coast from May 2008 to August 2009. ***p < 0.001, **p < 0.01, *p < 0.05

	Diatoms		Dinoflagellates		Euglenophytes		Chlorophytes		Cryptophytes		Prasinophytes		Prymnesiophytes		Cyanobacteria	
	CW	MW	CW	MW	CW	MW	CW	MW	CW	MW	CW	MW	CW	MW	CW	MW
T[a]	-0.16	-0.32*	0.45**	0.09	-0.17	-0.19	-0.35*	-0.13	-0.28*	-0.16	0.22	0.00	-0.39**	0.27	0.60***	0.72***
Salinity	0.03	-0.11	0.13	0.00	-0.06	0.05	-0.31*	-0.03	-0.18	0.02	0.10	0.39**	-0.14	-0.21	0.16	-0.05
DO	-0.16	0.21	-0.19	-0.14	-0.14	-0.13	0.05	0.05	-0.03	-0.24	-0.25	0.08	0.35*	-0.04	-0.41**	-0.27
NH_4^+	-0.13	0.00	-0.04	0.34*	0.20	-0.05	0.50***	0.09	0.44**	0.06	-0.15	-0.02	0.23	0.12	-0.11	-0.17
DIN	0.03	0.13	-0.15	-0.26	0.23	0.01	0.24	0.18	0.62***	0.28	-0.34*	0.03	0.02	0.04	-0.34**	-0.37**
DSi	-0.05	-0.01	-0.18	0.13	0.21	-0.12	0.22	0.15	0.40***	0.08	0.06	0.06	0.03	0.14	-0.38**	-0.19
DIP	-0.05	-0.09	0.16	0.13	0.27	0.00	0.07	0.03	-0.04	-0.08	0.07	0.15	0.03	0.17	0.12	0.01
PT	0.11	-0.32	-0.03	-0.10	-0.06	-0.02	0.46**	0.21	0.09	0.25	0.08	0.03	0.02	0.18	0.04	0.32
DIN:DIP	0.08	0.14	-0.14	-0.25	-0.03	0.00	0.13	0.10	0.50***	0.23	-0.32*	-0.06	-0.05	-0.13	-0.27*	-0.20
DSi:DIP	0.05	0.06	-0.21	-0.07	-0.12	-0.05	0.09	0.01	0.30*	0.09	0.00	0.09	-0.03	-0.07	-0.31*	-0.09
DSi:DIN	-0.07	-0.06	0.07	0.31*	-0.09	-0.12	-0.10	-0.10	-0.43**	-0.15	0.43**	0.00	0.01	0.00	0.11	0.14

The analysis of the seasonal variation revealed that in MW, diatom relative ($p < 0.05$) and absolute ($p < 0.001$) abundances were significantly higher in the wet season, while cyanobacteria abundances were higher in the dry season ($p < 0.001$). The other groups did not show any significant differences between seasons ($p > 0.05$). In CW, absolute abundance of all the phytoplankton groups was significantly higher in the wet season, except prasinophyte abundance which did not show any difference ($p > 0.05$). However, relative abundances of dinoflagellate ($p < 0.01$) and cyanobacteria ($p < 0.001$) were higher in the dry season and both showed a direct correlation with temperature (Table 3).

4 Discussion

The Serpis River is a typical Mediterranean river subjected to the hydrologic singularities of the Mediterranean climate and fluvial regime which cause flow, physical and chemical conditions to change dramatically on a seasonal basis (Torrecilla et al., 2005). During summer, river average flow was 0 $m^3 s^{-1}$ and higher flows were linked to periodic releases from the Beniarrés reservoir to satisfy crop irrigation needs. This reservoir suffers severe eutrophication problems, especially during summer and it was declared a sensitive zone according to the 91/271/EEC Directive (Molinos-Senante et al., 2011).

Artificial regulation of Mediterranean rivers and intensive abstractions for agriculture cause moderate correlation between river flow and rain episodes (Struglia et al., 2004). Despite the river flow seasonality, salinity values in the receiving waters did not show the same seasonal variation (seasonal differences were not significant) and were not correlated with river flow. The absence of significant correlation is attributed to river regulation. However, seasonal salinity changes, though not significant, were observed during the dry season when values of CW and MW were more similar (Fig. 4) and indicated reduced river discharge. On the other hand, correlation between salinity and rain was significant. Measures of river flow were taken at the last gauging stations in the direction of flow and about 10 km from the river mouth (Vernissa and Font En Carrós). Rain events generated a significant river flow downstream of these stations, which also received combined sewer overflows (CSO) from the 15 million urban square meters located in the lower basin. The seasonal variability of both rain and flow explained the seasonal variability in nutrient concentration patterns, which were correlated with either rain and flow (NH_4^+, DIN and DSi) or with rain only (TP) (Table 1).

The agricultural non-point sources generate the major part of DIN in the Mediterranean rivers basins (Torrecilla et al., 2005; Falco et al., 2010). In consequence, the DIN seasonal variation between the wet and the dry periods can be explained as agricultural mobilization by hydrological runoff from the catchment surface during rain events. This variation is also typical in other basins where agricultural land dominates the basin land uses (Cugier et al., 2005; Jarvie et al., 2008).

Chemical weathering of land silicates is the main process that supplies dissolved and particulate silicate to rivers, thus, higher DSi concentration in the wet period was also expected. In addition, groundwater discharges in the study area (wet period), are also characterized by high silica levels due to the lixiviation of biogenic silica from the wetland species of *Gramineae,* which are characterized by high silica content (typically 10-15% dry shoot weight) to the aquifer (Conley, 2002; Sebastia, unpublished data).

Phosphorus dynamics were more complex. DIP concentration was not correlated with river flow, did not present a clear seasonality and was not significantly different to that observed at MW. In the study area, higher phosphorus levels have been observed in surface irrigation channels during spring and have been attributed to diffuse sources, because they coincided with the period of phosphorus fertilizer application (Sebastia et al., 2012). During the low-flow period, phosphorus contributions from agricultural diffuse sources (mobilized by hydrological runoff) are minimal and the major source is therefore likely to be derived from point sources (Jarvie et al., 2008). In the Serpis River the low-flow period coincided with summer, when the population increase due to tourist activity causes a higher wastewater flow, which exceeds the WWTP capacity. In this period, discharges of partially treated effluent through the overflow channels to the Serpis River have been observed (Sebastia, unpublished data). However, phosphorus is the main potentially limiting nutrient, and any phosphorus discharge is rapidly consumed, which can cause the above described seasonal variations to go undetected (Falco et al., 2010).

On the other hand, total phosphorus correlated with rain showed a seasonal variation similar to that of DIN and DSi, with higher values during the wet period, and was significantly higher in coastal waters. This highlighted the variability of phosphorus inputs, which were not detected when DIP levels were observed. As described above, DIP phosphorus inputs are rapidly consumed and this translates into biomass increase (total chlorophyll *a* concentration) which is correlated with TP increases ($p <$

0.05). This has been described for other coastal ecosystems with potential phosphorus limitation (Falco et al., 2010).

According to the 91/271/EEC Directive Annex II, the coastal waters of the study area meet the requisites of a sensitive area: coastal waters with poor water exchange and which receive large quantities of nutrients, though it has not been declared as such. In NE Spain the delta of the Ebro River was declared a sensitive zone (on 10 July 2006 Resolution, BOE nº 179, 2006-07-28). The Ebro River is the longest European river (928 km) that empties into the Mediterranean Sea, it has the second largest basin (88 835 km^2) and the third largest flow (mean annual discharge 384 m^3 s^{-1} (Falco et al., 2010). Despite the evident differences in river size and discharge, it presents some similarities with the Serpis River: it is a highly regulated Mediterranean river; low discharge occurs from July to October; the main activity in the basin is agriculture (mainly vineyards, orchards and corn crops); and it receives wastewater discharges near the mouth. These similarities allowed us to establish a comparison of the nutrient seasonal variability in the receiving waters of the two rivers.

In Falco et al. (2010), the mean and standard deviations for nutrients at sea (S) were presented for each season of the year. For comparison, autumn, winter and spring were grouped as the wet season, as in Table 4. For the dry period, all nutrient concentrations were higher in the coastal waters of the Ebro River (Table 4). However, for the wet period, the average nutrient values of coastal waters of the Serpis River were even higher than those observed for the Ebro River in some seasons (e.g. average DSi for the Serpis wet period was higher than the average DSi for the Ebro spring period, and DIP and TP were higher than the average for the Ebro winter period). The aim of this comparison is to illustrate the importance of rain episodes in Mediterranean rivers of scarce flow. Despite the different magnitude of average annual flow of these two rivers, mean annual discharge of 3 $m^3 s^{-1}$ for the Serpis (Pulido-Velázquez et al., 2011) and 384 $m^3 s^{-1}$ for the Ebro (Falco et al., 2010), the nutrient concentration in the receiving coastal waters is comparable.

Table 4 Means (M) and standard deviation (SD) nutrients in receiving waters of the Serpis River (SR) and the Ebro River (ER) for wet and dry periods

		NH_4^+		**DIN**		**DSi**		**DIP**		**TP**	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Dry period	SR	0.5	1.0	3.5	3.2	2.2	1.2	0.05	0.05	0.47	0.25
	ER	5.5	4.9	9.7	9.0	11.9	9.6	0.23	0.24	0.83	0.25
Wet period	SR	0.7	0.9	9.0	13.3	4.9	3.8	0.07	0.09	0.42	0.21
	ER (autumn)	7.1		9.0		9.2		0.38		0.91	
	ER (winter)	1.8	0.8	15.6	9.1	6.2	4.3	0.03	0.01	0.21	0.04
	ER (spring)	1.2	0.5	15.2	18.2	1.4	1.6	0.11	0.14	0.92	1.06

On the other hand, all samples from MW were classified into one cluster which was characterized by the highest salinity and the lowest nutrient concentrations and variability. The range of nutrient concentrations (DIN, DSi and DIP) was of the order of those observed in previous studies further north in the Western Mediterranean Sea, 5 km from the coast (Olivos et al., 2002). As in CW, phosphorus was the main potentially limiting nutrient, but in MW the secondary potentially limiting nutrient was nitrogen, which agrees with the observations of Estrada (1996) for the Mediterranean Sea.

Coastal and marine waters also showed significant differences in phytoplankton biomass and relative group abundances. Total biomass (chl-*a*) and absolute abundance of phytoplankton groups was higher in coastal waters due to higher nutrient availability. The seasonal cycle showed maximum phytoplankton abundances in spring and minimum abundances in summer which is typical in coastal and marine waters (Garmendia et al., 2011).

In marine waters, abundance of diatoms was higher in spring and relative abundance of small cells (cyanobacteria and flagellates) increased in summer (Garmendia et al., 2011; Latasa et al., 2010). Cyanobacteria attain maximum growth rates at temperatures higher than those for diatoms and also prefer for more oligotrophic conditions (Latasa et al., 2010), in this study they were inversely correlated with DIN and DSi concentration. Maximum flagellate abundance was observed from November to December when silica was the potentially limiting nutrient after phosphorus.

In coastal waters, relative diatom abundance was lower than in marine waters and did not show the typical seasonal cycle of maximum abundance in spring and minimum abundance in summer. Minimum diatom abundance was observed after rain episodes as they were replaced by increases in flagellate (euglenophyte, chlorophyte and cryptophyte) and prymnesiophyte abundances. On the other hand, maximum diatom abundance was observed on 20 July 2008, with a 77% contribution to total chl-a. High diatom abundances during summer have been observed in other estuaries which receive wastewater discharges and are related to a continuous availability of silica (Garmendia et al., 2011). The negative correlation of chlorophytes with salinity (Table 3) showed that this group was characteristic of freshwater. Cryptophytes are a group characteristic of eutrophic conditions (Latasa et al., 2010); as such they were positively correlated with NH_4^+, DIN and DSi. Cryptophytes were also inversely correlated with the DSi:DIN ratio (Table 3), so they outcompeted diatoms when this ratio value was lower, mainly after rain episodes. Dinoflagellate, prasinophyte and prymnesiophyte relative abundances were higher in coastal waters than in marine waters. Dinoflagellates and prasinophytes have been identified as groups with preference for eutrophic conditions, but prymnesiophytes have been reported as a mesotrophic group (Latasa et al. 2010). Dinoflagellate blooms have been reported in estuaries and near-shore regions and are typically responsible for harmful algal blooms (Cugier et al., 2005; Latasa et al., 2010). In the study area, a higher relative dinoflagellate abundance was observed during the dry season, when DIN:DIP and DSi:DIP ratios were smaller and river discharge is reduced and mainly due to partially treated effluent. In the study area, prymnesiophytes were abundant throughout the year and did not show any significant difference between the wet and the dry period.

5 Conclusions

In Mediterranean basins, agricultural land use is important and contributes to the high nutrient loads delivered by rivers, especially during the wet season; together with combined sewer overflows. During the dry season, nutrients from freshwater discharges of partially treated effluents from wastewater treatment plants (WWTPs) gain importance given the reduced river flow. The results from this study show that nutrients and phytoplankton biomass are higher in coastal waters receiving Mediterranean river inputs. Nutrient inputs delivered through the river altered the typical seasonal cycle of diatoms in the receiving waters, where flagellates groups become more important mainly after rain episodes. In addition, phosphorus was shown to be the main potential limiting nutrient for phytoplankton growth. Although agriculture can be a significant phosphorus source during the fertilization period, wastewater discharges are the most important source.

In the Mediterranean region, the most common measures used to achieve the Water Framework Directive (WFD) quality targets for rivers and reservoirs involve increasing the quality of effluent produced from WWTPs (Molinos-Senante et al., 2011) in accordance with the 91/271/EEC Directive. Such measures have been recommended by the CHJ for the WWTPs discharging upstream the Beniarrés reservoir in the Serpis River Basin. However, in coastal WWTPs, which discharge effluent through submarine outfall, the main problem is not the quality of treated effluent but the CSO discharges, directly near the river mouths and subsequently into receiving coastal waters during rain episodes. The importance of this problem is recognized in Annex I of the 91/271/EEC Directive, but the adoption of preventive measures is conditioned by economic considerations.

Before implementing any management measures, the use of simulation models would be recommended to ensure that these measures have the anticipated effects on eutrophication problems. Taking into account that both agricultural land use and WWTP discharges are important sources of nutrients, different scenarios should be explored (Cugier et al., 2005): e.g. different percentages of reductions in agricultural nutrient inputs and different percentages of reduction in CSO. After selecting the best management option, analyzing the cost of this in the manner proposed by Molinos-Senante et al. (2011) would help to determine whether the costs are excessive or not. Management decisions should take into account that only reductions in CSO and partially treated summer effluent are likely to be measurable in the short term, while a reduction in diffuse agricultural sources, especially nitrogen, is only likely to give results in the long term due to the aquifer higher residence time (Cugier et al., 2005; Sebastiá et al., 2012).

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Capítulo 6: Estimation of chlorophyll *a* on the Mediterranean coast using a QuickBird image

1 Introduction

Coastal watersheds support more than 75% of the human population and are sites of large increases in nutrient loading associated with urban and agricultural expansion. Increased nutrient loading has led to eutrophication problems which symptoms include increased algal bloom activity (including harmful taxa), accumulation of organic matter, and excessive oxygen consumption (hypoxia and anoxia) (Paerl, 2006). In order to assess the eutrophication risk, chlorophyll *a* (chl-*a*) concentration, which is a proxy of phytoplankton biomass, has been used in monitoring programs such as the established by the European Water Framework Directive (WFD) (2000/60/EC). However, monitoring data characterizing the biological elements is imprecise due to spatial variations, temporal variations and sampling and analytical errors. Carstensen (2007) pointed out the need of sufficient monitoring data and the improvement of indicator bias and precision through modeling and further development of measurement techniques. In this sense, satellite monitoring is an alternative and efficient technology for water quality monitoring that can aid the application of these monitoring programs considerably (Chen et al., 2004). Satellite sensor and airborne images have been extensively used to assess water quality parameters such as temperature, chlorophyll *a*, turbidity and coloured dissolved organic matter (Oyama et al., 2009; Zhang et al., 2009; Santini et al., 2010). While conventional water quality sampling is time-consuming, expensive and limited with the numbers of stations, remote sensing provides a synoptic view not otherwise attainable at a relatively low cost (Liu et al., 2003; Chen et al., 2010). These advantages are especially important in environments with a high degree of variability in physico-chemical characteristics (i.e., salinity, temperature and oxygen) and nutrient inputs that is reflected in the ecological assemblage (Elliot and Quintino, 2007; Maier et al., 2009). Among these environments, coastal and estuarine ecosystems represent one of the most relevant examples.

In remote sensing oceanic waters are classified into one of two types: Case 1 or Case 2 (Morel and Prieur, 1977). By definition, Case 1 waters are those waters in which phytoplankton (with their accompanying and covarying retinue of material of biological origin) are the principal agents responsible for variations in optical properties of the water. On the other hand, Case 2 waters are influenced not just by phytoplankton and related particles, but also by other substances, that vary independently of phytoplankton, notably inorganic particles in suspension and dissolved organic matter (IOCCG, 2000). Case 1 waters have been widely studied using ocean colour sensors with low spatial resolution image (around 1km), such as SeaWiFS and MERIS (Djavidnia et al, 2010; Maritorena et al., 2010). This scale is appropriate for oceans but insufficient for monitoring case 2 waters, which are mostly coastal waters and lakes. In this case, terrestrial observation satellites with moderate spatial resolutions such as LANDSAT, Terra ASTER (spatial resolution lower than 30 m) and MERIS (Liu et al., 2003; Domínguez et al., 2010; Domínguez et al., 2011; Focardi et al., 2009; Oyama et al., 2009; Song et al., 2011) and high spatial resolution such as IKONOS (Ekercin, 2007; Ormeci et al., 2009) and QuickBird (Wheeler et al., 2012) have been used. Gohin et al. (2008) tested the capacity of using satellite data for assessing the eutrophication risk of coastal bodies according to the WFD with SeaWiFS and they found that the quality of the chl-*a* satellite estimation decreased with the distance to the coast. The strong chl-*a* gradient near shore was indicated as one of the possible causes of this loss of sensitivity and the use of a moderate resolution satellite was recommended. However, coastal water bodies may be very narrow to be studied from these satellites, according to the WFD definition (Gohin et al. 2008). To overcome this disadvantage further research based on satellite images of high spatial resolution is necessary for detecting and portraying complex spatial distributions of chlorophyll *a*, such as changes of chl-*a* near shore in small areas. These changes are mainly due to nutrient inputs from point (streams, submarine outfall) and diffuse (urban and agricultural runoff) sources. Quickbird sensor has been successfully used for benthic habitat mapping (Mishra et al., 2006) and for littoral remote bathymetry (Adler-Golden et al. 2005). However, research with Quickbird image for estimating water chlorophyll-*a* concentration has been conducted mainly in inland waters (Wheeler et al., 2012).

The Gandia coast is an ecologically and economically important coastal area and is a representative aquatic region of Spain´s Mediterranean coast. However, no previous research has been developed in this area with satellite images. In this study high spatial resolution images are tested to map the coastal gradient of chlorophyll-*a*.

The objectives of this research were as follows: (1) to develop and validate a linear regression model to estimate chlorophyll *a* concentration with a QuickBird image; (2) to analyze the spatial variation of chlorophyll *a* concentration at high scale; and (3) to explore the feasibility of using remote sensing techniques to monitor small and narrow areas with high variability.

2 Materials and methods

2.1 Study area

The study area (Fig. 1) is located in the southernmost sector of the Valencian Gulf (Mediterranean Sea) and it is defined by a 10 x 4.5 km rectangle which delimits the Gandia city coastline. The flat bottom morphology of this area (see isobaths in Fig. 2) is characterized by well graded sands and the absence of benthic vegetation such as seagrasses or macroalgae. Gandia is a populous coastal city with 1314 inhabitants/km^2, whose population triples in summer owing to beach tourism. 53% of the Gandia coast is considered as an urban area, including a small commercial, fishing and recreational harbour.

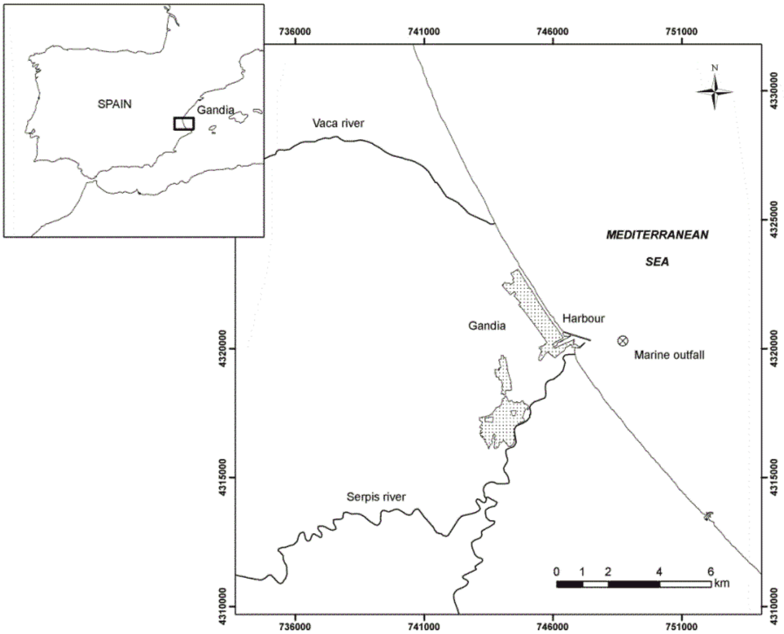

Figure 1 Location of the study area

The study area receives freshwater inputs from point and diffuses sources that are rich in nutrients. At the northern end is the Vaca river mouth (which is also named Xeraco river), which is a small river, 16.6 km in length, with a low slope. During the sampling period its flow was non existent. At the southern end the Serpis river flows into the Mediterranean. This river drains a basin of 752.8 km^2 and is 74.5 km in length. These rivers have a Mediterranean regimen characterized by a high seasonality, with a dry period during summer, and a wet period with episodes of torrential rain, mainly in autumn (Garófano et al., 2009). Another point source is the submarine outfall (1900 m in length), which discharges municipal wastewater from the treatment plant of Gandia. The alluvial plain next to the study area is 3 km wide and was totally occupied by the Safor wetland and crops until the seventies. Nowadays, this area shares its agricultural activity, mainly citrus fruits and vegetables, with the tourism of the urban areas. Due to the shallow phreatic level, freshwater is continuously pumped from the wetland to the Gandia Harbour to avoid crop and urban area flooding. Diffuse sources in the study area come from the groundwater discharge of the Plana Gandia-Denia detritic aquifer, quantified at 66 Hm^3 $year^{-1}$ (2.1 m^3 s^{-1}) (Ballesteros-Navarro, 2003), although our sampling period was in the dry season, so discharge would have been lower than average.

2.2 Field sampling and laboratory analysis

Field work was timed to coincide with the acquisition of the QuickBird image on July 16, 2009, at 10:56 GMT. Weather conditions during the image acquisition included cloudless skies over the study area and low wind speed, less than 2 km h^{-1}. The water samples were collected for each site at 0.5 m beneath the water surface within 1.5 h of the satellite overpass. For model development 16 samples were collected. (in Fig. 2 points numbered from 1 to 16). An independent data set was collected at 7 sampling points (in Fig. 2 points numbered from 38 to 44), and was used to validate the performance of the selected algorithm. For mapping nutrient water surface distribution, 21 extra samples were collected (points location not shown in fig. 2, samples numbered from 17 to 37). The coordinates of the sampling points were determined using a global positioning system (GPS) model Garmin 60C with an accuracy of 3-5 m. Subsequently chlorophyll-*a* (chl-*a*) was measured in samples used for model development and validation. Salinity, suspended solids and nutrients (nitrates, phosphates and silicates) were measured in all the samples. Chl-*a* concentration was selected for remote sensing analysis, while other parameters were used as secondary analysis.

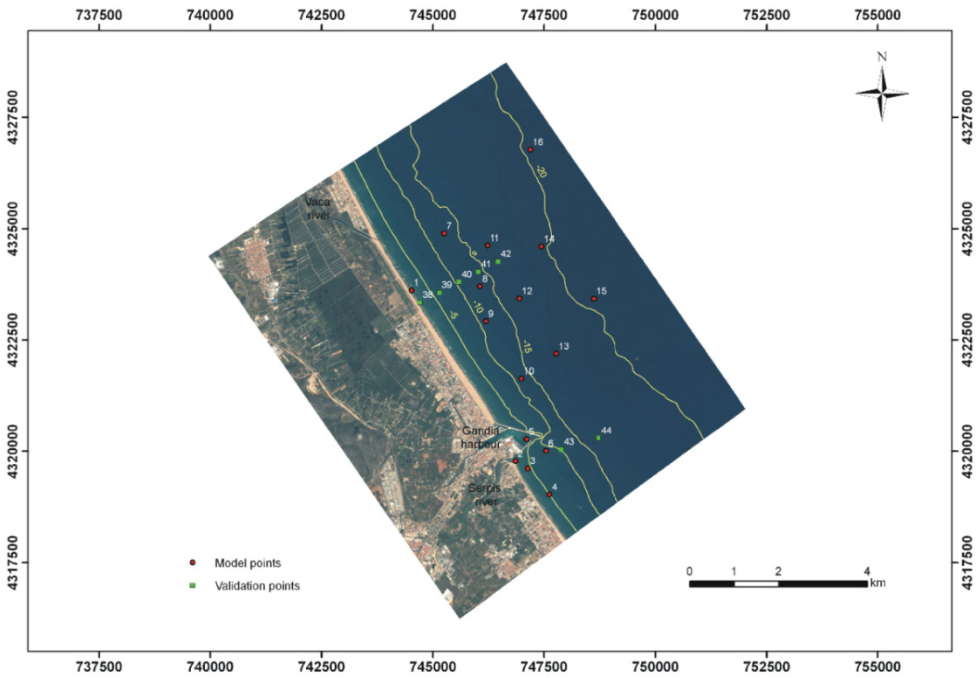

Figure 2 Location of the sampling points for model development and validation

Samples for chlorophyll *a* were filtered on GF/F fiberglass filters (25 mm diameter). The chl-*a* was extracted using acetone (100%) and was measured using reverse-phase high-performance liquid chromatography (HPLC). The HPLC method employed was that proposed by Wright et al. (1991) slightly modified as per Hooker et al. (2000). Nutrients were analyzed colorimetrically using the method of Aminot and Chaussepied (1983). Salinity was determined by means of an induction conductivity meter Multi 340i/SET WTW.

2.3 **Satellite image and model development**

The remotely sensed data used for this study was a high resolution QuickBird multispectral image ordered from Digital Globe Corporation. This sensor has four multispectral bands with a 2.4 m spatial resolution. The wavelength of the respective bands is 0.45–0.52 μm (B1: blue); 0.52–0.60 μm (B2: green); 0.63–0.69 μm (B3: red); 0.76–0.90 μm (B4: near infrared). Prior to delivery, the imagery was radiometrically and geometrically corrected and rectified to the World Geodetic System 1984 (WGS84) datum and the Universal Transverse Mercator (UTM) zone 30 coordinate system. To improve the positional accuracy, 39 control points were selected using a rectified airborne image with a pixel size of 0.5 m. The root mean square error (RMSE) was 0.49 m. The digital numbers (DN) recorded at the sensor were converted to satellite radiance using the technical note from Digital Globe (Krause, 2003). Then, the module QUAC of ENVI 4.7 (ITT Visual Information Solutions) was applied to the radiance images to eliminate the atmospheric effects (Bernstein et al., 2005). In addition, to remove the influence of depth on bottom reflectance upon chlorophyll-*a* retrievals, we calculated depth-invariant bottom indexes from each pair of reflectance bands as described in Green et al. (2000). This technique is only suitable where water clarity is good, such as in the study area. In this method a group of pixels distributed in shallow and deep areas with the same bottom type are selected. Then, a bi-plot is created from the reflectance values of two bands considering all possible band combinations. Six bi-plots are created: band1-band2; band1-band3; band1-band4; band2-band3; band2-band4; band3-band4. The slope of each bi-plot represents the ratio of attenuation co-efficients, ki/kj (equation 1), between bands (Green et al., 2000).

$$k_i / k_j = a + \sqrt{(a^2 + 1)} \quad (1)$$

Where

$$a = \frac{\sigma_{ii} - \sigma_{jj}}{2\sigma_{ij}}$$

σ_{ii} is the variance of the band i, σ_{jj} the variance of the band j, σ_{ij} is the covariance between bands i and j. From k_i/k_j, a depth-invariant index (equation 2) was calculated, which represents the y- intercept of the equation of a straight line

$$Depth - in\,\mathrm{var}\,iantindex_{ij} = \ln(radiance_i) - \left(k_i / k_j\right) \cdot \ln\left(radiance_j\right) \quad (2)$$

Where, i and j correspond to each band pair considered and (ki/kj) to the attenuation coefficient for the same band pair, and reflectance bands after applying an atmospheric correction. To calculate these indexes 180 pixels of each band were selected in shallow and deep areas with sand bottom type. The following ratios of attenuation coefficients for band pairs were calculated: k1/k2, k1/k3, k1/k4, k2/k3, k2/k4, and k3/k4. From these coefficients, six new images from each pair of spectral bands (hereafter referred to as Depth-invariant index) were generated (further information of this method can be found in Green et al., 2000). The average digital number of pixels (a 3x3 window) surrounding the sample pixel was used in order to remove errors resulting from GPS measurements in the field work (Oyama et al., 2009; Zhengjun et al., 2008; Nas et al., 2009).

A linear regression analysis was conducted between chlorophyll-*a* logarithm and depth-invariant indexes. When monitoring water quality, general methods are to find the best band combination. Thus the optimal index was judged by R^2 and RMSE (Root Mean Square Error) based on the comparison of simulated model outputs and actual observations (Zhang et al., 2009). An independent data set, collected at 7 sampling points in the study area, was used to assess the performance of the tuned model.

3 Results and Discussion

Results from field samples measurements (salinity, nutrients and chlorophyll-*a*) are shown in Table 1. In this table chl-*a* values obtained from QuicBird image are also included.

Different approaches can be used to estimate chlorophyll *a* from satellite data. The empirical approach, used in this study, is based on the development of a linear regression analysis between satellite image and measured water chl-*a*. This approach has been widely used by many researchers (Zhengjun et al, 2008; Ormeci et al., 2009) and it has proven to be very effective in Case 1 waters (IOCCG, 2000). Despite coastal waters are mainly classified as Case 2 waters, according to Lee and Hu (2006), in summer, the Mediterranean shows values characteristic of Case 1 waters. The present study was carried out during Case 1 waters conditions, when freshwater discharges were minimal and average suspended solids values were 12 mg L^{-1}.

For linear regression analysis, the best model was obtained when B1 and B3 bands were used. When bottom reflectance correction was not performed the R^2 and RMSE values of the model developed were 0.76 and

0.61 mg m^{-3} respectively. After applying this correction the model estimation improved. The best model developed with the depth-invariant index$_{13}$ showed a R^2 of 0.89 and a RMSE of 0.38 mg m^{-3}. This result reveals the importance of applying the bottom reflectance correction. The bottom corrected model was selected for mapping chl-*a* concentration in the study area and its equation was (see Fig. 3):

$$\log chl - a = -16.33 + 4.00 * k_{13}$$

In order to validate the applicability of the selected model, we used an independent data set of chlorophyll *a* concentration, which ranged from 0.21 mg m^{-3} to 1.86 mg m^{-3}. These values fell into the range of chl-*a* concentration used to calculate the model. Measured and estimated chl-*a* showed a great degree of concordance close to the 1:1 line (Fig. 4), with a highly significant linear relationship (R^2= 0.90). The correlation obtained both for model development and validation is higher than the 70% correlation considered a good fit (Gregg and Casey, 2003; Santamaría-del-Ángel et al., 2010).

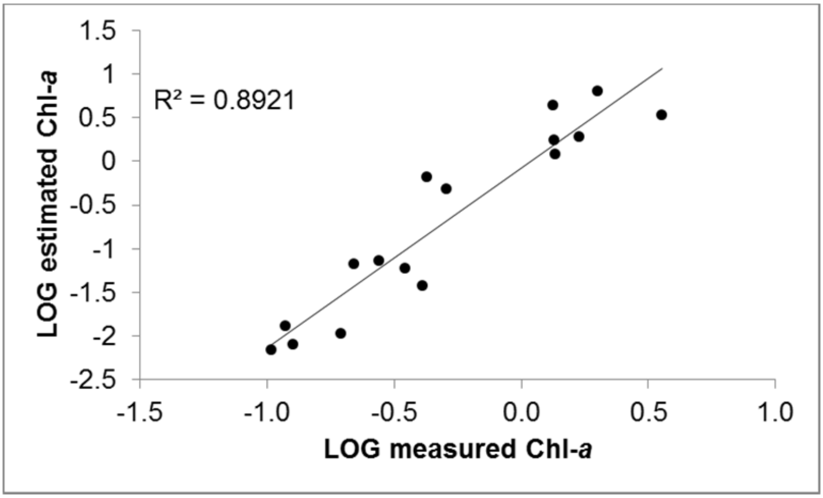

Figure 3 Comparison between satellite estimated and in situ measured chl-*a* concentrations for model development (1:1 line shown for reference)

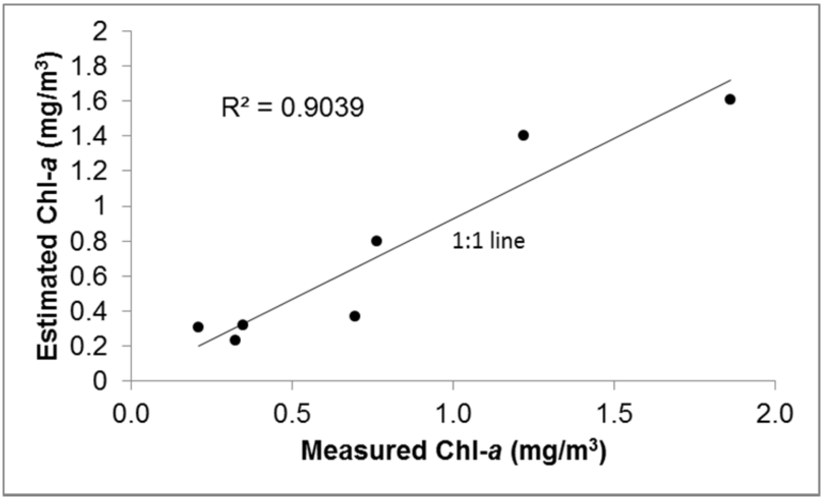

Figure 4 Comparison between satellite estimated and in situ measured chl-*a* concentrations for model validation (1:1 line shown for reference)

The estimated chlorophyll *a* concentration map is showed in Fig. 5. Chl-*a* values range from 3.58 mg m^{-3} in sampling point number 5 located in the Gandia Harbour, to values around 0.10 mg m^{-3} in the sampling points furthest from the coastline (Table 1). It is important to highlight that the largest chl-*a* variation occurs in the first kilometer from the coastline, decreasing from values higher than 5 mg m^{-3} on the coast to values lower than 1 mg m^{-3}. This chl-*a* variation can be explained by the salinity and nutrient distribution showed in Fig. 6, which are linked to the surface and subterranean freshwater discharges described below.

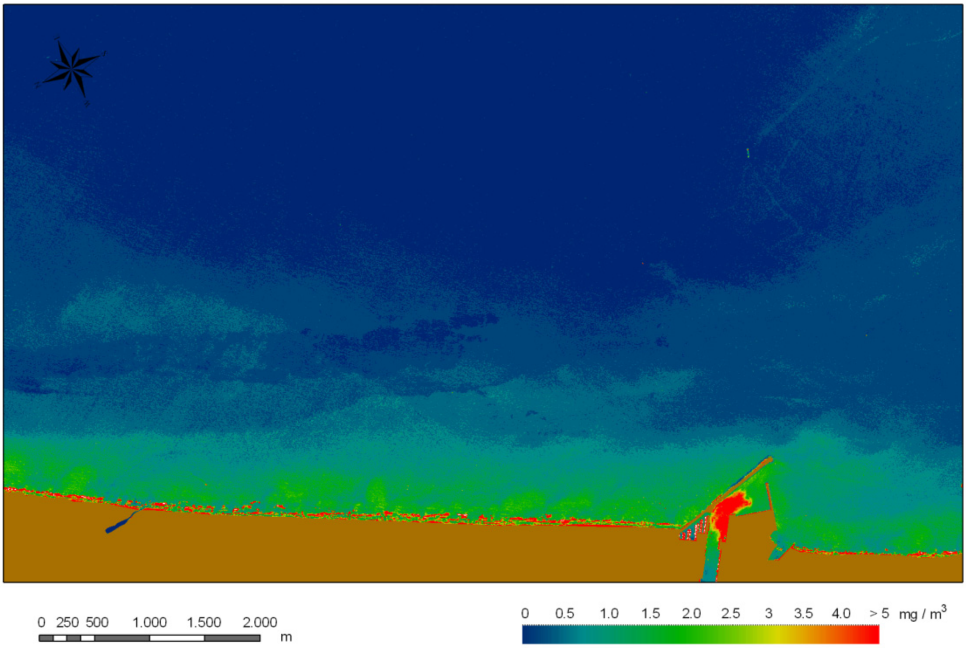

Figure 5 Estimated chlorophyll *a* (mg m^{-3}) concentration map

Fig. 6 a) shows distribution of surface water salinity. Three zones show a salinity decrease: one in the north of the study area at the mouth of the River Vaca, one in the port, and the third one south of the River Serpis. The Vaca has reduced or non flow all year long, which, together with the low slope, contributes to the formation of a littoral sand spit that prevents river outflow. During July 2009 this sand spit was more than 30 m wide, so the salinity decrease could only be attributed to groundwater flow. Port salinity decrease is due to freshwater inputs from the Safor wetland, with a 0.3 m^3

s^{-1} flow during the sampling period. However, groundwater inputs have also been observed in the port (unpublished data). South of the Serpis river mouth, the salinity decrease was more accentuated. During July 2009 this river flow was below its minimum ecological flow, 0.1 m^{3} s^{-1} (Garófano et al., 2009). Discharges from the wastewater treatment plant must be added to this river flow value. It should be highlighted that the submarine outfall discharges (0.7 m^{3} s^{-1}) were diluted because no changes in surface water salinity were detected nearby. In the surf zone, salinity was always lower than in deeper water probably due to the groundwater inputs.

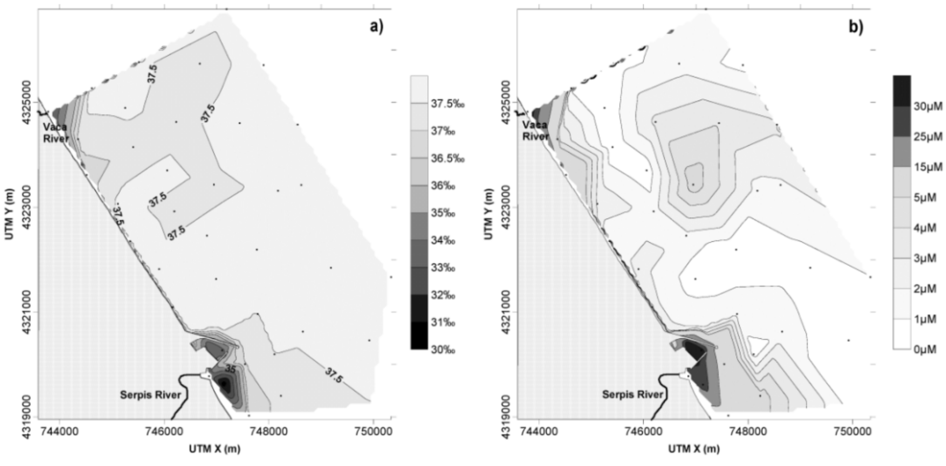

Figure 6 Distribution of nutrients in the surface waters. (a) Salinity (b) Nitrates concentration (µM)

Table 1 Summary of salinity (S), chlorophyll *a* (Chl-*a*) measured with HPLC (M) and estimated from image (E), and nutrient measurements (nitrates, phosphates and silicates) in the study area

Sampling point	X UTM	Y UTM	Salinity	Chl-*a* ($mg\ m^{-3}$)		Nitrates (μM)	Phosphates (μM)	Silicates (μM)
				M	E			
1	744520	4323614	36.7	1.33	1.91	8.82	0.13	2.7
2	746858	4319776	33.8	1.69	1.32	27.74	0.18	12.7
3	747136	4319615	30.1	2.00	2.23	29.90	0.08	19.2
4	747622	4319025	36.6	1.35	1.28	8.45	0.05	4.2
5	747107	4320268	33.5	3.58	1.69	33.83	0.04	9.9
6	747542	4320007	37.0	1.36	1.09	8.72	0.08	1.1
7	745254	4324892	37.6	0.22	0.31	0.09	0.07	0.7
8	746058	4323701	37.6	0.28	0.32	0.32	0.04	0.7
9	746194	4322920	37.4	0.42	0.83	1.56	0.07	0.8
10	746991	4321630	37.5	0.51	0.73	0.42	0.02	0.8
11	746230	4324624	37.3	0.41	0.24	3.23	0.07	0.9
12	746950	4323430	37.4	0.35	0.29	5.62	0.06	1.5
13	747772	4322191	37.8	0.20	0.14	0.15	0.07	0.5
14	747442	4324601	37.8	0.12	0.15	3.97	0.06	0.8
15	748620	4323424	37.7	0.10	0.12	1.86	0.05	0.5
16	747193	4326775	37.7	0.13	0.12	1.69	0.02	0.8

Fig. 6 b) and Fig. 7 a) and b) shows nitrate, silicate and phosphate surface distribution respectively. Nitrates and silicates showed higher values where salinity was lower. In Spain, it is usual to find high nitrate values in freshwater. Spain is a country with a strong tradition of farming and livestock, and water resources contain increasing levels of nitrate. This is mainly due to the abuse of fertilizers, poor management of livestock waste, and to a lesser extent, domestic wastewater (Pinilla, 1997). Silicates are associated with freshwater inputs due to the weathering of soil and rocks, so inputs of this nutrient depends more on geological formations than on any anthropogenic influence (Nedwell et al., 1999). Phosphate levels were lower than 0.2 μM in all of the study area. In the Mediterranean Sea, phosphorus is the limiting nutrient for phytoplankton growth, which is normally the case in freshwater ecosystems, and contrasts with other seas, where it is nitrogen (Krom et al., 2004). Phosphates (fig. 7 d) show higher values near the coast in the north and the south of the study area, and also in a deeper less well-defined zone. This spatial distribution could be because dissolved phosphate concentration can be notoriously modified as a result of adsorption/desorption and reduction/oxidation processes, as well as by biological assimilation (Howarth et al., 1995).

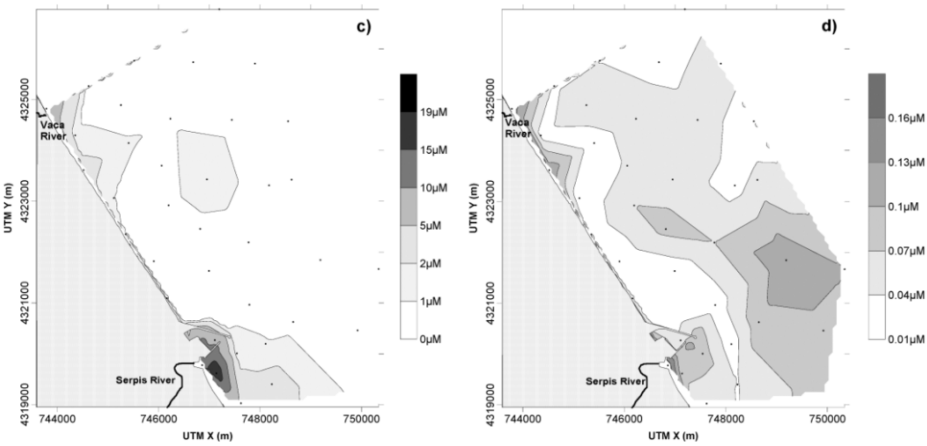

Figure 7 Distribution of nutrients in the surface waters. (a) Silicates concentration (µM) (b) Phosphates concentration (µM)

Estimated chlorophyll *a* distribution is closely related with the nutrient inputs described above, with the exception of the high phosphate levels in the deeper zone where chlorophyll *a* levels are lower than on the coast. According to Fang et al. (2006) and Smith (2006) this disparity can be explained because different phosphate levels and irradiance stimulate the growth of different phytoplankton groups. Depending on the chlorophyll cellular quote of these groups chlorophyll *a* concentration can finally be lower despite the higher phosphorus availability. An independent research was conducted simultaneously in this study area to analyze the spatial variation of nutrients, chlorophyll *a* and phytoplankton groups. Its results confirm the spatial distribution of chl-*a* obtained with the Quickbird image (Sebastiá et al., 2012; Capítulos 4 y 5 de la presente memoria de tesis).

4 Conclusions

The results of this study show how chlorophyll *a* estimation and mapping for the Gandia coast (Western Mediterranean) can be obtained using the depth-invariant $index_{13}$ of a Quickbird image (R^2 = 0.89). The most important result of this study is on the feasibility of high spatial resolution Quickbird image to detect the high chlorophyll *a* gradient of coastal areas. Despite the restrictive spectral resolution of this sensor, its high spatial resolution (2.4 m) makes it suitable for chlorophyll *a* mapping in this type of areas. Compared to traditional field measurements and laboratory analysis, QuickBird data can provide detailed spatial distribution information on the ecological status of water bodies and multi-temporal evaluation at a relatively low cost, which makes it suitable for monitoring programs such as the WFD one. In spite of the good results obtained, further research could be required to extend the approach applied in this study to more scenes (other days and other areas).

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Capítulo 7: Discusión general

1 Consideraciones metodológicas sobre el análisis de pigmentos y la clasificación taxonómica

Los análisis de pigmentos fotosintéticos realizados en esta tesis, han seguido las recomendaciones del monográfico “Phytoplankton pigments in Oceanography” publicado por la UNESCO en 1997. Recientemente, se ha publicado una nueva monografía “Phytoplankton pigments: characterization, chemotaxonomy, and applications in oceanography” (Roy et al., 2011) que es la continuación de aquella primera monografía. En los últimos años el desarrollo de nuevos métodos de HPLC, desarrollados a partir de los originales, ha permitido reconocer la importancia taxonómica y fisiológica de algunos pigmentos que no habían sido separados anteriormente. Sin embargo, ninguno de los métodos desarrollados es perfecto y el mejor es aquel que se adapte a las necesidades analíticas del problema en cuestión (Garrido et al., 2011). El método de HPLC utilizado en esta tesis es el de Wright et al. (1991) el cual fue recomendado en la primera monografía y sigue siendo utilizado actualmente (Garrido et al., 2011).

La clasificación taxonómica del fitoplancton es un campo del conocimiento en continua evolución (Jeffrey et al., 2011). En la presente tesis se han tenido en cuenta los pigmentos característicos de los grupos fitoplanctónicos reconocidos en la monografía de 1997. La aplicación del programa La Marjal CHEMTAX según la metodología descrita por Latasa (2007) ha permitido la clasificación taxonómica del fitoplancton de las muestras de aguas costeras de forma satisfactoria. Los recuentos por microscopía invertida de muestras representativas (Higgins et al., 2011) han permitido identificar previamente los grupos de fitoplancton presentes, lo que ha contribuido a la evaluación cualitativa de los resultados de CHEMTAX.

2 Variación espacial de los nutrientes en los casos de estudio analizados

Caso de estudio 1: en el Puerto de Gandía se ha observado un gradiente longitudinal decreciente en la concentración de nutrientes desde los puntos de descarga de agua dulce de las acequias que drenan La Marjal de La Safor hacia el mar. Este gradiente de concentraciones ha permitido separar las muestras en dos conglomerados. El conglomerado A incluye las muestras tomadas en las acequias, así como las muestras tomadas desde 0 hasta 0.75 m de profundidad de los puntos situados inmediatamente después de los puntos de vertido (P2 y P5). El conglomerado B incluye las muestras tomadas a más de 0.75 m en P2 y P5, así como todas las muestras tomadas en el resto de puntos situados en el transecto longitudinal (P6, P7 y P8). No se han observado diferencias significativas en la concentración de nitrógeno disuelto inorgánico (DIN) y sílice disuelta (DSi) entre los dos escenarios comparados (primavera y verano). El nitrato es la forma dominante de nitrógeno en todos los puntos y profundidades de muestreo, y los valores más elevados se han observado en las acequias con valores superiores a 200 µM en ambas estaciones. La ausencia de diferencias significativas se atribuye al origen del agua, que proviene del acuífero de la Plana de Gandía-Denia (directamente por bombeos o indirectamente como retornos de riego), el cual tiene elevadas concentraciones de ambos nutrientes. Los niveles de fósforo inorgánico disuelto (DIP) son por lo general inferiores a 0.32 µM (0.01 mg L^{-1}), valor fijado como umbral para la recuperación de lagos someros eutrofizados (Villena y Romo, 2003). Los niveles más altos DIP (0.30 µM) han coincidido con el período de abonado fosfórico que se realiza en dosis única al inicio de la primavera (Legaz y Primo-Millo, 1988). Sin embargo, se ha detectado un incremento puntual del DIP en verano

(0.97 μM) que se ha asociado a las descargas de aguas residuales no tratadas. El principal nutriente potencialmente limitante es el fósforo tanto a lo largo del gradiente longitudinal como en ambos escenarios, con ratios muy alejados de la relación de Redfield. En el conglomerado A el ratio medio observado DIN:DIP es de 1968 y el DSi:DIP es de 476, mientras que en el conglomerado B los valores son inferiores, DIN:DIP medio de 399 y DSi:DIP medio de 316, pero siguen siendo muy elevados.

Casos de estudio 2 y 5: en las aguas costeras (CW), con elevada influencia de los aportes terrestres (playa de Venecia), se han observado concentraciones de nutrientes superiores a las halladas en aguas marinas (MW). La concentración de DIN en CW varía entre 0.1 y 50.5 μM, mientras que en MW varía entre 0.1 y 8.0 μM. La concentración de DSi en CW varía entre 0.5 y 16.3 μM, mientras que en MW varía entre 0.2 y 4.5 μM. Por lo que respecta al DIP la diferencia es menor, siendo el valor máximo observado 0.43 μM en CW y 0.23 μM en MW. En ambas zonas se ha observa una mayor concentración de DIN, de DSi y de fósforo total (TP) en el período lluvioso (otoño a primavera). En cambio, no se ha observado ningún patrón temporal claro en los niveles de DIP. Esto se atribuye a que es el principal nutriente potencialmente limitante a lo largo del período de estudio en ambas áreas, por lo que cualquier aporte es aprovechado rápidamente por el fitoplancton e incorporado en forma de biomasa (Falco et al., 2010). Al analizar con mayor resolución espacial la zona de la desembocadura del río Serpis (playa de Venecia) (caso de estudio 2), se halla un área localizada que no presenta diferencias significativas en los niveles de DIN y DSi medidos en primavera (período húmedo) y verano (período seco). La concentración media observada de ambos nutrientes se aproxima a 20 µM. En cambio, sí que se han detectado diferencias en la concentración de DIP. En verano de 2008 se han medido niveles máximos de 0.86 µM, que se han relacionado con un vertido de aguas residuales parcialmente tratadas a través del aliviadero del emisario submarino que vierte directamente al río Serpis. Este vertido se produce porque el aumento de población durante el verano genera un caudal de aguas residuales que supera la capacidad de tratamiento de la planta (60000 m^3 $día^{-1}$). También en esta área el fósforo es el principal nutriente potencialmente limitante en prácticamente todos los puntos y profundidades de muestreo. En abril de 2009 y julio de 2009, los valores mínimos del ratio DIN:DIP fueron 65 y 40 respectivamente, y los mínimos del ratio DSi:DIP fueron 15 y 44 respectivamente. En julio de 2008 se observaron algunos valores de los ratios DIN:DIP y DSi:DIP por debajo de la relación de Redfield, siendo los valores mínimos observados de 7 para ambos ratios.

Caso de estudio 3: en la zona de influencia del emisario submarino, los principales aportes de agua dulce se producen durante el verano, debido al aumento de la población y de las aguas residuales. La concentración de DIN es superior en primavera (valor medio observado 7.08 μM), al igual que en los casos anteriores, cuando la forma dominante es el nitrato. En cambio, en Julio de 2008 cabe destacar la dominancia de la forma amoniacal que representa aproximadamente un 57% del DIN. La concentración de DSi no presenta diferencias significativas entre primavera y verano, variando entre 1.07 y 19.20 μM. En esta zona el muestreo con alta resolución espacial no aporta información relevante acerca del nutriente potencialmente limitante. La dilución con las aguas oligotróficas características del Mediterráneo (el emisario se halla en una zona con aproximadamente 18 m de columna de agua) hace que este varíe de un punto de muestreo a otro y en un mismo punto según la profundidad de toma de muestra. No se han detectado cambios significativos en la calidad del agua, al igual que en otros estudios (Aguilera et al., 2001; Juanes et al., 2005). Sin embargo, el funcionamiento irregular del emisario y la evacuación de efluentes parcialmente tratados, cuando se excede la capacidad de la planta de tratamiento (60000 m^3 $día^{-1}$), provoca mayores aportes de nutrientes y materia orgánica en la desembocadura del río Serpis.

Caso de estudio 4: en la playa del Ahuir se observan mayores concentraciones de DIN (máximo 8.36 µM) y DSi (máximo 9.41 µM) en el muestreo de primavera, que se relacionan con los aportes difusos de agua subterránea desde el acuífero de la Plana de Gandía-Denia. La forma dominante de DIN en esta zona es el nitrato, el cual se relaciona con el uso de fertilizantes en el suelo agrícola del área de estudio. Los elevados niveles de DSi se han relacionado tanto con la disolución química de los silicatos de origen geológico, como con la sílice de origen biogénico, especialmente importante en el acuífero de la Plana debido al depósito de sílice de la vegetación de La Marjal (Conley, 2002). Por otro lado, los niveles de DIP son significativamente más elevados en verano (máximo 0.23 µM), mientras que en primavera se hallan por debajo del límite de detección del análisis (< 0.001 µM), de modo que el fósforo es el principal nutriente potencialmente limitante en primavera. En verano se produce un potencial limitación de nitrógeno que se atribuye a la reducida descarga del acuífero, el cual puede llegar a tener problemas de intrusión marina durante el verano (Ballesteros-Navarro, 2003).

Al comparar los distintos casos de estudio las mayores concentraciones de nutrientes se observan en los canales que vierten las aguas de La Marjal al Puerto de Gandía y en el interior de este, así como en el río Serpis y su desembocadura (playa de Venecia). Los canales de riego y el río Serpis, aunque en si mismos constituyen fuentes puntuales de nutrientes a los ecosistemas costeros, actúan a su vez como colectores tanto de fuentes difusas (escorrentías superficiales y subterráneas) como de fuentes puntuales (vertidos de aguas residuales). Las escorrentías superficiales y subterráneas se caracterizan por elevadas concentraciones de nitratos y sílice, dado el importante uso agrícola en el área de estudio y el origen de la sílice descrito. Además, en ambos casos se han detectado descargas irregulares de aguas residuales, que provocan aumentos en la concentración de fósforo.

En la zona no urbana de La Marjal de La Safor, existen viviendas ilegales dispersas no conectadas al sistema de alcantarillado, que vierten a fosas sépticas o directamente a los canales de riego. Por otra parte, el río Serpis recibe descargas de aguas residuales en dos escenarios distintos. Durante los episodios de lluvia del período húmedo se produce el desbordamiento del sistema colector de aguas residuales, que es un sistema mixto que no separa pluviales y fecales. Durante el período seco, el incremento de población genera un aumento del caudal de aguas residuales que puede superar la capacidad de tratamiento de la planta depuradora (60000 m^3 $día^{-1}$), por lo que se alivian efluentes parcialmente tratados al río.

La desembocadura de los canales de riego y del río Serpis en zonas con un menor intercambio con el mar, el Puerto y la playa de Venecia, permite una mayor acumulación de estos nutrientes. Mientras que en otras zonas como la playa del Ahuir, zona costera abierta, o el emisario submarino, situado en una zona con aproximadamente 18 m de profundidad, los aportes de agua de origen terrestre se diluyen en mayor medida.

3 Variación espacial de la abundancia y composición del fitoplancton en los casos de estudio analizados

Caso de estudio 1: en el Puerto de Gandía se observa un gradiente longitudinal decreciente de clorofila *a* desde los puntos de vertido de agua dulce hacia el mar. Los valores máximos se midieron en el punto de muestreo P5 con 8.8 µg L^{-1} en primavera y en P2 con 11.5 µg L^{-1} en verano, ambos puntos situados inmediatamente después de los puntos de vertido. Los valores mínimos se midieron en el punto P8, situado fuera del puerto, siendo de 1.4 µg L^{-1} en primavera y 1.1 µg L^{-1} en verano. Las diferencias observadas en la clorofila *a* entre los dos escenarios analizados (primavera y verano) no fueron significativas. Su concentración es similar a la observada en otras zonas estuarinas eutróficas (Rodríguez et al., 2003; Seoane et al., 2005). En los canales de riego se observa una mayor abundancia de diatomeas en primavera, cuando representan un 23% del total de clorofila *a* en P1, un 40% en P3 y un 53% en P4. Mientras que en verano su abundancia disminuye (0% P1, 16% P3 y 23% P4) y es remplazada por un aumento de los flagelados (78% P1, 75% P3 y 77% P4). En el interior del puerto predominan en ambos escenarios flagelados característicos de sistemas eutrofizados, como euglenas, dinoflagelados y criptofíceas (Çelik y Ongun, 2007; Latasa et al., 2010; Seoane et al., 2005). En el puerto la contribución de las cianobacterias al total de clorofila *a* representa menos del 1% en primavera. En verano su abundancia aumenta significativamente y se detectan dos poblaciones, una típica de agua dulce con un máximo del 23% del total de clorofila *a* en el punto P5, y otra marina con un máximo del 34% en P7 (0.75 m). En el punto situado fuera del puerto (P8), la composición del fitoplancton difiere de la observada en el interior y predominan grupos característicos de aguas más oligotróficas, primnesiales y cianobacterias (Latasa et al., 2010). La abundancia de primnesiales es mayor en primavera, cuando representan el

73% (0.05 m de profundidad) del total de clorofila *a*, mientras que en verano constituyen el 34% (0.05 m). Es en verano cuando las cianobacterias marinas alcanzan una contribución máxima al total de clorofila *a* del 39%.

Resto de casos de estudio: de forma general, en el resto de zonas de estudio analizadas (desembocadura del Serpis (playa de Venecia) playa del Ahuir, zona del emisario submarino, punto situado en aguas costeras (CW) y punto situado en aguas marinas (MW)), se observan los valores más elevados de clorofila *a* en primavera, cuando las diatomeas son el grupo dominante y en general contribuyen en más del 50% al total de clorofila *a*. En verano, la abundancia de diatomeas es menor, y se observan abundancias más elevadas de flagelados y cianobacterias. Esta variación estacional ha sido observada en otras zonas costeras y se relaciona con la disminución de los nutrientes (nitrógeno y sílice) y con el aumento de temperatura durante el verano (Garmendia et al., 2010).

La zona con mayores niveles de clorofila *a* es el área de la desembocadura del Serpis (playa de Venecia), mientras que los menores niveles se detectan en la zona del emisario. Los altos niveles observados en la playa de Venecia se atribuyen a los aportes de nutrientes del río Serpis y al reducido intercambio con el mar. El río Serpis es un río Mediterráneo caracterizado por un período seco durante el verano. En este período, el caudal registrado en las estaciones de aforo de la Confederación Hidrográfica del Júcar (CHJ), situadas a aproximadamente 10 km de la desembocadura, es nulo o casi nulo. Sin embargo, el funcionamiento irregular del emisario submarino y el alivio de efluentes parcialmente tratados al río, genera un aporte de nutrientes en verano que permite mantener una abundancia relativa de diatomeas que no difiere significativamente de la observada en primavera (al comparar los dos escenarios analizados: muestreo de primavera y muestreo de verano). Los resultados observados en esta zona son similares a los descritos por Garmendia et al. (2010) en el estuario de Oka, el cual también recibe descargas de aguas residuales.

Al analizar los resultados obtenidos de los muestreos quincenales (aproximadamente) realizados en el punto situado cerca de la desembocadura del río Serpis (7.5 m de profundidad) (identificado como CW en la memoria de tesis, capítulo 5), se detecta que el comportamiento estacional de las diatomeas no coincide con la característica evolución con máxima abundancia en primavera y mínima en verano (Garmendia et al., 2010). Aunque sí que se observa una abundancia relativa de diatomeas superior al 40% del total de clorofila *a* en la primavera de 2009, fuera del período primaveral se observan abundancias superiores; por ejemplo, la máxima abundancia de diatomeas se registró el 20 de junio de 2008 con una abundancia relativa del 77%. Por otra parte, las disminuciones en la abundancia de diatomeas a lo largo del período de estudio (mayo de 2008 a agosto de 2009), se producen al registrarse aumentos significativos de flagelados (euglenas, clorofíceas y criptofíceas) y primnesiales, los cuales están correlacionados con los episodios de lluvia, que se producen principalmente en el período lluvioso (primavera, otoño, invierno). En verano, período seco, se detectan mayores abundancias de dinoflagelados.

Es importante destacar que en los recuentos microscópicos de muestras representativas se ha detectado especies y géneros potencialmente formadores de floraciones nocivas (HABs, Harmful Algal Blooms) en el interior del puerto de Gandía, como los dinoflagelados *Dinophysis caudata*, *Ceratium furca*, *Prorocentrum micans*, *Gymnodinium* spp., *Heterocapsa* sp., *Scrippsiella* spp.; diatomeas del género *Amphora* spp. Y *Pseudonitzschia* spp.; y euglenas del género *Eutreptiella* sp. El dinoflalgelado *Dinophysis caudata* se ha detectado también en la desembocadura del río Serpis (playa de Venecia), así como dinoflagelados del género *Alexandrium* sp. Entre estas especies, *Dinophysis caudata* es responsable de la síntesis de la toxina DSP (Diarrheic Shellfish Poisoning), especies del género *Alexandrium* sp pueden sintetizar la toxina PSP (Paralytic Shellfish Poisoning), y las diatomeas del género *Pseudo-nitzschia* spp. la toxina ASP (ácido domoico) (Vila et al. 2001).

4 Generalización de los resultados

En primer lugar es importante destacar que no existe ningún estudio previo publicado en el área de estudio en la que se desarrolla la presente Tesis Doctoral, situada al sur del Golfo de Valencia.

En el mar Mediterráneo noroccidental se desarrollaron los estudios de Olivos et al. (2002) y Flo et al. (2011), los cuales analizan la variabilidad espacial y temporal de las características físico-químicas de las aguas costeras y su interacción con las variables biológicas. En estos estudios la variabilidad espacial se discute principalmente en base a la distancia a la costa, clasificando las aguas costeras en CIW 0-200 m desde la línea de costa, CNW 200-1500 m de la línea de costa y COW situadas a más de 1500 m de la costa (Flo et al., 2011). La única variable biológica estudiada es la clorofila *a*, la cual se utiliza como parámetro indicador de la biomasa fitoplanctónica.

En esta Tesis la variabilidad espacial se ha analizado principalmente en base a las distintas fuentes de entrada de los nutrientes y a la morfología de la zona costera (zonas abiertas vs. zona cerrada). Además, se ha tenido en cuenta no sólo la clorofila *a*, sino la composición pigmentaria de las muestras de agua, la cual permite realizar la clasificación taxonómica del fitoplancton y estudiar las relaciones de las distintas variables físico-químicas con cada uno de los grupos fitoplanctónicos identificados.

En base al análisis especial realizado en función de las distintas entradas de nutrientes y la morfología costera, se recomienda la inclusión de puntos de control de la calidad de las aguas en zonas de débil intercambio con el mar. Esto es especialmente importante en mares micromareales como el Mediterráneo, y coincide con las observaciones de la EEA (1999) y Flo et al. (2011). Los problemas originados en estas zonas pueden extenderse a zonas más amplias, y además afectar a la economía local, al perjudicar a sectores como el turismo o la acuicultura (Vila et al., 2001; CIESM, 2010). Además, se recomienda que los planes de vigilancia ambiental de instalaciones portuarias incluyan el monitoreo de la calidad de las aguas en las zonas adyacentes que se vean afectadas por una reducción de su intercambio con el mar. Estas zonas son especialmente susceptibles a la formación de floraciones nocivas de fitoplancton (Vila et al., 2001).

La utilización conjunta de la técnica de cromatografía líquida de alta resolución (HPLC) aplicada al análisis de pigmentos fotosintéticos marcadores y el programa CHEMTAX, ha permitido elucidar la composición del fitoplancton, y en consecuencia analizar las relaciones descritas. Es destacable que los parámetros necesarios para caracterizar el estado ecológico de las masas de agua según la Directiva Marco del Agua (DMA) incluyen no sólo la clorofila *a* sino también los grupos fitoplanctónicos. La utilización del sensor de alta resolución espacial QuickBird, ha permitido observar el gradiente de clorofila *a* típico de las áreas costeras y descrito por Flo et al. (2011) para el Mediterráneo noroccidental. Así, el mapa de distribución de la clorofila *a* obtenido corrobora los resultados obtenidos con el muestreo tradicional para el mismo escenario (julio 2009). La aplicación exitosa de estas técnicas es un paso más en la validación del posible uso de técnicas de análisis avanzadas en los programas de monitoreo y gestión ambiental, como el de la Directiva Marco del Agua.

Para poder desarrollar estrategias de gestión apropiadas es necesario conocer en primer lugar como funcionan los ecosistemas. En esta Tesis Doctoral se ha analizado principalmente la relación entre nutrientes y grupos fitoplanctónicos. Este análisis es fundamental para poder tomar decisiones que afecten a la reducción de los aporte de nutrientes, sin este análisis se pueden adoptar costosas medidas que no obtengan el resultado esperado. Así, en el área de estudio, el Puerto de Gandía presenta características de un estuario eutrofizado debido a las entradas de nutrientes que provienen principalmente de la actividad agrícola, la cual supone un 48% del uso del suelo en la cuenca vertiente. En esta área ya se están adoptando medidas para reducir está fuente de nutrientes, a través de la aplicación del código de buenas prácticas agrícolas. Sin embargo, dados los niveles de nitratos hallados en el acuífero que alimenta La Marjal (> 50 mg L^{-1}), y cuyos drenajes se vierten en el puerto, se hace necesaria la aplicación de otras medidas. Estas medidas son la reducción de las entradas de fósforo que provienen en parte de descargas de aguas residuales no tratadas. Esta estrategia de gestión se ha propuesto en otros sistemas similares caracterizados por una limitación potencial del fósforo como La Albufera (Valencia, España) (Villena and Romo, 2003) y El Mar Menor (Murcia, España) (García-Pintado et al., 2007).

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Capítulo 8: Conclusiones

A continuación se resumen las principales conclusiones obtenidas en función de los objetivos propuestos.

Respecto a la metodología utilizada:

- El método de análisis de pigmentos fotosintéticos marcadores con HPLC desarrollado por Wright et al. (1991), asociado con la utilización del programa CHEMTAX según la metodología propuesta por Latasa (2007) ha permitido caracterizar la composición pigmentaria de muestras de agua de la zona costera y determinar la composición de la comunidad fitoplanctónica
- El diseño de campañas de muestreo, la toma de muestras y su análisis ha permitido establecer la variabilidad espacial de los grupos fitoplanctónicos en función de los aportes de nutrientes y del comportamiento de las distintas masas de agua de la zona costera estudiada
- Se ha desarrollado un modelo para la estimación de la clorofila *a* con el sensor de alta resolución espacial QuickBird que ha permitido detectar y caracterizar la elevada variabilidad espacial de

la biomasa fitoplanctónica en el área de estudio. La aplicación de este modelo en julio de 2009 ha generado un mapa de distribución de la clorofila *a* que coincide con el análisis espacial realizado con las técnicas tradicionales de muestreo y análisis. El uso de satélites de menor resolución espacial no habría permitido observar la variabilidad espacial del área de estudio.

Respecto a las fuentes de nutrientes y su variabilidad espacial:

- El uso del suelo en la zona de estudio condiciona los aportes de nutrientes. Los principales usos son el agrícola y el urbano, los cuales en el caso de la cuenca vertiente a la instalación portuaria de Gandía suponen un 48% y un 16% del total, según el análisis de usos del suelo realizado.
- La actividad agrícola es la principal fuente de nitratos. La concentración de nitratos en el área de estudio es mayor durante el período húmedo en todas las áreas de estudio, debido a los aportes continentales.
    - La mayor concentración de nitratos se observa en el interior de la instalación portuaria de Gandía (caso de estudio 1) con valores superiores a los 200 µM debido al

aporte de las acequias que drenan La Marjal de La Safor.

- En la desembocadura del río Serpis (caso de estudio 2) los valores medidos se aproximan a 50 µM. En esta área la fluctuación en los niveles de nitratos se correlaciona de forma significativa con los episodios de precipitación y con el caudal del río Serpis.
- En la playa del Ahuir (caso de estudio 4), la máxima concentración de nitratos observada es de 8.36 µM. En esta área no existen aportes de cursos superficiales de agua y el aporte de agua dulce se produce por entradas difusas desde el acuífero de la Plana de Gandía-Denia. Además, se trata de un área costera abierta en la que se produce una mayor dilución de los aportes continentales.
- En el área de vertido del emisario submarino de la estación depuradora de aguas residuales de Gandía (caso de estudio 3), la concentración media de nitratos observada en primavera es de 6.88 µM. Mientras que en las otras zonas estudiadas el nitrato es la forma de nitrógeno dominante en todas las campañas de

muestreo, en el emisario se observa una predominancia de la forma amoniacal en julio de 2008, cuando el amonio representa aproximadamente un 57% del nitrógeno disuelto inorgánico (DIN).

  - En el área de aguas marinas (MW, caso de estudio 5) el nitrato también es la forma dominante de DIN, y los valores máximos observados fueron de 7.10 µM.

— Los niveles de sílice observados en el área de estudio se atribuyen a la disolución de la sílice de origen geológico, pero también a la de origen biogénico, cuya fuente principal se halla en la vegetación natural y la materia orgánica acumulada en el suelo de La Marjal de La Safor. En general, los niveles de sílice observados en las aguas receptoras son elevados si se comparan con zonas costeras de similares características situadas más al norte en el Mediterráneo occidental. Los mayores niveles de sílice se observan también durante el período lluvioso.

— La actividad agrícola se relaciona también con el aporte de fósforo. En este caso se ha establecido una conexión en el puerto de Gandía entre los niveles de fósforo más elevados en primavera (máximo 0.30 μM) con el período de abonado de los cítricos, cultivo dominante en la cuenca vertiente.

— El uso urbano del suelo en el área de estudio genera aguas residuales que son tratadas en la estación depuradora de aguas residuales (EDAR) de Gandía. El efluente tratado es vertido al mar a través de un emisario submarino situado a 1900 m de la línea de costa. Sin embargo, se han observado ciertas anomalías en la evacuación de las aguas residuales que se asocian a un aporte de nutrientes, entre los que destaca el fósforo por su importancia como nutriente potencialmente limitante en la mayoría de situaciones estudiadas.

  - En la zona no urbana de La Marjal de La Safor existen viviendas dispersas, cuya ubicación ha sido localizada en las fotografías áreas de la zona, que no están conectadas a la red general de alcantarillado. Estas viviendas vierten las aguas residuales directamente a las acequias de riego o bien a fosas sépticas. El uso de estas viviendas como segunda residencia durante el período estival se ha vinculado a la detección de niveles elevados de fósforo disuelto inorgánico (DIP) (0.97µM) en julio de 2009 en la acequia Nova-Ahuir.

- Durante los episodios de lluvia se supera la capacidad del colector de aguas residuales, dado que en el área de estudio existe un sistema de alcantarillado combinado de fecales y pluviales. En estos casos se vierten caudales mixtos (fecales y pluviales) al río Serpis a través de los aliviaderos del colector
- El aumento de la población durante el verano debido al turismo residencial en el área de estudio genera un aumento de caudal de aguas residuales que supera la capacidad máxima de la EDAR de Gandía (60000 m^3 $día^{-1}$). En esta época se detectan descargas de efluentes parcialmente tratados directamente al río Serpis a través del aliviadero del conducto del emisario submarino.
- No se ha podido encontrar una correlación significativa del DIP con los aportes de aguas residuales descritos en el área de la desembocadura del Serpis ya que, dada la condición del fósforo como primer nutriente potencialmente, cualquier aporte de DIP es rápidamente

consumido por el fitoplancton. Sin embargo, la concentración de fósforo total sí que ha sido correlacionada con los episodios de lluvia, y presenta una variación temporal similar a la del nitrógeno inorgánico disuelto y de la sílice disuelta.

Respecto a la variabilidad de las comunidades fitoplanctónicas:

— Los valores más elevados de biomasa fitoplanctónica (clorofila *a*) se han detectado en primavera, y se han correlacionado con la mayor disponibilidad de nutrientes respecto al verano (período seco). Excepto en las muestras tomadas en las acequias del Molí y de Nova-Ahuir donde los mayores niveles de clorofila *a* se han medido en verano, siendo de 10.2 y 4.5 μg L^{-1} respectivamente. Así como en el río Serpis donde se han medido valores superiores a 70 μg L^{-1} en julio de 2008 similares a los medidos en primavera de 2009.

— Los valores más elevados de clorofila *a*, exceptuando las muestras tomadas en cursos de agua dulce, se han observado en el interior del puerto a Gandía en los puntos situados inmediatamente después de los vertidos de las acequias (máximo de 8.8 μg L^{-1} en

P5 punto situado después del vertido de las acequias del Rei y Nova-Ahuir) y en la desembocadura del río Serpis. Estos mayores niveles se han relacionado con las descargas de fuentes puntuales (acequias y río Serpis) y con el menor intercambio de estas áreas con el mar.

- En el puerto de Gandía se ha descrito una variación longitudinal y vertical de la composición fitoplanctónica debido a la influencia de los aportes de agua dulce de las acequias que drenan La Marjal de La Safor. En el interior del puerto se observa una dominancia de los flagelados característicos de sistemas eutrofizados, como euglenas, dinoflagelados y criptofíceas tanto en primavera como en verano. Mientras que en el punto situado fuera del puerto predominan grupos característicos de aguas más oligotróficas, como primnesiales y cianobacterias.
- En la playa del Ahuir y en el área de influencia del emisario submarino, se ha observado una mayor abundancia de diatomeas en primavera, que es sustituida por un aumento de los flagelados y las cianobacterias en verano.
- En la desembocadura del río Serpis la variación en la abundancia de diatomeas no responde al ciclo estacional descrito en las otras

áreas de estudio, sino que se relaciona con las variaciones de la población de flagelados que dependen a su vez de las descargas de agua dulce del río Serpis.

Respecto a las medidas recomendadas para mejorar la calidad de las aguas:

— En la actualidad se están implementado medidas dirigidas a reducir los aportes de nutrientes procedentes de la agricultura de acuerdo con el código de buenas prácticas agrícolas. Se recomienda la vigilancia del cumplimiento de estas medidas. No obstante, dada la elevada concentración de nitratos en el acuífero de la Plana de Gandía-Denia, se prevé que los resultados de su aplicación no sea inmediato.

— Se recomienda adoptar medidas dirigidas a reducir los aportes de nutrientes de las aguas residuales. Estas medidas son especialmente importantes dado el carácter del fósforo como principal nutriente potencialmente limitante del desarrollo de la biomasa fitoplanctónica, y dada la prevalencia de esta fuente en el aporte de fósforo.

— Se recomienda incluir en los planes de vigilancia ambiental de instalaciones portuarias el monitoreo de la calidad de las aguas en las zonas adyacentes que se vean afectadas por una reducción de su intercambio con el mar

Capítulo 9: Futuras líneas de investigación

El modelo aplicado para la estimación de clorofila *a* con el sensor QuickBird, ha sido desarrollado y validado en una única zona y en una época concreta (verano). Se trata de una primera aproximación al uso de un sensor de alta resolución espacial. Dados los buenos resultados obtenidos, se abren dos nuevas líneas de investigación:

1. Evaluar el modelo en distintas zonas y escenas
2. Comparar las estimaciones de clorofila *a* realizadas con QuickBird con otros satélites de menor resolución espacial pero mayor resolución espectral

El Plan Nacional de Calidad de las Aguas: saneamiento y depuración 2007-2015, identifica como uno de los retos para un futuro inmediato la importancia del control de la contaminación producida por descargas de sistemas unitarios, provocadas por el alivio de la mezcla de agua residual con pluvial durante tormentas. De acuerdo con la Directiva 91/271/CEE, la cual señala en el Anexo I acerca de los requisitos de las aguas residuales urbanas, que el diseño, construcción y mantenimiento de los sistemas colectores deberá realizarse de acuerdo con los mejores conocimientos técnicos que no redunden en costes excesivos, en especial por lo que respecta a la restricción de la contaminación de las aguas receptoras por el desbordamiento de las aguas de tormenta. Sin embargo, estas medidas tienen un elevado coste económico por lo que en el anterior plan (1995-2005) fueron pocas las que se llevaron a término. Dada la importancia de los aportes del sistema unitario en nuestra área de estudio, se plantea una nueva línea de investigación, que consistiría en:

1. Modelar la contribución de los aportes de aguas residuales y de nutrientes de origen agrícola, testando diferentes escenarios. El objetivo sería analizar si las medidas propuestas tendrían el efecto esperado en las concentraciones de nutrientes y abundancia de los grupos fitoplanctónicos en las aguas receptoras.

Anexos

Anexo I Cromatogramas

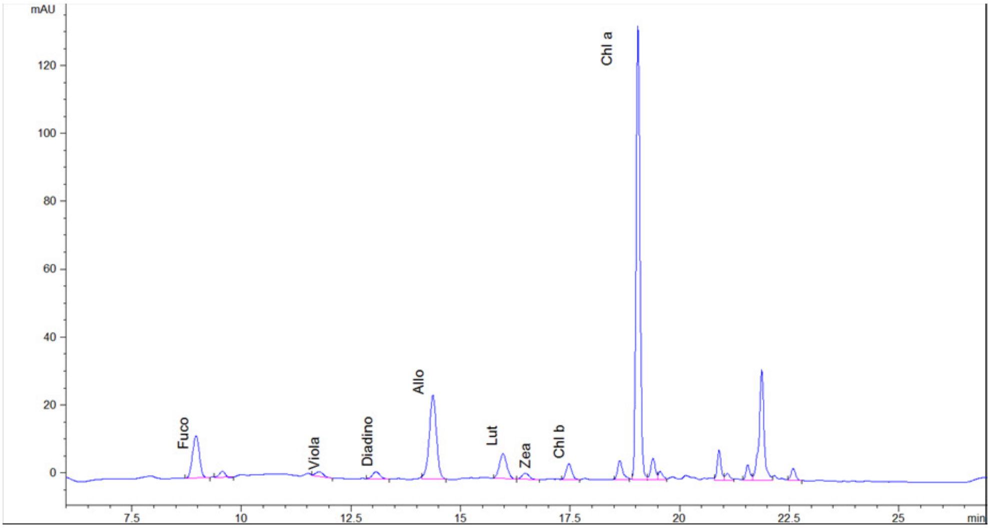

Figura 4 Cromatograma correspondiente a la muestra P1-julio-2008 (Acequia del Molí, muestreo Puerto de Gandía, Capítulo 3 del presente documento)

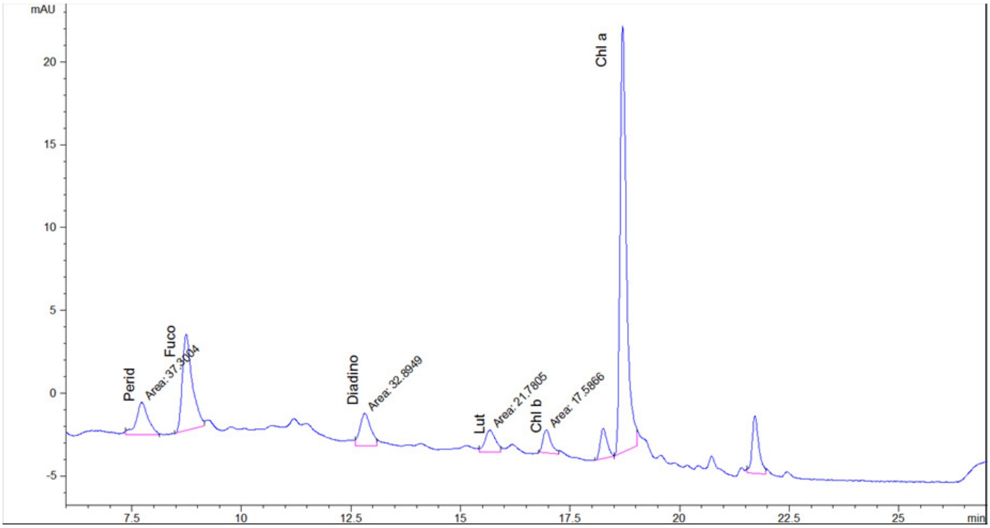

Figura 5 Cromatograma correspondiente a la muestra P3-abril-2009 (Acequia del Rei, muestreo Puerto de Gandía, Capítulo 3 del presente documento)

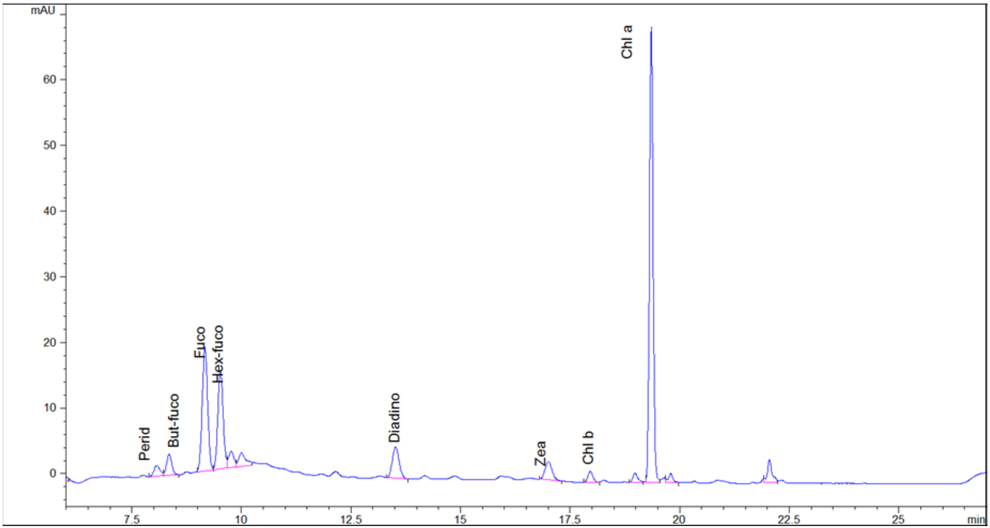

Figura 6 Cromatograma correspondiente a la muestra A6-julio-2008 (Muestreo transecto de la playa del Ahuir, Capítulo 4 del presente documento)

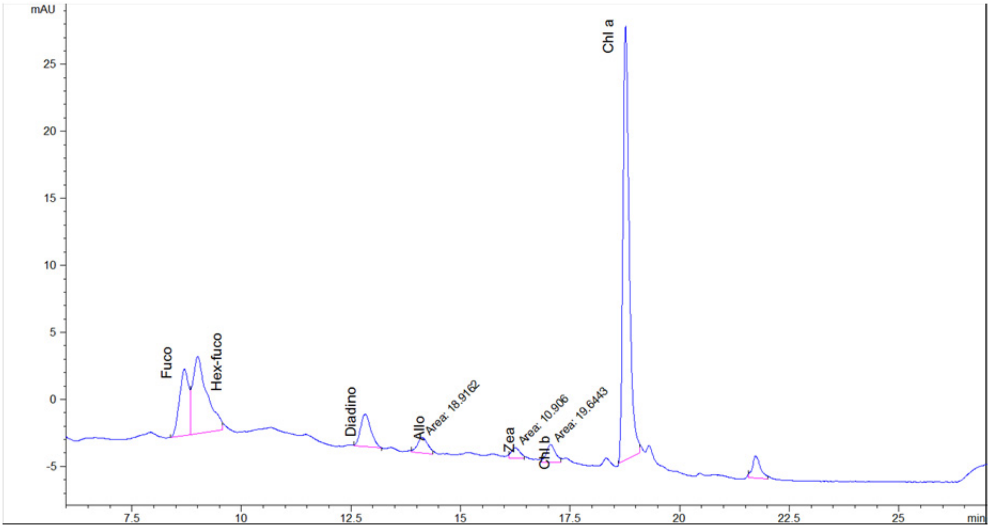

Figura 7 Cromatograma correspondiente a la muestra A6-abril-2009 (Muestreo transecto de la playa del Ahuir, Capítulo 4 del presente documento)

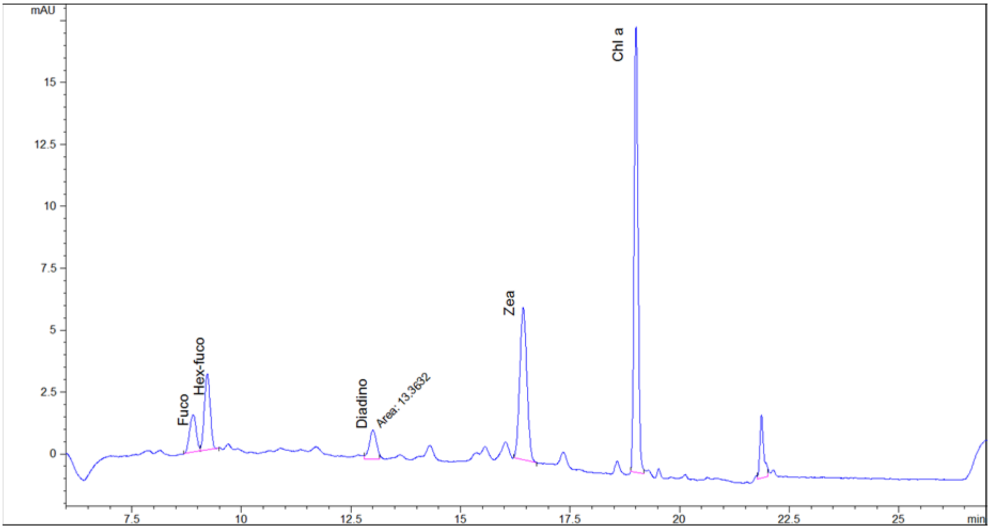

Figura 8 Cromatograma correspondiente a la muestra O1-julio-2008 (Muestreo Emisario submarino EDAR de Gandía, Capítulo 4 del presente documento)

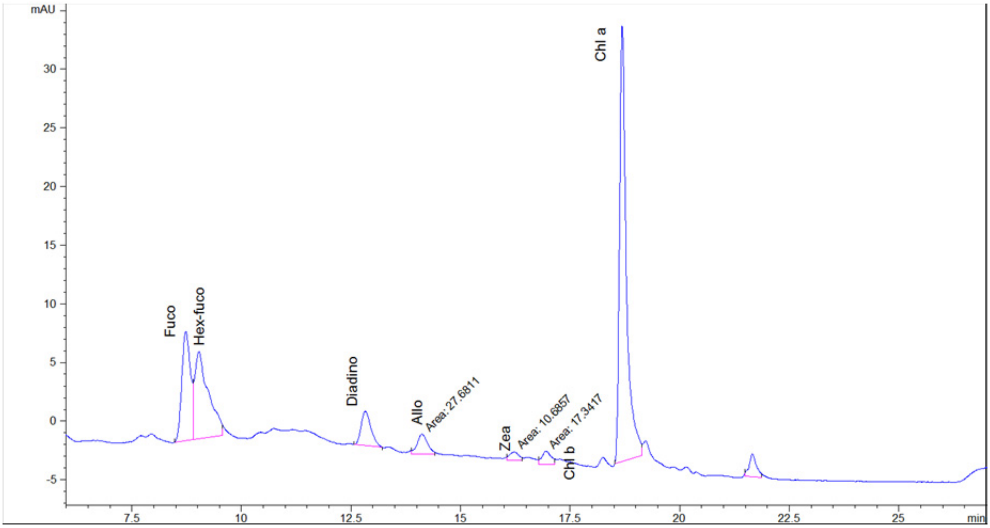

Figura 9 Cromatograma correspondiente a la muestra O1-abril-2009 (Muestreo Emisario submarino de la EDAR de Gandía, Capítulo 4 del presente documento)

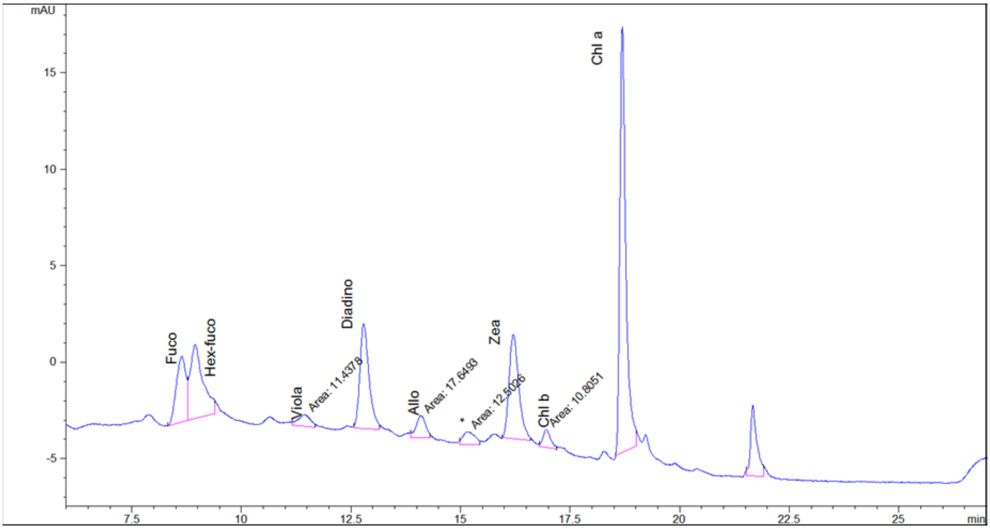

Figura 10 Cromatograma correspondiente a la muestra O2-julio-2009 (Muestreo Emisario submarino de la EDAR de Gandía, Capítulo 4 del presente documento)

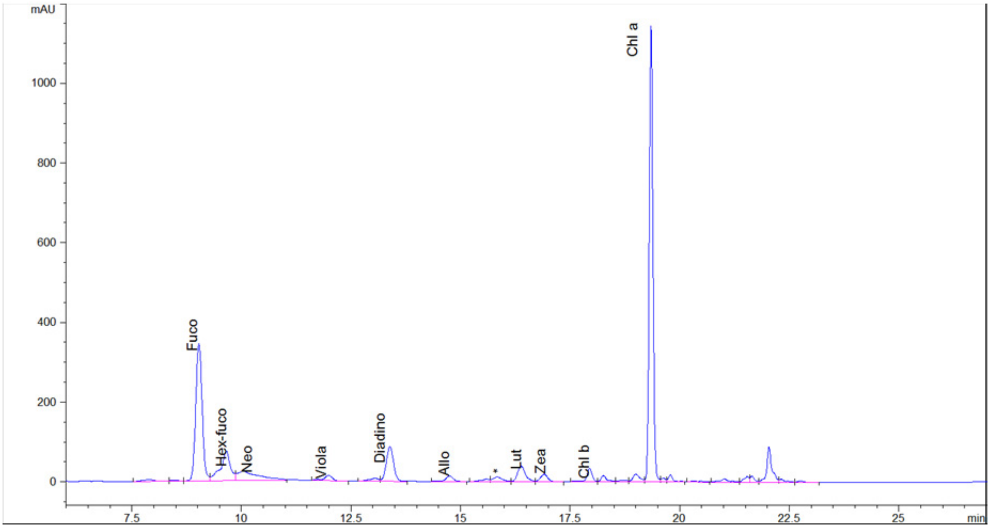

Figura 11 Cromatograma correspondiente a la muestra del río Serpis-julio-2008 (Muestreo playa de Venecia y pluma del río Serpis, Capítulo 4 del presente documento)

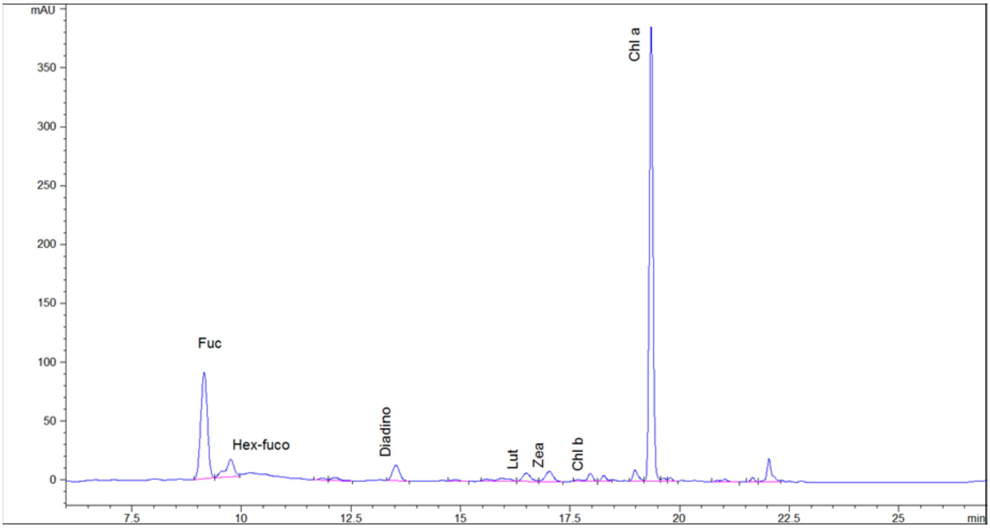

Figura 12 Cromatograma correspondiente a la muestra V5-julio-2008 (Muestreo playa de Venecia y pluma del río Serpis, Capítulo 4 del presente documento)

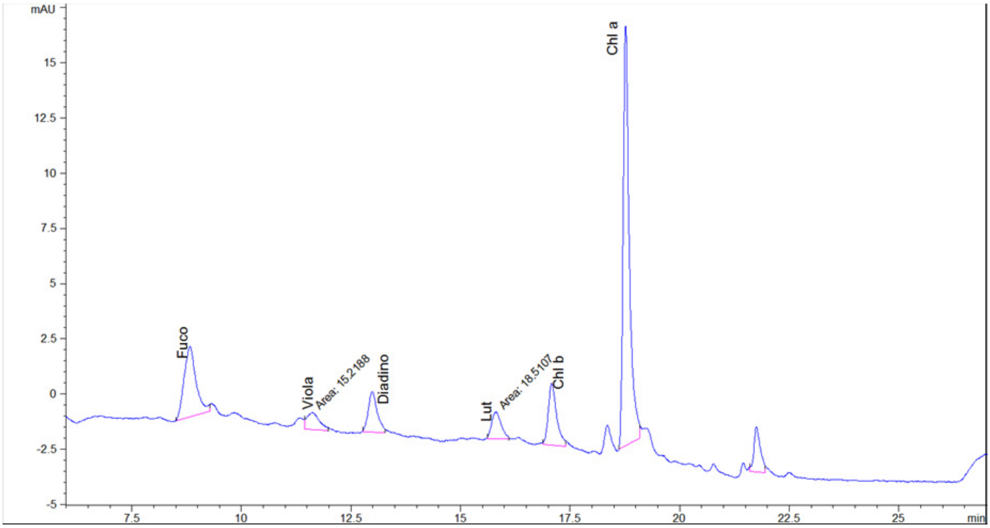

Figura 13 Cromatograma correspondiente a la muestra V5-abril-2009 (Muestreo playa de Venecia y pluma del río Serpis, Capítulo 4 del presente documento)

Anexo II Matrices ratio finales CHEMTAX

Tabla 1 Matriz final de ratios obtenida con CHEMTAX para los conglomerados del Capítulo 3 de esta memoria de tesis “Influence of nutrient inputs from a wetland dominated by agriculture on the phytoplankton community in a shallow harbour at the Spanish Mediterranean coast”

	Per	19’But	Fuc	19’Hex	Neo	Pras	Viol	Allo	Lut	Zea	Chl b
Diatoms											
Cluster1	-	-	0.456	-	0.001	-	-	-	-	-	-
Cluster2	-	-	0.704	-	0.003	-	-	-	-	-	-
Dinoflagellates											
Cluster1	0.582	-	-	0.014	-	-	-	-	-	-	-
Cluster2	0.540	-	-	0.080	-	-	-	-	-	-	-
Euglenophytes											
Cluster1	-	-	-	-	0.001	-	-	-	-	-	0.098
Cluster2	-	-	-	-	0.003	-	-	-	-	-	0.056
Chlorophytes											
Cluster1	-	-	-	-	0.039	-	0.064	-	0.185	0.029	0.323
Cluster2	-	-	-	-	0.016	-	0.097	-	0.029	0.023	0.059
Cryptophytes											
Cluster1	-	-	-	-	-	-	-	0.193	-	-	-
Cluster2	-	-	-	-	-	-	-	0.703	-	-	-
Prasinophytes											

	Per	19’But	Fuc	19’Hex	Neo	Pras	Viol	Allo	Lut	Zea	Chl b
Cluster1	-	-	-	-	0.004	0.246	0.019	-	0.009	0.024	0.112
Cluster2	-	-	-	-	0.005	0.131	0.092	-	0.008	0.025	0.104
Prymnesiophytes											
Cluster1	-	0.005	0.089	0.222	-	-	-	-	-	-	-
Cluster2	-	0.008	0.135	0.230	-	-	-	-	-	-	-
Cyanobacteria											
Cluster1	-	-	-	-	-	-	-	-	-	0.427	-
Cluster2	-	-	-	-	-	-	-	-	-	0.706	-

Per: peridinin; 19’But: 19’-butanoyloxyfucoxanthin; Fuco: fucoxanthin; 19’Hex: 19’-hexanoyloxyfucoxanthin; Neo: neoxanthin; Pras: prasinoxanthin; Viol: violaxanthin; Allo: alloxanthin; Lut: lutein; Zea: zeaxanthin; Chl b: chlorophyll *b*. –: pigmento no presente en el grupo fitoplanctónico

El cluster 1 incluye las muestras de los canales de riego (P1, P3 y P4) y las muestras de P2 a P5 (de 0 a 0.75 m de profundidad). El cluster 2 incluye el resto de muestras

Tabla 2 Matriz final de ratios obtenida con CHEMTAX para los conglomerados del Capítulo 4 de esta memoria de tesis "Effects of freshwater inputs on the receiving waters of a Mediterranean coastal area: nutrients and phytoplankton analysis"

	Per	19'But	Fuc	19'Hex	Neo	Pras	Viol	Allo	Lut	Zea	Chl b
Diatoms											
Cluster1	-	-	0.169	-	0.001	-	-	-	-	-	-
Cluster2	-	-	0.246	-	0.000	-	-	-	-	-	-
Dinoflagellates											
Cluster1	0.524	-	-	0.169	-	-	-	-	-	-	-
Cluster2	0.231	-	-	0.008	-	-	-	-	-	-	-
Euglenophytes											
Cluster1	-	-	-	-	0.013	-	-	-	-	-	0.539
Cluster2	-	-	-	-	0.001	-	-	-	-	-	0.317
Chlorophytes											
Cluster1	-	-	-	-	0.071	-	0.030	-	0.130	0.057	0.091
Cluster2	-	-	-	-	0.001	-	0.090	-	0.176	0.002	0.198
Cryptophytes											
Cluster1	-	-	-	-	-	-	-	0.129	-	-	-
Cluster2	-	-	-	-	-	-	-	0.617	-	-	-
Prasinophytes											
Cluster1	-	-	-	-	0.068	0.090	0.114	-	0.027	0.073	0.147
Cluster2	-	-	-	-	0.086	0.094	0.045	-	0.020	0.012	0.298
Prymnesiophytes											
Cluster1	-	0.020	0.220	0.405	-	-	-	-	-	-	-
Cluster2	-	0.003	0.140	0.185	-	-	-	-	-	-	-

Cyanobacteria											
Cluster1	-	-	-	-	-	-	-	-	-	0.443	-
Cluster2	-	-	-	-	-	-	-	-	-	0.263	-

Per: peridinin; 19'But: 19'-butanoyloxyfucoxanthin; Fuco: fucoxanthin; 19'Hex: 19'-hexanoyloxyfucoxanthin; Neo: neoxanthin; Pras: prasinoxanthin; Viol: violaxanthin; Allo: alloxanthin; Lut: lutein; Zea: zeaxanthin; Chl b: chlorophyll *b*. –: pigmento no presente en el grupo fitoplanctónico

Tabla 3 Matriz final de ratios obtenida con CHEMTAX para los conglomerados del Capítulo 5 de esta memoria de tesis "Nutrients and phytoplankton dynamics in the receiving waters of a Mediterranean river: analysis of the wet and the dry season"

	per	but	fuc	hex	neo	pras	viol	allo	lut	zea	chl_b
Diatoms											
Cluster 1	-	-	0.1753	-	0.0007	-	-	-	-	-	-
Cluster 2	-	-	0.2636	-	0.0000	-	-	-	-	-	-
Cluster 3	-	-	0.2634	-	0.0000	-	-	-	-	-	-
Dinoflagellates											
Cluster 1	0.5275	-	-	0.1095	-	-	-	-	-	-	-
Cluster 2	0.2221	-	-	0.1307	-	-	-	-	-	-	-
Cluster 3	0.2628	-	-	0.0933	-	-	-	-	-	-	-
Euglenophytes											
Cluster 1	-	-	-	-	0.0015	-	-	-	-	-	0.2965
Cluster 2	-	-	-	-	0.0052	-	-	-	-	-	0.4234
Cluster 3	-	-	-	-	0.0005	-	-	-	-	-	0.3575
Chlorophytes											
Cluster 1	-	-	-	-	0.1990	-	0.0310	-	0.0226	0.0503	0.0638
Cluster 2	-	-	-	-	0.3895	-	0.0162	-	0.0205	0.0411	0.0250
Cluster 3	-	-	-	-	0.5452	-	0.0001	-	0.0569	0.0042	0.0064
Cryptophytes											
Cluster 1	-	-	-	-	-	-	-	0.3136	-	-	-

	per	but	fuc	hex	neo	pras	viol	allo	lut	zea	chl_b
Cluster 2	-	-	-	-	-	-	-	0.3561	-	-	-
Cluster 3	-	-	-	-	-	-	-	0.3600	-	-	-
Prasinophytes											
Cluster 1	-	-	-	-	0.0535	0.1721	0.1057	-	0.0823	0.0973	0.1631
Cluster 2	-	-	-	-	0.0068	0.0428	0.2202	-	0.0250	0.0474	0.0271
Cluster 3	-	-	-	-	0.0093	0.1205	0.1751	-	0.0592	0.0804	0.1070
Prymnesiophytes											
Cluster 1	-	0.0447	0.0056	0.4331	-	-	-	-	-	-	-
Cluster 2	-	0.0267	0.0844	0.1833	-	-	-	-	-	-	-
Cluster 3	-	0.0265	0.0860	0.1818	-	-	-	-	-	-	-
Synechococcus											
Cluster 1	-	-	-	-	-	-	-	-	-	0.4605	-
Cluster 2	-	-	-	-	-	-	-	-	-	0.4033	-
Cluster 3	-	-	-	-	-	-	-	-	-	0.4551	-

Per: peridinin; 19'But: 19'-butanoyloxyfucoxanthin; Fuco: fucoxanthin; 19'Hex: 19'-hexanoyloxyfucoxanthin; Neo: neoxanthin; Pras: prasinoxanthin; Viol: violaxanthin; Allo: alloxanthin; Lut: lutein; Zea: zeaxanthin; Chl b: chlorophyll *b*. –: pigmento no presente en el grupo fitoplanctónico

Para el cluster 4 de este capítulo se ha utilizado la matriz de ratios del Cluster 1 del Capítulo 3, Puerto de Gandía, por presentar similares características. Además, el número de muestras de este cluster (n = 4) era insuficiente para calcularlo por separado con CHEMTAX