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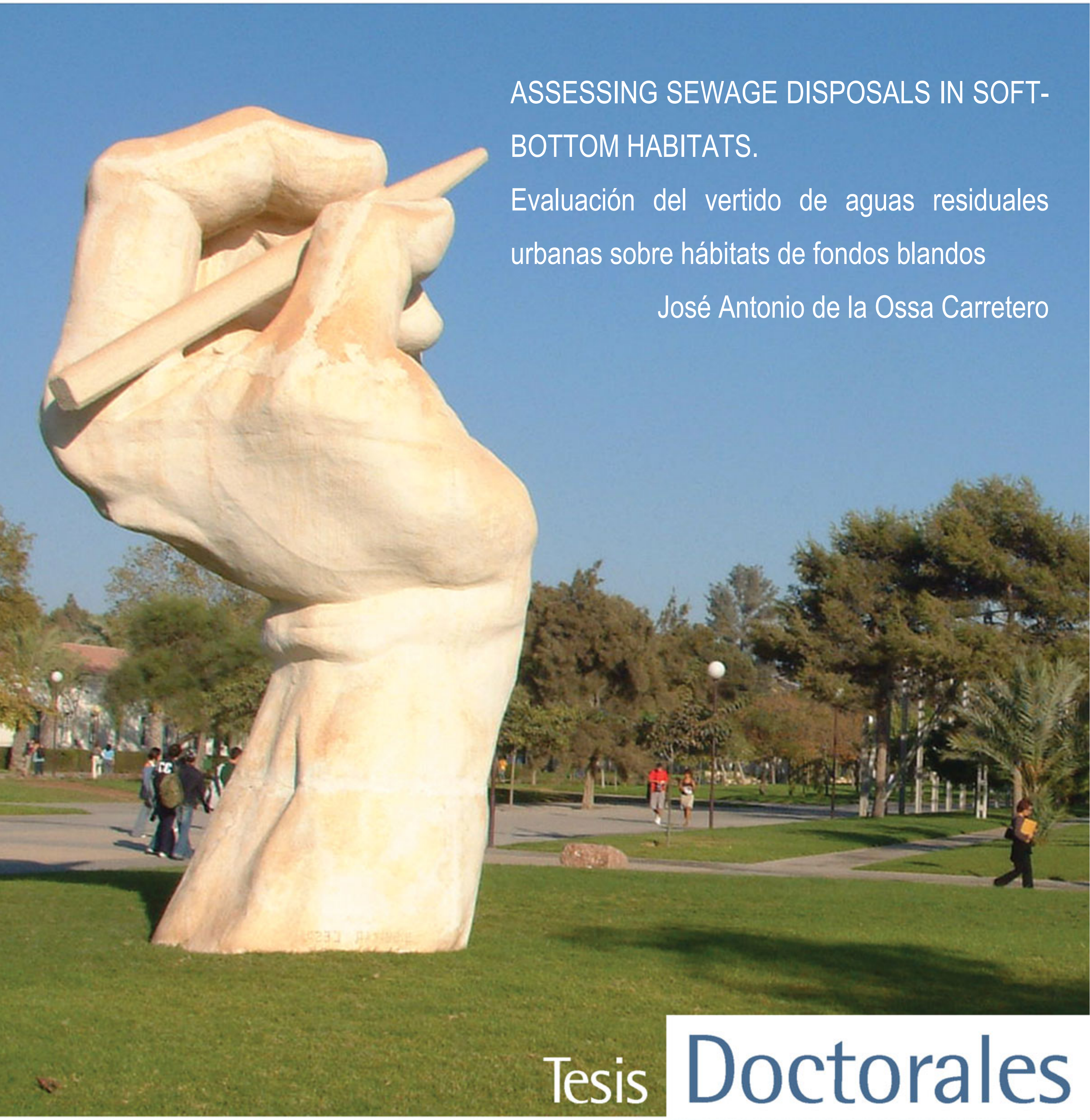

ASSESSING SEWAGE DISPOSALS IN SOFT-BOTTOM HABITATS.

Evaluación del vertido de aguas residuales urbanas sobre hábitats de fondos blandos

José Antonio de la Ossa Carretero

Tesis Doctorales

UNIVERSIDAD de ALICANTE

Universitat d'Alacant
Universidad de Alicante
Departament de Ciències del Mar i Biologia Aplicada
Departamento de Ciencias del Mar y Biología Aplicada

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Doctoral thesis
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Memoria presentada para optar al grado de Doctor, mención Doctor Europeo, en la Universidad de Alicante por

JOSE ANTONIO DE LA OSSA CARRETERO

ALICANTE, 2011

Universitat d'Alacant
Universidad de Alicante

Departament de Ciències del Mar i Biologia Aplicada
Departamento de Ciencias del Mar y Biología Aplicada

Memoria presentada por **JOSE ANTONIO DE LA OSSA CARRETERO** para optar al título de Doctor en Ciencias del Mar en el Departamento de Ciencias del Mar y Biología Aplicada de la Universidad de Alicante, bajo la dirección de **DR. JOSÉ LUIS SÁNCHEZ LIZASO** y de **DRA. FRANCISCA GIMÉNEZ CASALDUERO.**

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Alicante.

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TESIS DOCTORAL

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2011

Director del Departamento de Ciencias del Mar y Biología Aplicada

José Luis Sánchez Lizaso

Universitat d'Alacant
Universidad de Alicante
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CERTIFICAN:

Que la memoria de la Tesis Doctoral "*Evaluación del vertido de aguas residuales urbanas sobre hábitats de fondos blandos*" presentada por **JOSE ANTONIO DE LA OSSA CARRETERO** ha sido realizada bajo su dirección en el Departamento de Ciencias del Mar y Biología Aplicada de la Universidad de Alicante.

Y para que conste a los efectos oportunos, firman en Alicante a 7 de noviembre del año dos mil once.

Directores de la tesis

Dr. José Luis Sánchez Lizaso **Dra. Francisca Giménez Casalduero**

Alicante.

Noviembre 2011

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A mis padres,

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y, como no,

a Gema

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Introducción general

1.1.- Antecedentes.

Las zonas costeras soportan una gran variedad de usos socioeconómicos, lo cual provoca un aumento de la población en las localidades cercanas al litoral. Estas zonas presentan una densidad de población muy alta, que supera hasta 2.5 veces el promedio total de la de los continentes donde se ubican. De hecho, el 35% de la población mundial habita a menos de 50 kilómetros de la costa, considerándose población directamente litoral, lo cual indica la tendencia del ser humano a desplazarse hacia estas zonas, atraído por las ventajas que representa la riqueza de sus recursos (Cifuentes Lemus *et al.*, 1991).

En los países costeros mediterráneos residen alrededor de 450 millones de personas y además esta región es visitada por más de 135 millones de turistas al año (EEA, 2001). Este gran desarrollo poblacional produce un incremento en las presiones e impactos antrópicos, lo que genera un aumento en el stress medioambiental en la zona litoral (Bald *et al.*, 2005). Entre las presiones producidas por el hombre, el vertido de aguas residuales urbanas es uno de los impactos más comunes y es considerado uno de los más importantes, debido a que representan el mayor volumen de los residuos descargados en el medio marino (Islam y Tanaka, 2004).

La alta densidad poblacional del Mediterráneo genera el vertido de un gran volumen de aguas residuales urbanas, centralizado en las 601 ciudades con población superior a 10000 habitantes (Stamou y Kamizoulis, 2009). Entre estas ciudades, 463 tienen algún tipo de depuración, de pretratamiento a tratamiento terciario, mientras que 138 de estas ciudades vierten sus aguas sin ningún tipo de tratamiento (UNEP/MAP, 2004). El vertido de aguas residuales sin tratar o apenas tratadas es, junto con el vertido de nutrientes desde la agricultura y acuicultura, el mayor problema relacionado con la eutrofización del Mar Mediterráneo (EEA, 2001). El incremento en los niveles de nutrientes (fósforo o nitrógeno) y de materia orgánica produce fenómenos de eutrofización, generando cambios en la estructura y funcionamiento de los ecosistemas marinos (Gray *et al.*, 2002).

La Directiva del Consejo 91/271/CEE, de 21 de mayo de 1991, sobre el tratamiento de las aguas residuales urbanas (UWWTD, 1991), tiene como objetivo evitar que la evacuación de aguas residuales tratadas de manera insuficiente provoque repercusiones negativas en el medio ambiente. Esta directiva obliga a los Estados Miembros a la instalación de sistemas colectores y un tratamiento apropiado para aguas residuales en las aglomeraciones con más de 2000 habitantes equivalentes. Los logros de esta normativa se integran dentro de la Directiva 2000/60/CE (WFD, 2000), conocida como Directiva Marco de Aguas (en adelante DMA), entre cuyos objetivos están prevenir el deterioro del estado de todas las masas de agua superficial y lograr un buen potencial ecológico y un buen estado químico antes del año 2015. Dentro de la legislación estatal, el Real Decreto Legislativo 1/2001 aprueba el texto refundido de la Ley de Aguas, que marca como principio general que la protección de las aguas marinas tendrá por objeto interrumpir o suprimir gradualmente los vertidos, las emisiones y las pérdidas de sustancias peligrosas prioritarias, con el objetivo último de conseguir concentraciones en el medio marino cercanas a los valores básicos por lo que se refiere a las sustancias de origen natural y próximas a cero por lo que respecta a las sustancias sintéticas artificiales.

De esta manera, el control de los vertidos de aguas residuales urbanas es imprescindible para la correcta valoración y gestión medioambiental requerida por la aplicación de esta legislación. Por ello, es necesario llevar a cabo un seguimiento de las áreas afectadas, tanto para detectar posibles efectos medioambientales adversos que conlleven el vertido de aguas insuficientemente tratadas, como para evaluar los beneficios de una mejora en la depuración de las aguas o una disminución del caudal vertido.

1.2.- Emisarios de la Comunidad Valenciana.

Actualmente, en la Comunidad Valenciana vierten aguas residuales 21 emisarios submarinos a lo largo de sus 518 kilómetros de litoral (figura 1.1). Estas aguas son previamente tratadas en estaciones depuradoras de aguas residuales (EDAR). Sin embargo, el nivel de depuración varía entre localidades, desde un simple pretratamiento, que únicamente permite la extracción de grandes sólidos en suspensión y la eliminación de compuestos no específicos, a tratamientos secundarios, donde la materia orgánica es oxidada y se reduce la concentración de nitrógeno y fósforo, o incluso terciarios, donde el agua es depurada mediante tecnologías de tratamiento avanzado, eliminando la presencia de sustancias contaminantes en los vertidos (Zarzo Martínez, 2008).

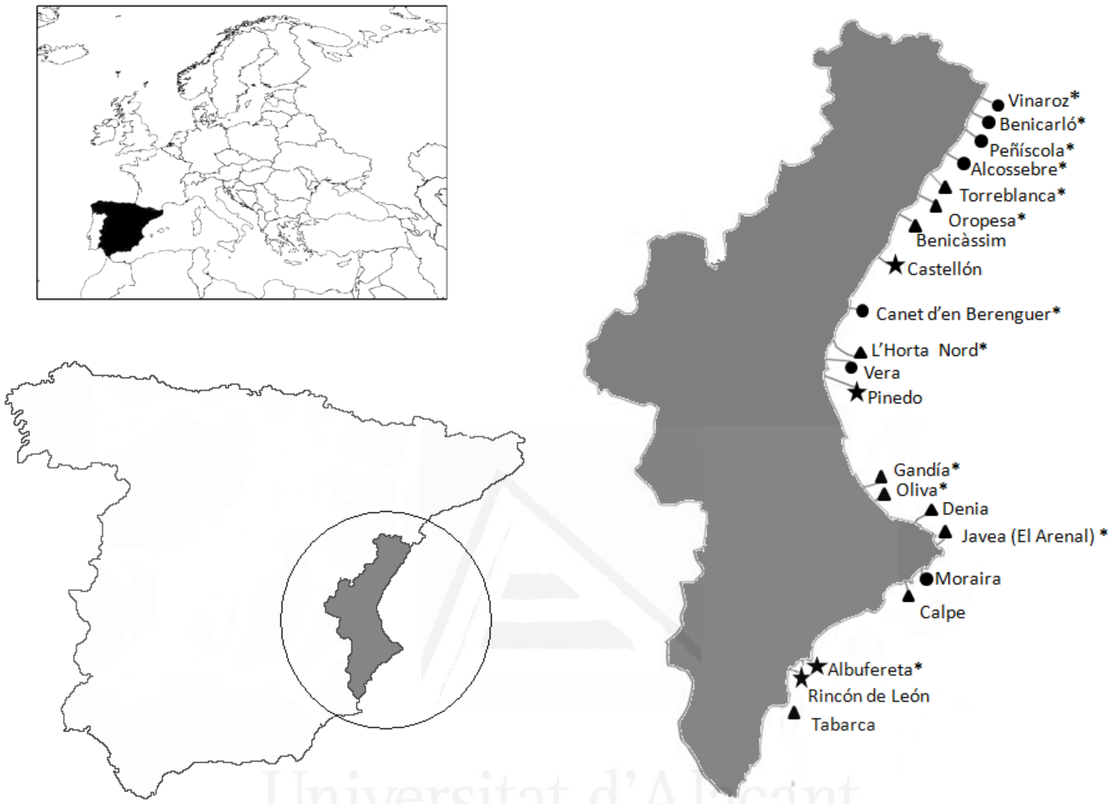


Figura 1.1. Emisarios de la Comunidad Valenciana (modificado de http://www.epsar.gva.es). Nivel de depuración: ●pretratamiento, ▲tratamiento secundario, ★tratamiento terciario, * Emisarios que vierten en fondos de arenas medias a finas.

La mayor parte de estos 21 emisarios que recogen las aguas procedentes de EDAR vierten sus aguas en zonas de fondos blandos a profundidades entre 7 m y 35 m. En estos fondos, la comunidad de arenas medias a finas de *Spisula subtruncata* (Pérès y Picard, 1964; Cardell *et al*., 1999; Sardá *et al*., 1999) es dominante en el Mediterráneo noroccidental, colonizando la zona sublitoral desde profundidades someras hasta 30 metros, tanto en áreas expuestas como en semiexpuestas (Sardá *et al*., 1999). Esta comunidad está habitualmente sujeta a múltiples presiones causadas por la actividad humana como puertos, extracción de arenas y por supuesto, el vertido de contaminantes y materia orgánica a través de emisarios de aguas residuales. De hecho, 12 de los emisarios presentes en la Comunidad Valenciana vierten sus aguas sobre este tipo de hábitat (figura 1.1). Estas comunidades son muy diferentes a otros ambientes más eutróficos, donde de forma natural hay un mayor contenido orgánico y un menor grado de oxigenación, lo que provoca que sean especialmente vulnerables a los vertidos de aguas residuales (Gray, 1992; Heip, 1995) y requieran de un seguimiento que valore el impacto generado por este tipo de efluentes.

1.3.- Valoración del impacto generado por el vertido de aguas residuales urbanas.

El vertido de aguas residuales urbanas supone la emisión de distintos contaminantes al medio marino. Estos contaminantes afectan tanto al agua receptora como al sedimento. Sin embargo, detectar la contaminación en la columna de agua puede ser complicado y valoraciones únicamente basadas en su estudio pueden ser insuficientes, debido a la rápida dilución o sedimentación de ciertos contaminantes (Mallín et al., 2007).

Por otro lado, el sedimento registra a largo plazo los efectos provocados por los vertidos antrópicos, de modo que su análisis es necesario para realizar una valoración completa de las condiciones ambientales de una zona cercana e influenciada por un vertido (Chapman *et al.*, 1996). Entre las razones principales que justifican la importancia del análisis de los sedimentos frente al análisis del agua, DelValls (2001) cita: i) muchos contaminantes presentan una baja solubilidad en el agua y presentan afinidad por materia particulada (Meiggs, 1980), ii) los compuestos contaminantes tienen un tiempo de residencia mayor en los sedimentos que en la columna de agua (Shea, 1988), iii) la mineralización de los contaminantes en el sedimento puede ser reversible, de modo que pueden ser liberados al agua desde el sedimento si alguna condición del medio cambia (Luoma *et al.*, 1992).

Diferentes aproximaciones y herramientas pueden ser empleadas para valorar el impacto producido por el vertido de aguas residuales urbanas en el sedimento. Entre estas herramientas se pueden diferenciar las basadas en el análisis de parámetros fisicoquímicos y las basadas en el empleo de indicadores biológicos o bioindicadores (Dauvin *el al*., 2010).

1.3.1.- Análisis de parámetros fisicoquímicos.

Entre los impactos generados por estos efluentes, la precipitación de los sólidos presentes en el vertido provoca variaciones en características del sedimento como la granulometría o el contenido de materia orgánica (Abessa *et al.*, 2005; Cotano y Villate, 2006). Además de aportar materia orgánica particulada y disuelta (Smith y Shackley, 2006), estos vertidos alteran la composición del sedimento (Cotano y Villate, 2006) al provocar una posible acumulación de compuestos orgánicos o inorgánicos (Phillips, 1978). La presencia de estas emisiones en el medio marino supone también una fuente de nutrientes. Un alto contenido de nutrientes puede provocar variaciones en ciclos biogeoquímicos, produciéndose situaciones de mineralización que posteriormente provocan un alto consumo de oxígeno dando lugar a procesos de anoxia (Gray *et al*., 2002).

Por otro lado, estos vertidos contienen una gran variedad de sustancias tóxicas: contaminantes orgánicos (PCBs, PAHs o estrógenos), bacterias, virus, protozoos, metales pesados… (Bothner *et al.*, 2002, Mallin *et al.*, 2007; Moon *et al.*, 2008). Entre estos contaminantes, los metales pesados están incluidos como una categoría de contaminantes de gran interés debido a sus efectos tóxicos a diferentes niveles biológicos (Gutiérrez-Galindo *et al.*, 1994). Su procedencia es variada (pequeñas industrias, talleres de automóviles, puertos, vertidos ilegales de aceites lubricantes, pinturas, pilas…) y en ocasiones se han detectado incrementos en las proximidades de vertidos de aguas residuales urbanas (Matthai y Birch, 2000; Bothner *et al.*, 2002; Chicón, 2006).

Sin embargo, el análisis de estos parámetros fisicoquímicos para la valoración ambiental de una zona presenta ciertos problemas derivados de la periocidad en las mediciones y la toma de datos de carácter puntual. Algunos vertidos muestran fluctuaciones en su caudal o concentración, y dado que la persistencia de algunos compuestos es limitada, las interpretaciones de estos análisis resultan inefectivas en algunos casos (Salas *et al.*, 2001); de modo que la medida de concentración que alcanzan las sustancias tóxicas en el sedimento o el grado de alteración en estos parámetros a veces no guarda relación directa con el daño producido al sistema (Bryan y Langston, 1992; DelValls, 2001). Hay situaciones en las que las variables fisicoquímicas o abióticas no detectan un efecto adverso aunque se esté produciendo. Por ejemplo, un contaminante puede estar presente en unas concentraciones no detectables aunque esté generando un efecto adverso, debido a que las técnicas analíticas actuales no permitan su detección. Otros aspectos como el gran número de posibles contaminantes, la posibilidad de que se produzcan efectos sinérgicos entre los contaminantes o la biodisponibilidad de ciertas toxinas, contribuyen a que analizar únicamente las medidas fisicoquímicas se aleje de una adecuada evaluación ecológica del medio (DelValls, 2001).

De esta manera, mientras que las variables químicas miden cambios en concentraciones de ciertas sustancias, o las variables físicas miden alteraciones en ciertos parámetros, una variable biológica permite la valoración del grado de contaminación al registrar la respuesta biológica del medio al contaminante (Wilhm y Dorris, 1968) o al impacto generado por dicha alteración (Sutherland, 1990).

1.3.2.- Empleo de indicadores biológicos.

El empleo de datos biológicos presenta ventajas para valorar el efecto de un impacto sobre el medio marino, dado que un bioindicador o indicador biológico permite integrar la existencia de ciertas condiciones resultantes de un grupo de factores bióticos o abióticos que son difíciles de medir individualmente (Goodseell *et al.*, 2009; Dauvin *et al.*, 2010). Diferentes tipos de comunidades y elementos biológicos se han empleado como bioindicadores para el seguimiento de vertidos de aguas residuales: plancton, ictiofauna, macroalgas, fanerógamas, fauna bentónica, foraminíferos… (Warwick, 1993 y referencias incluidas). De modo que los cambios en la dinámica poblacional o la respuesta de estos organismos son empleados para evaluar el estado ambiental de la zona afectada.

Entre todas las posibilidades, el estudio de comunidades bentónicas es el más conocido y es considerado uno de los más adecuados, debido a su capacidad de reflejar impactos y a la facilidad de su estudio (Warwick, 1993). Tras un largo periodo de estancamiento, su empleo en seguimiento ambiental ha aumentado en los últimos años por el establecimiento de la normativa previamente citada, la cual requiere de nuevas herramientas que permitan evaluar el estado ecológico de las aguas marinas (Dauvin *et al.*, 2010).

Las comunidades bentónicas presentan una serie de características que las hacen especialmente sensibles a responder a la presencia de este tipo de vertidos: i) los organismos bentónicos son sedentarios y con baja movilidad, de modo que se ven afectados por variaciones locales (Oslgard y Gray, 1995; Rosenberg, 2001); ii) muchas especies residen en la interfase de sedimento y agua, lugar donde se concentran muchos contaminantes; iii) es una comunidad muy diversa, presenta taxones con distinta tolerancia a estados de stress, de modo que responden a cambios ambientales dependiendo del nivel sensibilidad o tolerancia de cada taxon (Ferraro y Cole, 1995; Paiva, 2001; Mendez, 2002; Lancellotti y Stotz, 2004); iv) el ciclo de vida de muchas especies consta de fases larvarias planctónicas, provocando que la comunidad se vea afectada tanto por el estado del agua como del sedimento; v) muchos taxones presentan ciclos de vida cortos, reduciendo el tiempo de respuesta a un contaminante; vi) tiene un papel importante en el ciclo de nutrientes y otras sustancias químicas entre el agua y el sedimento (Gray, 1980; Philips y Segar, 1986; Gray *et al.*, 1988; Weston, 1990), vii) algunas especies tienen importancia comercial o son fuente de alimentación de eslabones tróficos superiores como la ictiofauna jugando un papel importante en la cadena trófica (DelValls *et al.*, 1998; Bigot *et al.*, 2006).

Todas estas características hacen de la comunidad bentónica una excelente herramienta para la detección y seguimiento de impactos antrópicos sobre el ecosistema marino. De modo que el estudio de las comunidades bentónicas proporciona una valoración instantánea de los efectos producidos por las perturbaciones, muchos de los cuales no son detectados por los análisis fisicoquímicos, dando una respuesta integrada al impacto que ha ocurrido durante el ciclo de vida de los organismos estudiados (Bigot *et al.*, 2006).

La respuesta de estas comunidades bentónicas a la presencia de fuentes de contaminación orgánica ha sido tradicionalmente explicada por el modelo de Pearson y Rosenberg (1978). En este modelo, se explica el comportamiento del número de especies, la abundancia y la biomasa de una comunidad bentónica a lo largo de un gradiente de enriquecimiento orgánico en el sedimento. Según este modelo, la biomasa total de organismos aumenta inicialmente cuando se produce un incremento de la carga de materia orgánica, lo cual coincide con el mayor número de especies. Tras este incremento, la biomasa y el número de especies disminuye, pudiendo detectarse un segundo aumento de biomasa. Por lo que se refiere al número de individuos, se produce un incremento al aumentar el enriquecimiento orgánico, coincidiendo con una mayor proliferación en el número de especies oportunistas. Tras este incremento, la abundancia disminuye rápidamente, con el descenso en la concentración de oxígeno.

Sin embargo, a pesar del gran número de artículos que han validado el modelo de Pearson-Rosenberg, no todas las comunidades bentónicas responden de la misma manera (Khan y Garwood, 1995; Karakassis *et al.*, 1999). De modo que el empleo de las comunidades bentónicas como herramienta de evaluación medioambiental está condicionado por ciertas dificultades. Entre estas, la variabilidad natural, tanto temporal como espacial, puede dificultar un correcto uso del bentos como bioindicador, lo que provoca que sea difícil distinguir entre fluctuaciones naturales y las debidas a la presencia de un impacto antropogénico (Buchanan y Moore, 1986). Aunque el vertido de aguas residuales puede provocar variaciones en parámetros del sedimento y en comunidades bentónicas, ambos aspectos presentan una alta variabilidad natural (Levin, 1992; Wiens *et al.*, 1993; Fraschetti *et al.*, 2005). El efecto de la contaminación sobre el ecosistema depende de una multitud de factores que pueden provocar variaciones en el sedimento y por lo tanto en las comunidades bentónicas: la profundidad, el hidrodinamismo de la zona, la situación geográfica, el tipo de sustrato sedimentario, la distribución de la fauna (DellValls, 2001). Esta variabilidad natural puede generar errores de interpretación a la hora de diferenciar cambios producidos por el nivel de contaminación (Ferraro *et al.*, 1991; Underwood, 1994; Chapman *et al.*, 1995). El éxito de un indicador dependerá de su capacidad para discriminar los cambios producidos por actividades antrópicas de los producidos por otros componentes naturales. Así es importante realizar distintos estudios a distintos rangos temporales y espaciales con el fin de clarificar la respuesta a la contaminación de las comunidades bentónicas.

La valoración del estado ecológico de las comunidades bentónicas se puede realizar analizando distintos componentes: i) estudio de la composición de la comunidad bentónica a niveles taxonómicos altos, permite una valoración integra de los cambios que se están produciendo; ii) cálculo de índices bióticos, obteniendo resultados fácilmente interpretables ya que nos dan una valor numérico comparable con un valor de referencia dentro de una escala establecida, iii) análisis de un grupo característico y especialmente sensible a la contaminación, iv) respuesta de una sola especie característica de la comunidad, cuya presencia, abundancia o comportamiento es representativa del estado ecológico de la comunidad.

1.3.2.1.- Estudio de la comunidad bentónica empleando niveles taxonómicos altos.

El empleo del bentos en estudios de evaluación ambiental puede ser reducido u omitido en ciertos casos debido al coste de muestreo e identificación o a que su empleo requiere taxónomos especializados (Bilyard, 1987; Dauvin *et al.*, 2003). Tradicionalmente, los estudios ecológicos se han llevado a cabo basándose en el principio de que cada especie presenta una sensibilidad diferente al impacto e identificando la composición de la comunidad a nivel de especie (Ferraro y Cole, 1995). En una situación de estrés mientras que unas especies son beneficiadas, incrementándose su abundancia, otras se ven perjudicadas, al disminuir su abundancia llegando incluso a desaparecer (Gray *et al.*, 1990). Sin embargo, la identificación de organismos bentónicos a nivel de especie puede llegar a ser muy costosa tanto en tiempo como en dinero, especialmente para ciertos grupos taxonómicos como los poliquetos de las familias Spionidae o Cirratulidae o los anfípodos de la familia Ampeliscidae (De Biasi *et al.*, 2003). Uno de los desafíos del uso de las comunidades bentónicas para el control de contaminación, es crear protocolos analíticos que sean fáciles de aplicar y accesibles para laboratorios con técnicas poco sofisticadas (Agard *et al.*, 1993). Por lo que es necesario desarrollar métodos para un uso factible de estas comunidades como herramienta de seguimiento. Métodos que sean efectivos a la hora de proteger recursos naturales e implementar la legislación ambiental (Thompson *et al.*, 2003; Ferraro *et al.*, 2006).

En 1985, Ellis, basándose en la posibilidad de detectar el efecto de la contaminación en las comunidades bentónicas a niveles altos de identificación taxonómica, desarrolla el concepto de Suficiencia Taxonómica. Desde la definición de este concepto, numerosos autores han llevado a cabo estudios relacionados (Help *et al.*, 1988; Herman y Heip, 1988; Gray *et al.*, 1988, 1990; Warkick, 1988, 1993; Austen *et al.*, 1989; Ferraro y Cole, 1990; Warwick *et al.*, 1990; Smith y Simpson, 1993; Somerfield y Clarke, 1995; Olsgard *et al.*,1997; Baldo *et al.*, 1999; Guerold, 2000; Dauvin *et al.*, 2003; Gomez Gesteira *et al.*, 2003; Anderson *et al.*, 2005). Muchos de ellos llegan a la conclusión de que el uso de niveles de identificación altos no supone una pérdida de información importante, de modo que se mantiene la capacidad de discriminar entre comunidades afectadas frente a comunidades no afectadas por una fuente de contaminación (Help *et al.*, 1988; Herman y Heip, 1988; Gray *et al.*, 1988, 1990; Warkick, 1988, 1993; Austen *et al.*, 1989; Ferraro y Cole, 1990; Warwick *et al.*, 1990; Agard *et al.*, 1993; Smith y

Simpson, 1993; Del-Pilar-Ruso *et al.*, 2007, 2010). La aplicación de esta aproximación reduce el coste en tiempo y dinero necesario para el análisis de las muestras, permitiendo el empleo de las comunidades bentónicas en estudios de seguimiento ambiental.

1.3.2.2.- Índices bióticos.

Con el fin de integrar la información del estado ecológico de las comunidades bentónicas en un sólo valor, se han desarrollado varios índices que permiten obtener resultados fácilmente interpretables. El número de índices desarrollados aumenta a partir del establecimiento de la DMA, entre cuyos requerimientos está determinar la situación ecológica de las aguas costeras. Estos índices permiten establecer escalas para la clasificación del Estado de Calidad Ecológica, empleando uno de los indicadores propuesto en esta directiva, los invertebrados bentónicos.

Estos índices pueden ser divididos en tres categorías: los índices basados en diversidad, los índices basados en grupos tróficos y los índices basados en grupos ecológicos (ver resumen en Diaz *et al.*, 2004). Muchos de los índices desarrollados en los últimos años corresponden a esta última categoría: AMBI (Borja *et al.*, 2000); BENTIX (Simboura y Zenetos, 2002), BOPA (Dauvin y Ruellet, 2007). Estos índices se basan en la clasificación de las especies en grupos ecológicos según su respuesta a la presencia de la contaminación, estableciendo: (i) especies sensibles, sólo sobreviven bajo ciertas condiciones medioambientales y desaparecen en zonas contaminadas o ante situaciones de cambios ambientales; (ii) especies tolerantes que no son sensibles a estas situaciones de stress; y (iii) especies oportunistas que son beneficiadas por estas situaciones, al ser capaces de explotar nuevos recursos o colonizar nuevos ambientes (Dauvin *et al.*, 2010).

Debido al gran número de índices desarrollados en los últimos años, Díaz *et al.* (2004) recomiendan realizar pruebas de los índices ya existentes, antes de proponer nuevos índices. El empleo de estos índices requiere un proceso previo de intercalibración que permita obtener resultados similares a la hora de clasificar el estado ecológico de un área (Borja *et al.*, 2009), este proceso puede ser comprobado estudiando el nivel de concordancia y correlación entre resultados obtenidos por distintos índices (Borja y Dauer, 2008). También es necesario realizar un proceso de validación que determine la eficacia de un índice frente a distintos tipos de impactos y en regiones (Borja *et al.*, 2009).

Entre los índices desarrollados, el AMBI ha sido ampliamente validado empleando distintas presiones antropogénicas en diferentes zonas geográficas (Borja *et al.*, 2003; Solís-Weiss *et al.*, 2004; Chenery y Mudge, 2005; Muniz *et al.*, 2005; Muxika *et al.*, 2005; Carvalho *et al.*, 2006; Quintino *et al.*, 2006; Dauvin *et al.*, 2007; Fleischer *et al.*, 2007), de modo que según estos estudios el índice AMBI responde eficazmente a distintas presiones como son: hipoxia, eutrofización, descargas de hidrocarburos, obras costeras, acuicultura ... Sin embargo, se necesita un alto grado de esfuerzo para la aplicación de este índice, puesto que requiere una clasificación taxonómica a nivel de especie para calcularlo. Por otro lado, el índice BOPA destaca por dos ventajas: 1) es independiente del protocolo de muestreo, ya que trabaja con proporción de organismos, y 2) es posible emplearlo con un esfuerzo taxonómico bajo, ya que se basa en la respuesta antagonista de sólo dos grupos taxonómicos fácilmente identificables: el orden Amphipoda frente a ciertas familias oportunistas de la clase Polychaeta (Pinto *et al.*, 2009). Ambas ventajas hacen del índice BOPA una herramienta a considerar para trabajos de seguimiento ambiental como es la evaluación del efecto del vertido de aguas residuales urbanas.

1.3.2.3.- Sensibilidad del orden Amphipoda.

El orden Amphipoda reúne ciertas características que le hace especialmente recomendable para incluirlo en estudios de evaluación de impactos: es un grupo abundante en fondos blandos, presenta una capacidad de dispersión y movilidad baja, viven en directo contacto con el sedimento y es muy sensible a los contaminantes comparado con otros grupos taxonómicos (Reish, 1993; Thomas, 1993; Gomez Geistera y Dauvin, 2000; Dauvin y Ruellet, 2009). De hecho, la premisa de que el orden Amphipoda muestra una alta sensibilidad a la contaminación frente a otros organismos bentónicos es ampliamente aceptada por numerosos autores (Rand y Petrocelli, 1985; Arvai *et al.*, 2002; Cesar *et al.*, 2004; Riba *et al.*, 2004; Dauvin y Ruellet, 2009). Lo cual además de posibilitar la aplicabilidad del índice BOPA, provoca que sean empleados en bioensayos y que un gran número de especies sean utilizadas en experimentos de ecotoxicología para distintos contaminantes.

Sin embargo, existe la posibilidad de que las distintas especies de un mismo género o grupo taxonómico tengan un amplio rango de tolerancia a la contaminación (Resh y Unzizker, 1975; Maurer, 2000). En el orden Amphipoda mientras que la especie *Ampelisca sarsi* sobrevivió, aunque con bajas abundancias, al vertido de petróleo del Amoco Cadiz, otras especies del mismo género desaparecieron completamente (Dauvin, 1998), y del mismo modo, a otras especies de anfípodos se les atribuye cierta tolerancia a la contaminación a pesar de la alta sensibilidad del grupo, como por ejemplo *Dexamine spinosa*, *Gamarella fucicola*, *Melita palmata* o el género *Corophium* (Glémarec y Hily, 1981; Borja *et al.*, 2000). Esto provoca que sea necesario comprobar la sensibilidad de las distintas especies de este orden a la presencia de los emisarios submarinos.

1.3.2.4.-Empleo de especies centinelas.

Entre las distintas especies de una comunidad, las especies centinelas son aquellas cuya presencia o abundancia pueden indicar posibles alteraciones en la comunidad (Dauvin *et al.*, 2010). Estas especies son consideradas representativas del estado de la comunidad bentónica de modo que pueden ser empleadas como bioindicadores. Del mismo modo que ocurre con las especies de anfípodos, es necesario establecer el grado de sensibilidad de estas especies antes de considerarlas como indicadores biológicos.

Sin embargo, la clasificación de las especies a lo largo de una escala de sensibilidad-tolerancia es una tarea complicada y es materia de debate (Labrune *et al.*, 2006), debido a que la respuesta de una especie depende del tipo de contaminación (Bustos-Baez y Frid, 2003) o del área geográfica (Grémare *et al.*, 2009). Estas incertidumbres hacen que sean necesarios estudios específicos que permitan evaluar el grado de sensibilidad de las especies más importantes de una comunidad, antes de ser empleadas como especies centinelas en una zona geográfica ante un determinado impacto.

El tanaidáceo *Apseudes latrielli* (Milne-Edwards, 1828), actualmente aceptado como *Apseudopsis latrielli* (Milne-Edwards, 1828) (Bird, 2001), es una especie muy habitual en las comunidades de fondos blandos del Mediterráneo y Atlántico oriental. Sin embargo, a pesar de esta amplia distribución y de presentarse en altas densidades en las comunidades de fondos de arenas finas a medias, su sensibilidad a la contaminación no está clara, ya que ha sido considerada tanto como una especie tolerante como una especie sensible. En este sentido, es importante conocer su respuesta a los distintos tipos de contaminación para de este modo evitar errores a la hora de emplearla como indicador biológico.

Por otro lado, *Spisula subtruncata* (da Costa, 1778) domina el poblamiento de bivalvos en las zonas de fondos blandos del Mediterráneo noroccidental, donde se suele producir el vertido de aguas residuales urbanas. Esta especie de bivalvo es muy común en las comunidades de arenas finas, y es considerado la mayor fuente de alimento para los peces demersales ligados a estos fondos, siendo importante como especie dominante en la estructura de la comunidad bentónica, especialmente durante primavera y verano (Fraschetti *et al.*, 1997). Por su abundancia resulta interesante y estudiar la respuesta de este bivalvo ante la presencia de emisarios, pudiendo ser utilizada como una especie centinela.

1.4.- Trabajos realizados.

1.4.1.- Estudio de la comunidad bentónica frente al análisis de parámetros fisicoquímicos.

- En un primer trabajo (**capitulo 2)** se analiza el efecto del vertido de aguas residuales sobre las características del sedimento y las comunidades bentónicas a niveles taxonómicos altos. Se discute la idoneidad de evaluar este tipo de contaminación por medio de un análisis de la composición de la comunidad bentónica frente al empleo de ciertos parámetros fisicoquímicos.

1.4.2.- Índices bióticos.

- En el **capítulo 3**, se contrastan los resultados obtenidos mediante la aplicación del índice BOPA con los obtenidos por uno de los índices más utilizados en el marco de la DMA: AMBI (AZTI's Marine Biotic Index). Para este análisis se emplea la base de datos MABES (Seine Estuary MAcroBenthos) y datos de la bibliografía publicada hasta noviembre del 2009. Esta comparación se lleva a cabo clasificando los datos según su origen: i) aguas de transición del Atlántico francés, ii) aguas costeras del Mediterráneo y iii) lagunas costeras del Mediterráneo. Con este trabajo se realiza una intercalibración del índice BOPA empleando los límites establecidos para el AMBI para la clasificación del Estado de Calidad Ecológica (Borja *et al.*, 2000).

- A continuación, en el **capítulo 4**, el índice BOPA es validado en las localidades afectadas por el vertido de aguas residuales, evaluando su idoneidad como herramienta para el seguimiento y gestión de estos vertidos.

1.4.3.- Orden Amphipoda.

- En el **capítulo 5** se realiza un análisis del estado del conocimiento taxonómico de este orden Amphipoda en la zona de estudio. Dado que, aunque este orden ha sido ampliamente estudiado en el Mediterráneo, el grado de conocimiento de su ecología y taxonomía en las costas de la Península Ibérica es todavía fragmentado (Jimeno y Turón, 1995; Bellan-Santini *et al.*, 1998).

- Posteriormente, en el **capítulo 6,** se comprueba la respuesta del orden a la presencia de estos vertidos con el fin de establecer el grado de sensibilidad de las distintas especies identificadas, relacionando su respuesta con aspectos de su ecología como su grupo trófico o su forma de enterramiento.

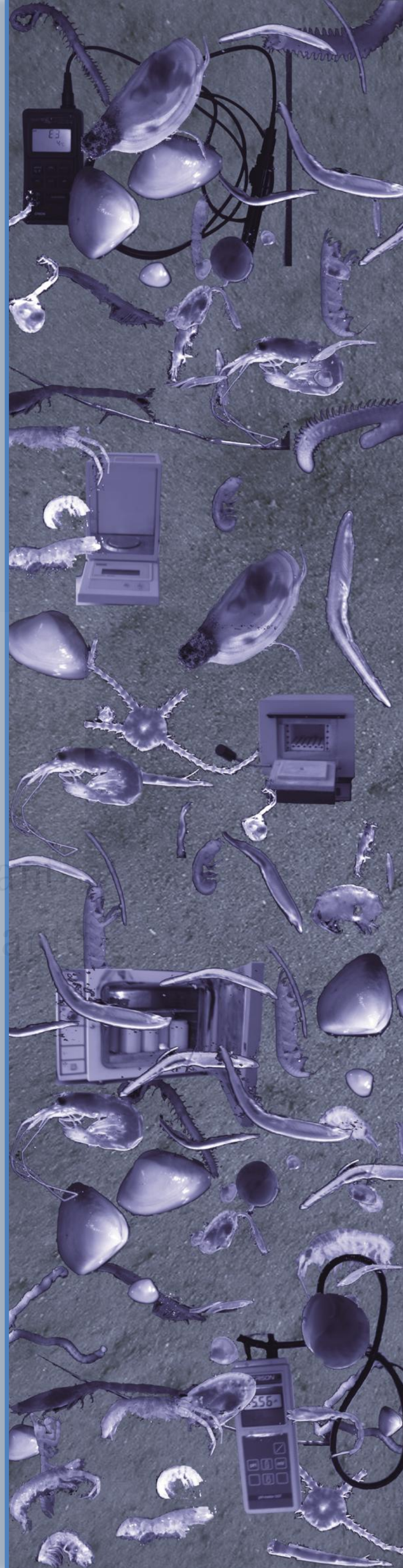
1.4.4.- Especies centinelas.

- En el **capítulo 7** se estudia el comportamiento poblacional del tanaidáceo *Apseudopsis latreillii* con el fin de clarificar su sensibilidad ante la presencia de los vertidos de aguas residuales urbanas.

- En el **capítulo 8** se evalúa el grado de sensibilidad del bivalvo dominante, *Spisula subtruncata*, al vertido de aguas residuales urbanas.

ANALYSIS OF BENTHIC COMMUNITIES *VERSUS* PHYSICOCHEMICAL CHARACTERISTICS

Estudio de la comunidad bentónica frente al análisis de parámetros fisicoquímicos



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CHAPTER 2

Assessing reliable indicators to sewage pollution in coastal soft-bottom communities

Evaluación de indicadores óptimos para el vertido de aguas residuales en comunidades costeras de fondos blandos

Abstract. Physicochemical characteristics of sediment and benthic communities were studied in the proximity of seven sewage outfalls with differences in flow and wastewater treatment in the Western Mediterranean Sea. Redox potential was the only abiotic parameter which showed a pattern related with distance to outfalls, whereas granulometry, percentage of organic matter, metal concentrations or pH did not show changes related with outfall presence. Benthic community analysis proved to be the most suitable monitoring tool. The results showed that the highest impacted stations corresponded with those closest to outfall with the highest flow and only pre-treatment, whilst a decrease of this tendency was detected in the locations where secondary treatment takes place. Meta-analysis showed a decrease of amphipods and tanaids abundance as well as redox potential, as the indicators with the clearest response to sewage presence.

Resumen. Se analizaron las características físico-químicas del sedimento y la comunidad bentónica de 7 localidades afectadas por vertidos de aguas residuales con diferentes caudales y nivel de depuración**.** El potencial redox fue el único parámetro abiótico con un patrón relacionado con la distancia al vertido, mientras que la granulometría, el porcentaje de materia orgánica, el pH o la concentración de metales no mostraron cambios debidos a la presencia de los vertidos. El análisis de la comunidad bentónica resultó ser la herramienta más eficaz. Los resultados reflejan que las estaciones más impactadas corresponden con las cercanas al vertido de mayor caudal y con menor nivel de depuración, pretratamiento. Mientras que esta tendencia disminuye en las localidades donde se realiza un tratamiento secundario. El metanálisis establece que el descenso de las abundancias de anfípodos y tanaidáceos, así como de potencial redox, son los indicadores con mejor respuesta a la presencia del vertido.

2.1.- Introduction.

The need for prevention of the environment from being adversely affected by the disposal of insufficiently-treated urban wastewater has led to the development of Urban Waste Water Treatment Directive 91/271/EEC (UWWTD, 1991). It is expected, that measures aimed at the protection of the coastal environment in conjunction with the additional benefits from the reuse of water will lead to an improvement in the level of wastewater treatment in the near future. Identification and characterisation of the locations affected by sewage discharge is necessary in order to detect the effectiveness of wastewater management.

Although in most European countries the residual waters are treated in wastewater treatment plants, some of them only use primary treatment (solids settlement) to reduce oils, grease, fats, sand, grit, and coarse solids. Disposal may still contain heavy metals, bacteria, and increased amounts of suspended particulate organic matter (Bothner *et al.*, 2002; Smith and Shackley, 2006). This effluent could produce physical and chemical changes in sediments that has been addressed by several authors (Bald *et al.*, 2005; Best *et al.*, 2007; Simboura and Reizopoulou, 2008).

On the other hand, the benthic communities often reflect the effects of pollution and they are widely used in the monitoring effects of marine pollution (Gray *et al.*, 1990). The relationships between benthic assemblages and the effect of contaminants have been described extensively in the literature (Pearson and Rosenberg, 1978; Warwick *et al.*, 1990; Estacio *et al.*, 1997; Borja *et al.*, 2006; Del-Pilar-Ruso *et al.*, 2007). However, the fauna composition is also affected by natural variables and requires specialized taxonomic and statistical expertise to analyse and interpret correctly its process in such way that benthic analysis could be reduced or omitted in certain cases, due to sampling and identification costs (Bilyard, 1987; Warwick, 1993; Dauvin *et al.*, 2003). This has led that classical approaches such as physicochemical water analyses were considered more favourable to achieve results within short timescales (Sánchez-Moyano *et al.*, 2006). A possible alternative to decreasing this cost problem and “natural noise” is to reduce taxonomic efforts in order to consider the Taxonomic Sufficiency, where the identification of taxa only needs to be carried to the taxonomic level necessary for the purpose of the study (Ellis, 1985). If the abundance and composition of taxa differ in polluted and unpolluted areas, little or no relevant information may be lost by identifying animals to higher taxa (Herman and Heip, 1988; Warkick, 1988, 1993; Gray *et al.*, 1990; Warwick *et al.*, 1990; Sánchez-Moyano *et al.*, 2006).

Along the Spanish Mediterranean Coast there are numerous sewage discharges. These disposal sites have been active for several decades, and represent a source of continuous pollution showing a marked increase in disposal during the summer period caused by seasonal population rise due to tourism. Several municipal treatment plants discharge treated wastewater with different degrees of sewage treatment and different flows. Most of these outfalls discharge at similar depth and into a similar benthic. It is characterized as medium-to-fine sand communities of *Spisula subtruncata*, one of the communities more frequently distributed in shallow soft-bottom non-vegetated areas from Western Mediterranean (Cardell *et al.*, 1999). Consequently, it is an ideal site for investigating the effect of pollutants (de-la-Ossa-Carretero *et al.*, 2009). Physicochemical characteristics of sediment and benthic communities affected by seven outfalls were analysed. The aims of this analysis were to assess sewage discharge effects through these two different approaches in order to establish general trends for a widely distributed community and to determinate the reliability of physical parameters and benthic fauna as indicators for the monitoring of the sewage pollution.

2.2.- Material and methods.

The study area was located off the Comunidad Valenciana Coast (NE Spain, Western Mediterranean), where seven locations affected by sewage outfalls were analyzed. These outfalls correspond to the villages of Vinaroz (location I), Benicarló (location II), Peñíscola (location III), Alcossebre (location IV), Torreblanca (location V), Gandia (location VI) and Oliva (location VII) (figure 2.1). Wastewater was discharged through submarine pipelines at a depth of approximately 15 m. Wastewater treatment plants from locations I, II, III and IV utilise only a pre-treatment process, which includes an automated mechanically raked screen, a sand catcher and grease trap. Whereas, secondary treatment, consisting of biological treatment of activated sludge, was implemented in locations V, VI and VII.

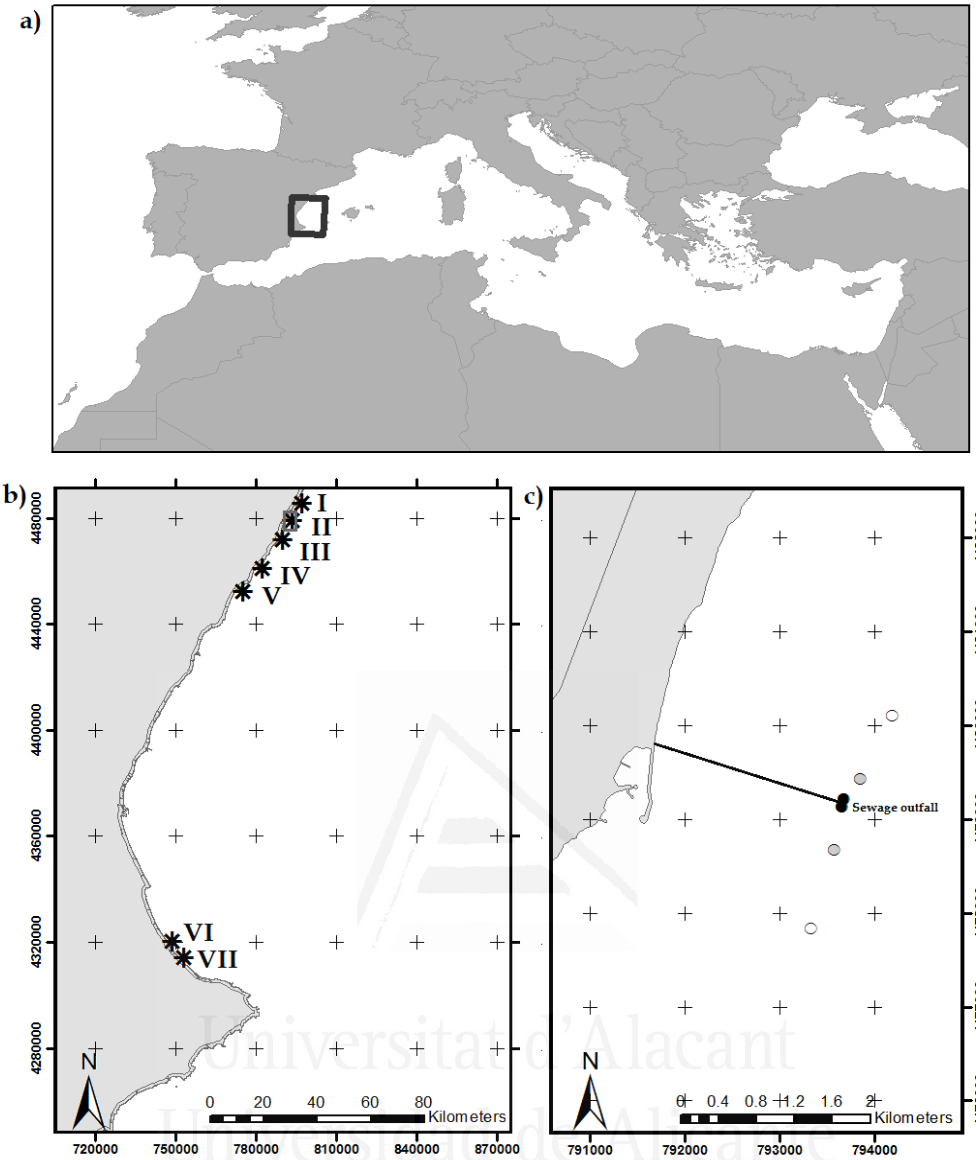


Figure 2. 1. a) Study area. Location of the seven pipelines b) Sampling stations of location II (colour of circle is related to distance to outfall (black: 0 m, grey: 200 m and white: 1000 m to outfall). UTM coordinate system. Grid zone 30 S.

Monthly data of flow and water quality of sewage disposal (suspended solids, BOD (biological oxygen demand), COD (chemical oxygen demand), phosphates, nitrates and turbidity) were provided us by CONSOMAR S.A and Entitat de Sanejament d´Aigues (table 2.1).

Table 2.1. Characteristics of the sewage outfalls analysed: outfall depth, sewage treatment, and monthly average of flow and measurements of water quality disposal (suspended solids, BOD (biologic oxygen demand), COD (chemical oxygen demand), phosphates, nitrates, conductivity and turbidity). Annual average of monthly samples. Data from CONSOMAR S. A. and Entitat de Sanejament d ´ Aigües.

Location	**Outfall depth (m)**	**Sewage treatment**	**Wastewater parameters**								
			pH	**Flow (m^3/month)**	**S.S. (mg/l)**	**BOD (mg/l)**	**COD (mg/l)**	**Pt (mg/l)**	**Nt (mg/l)**	**Cond.**	**Turb. (NTU)**
I	15.81	Pre-treatment	7.54	226799	262.47	358.61	624.08	55.58	11.11	2609	217.53
II	14.58	Pre-treatment	7.58	502612	266.72	209.72	479.17	30.87	12.98	10539	153.36
III	15.5	Pre-treatment	7.39	286127	171.44	112.72	236.64	16.70	4.04	6787	115.39
IV	14	Pre-treatment	7.45	54190	228.92	252.22	448.53	41.56	6.59	2382	154.92
V	14	Biological treatment	7.56	43256	9.92	10.31	34.83	10.20	1.02	3050	5.44
VI	16.84	Biological treatment	7.63	1358387	10.36	12.97	37.39	14.73	2.57	4688	4.67
VII	15.5	Biological treatment	7.64	118423	13.53	19.14	41.25	16.44	3.33	1503	9.83

For each location, three distances from the discharge (0, 200 and 1000 m) were sampled, with two sites, following the coastline in order to keep a constant depth at each location. All samples were collected in July during five consecutive years (2004 to 2008). Four Van Veen grab samples (400 cm^2) were obtained at each station. Three samples were sieved through a 0.5 mm screen, and preserved in 10% formalin for the study of the benthic community. Other sample was used to characterize the sediment. Grain size analysis was assessed by standard sieve fractionation (Holme and McIntyre, 1984). Redox potential and pH were analysed using a CRISON 507 pH meter. Organic content of dry sediment was estimated as the loss of weight after ashing. Granulometric analysis was performed all years- whereas redox potential, pH, and organic content were analysed from 2005 to 2008. Redox potential, pH, and organic content values were examined using 3-factor analyses of variance (ANOVA) with distance, location and year as factors. Prior to ANOVA, the homogeneity of variance was tested using Cochran's test. Data were $\sqrt{X+1}$ transformed when variances were significantly different, and if variance remained heterogeneous, untransformed data were analysed by reducing significance level. SNK test (Student-Newman-Keuls) was used to determine which samples were implicated in the differences.

During the last campaign (2008), heavy metal concentrations (Cd, Cu, Pb, As, Zn, Cr and Ni) were analysed. Sediment samples were digested with acid HNO_3 solution, and three replicates were analysed by inductively coupled plasma mass spectroscopy THERMO ELEMENTAL VG PQ. Heavy metal concentrations were examined using 2-factor analyses of variance (ANOVA) with distance and location as factors.

Changes in benthic community were analysed using high taxonomic level, in order to consider the Taxonomic Sufficiency (Ellis, 1985), although species composition was considered when it comes to interpret results. Non-parametric multivariate techniques were used to compare abundance of different taxonomic groups present at each station from the study area. All multivariate analyses were performed using the PRIMER version 6 statistical package (Clarke and Warwick, 1994). Triangular similarity matrices were calculated through the Bray-Curtis similarity coefficient using abundance values. The values were previously dispersion weighted in order to reduce “noise” produced by taxa whose distribution is erratic and whose abundance shows high variance between replicates (Clarke *et al.*, 2006). Graphical representation of multivariate patterns of infaunal assemblages was obtained by non-metric multidimensional scaling (nMDS). ANOSIM was used to test the differences between distances at each location. Similarities percentage analyses (SIMPER) of abundances was used to determine the infaunal groups with higher percentage contribution in dissimilarity between distances. Analyses of variance (ANOVA) with distance, location and year as factors were used in order to test differences in abundance of benthic groups indicated by the SIMPER analysis.

BEST procedure was used to determine the parameter combination most correlated with benthic changes between sampled stations, in order to link benthic community analyses to sediment and wastewater variables. Spearman correlation between similarity matrices of samples of outfall points based on the abundances of benthic community, data of sediment (granulometric analysis, redox potential, pH, and organic content) and wastewater data (flow, suspended solids, BOD, COD, phosphates, nitrates and turbidity) were determined.

Meta-analysis was applied in order to assess which parameter, among physical characteristics and abundance of taxa, responds better to sewage presence. Meta-analysis is a set of methods designed to synthesis the results of disparate studies (Hedges and Olkin, 1985), in this case different locations and sampling years. For meta-analysis of studies with continuous measures, such as physical parameters or benthos abundance, a standardized difference between treatments means is typically used (Cooper and Hedges, 1994). We used Hedges'g statistic (Hedges and Olkin, 1985) as measure of effect size (standardized differences in mean of sediment parameters or taxa abundance between outfalls sites and sites at 1000 meters to the outfall).

2.3.- Results.

The study area was characterized by fine-sand sediments (0.125mm-0.25mm). There was a low variability of the granulometry which did not seem to be related to outfall presence (figure 2.2). Only punctual changes were detected, such as increases of coarse sand at 200 meters to the outfall in location I in 2004 and at 1000 meters to the outfall in location VII in 2008; increases of mud at 1000 meters to the outfall in location II in 2004 and increases of medium sand in some stations in year 2006 (figure 2.2).

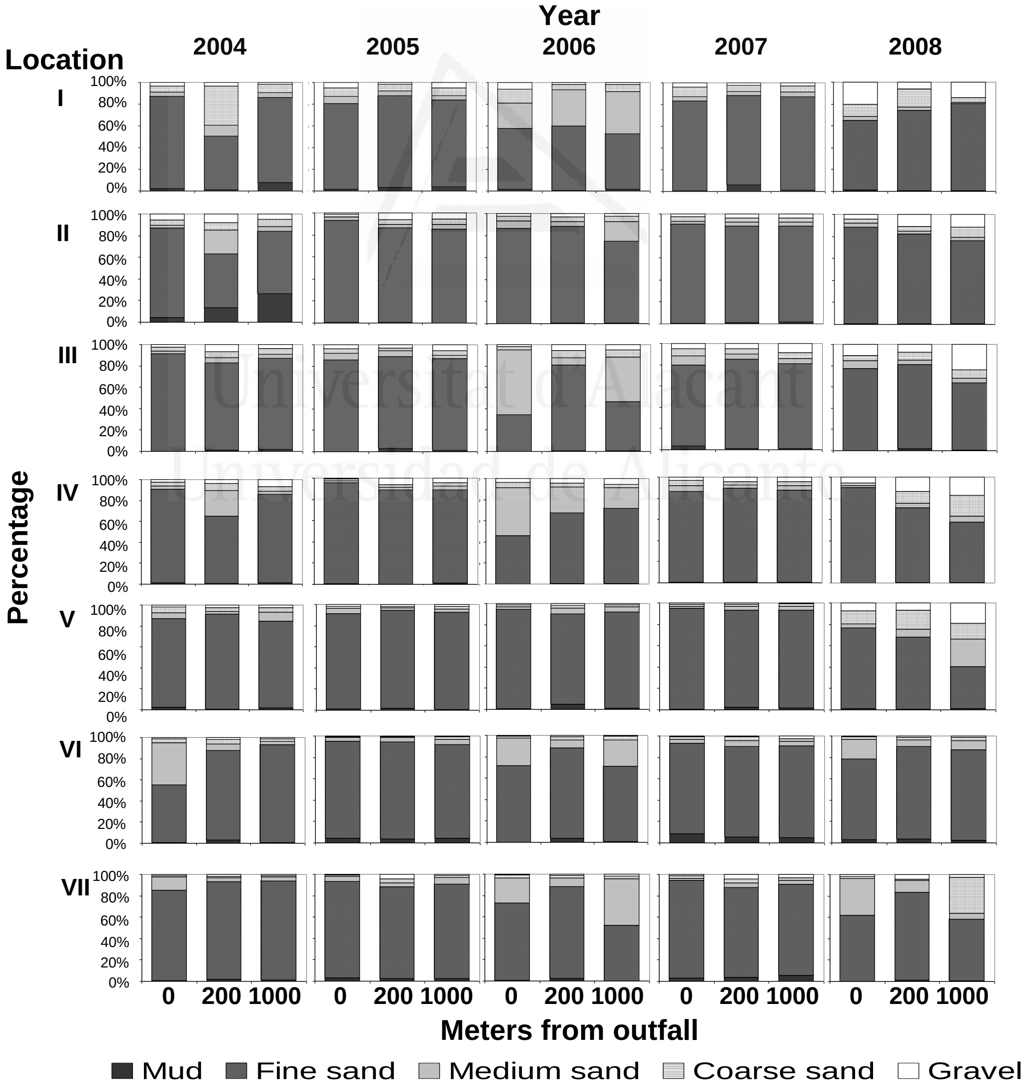


Figure 2.2. Grain size distribution for the three distances (0, 200 and 1000 m) at each location (I, II, III, IV, V, VI and VII) for five years (2004, 2005, 2006, 2007 and 2008).

With regard to the other analysed parameters (figure 2.3), only redox potential showed a pattern related to distance to outfall. In this way, significant differences (table 2.2) were detected for interaction between distance and location due to the reduced conditions of the sediment in stations closer to the location II outfall. Significant differences were also detected for interaction between location and year. These differences were due to changes in redox potential values among locations in years 2005 and 2006.

Table 2.2. Results of ANOVA for sediment parameters (redox potential, pH and % organic matter) for the factors location, distance and year. df: degrees of freedom, MS: medium squares, F of each factor =MS factor/MS residual. Levels of significance: ns no significant difference, *p < 0.05, **p < 0.01 and ***p < 0.001.

Transformation: dash (–) indicates that there is no transformation; plus (+) indicates that there is no homogeneity of variance and levels of significance were *p < 0.01; **p < 0.001, *** p < 0.0005.

	Source	**Df**	**MS**	**F**	**P**
Redox potential (mV)	Distance	2	52320.46	9.51	*
	Location	6	19071.37	2.43	ns
	Year	3	22707.22	6.27	***
	Dis.x Loc	12	9503.45	2.53	*
	Dis.xYear	6	5502.93	1.52	ns
	Loc.xYear	18	7848.29	2.17	*
	Dis.xLoc.xYear	36	3755.83	1.04	ns
	RES	84	3624.35		
	Transformation	-			
Ph	Distance	2	0.0602	3.39	ns
	Location	6	0.0237	0.44	ns
	Year	3	0.2599	15.71	***
	Dis.x Loc	12	0.0184	1.18	ns
	Dis.xYear	6	0.0178	1.07	ns
	Loc.xYear	18	0.0541	3.27	***
	Dis.xLoc.xYear	36	0.0155	0.94	ns
	RES	84	0.0165		
	Transformation	-			
% Organic matter	Distance	2	8.8624	0.63	ns
	Location	6	28.1208	1.77	ns
	Year	3	128.7655	178.45	***
	Dis.x Loc	12	8.5683	0.89	ns
	Dis.xYear	6	13.9597	19.35	***
	Loc.xYear	18	15.8758	22	***
	Dis.xLoc.xYear	36	9.6692	13.4	***
	RES	420	0.7216		
	Transformation	+			

With regard to pH, significant differences (table 2.2) were only detected for the interaction between location and year due to the higher values obtained in 2008 in locations II and V. In the same way, percentage of organic matter did not clearly correlate with distance to the outfall, but significant differences for interaction distance, location and year were detected (table 2.2). Differences among distances were due to an organic matter increase, at 0 meters to the outfall in location I in 2006 and in location III in 2006, and to a decrease in the station close to the outfall in location V in 2007. The higher percentages obtained in locations I, III, VI and VII in 2006 produced differences among locations.

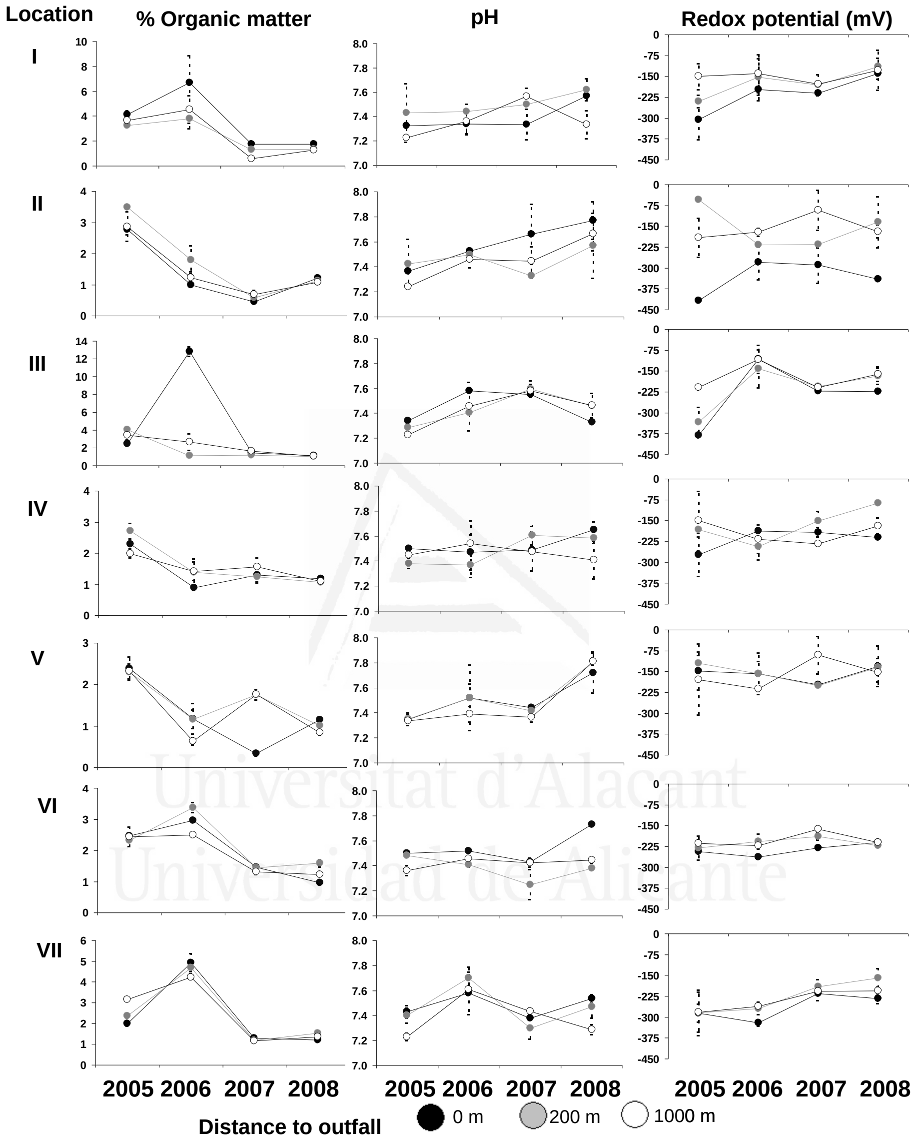


Figure 2.3. Sediment parameters (Redox potential, pH and % organic matter) for three distances to the outfall and four years in the seven locations: I, II, III, IV, V, VI and VII.

Increase in heavy metal concentrations were not clearly detected in stations closest to the outfalls (figure 2.4, table 2.3), only Cu and Cd increased significantly at outfalls from locations IV and II, respectively. In most of the cases the opposite pattern was observed, with a decrease of metal concentrations that was detected at stations sited 0 meters to the outfalls producing a significant interaction between distance and location.

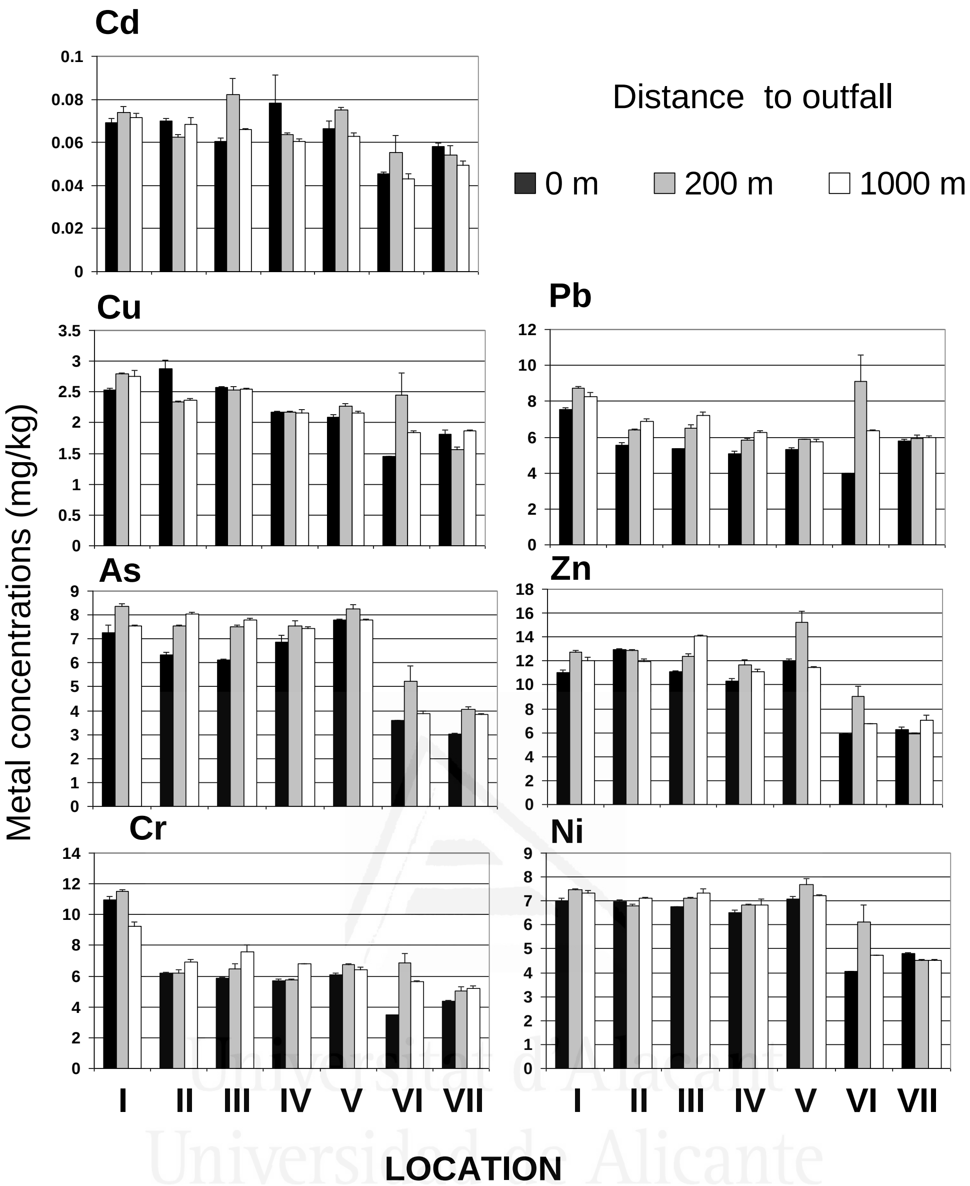


Figure 2.4. Heavy metal concentrations for three distances to the outfall in the seven locations: I, II, III, IV, V, VI and VII.

Table 2.3. Results of ANOVA for heavy metal concentrations for the factors distance and location. df: degrees of freedom. F of each factor =MS factor/MS residual. Levels of significance: ns no significant difference, *p < 0.05, **p < 0.01 and ***p < 0.001.

Transformation: plus (+) indicates that there is no homogeneity of variance and levels of significance were *p < 0.01; **p < 0.001, *** p < 0.0005

		Cd	**Cu**	**As**	**Cr**	**Pb**	**Zn**	**Ni**
Source	**df**	**F** ^{p}	**F** ^{p}	**F** ^{p}	**F** ^{p}	**F** ^{p}	**F** ^{p}	**F** ^{p}
Distance	2	4.1 ^{ns}	1.65 ^{ns}	65***	30.35***	33.47***	33.5***	12.95***
Location	6	13.78***	43.87***	381***	210.06***	19.09***	191.1***	117.41***
Dis.x Loc	12	2.5*	7.66***	5.19***	15.01***	7.02***	10.16***	5.35***
RES	105							
Transformation		+	+	+	+	+	+	+

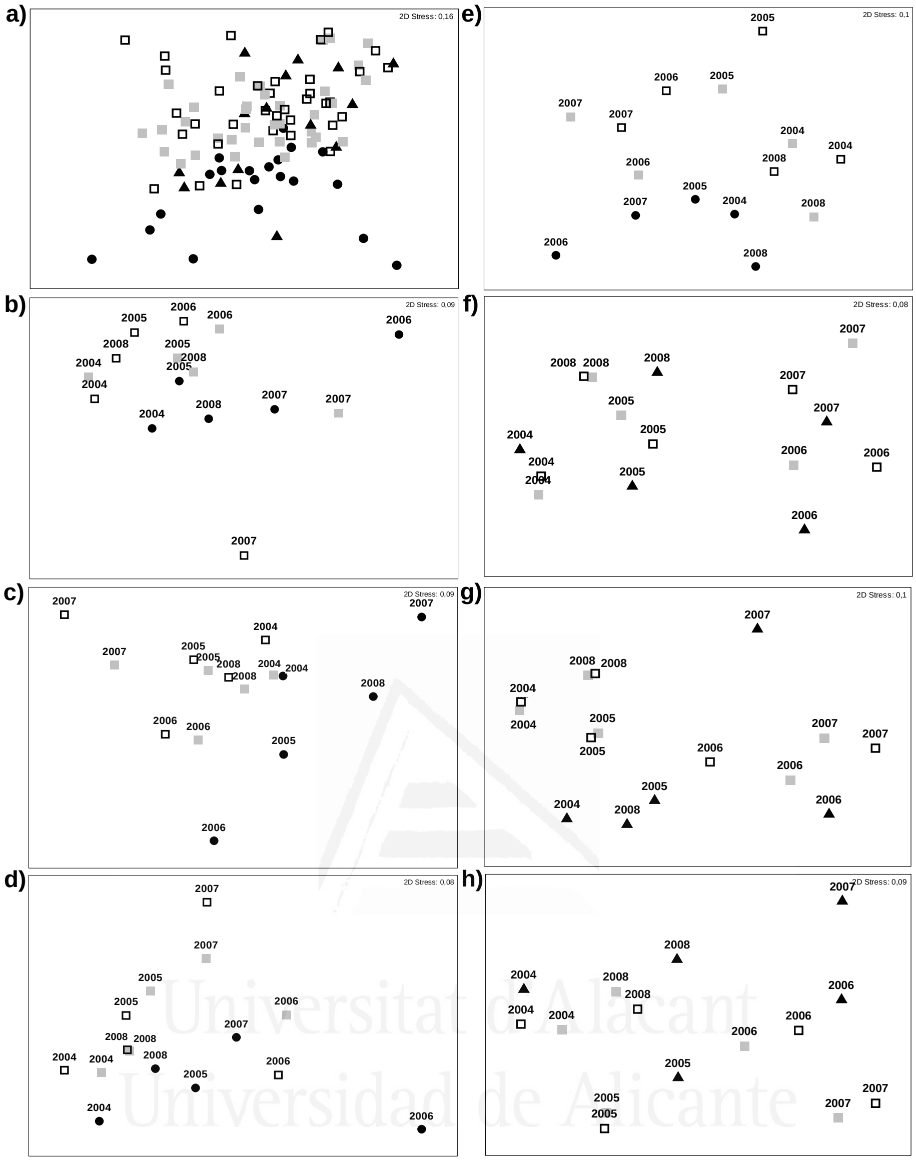


Figure 2.5. nMDS ordination of infauna abundance (indiv./m^2) and associated stress values for a) all locations and each location b) I, c) II, d) III, e) IV, f) V, g) VI and h) VII. Three distances to the outfall (0, 200 and 1000 m) and five years (2004, 2005, 2006, 2007 and 2008). Colour and shape was related to distance to outfall and different treatment levels: ●: 0 m to outfall with pre-treatment, ▲: 0 m to outfall with biological treatment, grey squares: 200 m and white squares: 1000 m to outfall.

Infaunal taxonomic composition showed a gradation of stations from sites further away to the outfalls to those closest in certain locations (figure 2.5 a). This segregation was observed in the locations where wastewater was only pre-treated (black circles in nMDS plots); especially in locations II and IV where this pattern was clear noticed (figure 2.5 c and e). On the other hand, stations corresponding to outfalls where wastewater was previously biological treated (black triangles in nMDS plots) were closer to stations at

200 m and 1000 m to the outfalls (grey and white squares in nMDS plots). This layout was clearly marked in location V (figure 2.5 f), where variability in community structure was related to a temporal pattern.

In order to establish differences between distances at each location, a two-way crossed ANOSIM with distance and year as factors was run. It showed significance difference between distance groups (Global R) in locations I, II and IV (table 2.4). Pairwise test (table 2.4) showed that differences attributed to distance were mainly due to differences detected between stations closest the outfall and sites at 200 m and 1000 m to the outfall. On the other hand, no significant differences were detected between 200 and 1000 meters, except in location I whose R value was lower.

Table 2.4. R statistics values and significance level of statistic (R^p) from Pairwise test of two way crossed ANOSIM of infauna abundance (indiv./m^2) for the factor distance to the outfall (0, 200 and 1000 m) across all years group at each location (I, II, III, IV, V, VI and VII). Levels of significance: ns no significant difference, *p < 0.05, **p < 0.01 and ***p < 0.001.

	Pairs of Distances			
Location	**0 m vs 200 m**	**0 m vs 1000 m**	**200 m vs1000 m**	**Global R**
I	0.40 *	0.65***	0.25*	0.41***
II	0.60 *	0.80***	0.25^{ns}	0.48***
III	1.00***	0.25^{ns}	-0.11^{ns}	0.24^{ns}
IV	0.70**	0.55*	0.20^{ns}	0.44***
V	0.15^{ns}	-0.30^{ns}	-0.40^{ns}	-0.13^{ns}
VI	1.00***	0.00^{ns}	-0.05^{ns}	0.20^{ns}
VII	0.55*	0.35^{ns}	-0.25^{ns}	0.20^{ns}

SIMPER showed that abundance of Amphipoda, Bivalvia, Polychaeta, Cumacea, Tanaidacea, Cephalochordata and Ophiuroidea were the taxa with higher average contribution to the overall dissimilarity between the stations closer to the outfalls and stations at 1000 meters to the outfall in locations I, II and IV (table 2.5). Most of these taxa obtained average dissimilarity contributions higher than standard deviation, except Bivalvia or Ophiuroidea that showed a high standard deviation, being less consistent discriminating taxa.

Table 2.5. Percentage of contributions (Contrib.%) and ratio average contributions/standard deviation (Diss/SD) from each taxa to the average Bray-Curtis dissimilarity and between pairs of distance groups (0 vs 1000 m) for locations I, II and IV. Taxa were ordered in decreasing mean contribution of the three locations.

Taxon	Location I		Location II		Location IV	
	Contrib.%	Diss/SD	Contrib.%	Diss/SD	Contrib.%	Diss/SD
Amphipoda	32.7	1.6	18.3	1.5	13.8	1.4
Bivalvia	15.7	0.7	16.5	0.9	22.4	1.0
Polychaeta	11.0	1.3	27.5	1.0	9.7	1.4
Cumacea	7.4	1.0	4.8	0.9	9.9	1.2
Tanaidacea	4.5	1.1	5.6	1.3	7.5	1.2
Cephalochardata	3.6	1.1	4.0	1.1	9.3	1.1
Ophiuroidea	5.1	0.9	4.8	0.8	3.4	1.1
Decapoda	2.9	1.1	2.6	1.3	3.8	1.1
Gasteropoda	2.7	0.9	2.6	1.0	3.8	1.5
Nematoda	1.9	0.7	3.1	0.7	3.6	0.8
Nemertea	2.7	1.2	1.9	1.2	3.1	1.1

Plotting mean abundance of the most relevant taxa, we observed the general trends of each main taxa with proximity to outfalls from locations where significance differences between distances were detected. The abundance of Polychaeta, Amphipoda, Tanaidacea, Cumacea, Bivalvia, Ophiuroidea and Cephalochordata for each year and distance from outfall of locations I, II and IV is shown in figure 2.6.

ANOVA detected significant differences in the interaction among the three factors in Polychaeta, Amphipoda, Bivalvia and Ophiuroidea (table 2.6). An increase of polychaetes abundance was detected in the stations closer to the outfall in location II (years 2007 and 2008), whereas amphipods, bivalves and ophiurids decrease certain years in this stations. Regarding to Amphipoda, this decrease was detected in locations I and II, all years except 2007, and in location IV, years 2004 and 2008. Bivalvia decreases near the outfall in locations I and II, 2007, and in location IV, 2005, while a decrease of ophiurids was detected in locations I and II, year 2004 and 2007 and in location IV, year 2007 (figure 2.6). In the case of Tanaidacea, significant differences were detected for interactions between the pairs of factors distance-year and location-year (table 2.6). Differences between distances were due to lower abundance in the stations closer to the outfall all years except 2007 (figure 2.6). Whereas, differences between locations were due to higher abundances obtained in locations IV with respect to locations I and II. With regard to Cumacea abundances, significant differences were detected in interactions distance-location, distance-year and location-year (table 2.6). Differences in interaction distance-location were due to a decrease in stations near outfalls in locations I and II (figure 2.6). This decrease was detected in years 2005, 2006 and 2008. Finally, abundance of Cephalochordata showed differences in the interaction (location and year). Whereas, the highest abundances were detected in years 2004, 2005 and 2008 in location IV, in year 2007 the abundances of Cephalochordata increased in location I and II (figure 2.6, table 2.6).

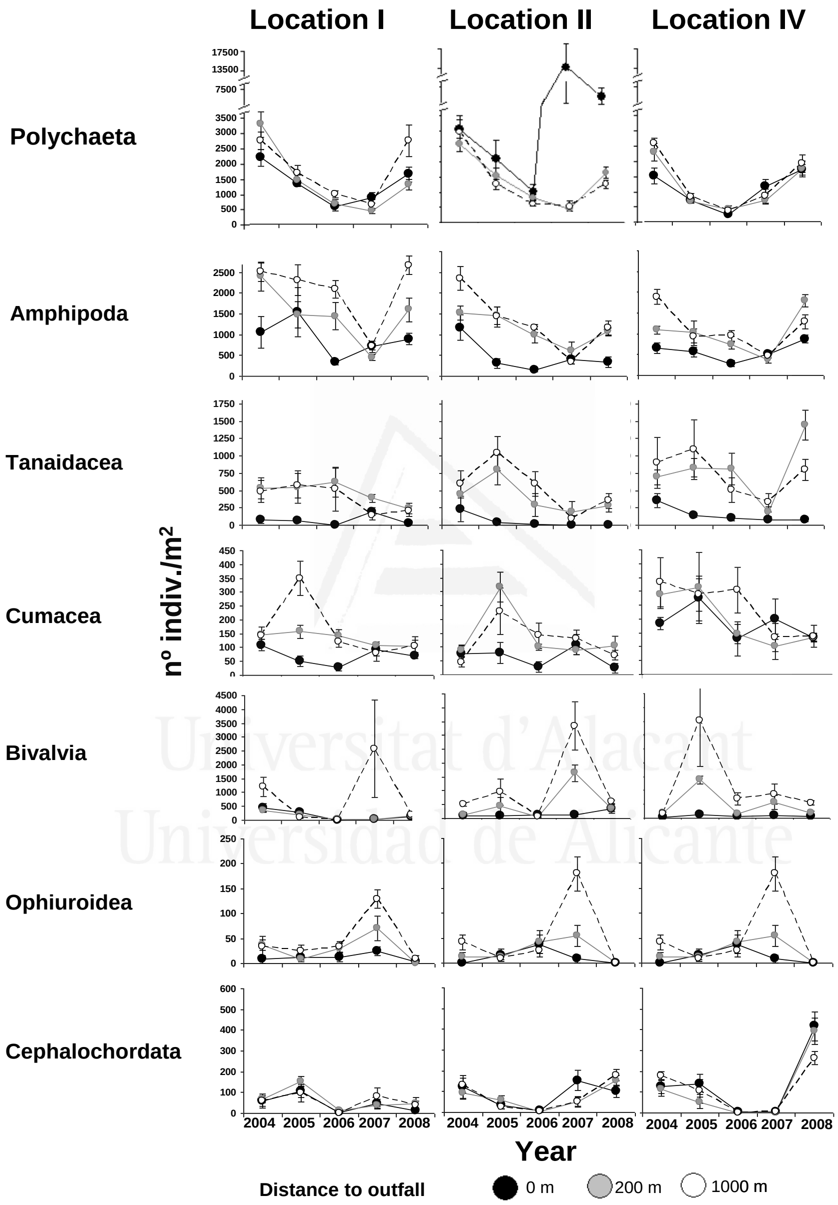


Figure 2.6. Mean abundances (indiv./m^2) and standard errors of Polychaeta, Amphipoda, Tanaidacea, Cumacea, Bivalvia, Cephalochordata and Ophiuroidea in locations I, II and IV, year (2004 to 2008) and distance to the outfall (0, 200 and 1000 m).

Table 2.6. Results of ANOVA for abundance (individuals/m^2) of Polychaeta, Amphipoda, Tanaidacea and Bivalvia for the factors location (I, II and III), distance from outfall (0, 200 and 1000 m) and year (2004, 2005, 2006, 2007 and 2008). RES = Residual., df: degrees of freedom, F of each factor =MS factor/MS residual. Levels of significance: ns no significant difference, *p < 0.05, **p < 0.01 and ***p < 0.001. Transformation: √(x+1), data were √ X+1 transformed when variances were significantly different; plus (+) indicates that there is no homogeneity of variance and levels of significance were *p < 0.01; **p < 0.001, *** p < 0.0005.

		Polychaeta	Amphipoda	Tanaidacea	Cumacea	Bivalvia	Ophiuroidea	Cephalochordata
Source	**df**	**F** ^{p}	**F** ^{p}	**F** ^{p}	**F** ^{p}	**F** ^{p}	**F** ^{p}	**F** ^{p}
Distance	2	1.50^{ns}	12.26**	27.81***	4.10*	5.37*	5.79 *	0.00 ^{ns}
Location	2	3.50 ^{ns}	10.90*	6.14*	4.83*	0.27 ^{ns}	2.69 ^{ns}	0.34 ^{ns}
Year	4	3.23 *	36.33***	10.39***	2.71*	7.70***	36.78 ***	49.05 ***
Dis.x Loc	4	2.94 ^{ns}	1.68 ^{ns}	1.26 ^{ns}	3.14*	0.27 ^{ns}	0.38 ^{ns}	0.84 ^{ns}
Dis.xYear	8	2.53 ^{ns}	4.89	3.12**	2.75**	3.46 **	3.76 ***	0.96 ^{ns}
Loc.xYear	8	1.56 ^{ns}	3.15**	3.09**	2.04*	4.67***	1.08 ^{ns}	19.92***
Dis.xLoc.xYear	16	1.82 *	2.00*	1.41 ^{ns}	0.99 ^{ns}	2.19**	3.66***	1.25 ^{ns}
RES	225							
Transformation		+	+	√(x+1)	√(x+1)	+	√(x+1)	√(x+1)

BEST procedure showed that percentage of mud; redox potential and percentage of organic matter were the combination of parameters of sediment most related to changes detected in fauna composition (table 2.7). However, all parameters obtained a weak correlation level and did not show significant correlation (Rho: 0.043, p: 0.91). Regarding wastewater parameters; phosphates, suspended solids, conductivity, nitrates and pH were the parameter combination best explaining community variability of stations closer to sewage outfalls. These combination obtained significant correlation. The parameters with the highest Spearman correlation level were phosphates (Rho: 0.258), suspended solids (Rho: 0.250) and DQO (Rho: 0.219); whereas ph (Rho: 0.086) and flow (Rho: 0.073) obtained the lowest correlation level.

Table 2.7. BEST results. Spearman correlation of benthic data with sediment and wastewater parameters.

	No.Vars	Correlation	Selections
Sediment parameters	3	0.043	% Mud; Redox potencial; % organic matter
	3	0.038	% medium sand; Redox potencial; % organic matter
	4	0.037	% Mud; % medium sand; Redox potencial; % organic matter
	2	0.037	Redox potencial; % organic matter
	2	0.035	% Mud
Wastewater parameters	5	0.333	Pt, suspended solids, conductivity, Nt pH
	5	0.330	Pt suspended solids DQO conductivity, pH
	4	0.330	Pt suspended solids conductivity, pH
	4	0.329	suspended solids conductivity, Nt pH
	4	0.327	suspended solids DQO conductivity, pH

Forest plots of the differences in physical parameters and taxa abundance between the stations sited at 0 m and at 1000 m to the outfall was displayed in figure 2.7. The clearest response to sewage presence was the decrease in amphipods and tanaids abundance. Among abiotic parameters, redox potential showed also a negative response though it had wide confidence intervals. A less clear negative response was observed in ophiuroids, bivalves and cumaceans abundance; whereas polychaetes and cephalochordates showed a slight positive response.

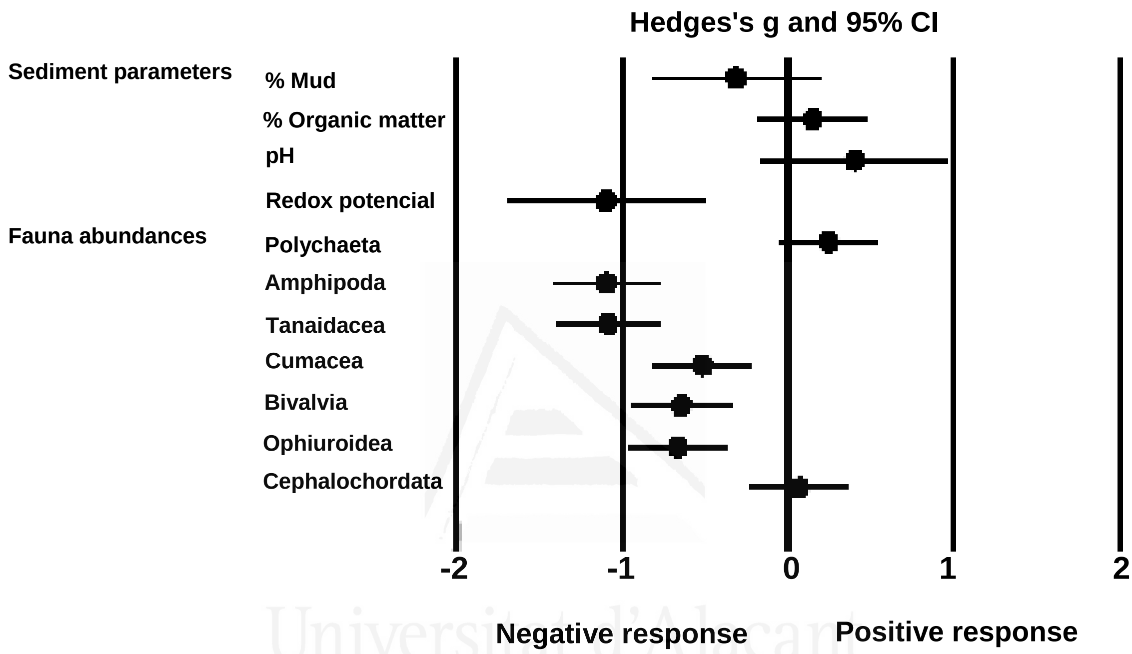


Figure 2.7. Forest plot of effect sizes for sediment parameters and taxa abundances (standardized differences in parameters and abundances between sited at 0 m and 1000 m) based on different locations and years. The vertical line represents no difference and the error bars are equivalent to 95% confidence intervals.

2.4.- Discussion.

Sewage discharges may alter the biochemical composition of sediments (Cotano and Villate, 2006), producing organic enrichment and physical changes towards finer grained sediment (Pearson and Rosenberg, 1978). Moreover, posterior degradation of the organic matter could lead to lower oxygen concentrations and hypoxia situations (Gray *et al.*, 2002). However, in this study granulometry, organic matter percentage and pH did not show any change related with sewage discharge. The medium-to-fine sand communities, such as the study area, are always influenced by high hydrodynamism and a low input of organic matter, which can partially or totally clean the bottom, by removing the surface layer and concealing the disposal effect (Cardell et al. 1999). Nevertheless, redox potential decreased in the outfall with higher flow and low treatment, showing a possible hypoxia situation near this disposal site. This effect in redox potential was not detected around outfalls marked by higher values of water quality. In the same way, heavy metal concentrations did not show a clear increase near disposals studied. According to Gonçalves and Souza (1997), urban wastewaters have a typical composition, with high contents of solids and nutrients and low concentrations of metals, hydrocarbons and pesticides. However, high heavy metal concentrations were detected in other outfalls, as in sewage disposals from Sydney (Matthai and Birch, 2000). These increases had been detected with presence of industrial activities, as photography (Bothner *et al.*, 2002), oil and chemical industries (Verlecar *et al.*, 2006) or mining activity (Riba *et al.*, 2004). Sewage from studied outfalls should not be mixed with industrial wastewaters, being mainly of domestic origin and avoiding high concentrations of metals. Moreover, sandy habitats influenced by high hydrodynamics, where the study was carried out, are not expected to accumulate toxic levels of contaminants.

Despite the fact that the physical analysis of sediment did not show a clear disturbance effect, benthic community analysis detected changes between stations close to outfalls from locations I, II and IV, and stations further afield. Assessment of patterns in structure of marine benthic assemblages has several advantages over other experimental or field methods for detection of anthropogenic disturbance (Elias *et al.*, 2005). The benthos can integrate conditions over a period of time rather than reflecting conditions just at the time of sampling, so benthic organisms should be more useful in assessing local effects in monitoring programs than classical approaches such as physico-chemical analyses of sediment. In fact, changes detected in this work are correlated with the flow of sewage disposal, treatment level and water quality. Significant differences between distance groups were not detected in locations III, V, VI and VII. Wastewater was previously treated by biological treatment in locations V, VI and VII, and although wastewater was only pre-treated in location III, water quality parameters showed better values in this location than in locations I, II and IV. Water quality of sewage disposal (suspended solids, phosphates, nitrates and turbidity) showed correlated differences with the variations in the benthic community structure. The highest impacted stations correspond with those closest to the outfall characterised by the highest flow of sewage effluents in which only pre-treatment is received, location II. Meanwhile, the community near the location V sewage outfall showed lowest changes related with distance to the outfall, since this is the location with the lowest flow and where secondary treatment takes place. At this location, water quality parameters of sewage disposal showed the best values where requirements for discharges from urban wastewater treatment plants which dump in sensitive areas, set by Directive 91/271 (UWWTD, 1991), were complied to (phosphates<2 mg/l, nitrates<15 mg/l, BOD<25 mg/l O_2, COD<125 mg/l $O_{2,}$ suspended solids<35mg/l) (de-la-Ossa-Carretero *et al.*, 2009).

Multivariate analysis appears to be an especially sensitive tool for detecting these changes (Warwick and Clarke, 1991; Clarke and Ainsworth, 1993; DelValls *et al.*, 1998; Del-Pilar-Ruso *et al.*, 2007). Benthic community and multivariate analysis allowed us to monitor the influence of sewage disposals, differentiating sites affected by sewage disposal and differentiating a decrease of this effect with an increase in wastewater treatment and a decrease of the flow discharged.

Among taxa which contributed in these differences, the crustacean groups -amphipods and tanaids- demonstrated higher sensitivity to sewage outfall presence. The increased abundance of amphipods as one moves away from the discharge site, suggests that the environment was less stressful, since amphipods are more sensitive to pollution than other marine species (Arvai *et al.*, 2002; Cesar *et al.*, 2004; Riba *et al.*, 2004; Dauvin and Ruellet, 2007). Tanaidacea assemblages of this study were dominated by *Apseudopsis latreillii*. Despite being an abundant and widely distributed crustacean species, *A. latreillii* response to pollution is not clear, since it has sometimes been reported as a tolerant species (Grall and Glémarec, 1997; Marín-Guirao *et al.*, 2005; de Juan *et al.*, 2007) and by others as a sensitive species (Sanz-Lázaro and Marín, 2006; Bouchet and Sauriau, 2008). The sensitivity to sewage pollution of this tanaid will be tested in chapter 7. As regards to cumaceans, they showed response to sewage disposals, thought its sensitivity was lower than in amphipods or tanaids, since its abundance only decreased near outfalls at locations I and II, where higher flows were registered.

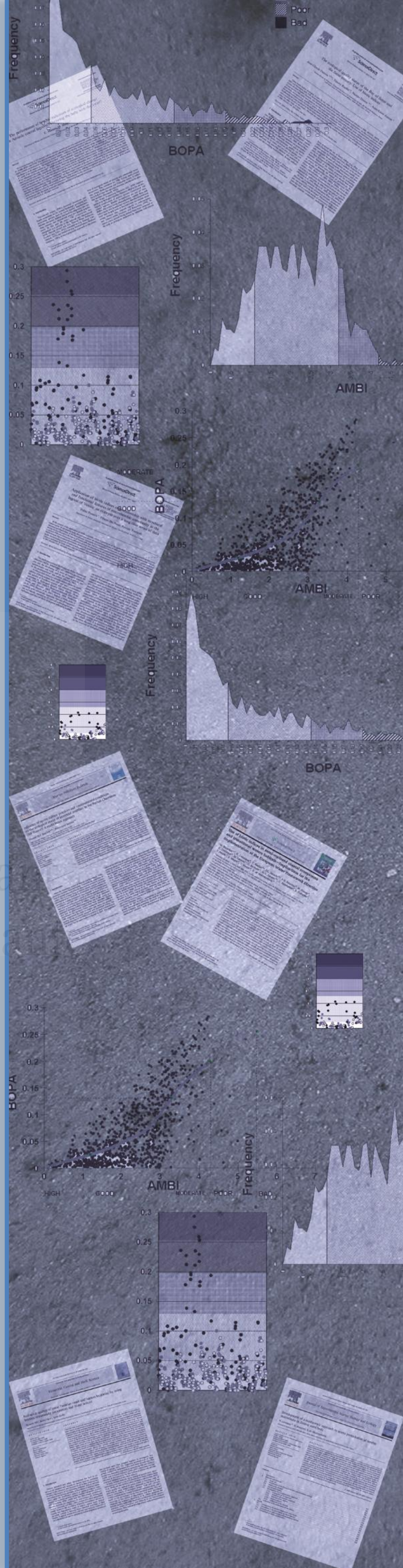
On the other hand, Polychaeta group contains both sensitive and tolerant species and they are found along the whole gradient from pristine to heavily disturbed areas

(Olsgard *et al.*, 2003). Some Polychaeta species showed significantly greater numbers of individuals at the sewage-affected sites while other species densities showed no difference or in some cases even a decrease (Dauer and Conner, 1980). In our study, it was found that an increase of abundance of Polychaeta in stations near the outfall from location II was due to an increase in the abundance of the family Dorvilleidae. Similarly, some Bivalvia species have been considered to be very tolerant to organic matter enrichment and other species avoided the polluted stations (Guerra-Garcia and Garcia-Gomez, 2004). *Spisula subtruncata* dominates bivalve populations in medium to fine sand communities from the Western Mediterranean. The decrease of Bivalvia abundances near outfalls in certain cases may be related with the dominance of *S. subtruncata*, the response of *S. subtruncata* populations to sewage outfall presence will be analysed in chapter 8. In the same way, Ophiuroidea showed sensitivity to sewage presence because a decrease of its abundance was detected near outfalls the year when higher densities where obtained in sites at 200 m and 1000 m to the outfall. However, temporal and spatial variability of both taxa could cause that their discriminating capability was not as consistent as other taxa. Finally, the Cephalochordata *Branchiostoma lanceolatum*, despite being considered a sensitive species, did not showed a clear response to sewage presence; in fact only interannual variations and variability among locations were obtained some years. These interannual changes were also detected in abundances of other taxa; however sewage effect was detected throughout the whole study period. Moreover sewage discharges did not vary from year to year, therefore these changes seem to be due to interannual natural variability. For this reason, assessing the impacts of sewage disposals requires that the outfalls and multiple control sites be sampled contemporaneously.

Meta-analysis allowed obtaining general patterns from large data sets from different locations and years. Amphipods and tanaids abundance and, with respect to abiotic parameters, redox potential showed the clearest response. Benthos has the advantage that it gives an integrated view of the long-term conditions at a site, whereas chemical and physical analytical methods provide only a 'snapshot' of conditions at the time of sampling (Saiz-Salinas, 1997). Chemical analysis could fail assessing outfall disposals, the large amount of possibilities: organic enrichment, heavy metals, chlorinated pesticides, PCB, PAH’s, ammonia, steroids … could complicate finding the correct variable or combination of variables affected by the disposal. In fact the only abiotic variable which showed certain response to outfall presence, redox potential, depends on biotic process as organic compounds decomposition.

BIOTIC INDICES

Índices bióticos



Publications.

De-la-Ossa-Carretero J.A., Dauvin, J.C., 2010. A Comparison of Two Biotic Indices, AMBI and BOPA/BO2A, for assessing the Ecological Quality Status (EcoQS) of Benthic Macro-invertebrates. *Transitional Water Bulletin* **4**: 12-24.

De-la-Ossa-Carretero J.A., Del-Pilar-Ruso Y., Giménez-Casalduero F., Sánchez-Lizaso J.L. 2009. Testing BOPA index in sewage affected soft-bottom communities in the north-western Mediterranean. *Marine Pollution Bulletin* **58**: 332-340.

CHAPTER 3

A Comparison of Two Biotic Indices, AMBI and BOPA/BO2A, for assessing the Ecological Quality Status (EcoQS) of Benthic Communities

Comparación de dos índices Bióticos, AMBI y BOPA/BO2A, para evaluar el Estado de Calidad Ecológica de comunidades bentónicas

Abstract. The results of two common indices were compared for the north-eastern Atlantic and Mediterranean Sea waters. Eight studies provided raw data permitting AMBI and BOPA to be compared. A total 922 data element was available, most of them from the Seine estuary (78%). The database was later divided into three sub-sets: French Atlantic Transitional Waters, Mediterranean Coastal Waters and Mediterranean Lagoons. Both indices' values demonstrated a strong correlation; however, the BOPA index had a tendency to overestimate the EcoQs compared to the values obtained from AMBI index, mainly due to discrepancies between ‘high’ and ‘good’ quality. New thresholds for BOPA index are proposed in order to reduce this overestimation.

Resumen. En este capítulo se comparan los resultados de dos índices bióticos utilizados tanto en el Atlántico nororiental y el mar Mediterráneo (AMBI y BOPA). A partir de ocho estudios ya publicados se emplean un total de 922 datos, procedentes en su mayor parte del estuario del Sena (78%). La base de datos se divide en tres grupos: aguas de transición del Atlántico francés, aguas costeras del Mediterráneo y lagunas costeras del Mediterráneo. Existe una fuerte correlación entre ambos índices, sin embargo el índice BOPA tiende a sobrestimar el estado ecológico, comparándolo con los resultados obtenidos por el índice AMBI. Esto es debido principalmente a discrepancias entre los estados ecológicos ‘alto’ y ‘bueno’. Con el fin de reducir esta sobrestimación, se propusieron nuevos límites para el índice BOPA.

3.1.- Introduction.

In order to achieve the objectives of the European Water Framework Directive (WFD, 2000), common definitions for water quality status needed to be established. Benthic macro-invertebrates make good ecological indicators because they are relatively sedentary and thus are unable to avoid deteriorating water/sediment quality. They have relatively long life-spans, show marked responses to stress depending on their species-specific sensitivity/tolerance levels and play a vital role in cycling nutrients and materials between the underlying sediment and the overlying water column (Borja *et al.*, 2000; Dauvin *et al.*, 2007). Thus, the composition and abundance of benthic macro-invertebrate fauna is one of the elements proposed in the WFD for assessing quality and for determining ecological status.

Ecological indicators are employed with the aim of supplying synoptic information about the state of ecosystems (Salas *et al.*, 2006). In response to WFD implementation in coastal aquatic ecosystems, several indices were developed to infer environmental status from the assessment of benthic community condition: AMBI (Borja et *al.*, 2000; Borja and Muxika, 2005), BENTIX (Simboura and Zenetos, 2002), BQI (Rosenberg *et al.*, 2004), BOPA (Dauvin and Ruellet, 2007), M-AMBI (Muxika *et al.*, 2007) and BO2A (Dauvin and Ruellet, 2009). These indices summarise the ecological status or the ecological quality of a water body and allow the results to be easily interpreted. However, because all organisms are not equally sensitive to all types of anthropogenic disturbances and are thus likely to respond differently to different types of perturbations, one biotic index is unlikely to be universally applicable (Dauvin *et al.*, 2007; Pranovi *et al.*, 2007; Afli *et al.*, 2008; Grémare *et al.*, 2009).

Following an initial study that showed the effectiveness of an opportunistic polychaeta/amphipod ratio for identifying oil spill events (Gomez-Gesteira and Dauvin, 2000), the BOPA index was created, modifying this ratio to allow estuarine and coastal communities to be divided into the five classes suggested by the European directive (Dauvin and Ruellet, 2007): ‘high’ for unpolluted sites, ‘good’ for slightly polluted sites, ‘moderate’ for moderately polluted sites, ‘poor’ for heavily polluted sites and ‘bad’ for extremely polluted or azoic sites. This BOPA index was originally calibrated using the AMBI index. It respects two main principles: 1) the Taxonomic Sufficiency principle, and 2) the principle of antagonism between sensitive species and opportunistic species. Opportunistic polychaetes are known to be resistant to, indifferent to or favoured by organically enriched sedimentary matter, whereas amphipods form an abundant and ecologically-important zoological group that is more highly sensitive to contaminated sediments than other benthic macro-invertebrates (Dauvin and Ruellet, 2009). Except some amphipod species of the genus *Jassa* Leach (Corophioidea: ischyroceridae) that are not counted as sensitive species because most of them are part of the EG IV on the AZTI list (www.azti.es). The main advantages of the BOPA index are its independence from sampling protocols using several meshes sizes and several surface units for expressing abundances, since BOPA uses frequency data and the proportion of organisms in each category (Pinto *et al.*, 2009). An additional advantage is the reduced need for taxonomic knowledge. After the initial BOPA proposition, Dauvin and Ruellet (2009) proposed adding Clittellata (i.e., Hirudinea and Oligochaeta) to the opportunistic polychaeta in order to adapt BOPA index for application in the freshwater sectors of transitional waters, thus creating the Benthic Opportunistic Annelida Amphipods index (BO2A). In coastal sediment, as the Clitellata are generally absent or weakly represented, the BO2A and BOPA values are strictly or very similar. The BOPA index has been applied in different situations, thus showing its effectiveness at distinguishing the presence of hydrocarbons (Gomez-Gesteira and Dauvin, 2000 Dauvin and Ruellet, 2007) and in certain zones, such as oyster culture areas (Bouchet and Sauriau, 2008) or harbours (Ingole *et al.*, 2009). However, this effectiveness is limited by an apparent overestimation of the ecological quality status (EcoQS) compared to the EcoQS from other indices. In order for the various indices to be successfully implemented, they must be intercalibrated to make them comparables, and this intercalibration must identify the level of agreement between methodologies (Borja *et al.*, 2007). For this reason, the EcoQS thresholds of the indices must be modified in order to produce the same, or at least similar, results for the assessment of the same area (Ruellet and Dauvin, 2007). The main objective of this chapter is to correctly intercalibrate the BOPA/BO2A EcoQS classifications with the AMBI classifications. In this study, we first review the results in the literature for ecological quality status (EcoQS) obtained with the BOPA index. Then, we propose new thresholds in order to adapt the EcoQS results produced by BOPA/BO2A for use in benthic coastal and transitional waters.

3.2.- Materials and methods.

In November 2009, we reviewed a total of 10 papers in which the BOPA index was employed in sites in the Atlantic Ocean and the Mediterranean Sea (table 3.1 provides a list of the papers reviewed). In these papers, both the BOPA index and the AMBI index were usually used to identify the ecological quality status (EcoQS) of macrobenthic communities. It was not always possible to have access to the raw data. Nevertheless, we managed to obtain the data for eight papers, which gave us a total of 1093 values for both the BOPA and AMBI indices. But 171 values corresponded to null BOPA values, i.e. BOPA = 0 when there were no opportunistic annelida or polychaeta or no sensitive amphipod species. After having excluded the null BOPA values, the remaining values which are taken into account in our comparison are 922 (table 3.2). The data extracted from the MABES database (Seine Estuary MAcroBenthos, available via the data administrator of the GIP Seine Aval: nbacq@seine-aval.fr) made up 78% of the data (table 3.2). Indices values were divided into three subsets according to where the samples on which the values were calculated were taken: French Atlantic transitional waters, Mediterranean lagoons and Mediterranean coastal waters.

Table 3.1. List of articles in which the BOPA index was employed.

Reference	Site	Applied Indices
Afli et al., 2008	Bizerte Lagoon, Bay of Tunis, Dkhila Coast (Tunisian Coast)	BOPA, AMBI, BENTIX, ITI
Bakalem et al., 2009	Bays of Fetzara, Jijel, Bejaia, Alger, Bou Ismail, Arzew & Oran (Algerian Coast)	BOPA, AMBI, mAMBI, BENTIX, H', ITI
Blanchet et al., 2008	Marennes-Olèron Bay, Arcachon Bay, Seine Estuary (Western French Coast)	BOPA, AMBI, BENTIX, BQI, H'
Bouchet and Sauriau, 2008	Pertuis Charentais (Western French Coast)	BOPA, AMBI, BENTIx, H'
Dauvin et al., 2007	Seine Estuary (Western French Coast)	BOPA, AMBI, BQI
de-la-Ossa-Carretero et al., 2009	Castellon Coast (Eastern Spanish Coast)	BOPA
Munari and Mistri, 2007	Orbetello, Padrogiano, Tortolì, San Teodoro, (Tyrrhenian lagoons)	BOPA, AMBI, FINE
Munari and Mistri, 2008	Venice, Scardovari, Goro, Gorino, Comacchio, Lesina, Oran (Adriatic coastal lagoons)	BOPA, AMBI, H', FINE
Pranovi et al., 2007	Venice Lagoon	BOPA, AMBI, mAMBI, BENTIX, H'
Lavesque et al., 2009	Arcachon Bay (Western French Coast)	BOPA, AMBI

Table 3.2. Number of available BOPA data elements in the different data sets, the number of null values (BOPA= 0, when there were no opportunistic polychaetes in the samples that had at least 20 individuals), and the identification of marine sub-section concerned. (N= numbers).

	N data	N null values	N of retained values in the analysis	Marine sub-section concerned
MABES Dauvin et al., 2007; Dauvin and Ruellet, 2007, 2009	864	142	722	French Atlantic transitional waters
Blanchet et al., 2008	24	6	18	French Atlantic transitional waters
Bouchet and Sauriau, 2008	15	0	15	French Atlantic transitional waters
Lavesque et al., 2009	12	0	12	French Atlantic transitional waters
Bakalem et al., 2009	101	5	96	Mediterranean coastal waters
Afli et al., 2008	31	12	19	Mediterranean coastal waters and Mediterranean lagoons
Munari and Mistri, 2007	30	4	26	Mediterranean lagoons
Pranovi et al., 2007	16	2	14	Mediterranean lagoons
Total	1093	171	922	

First, we determined the degree of correlation between values for both indices using Pearson coefficient for total area and each area sub-section. The significance of the correlation was set at 0.01. Then, we looked at the EcoQS classifications recorded for each site for both indices. These classifications were ranked from 1 to 5, from ‘high’ status to ‘bad’ status, giving us a numerical EcoQS value. We compared these values using the ratio, EcoQS (BOPA)/EcoQS (AMBI), to validate the EcoQS classifications of both indices. When this ratio is <1, BOPA had overestimated the EcoQS compared to AMBI; when this ratio is >1, BOPA had underestimated the EcoQS compared to AMBI.

The frequency distribution of the BOPA and AMBI EcoQS values obtained from the compiled papers were plotted in order to obtain an overview of the probability of belonging to each EcoQS category for the BOPA and AMBI indices.

Linear regression was initially considered to calculate new BOPA thresholds values. However, while AMBI follows the normal law, BOPA follows an exponential law (Ruellet and Dauvin, 2007), and for that reason, it is not possible to use linear regression. Thus, the new BOPA thresholds were calculated from the AMBI thresholds using non-parametric regression through a pairwise comparison of both indices. Non-parametric regression can be used when the hypotheses of the more traditional regression methods cannot be verified or when the main interest is the predictive quality of the model and not its structure (Härdle, 1992). Finally, new threshold values were applied to database, and the percentages of each EcoQS were calculated.

Weighted Kappa analysis (Cohen, 1960; Landis and Kosch, 1977) was used to analyse the agreement between the indices for both the previous BOPA thresholds and the new thresholds proposed in this paper. The methodology proposed by Borja *et al.* (2007) was employed. The equivalence table from Monserud and Leemans (1992) was used to establish the level of agreement of the two indices. In addition, since the importance of misclassification is not the same between close categories (e.g., between 'high' and 'good', or 'poor' and 'bad') as between distant categories (e.g., between 'high' and 'moderate', or 'high' and 'bad'), they chose to apply Fleiss-Cohen weights (Fleiss and Cohen, 1973) to the analysis to decrease importance of misclassification between close categories and increase importance between distant categories. The percentage of correspondence was also calculated for each threshold system.

3.3. - Results.

The Pearson coefficient showed a significant strong positive correlation between both indices in each area sub-section (table 3.3). The highest value of Pearson coefficient was obtained for Mediterranean coastal waters, while the lowest value was obtained for Mediterranean lagoons. Despite this correlation, the BOPA index had a general tendency to overestimate EcoQS compared to the classification obtained from AMBI index (figure 3.1). The BOPA provided better EcoQS for 87.5% of the sites. It underlined great differences in the Tyrrhenian and the Adriatic lagoons, and some sites on the Pertuis Charentais, Tunisian Coast and Algerian coast (Bejaia) had similar or worse EcoQS than the values provided by AMBI.

Table 3.3. Pearson coefficient values and significance level for the BOPA and AMBI indices for the total area and each area sub-section.

BOPA / AMBI	Pearson coeficient	Sig. level	N
Total	0.707	0.000	922
Atlantic transitional waters	0.701	0.000	767
Mediterranean coastal waters	0.878	0.000	110
Mediterranean lagoons	0.605	0.000	45

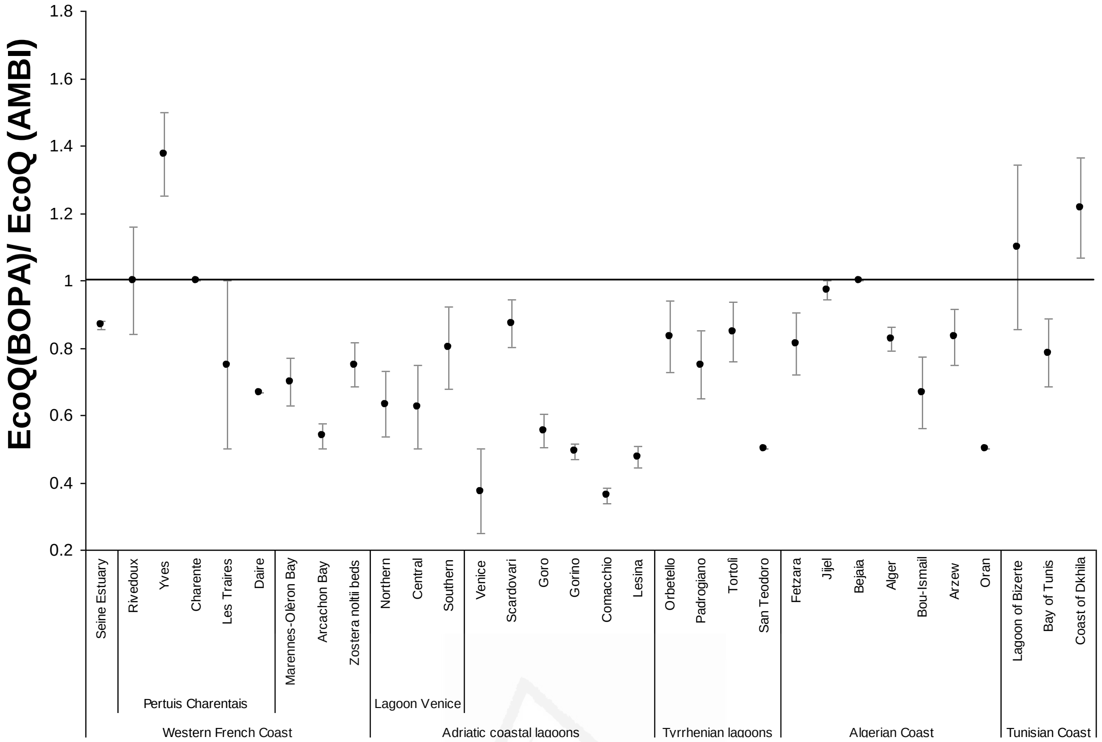


Figure 3.1. Mean and standard error of the ratio of both indices' EcoQS classifications [EcoQS (BOPA)/EcoQS (AMBI)]. When this ratio is <1, BOPA has overestimated the EcoQS compared to AMBI; when this ratio is >1, BOPA has underestimated the EcoQS compared to AMBI.

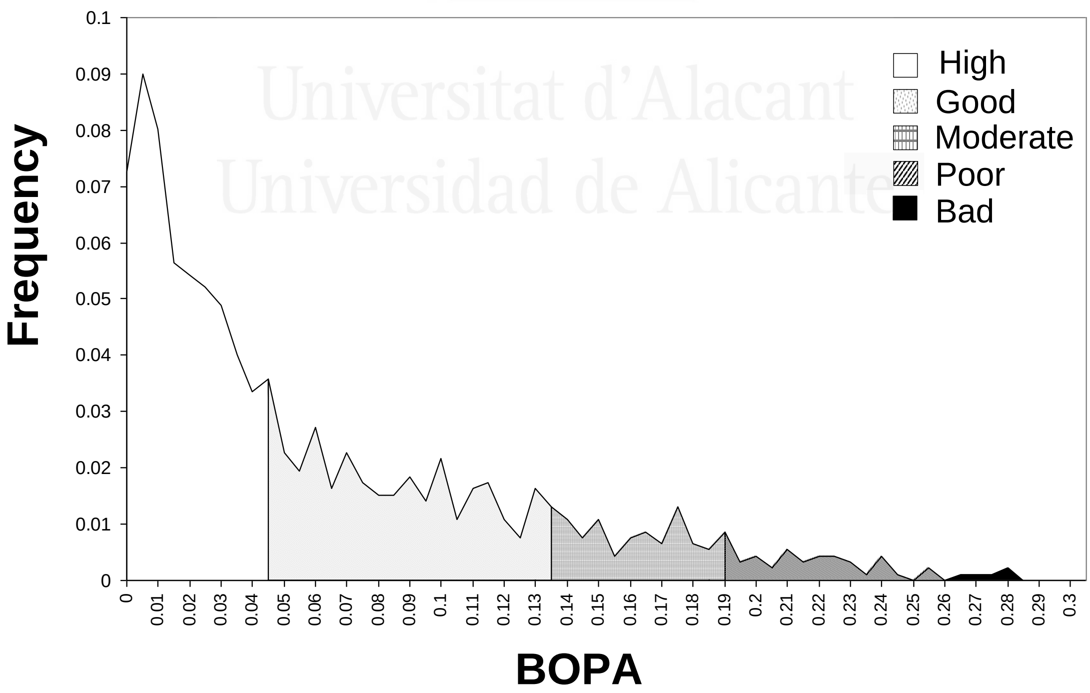


Figure 3.2. Frequency distribution of BOPA values. Thresholds taken from Dauvin and Ruellet (2007).

Our analysis of the frequency distribution of the BOPA values (figure 3.2) highlighted a tendency to classify sites as 'high': 54% of the samples were classified as 'high', whereas only 13.3% were classified as 'moderate', 'poor' or 'bad'. On the other hand, the AMBI index (figure 3.3) tended to classify sites as 'good' (70.6%), with only 10.3% being classified as 'moderate', 'poor' or 'bad'. Given these results, it would seem that overestimation of the BOPA index was caused by the thresholds between 'high' and 'good' status.

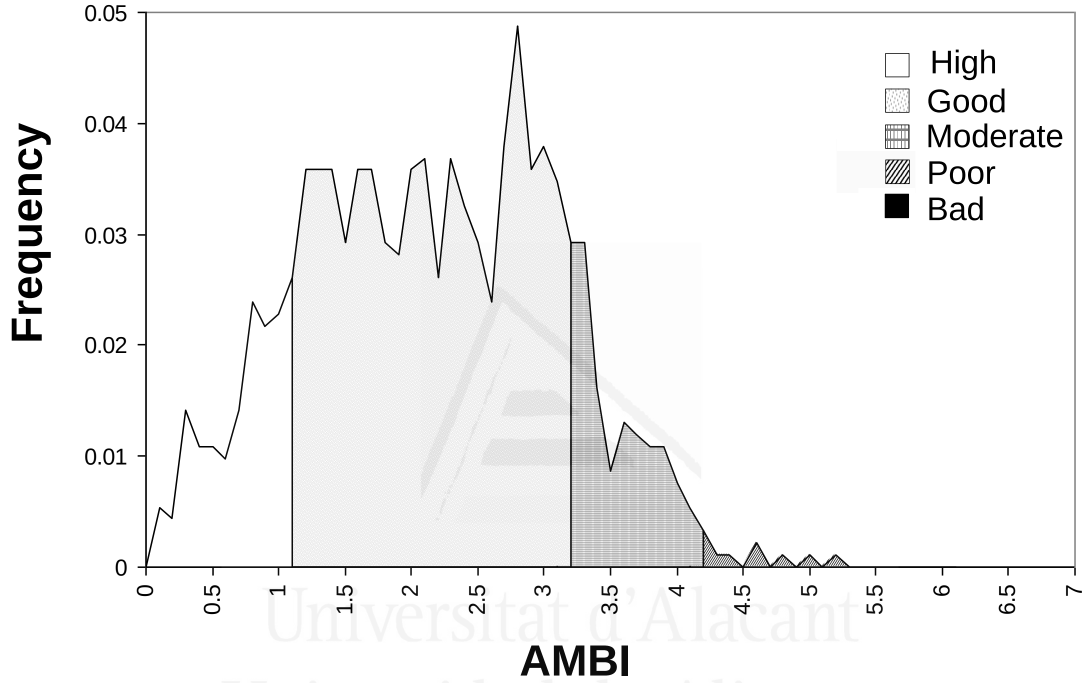


Figure 3.3. Frequency distribution of AMBI values. Thresholds taken from Borja et al. (2000).

Using the values predicted for the new thresholds with non-parametric regression (figure 3.4) demonstrated that the greatest change occurred in the limit between 'high' and 'good', whereas the other limits scarcely changed. Consequently, though the limits between 'good' and 'moderate' and 'poor' and 'bad' decreased slightly, the limit between 'moderate' and 'poor' got slightly bigger (table 3.4).

By applying these new thresholds, we obtained changes in the percentages of each EcoQS category (figure 3.5). Using these new thresholds, we obtained more similar EcoQS category frequencies for AMBI and BOPA. The percentage of samples classified as 'high' was reduced to 35.36%, while the percentage of 'good' samples increased to 48.7%. Still, differences between the classifications resulting from both indices remain, especially for Atlantic transitional waters and for Mediterranean lagoons, where AMBI continues to show higher percentages of good EcoQS. On the other hand, similar percentages of EcoQS categories for both indices were found for Mediterranean coastal waters. The results of Kappa agreement analysis for both indices (table 3.5) indicate an increase in the Kappa values, except for Mediterranean lagoons, and increase in the percentage of matching using the new thresholds proposed. The increase in matching was around 10% for the coincidence classifications for the total values.

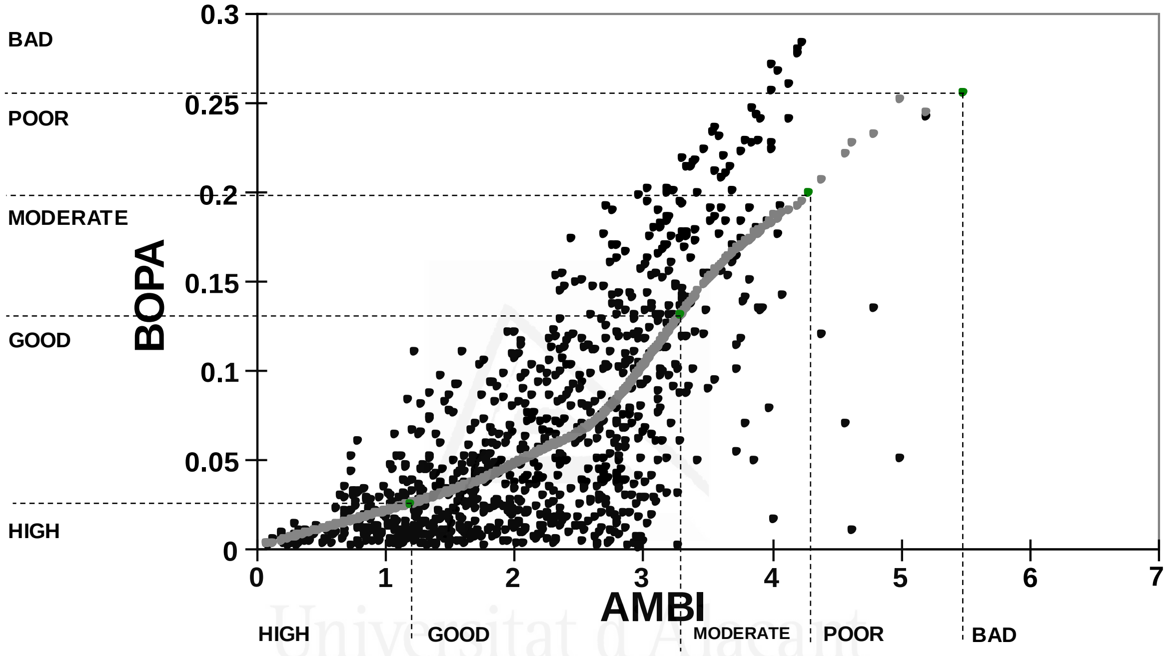


Figure 3.4. Non-parametric regression (grey points) between the BOPA and AMBI values (black points). New thresholds for the BOPA index were predicted based on the AMBI thresholds (Borja et al., 2000).

Table 3.4. BOPA thresholds from Dauvin and Ruellet (2007) and the new BOPA thresholds proposed.

	Thresholds from Dauvin and Ruellet (2007)	New thresholds proposed
High-Good	0.04576	0.02452
Good-moderate	0.13966	0.13002
Moderate-poor	0.19382	0.19884
Poor-bad	0.26761	0.25512

The Kappa values indicate that the agreement between the two indices remained similar, except for Mediterranean coastal waters where the increase was greater. Despite obtaining higher matching percentages with the new thresholds proposed, Mediterranean lagoons had lower Kappa values due to an increase of misclassifications in the 'high' EcoQS category.

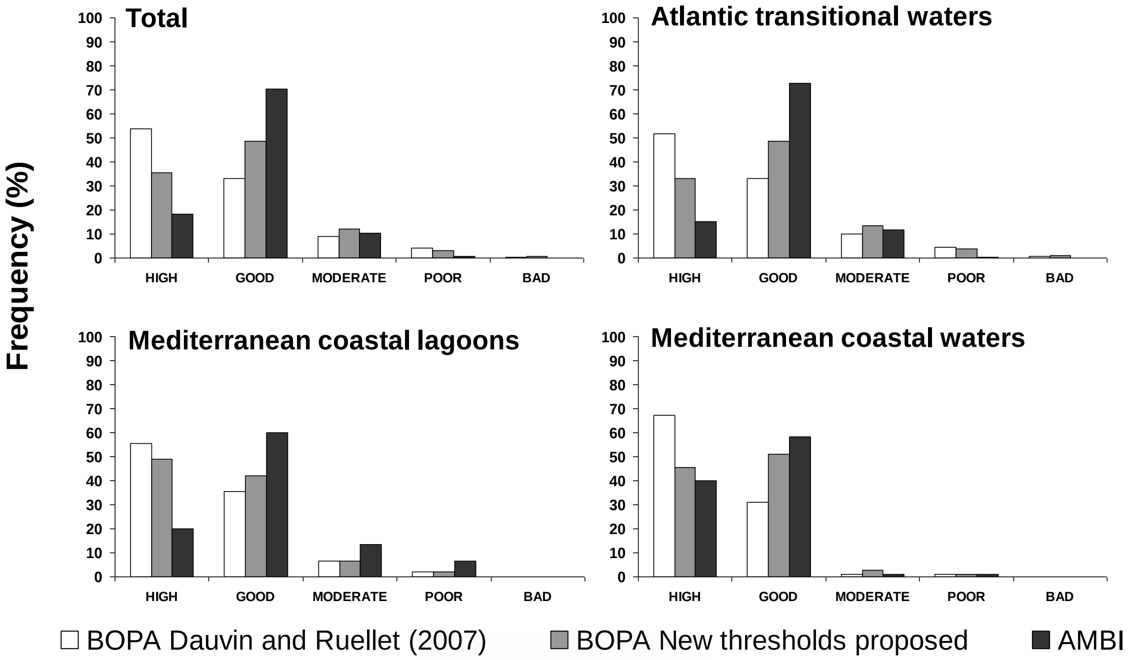


Figure 3.5. Percentage of each Ecological Quality Status (EcoQS) derived from BOPA (thresholds from Dauvin and Ruellet (2007) and this article) and AMBI for the total area and each area sub-section.

Table 3.5. Kappa values, levels of agreement, and percentage of correspondence between AMBI and BOPA were calculated for the thresholds proposed by Dauvin and Ruellet (2007) and new thresholds proposed in this paper for total area and each area sub-section.

BOPA / AMBI	Kappa analysis		% matching
	Kappa value	Level of agreement	
Total			
Thresholds proposed by Dauvin and Ruellet (2007)	0.56	Good	50.54%
New thresholds proposed in this paper	0.59	Good	61.28%
Atlantic transitional waters			
Thresholds proposed by Dauvin and Ruellet (2007)	0.56	Good	47. 98%
New thresholds proposed in this paper	0.59	Good	59.19%
Mediterranean lagoons			
Thresholds proposed by Dauvin and Ruellet (2007)	0.38	Low	48.89%
New thresholds proposed in this paper	0.37	Low	51.11%
Mediterranean coast			
Thresholds proposed by Dauvin and Ruellet (2007)	0.74	Very good	69.09%
New thresholds proposed in this paper	0.81	Very good	80%

3.4.- Discussion.

In spite of its initially demonstrated effectiveness for identifying polluted areas (Gomez-Gesteira and Dauvin, 2000; Dauvin and Ruellet, 2007; Bouchet and Sauriau, 2008; Ingole *et al.*, 2009), the BOPA index appears to regularly overestimate the EcoQS values compared to AMBI index, ranking the sites in the same order but disagreeing on the precise EcoQS level for each site. AMBI and BOPA don't use the same model of sensitivity/tolerance to pollution. The BOPA index (Dauvin and Ruellet, 2007) uses an opportunistic polychaete/amphipod ratio (Gomez-Gesteira and Dauvin, 2000), then the BO2A uses an opportunistic annelida/amphipod ratio, while AMBI (Borja *et al.*, 2000) uses a theoretical model in which the various species are divided into five ecological groups (EG) according to their sensitivity to organic pollution. However, the values for both BOPA and AMBI indices showed high correlations in the various sub-sections, especially in Mediterranean coastal waters. The fact that most of the amphipods, with the exception of one genus (*Jassa*), belong to EG1 and all the opportunistic polychaetes belong to EG4 and EG5 (see www.azti.es) could justify the variability of AMBI compared to BOPA. In other words, the high correlation between BOPA and AMBI could be due to the fact that both indices classify the taxa in the same way: opportunistic polychaetes (BOPA group) belong to EG4 and EG5 (AMBI group), and most of amphipods (BOPA group) belong to EG1 (AMBI group). Despite this high correlation and the fact that the BOPA index was calibrated using the AMBI thresholds (Dauvin and Ruellet, 2007), the BOPA index overestimated the EcoQS, mainly because it classified most of the samples as "high" EcoQS while AMBI classified them as "good". The two indices had different frequency distributions (Ruellet and Dauvin, 2007) due to the laws that the indices follow: BOPA follows an exponential law, which tends to produce low values and thus 'high' EcoQS, and AMBI follows a normal law, which tends to produce higher values and thus 'good' EcoQS. Comparing and inter-calibrating these indices for a large number of sites could probably overcome this problem (Dauvin *et al.*, 2007; Ruellet and Dauvin, 2007; Bouchet and Sauriau, 2008). Adjusting the limits between the EcoQS categories would insure better agreement between the methods (Borja *et al.*, 2007) and produce the same EcoQS assessment for a given zone (Ruellet and Dauvin, 2007). These adjustments should partially resolve the overestimation problem. The new BOPA thresholds proposed in this paper which can be also adopted for the BO2A produced more limited 'high' and 'good' EcoQS classifications, which increased the conformity of the BOPA results with those produced by AMBI, thus resulting in a better agreement in Mediterranean coastal waters and in French Atlantic transitional waters. Nonetheless, the misclassification of these close categories (‘high’ and ‘good’) remained due to the inherently different mathematical nature of the indices. AMBI produces a greater range of the ‘good’ EcoQS (Simboura and Reizopoulou, 2007), while BOPA tends to produces low values and thus ‘high’ EcoQS, especially in environments where amphipod species are abundant and diverse. For example, Bakalem *et al.* (2009) did not find any ‘moderate’ EcoQS in Algeria's shallow fine sand communities with the BOPA index due to this community's high diversity of amphipods. Despite the fact that opportunistic polychaetes represent the majority (over 70%) of the opportunistic benthic macro-invertebrate species on the list used by AMBI (www.azti.es), there are situations in which opportunistic polychaetes are only present in small numbers, if they exist at all. In fact, 18% of BOPA values in the literature were 0, due to the complete disappearance of this taxa. If little to no opportunistic polychaete is in the samples, BOPA produces low or null values, thus classifying samples as ‘high’ EcoQS. In order to remedy this problem, Dauvin and Ruellet (2009) proposed Benthic Opportunistic Annelida Amphipods index (BO2A), which adds clitellata to the opportunistic polychaete. This addition permits BO2A to be used all along the estuarine continuum. Moreover, although both indices do not produce identical EcoQS for all the samples, as do BENTIX and AMBI (Ruellet and Dauvin, 2007), the mean EcoQS calculated for a given body of water can be identical. Nevertheless, some particular situations, i.e. when non-polychaeta opportunistic species dominated such as the bivalve *Corbula gibba*, show divergence between high values of AMBI given a ‘moderate’ to ‘poor’ EcoQS and low values of BOPA or BO2A corresponding to ‘good’ or ‘high’ EcoQS. But, these cases remain limited in particular environment like in the harbours (see Bakalem *et al.*, 2009). Among the remaining BOPA misclassifications, those between close categories are not as important as those between distant categories. Although the WFD requires a five-category quality classification system, for environmental managers and policymakers, the most important boundary is that between ‘moderate’ and ‘good’ (Munari and Mistri, 2007). Diagnostic discordances between acceptable and non-acceptable situations were lower than 10%. However, the situation is different for Mediterranean lagoons, where the agreement between the two indices continued to be slight. Given the high variability of environmental parameters in these lagoons (e.g., salinity, dissolved oxygen, temperature), the species living in such environments adapt to this variability (Cognetti, 1992) and become tolerant of changes. In addition, the common presence of macroalgae, which could be related to contamination problems (Pranovi *et al.*, 2007), is associated with high amphipod abundance, and this is probably sufficient for BOPA to increase the ecological quality of the site (Munari and Mistri, 2008). On the other hand, AMBI index showed a poor discriminating power classifying some polluted Mediterranean lagoons (Pranovi *et al.*, 2007, Simboura and Reizopoulou, 2008), and since it was used to recalibrated BOPA, it could led some misclassifications. Finally, because the BOPA index is not based on the same ecological model of species sensitivity/tolerance to increasing organic matter input, like AMBI or BENTIX, BOPA can provide new information to a multi-index approach. The BOPA/BO2A index also limits the taxa misclassification caused by too many ecological groups (Ruellet and Dauvin, 2007): amphipods are unanimously recognized as being sensitive to organic enrichment and the list of opportunistic annelida is rarely contested, whereas species sensitivity/tolerance levels vary depending on the region (Grémare *et al.*, 2009). In contrast with the other biotic indices, the BOPA/BO2A index proved to be relatively independent of the habitat characteristics in such areas as Atlantic transitional waters (Blanchet *et al.*, 2008).

CHAPTER 4

Testing BOPA index in sewage affected soft bottom communities

Comprobación del índice BOPA en comunidades de fondos blandos afectadas por vertidos de aguas residuales

Abstract. The implementation of the European Directive 2000/60/EC has produced the development of several biotic indices based on benthic communities. These indices try to summarise ecological quality status of different communities. However, a universal index that works in all situations is difficult to establish, because there are several sources of variation. Therefore, there is the need for testing and validation of these indices which is required for making management decisions on different scales, and in different regions and communities. In this chapter, we test BOPA index in five locations affected by sewage disposal. BOPA index provides a valuable overview of the gradient status of a benthic environment, discriminating between stations more affected by discharge. Nevertheless, BOPA index, used to establish the ECOlogical Quality Status, seemed to overestimate the status inspite of thresholds proposed in last chapter.

Resumen. La aplicación de la Directiva Europea 2000/60/CE ha provocado el desarrollo de numerosos índices basados en organismos bentónicos. Estos índices tratan de resumir el estado ecológico de distintas comunidades. Sin embargo, es difícil crear un índice universal que trabaje en todas las situaciones posibles, puesto que hay demasiadas fuentes de variabilidad. Por lo tanto, es necesario comprobar y validar estos índices a distintas escalas, regiones y en distintas comunidades. En este capítulo, utilizamos el índice BOPA, en cinco localidades afectadas por el vertido de aguas residuales urbanas. El índice BOPA proporciona una perspectiva útil del estado de la comunidad bentónica, diferenciando las estaciones más afectadas por los vertidos. Sin embargo, al emplearlo para establecer el Estado de Calidad Ecológica, el índice parece sobreestimar la calidad a pesar de los nuevos límites propuestos en el capítulo anterior.

4.1.- Introduction.

Due to the increase in pressure on aquatic ecosystems which in turn is a consequence of continuous population growth, the European Parliament on 23 October 2000 established a framework for the Community to protect waters, the European Directive 2000/60/EC (WFD, 2000). One object of the Directive 2000/60 is the protection and improvement of the aquatic environment with the progressive reduction of discharges and emissions. To comply, Member States have to ensure that the highest ecological and chemical status possible is achieved, given impacts that could not reasonably have been avoided due to the nature of human activity or pollution.

A common source of pollution in coastal marine environments is sewage discharges that are often released via outfall into shallow subtidal habitats (McIntyre, 1995; Koop and Hutchins, 1996). This source is regulated by the Urban Waste-water Treatment Directive (91/271/EEC) (UWWTD, 1991), which established that there is a general need for secondary treatment of urban waste water to prevent the environment being adversely affected by the disposal of insufficiently-treated urban waste water. Member States will monitor and carry out any other relevant studies to verify that the discharge or disposal does not adversely affect the environment. Therefore, identification and characterisation of the locations affected by sewage discharge is necessary for efficient urban waste water management.

For the implementation of both directives it is necessary to have new tools to assess the anthropogenic impacts on marine habitats (Borja *et al*., 2003). This necessity has led to the development of different indices based on soft-bottom communities, which summarise ecological status and ecological quality. The relationships between benthic macrofaunal assemblages and the effect of contaminants on them have been described extensively in the literature (Pearson and Rosenberg, 1978; Gray and Mirza, 1979; Dauvin, 1982; Warwick *et al.*, 1990; Simboura *et al.*, 1995; Estacio *et al.*, 1997; Ellingsen, 2002; Morrisey *et al.*, 2003; Guerra-García and García-Gómez, 2004).

Several biotic indices have been developed with the aim of standardizing the use of benthic communities in order to establish marine habitat quality. Some of these indices are based on the classification of species (or groups of species) in several ecological groups representing specific sensitivity levels to disturbance. Two of the most widely used developed indices are AMBI (Borja *et al.*, 2000) and BENTIX (Simboura and Zenetos, 2002). Both these indices require classification to species level. However, this operation is labour intensive and time-consuming, especially for certain difficult groups such as spionid or cirratulid polychaetes, ampeliscid amphipods, etc. (De Biasi *et al.*, 2003). Nevertheless there are others, such as BOPA index, in which the taxonomic effort is reduced. After an initial proposal which had only considered the ratio amphipod/opportunistic polychaetes (Gomez Gesteira and Dauvin, 2000), BOPA has since been created and applied to the soft-bottom communities in the English Channel leading to the proposal of a modified index (Dauvin and Ruellet, 2007). BOPA index is based on ratio opportunistic polychaetes and amphipods (except the genus *Jassa*). Opportunist polychaetes are resistant, indifferent or favoured by organically enriched sedimentary matter, whereas amphipods form a particular zoological group which is sensitive to significant increases in organic matter. The main advantages of this index, as well as the reduced taxonomic knowledge, are its independence of sampling protocols, its use of mesh sieves and of the surface unit chosen to express abundances, since this uses frequency data and the proportion of each category of organism (Pinto *et al.*, 2009).

This index has already been used for monitoring the impact of pollution on different macrobenthic communities (Quintino *et al.*, 2006; Dauvin *et al.*, 2007; Munari and Mistri, 2007, 2008; Pranovi *et al.*, 2007; Afli *et al.*, 2008; Blanchet *et al.*, 2008; Bouchet and Sauriau, 2008). However, a biotic index is unlikely to be universally applicable, because not all organisms are equally sensitive to all types of anthropogenic disturbances and thus are likely to respond differently to different types of perturbations (Dauvin *et al.*, 2007; Pranovi *et al.*, 2007; Afli *et al.*, 2008). Indeed, the WFD recommends the use of reference sites to assess temporal changes in the ecological quality of water types under study, therefore these indices are required to test for departure from a reference or control situation (Quintino *et al.*, 2006). It is interesting to assess the applicability, in terms of discriminating capacity, of biotic indices for environments in different state of conservation.

In this study we apply BOPA index, with the criteria described by Dauvin and Ruellet (2007), to locations affected by sewage disposal by testing its ability to detect this impact and allowing us to come up with a future calibration for Mediterranean medium-to-fine-sand communities. These communities characterize shallow sublittoral soft-bottoms in the north-western Mediterranean Sea (Pérês and Picard, 1964; Cardell *et al.*, 1999; Sardá *et al.*, 1999, 2000) and these communities are common off the coast of Castellon (NE Spain). Several municipal treatment plants dump treated water into this area with a constant depth and uniform benthic community. Consequently, it is a site, with established pollution gradients, which is ideal for investigating the links between macrofaunal assemblages and the effect of contaminants.

4.2.- Material and methods.

The study area is located off the Castellon Coast (NE Spain); five locations affected by sewage outfalls along 40 km of coast were analyzed (locations I to V in chapter 2, figure 2.1). The minimum distance between outfalls is 7.62 km and the maximum 11.03 km. These outfalls correspond to the villages of Vinaroz (location I), Benicarló (location II), Peñiscola (location III), Alcossebre (location IV) and Torreblanca (location V). The mean length of the pipelines is 2138 m and the mean sewage outfall depth is 14.8 m (See table 2.1 and figure 2.1 in Chapter 2). Sampling method was previously described in chapter 2.

Total infaunal abundance was counted (see chapter 2); differentiating abundance of Amphipoda and abundance of oportunistic polychaete. We considered Capitellidae, Cirratullidae and Spionidae as opportunistic polychaetes families since their tolerance are usualy accepted, as well as Dorvillidae because of dominance of opportunistic *Ophryotrocha* genus (Dauvin and Ruellet, 2007; Glémarec and Hily, 1981; Gomez Gesteira and Dauvin, 2000; Hilbig, 1995; Karakassis *et al.*, 2000; Pearson and Rosenberg, 1978).

Using these data we calculated BOPA index for each replicate:

$$\mathrm{BOPA} = \log\left(\frac{fp_{op}}{fa+1}+1\right)$$

Where f_{pop} is opportunistic polychaete frequency and f_a is amphipod (excluding the opportunistic *Jassa* amphipods) frequency (Dauvin and Ruellet, 2007)

Besides sediment parameters analysed in chapter 2; salinity, redox potential, dissolved O_2 and pH of seawater was also measured in the year 2006 using a multiparametric probe Hydrolab H20.

BOPA values for each location were examined using 3-factor analyses of variance (ANOVA) with distance and location as fixed factors and year random as factor.

Correlation between BOPA values and data of wastewater (flow, suspended solids, BOD, COD, phosphates, nitrates and turbidity), abiotic factors for sediment (granulometric analysis, organic matter, pH) and seawater (pH, redox potential., temperature, salinity) were determined using Pearson Correlation, testing probability of each correlation coefficient with Bonferroni test.

The BOPA values were used to assign studied samples to the five (ECOlogical Quality Status) classes suggested by WFD: 'high' for unpolluted sites, 'good' for slightly polluted sites, 'moderate' for moderately polluted sites, 'poor' for heavily polluted sites and 'bad' for extremely polluted or azoic sites. In this way, classifications obtained with boundaries ranked by Dauvin and Ruellet (2007) and boundaries proposed in the previous work were compared (de-la-Ossa-Carretero and Dauvin, 2010).

4.3.- Results.

The ANOVA results showed significant differences in BOPA values in the interaction among the three factors (table 4.1). Differences among distances were due to the increase of BOPA index values for distance 0 m (figure 4.1). The highest values of BOPA were obtained at 0 m from outfall at location II, whereas the lowest values were obtained in the location IV at 1000 m from the outfall. A significant increase of BOPA was detected in the stations closer to the outfall in location I (years 2005, 2006 and 2007), location II (all years), location III (year 2006) and location IV (years 2006 and 2007), whereas there were not differences for factor distance in location V. Differences among locations were detected mostly due to the higher values of BOPA index at 0 m to outfall in location II.

Table 4.1. Results of ANOVA for BOPA values in each location for the factors distance, year and location. df: degrees of freedom, MS: medium squares, F of each factor =MS factor/MS residual because all the factors are orthogonal. Levels of significance: ns no significant difference, *p < 0.05, **p < 0.01 and ***p < 0.001.

	Source	DF	MS	F	P
BOPA	Distance	2	0.0822	47.73	***
	Location	4	0.0262	5.55	**
	Year	4	0.0099	12.86	***
	Dis.xLoc.	8	0.0286	14.55	***
	Dis.xYear	8	0.0017	2.23	*
	Loc.xYear	16	0.0047	6.11	***
	Dis.x Loc.x Year	32	0.0020	2.54	***
	RES	375	0.0008		
	TOT	449			

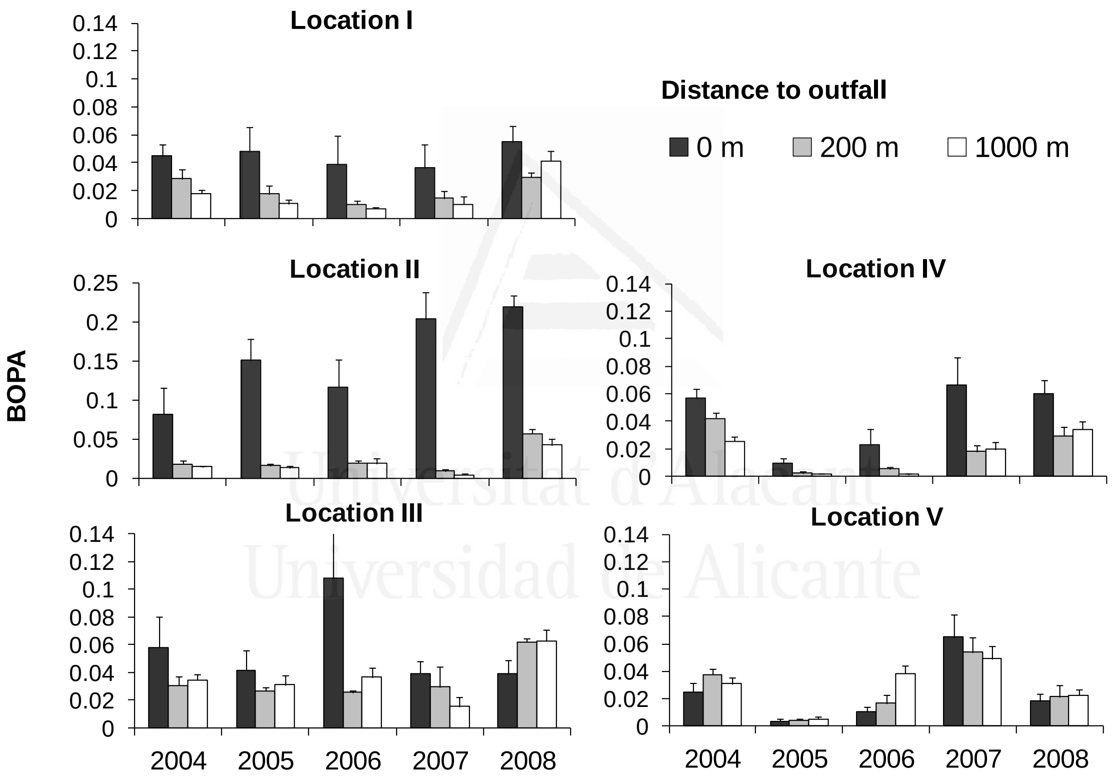


Figure 4.1. Means and standard errors of BOPA values for each location (I, II, III, IV and V), each year (2004, 2005, 2006, 2007 and 2008) and each distance to outfall (0 m, 200 m and 1000 m).

Measurement of water quality showed better values in location V, where values of flow, turbidity, suspended solids, BOD, COD, phosphates and nitrates were the lowest (see chapter 2 table 2.1); whereas the other locations showed worse values from which location II showed the highest flow and in location IV the lowest. The values of studied seawater parameters were presented in table 9.2 and sediment parameters were studied in chapter 2 (table 2.2, figures 2.2, 2.3 and 2.4)

Table 4.2. Means and standard errors of seawater parameters (pH, temperature, salinity, dissolved O_2 and potential error), in each location and distance from outfall.

	pH	**Temperature (ºC)**	**Salinity**	**Dissolved O_2 (%)**	**Pot. Redox (mV)**
Location I					
0 m	8.24±0.00	20.05±0.01	38.39±0.00	101.55±0.02	52.00±4.47
200 m	8.26±0.01	20.08±0.07	38.29±0.04	104.05±1.54	40.5±12.75
1000 m	8.27±0.00	20.11±0.11	38.25±0.02	104.15±1.05	46.00±7.11
Location II					
0 m	8.4±0.00	21.04±0.01	38.35±0.02	105.35±1.54	14.5±10.96
200 m	8.4±0.01	20.89±0.03	38.49±0.04	100.75±0.65	41.5±2.91
1000 m	8.4±0.02	21.02±0.01	38.49±0.04	99.25±0.11	50.0±2.68
Location III					
0 m	8.27±0.00	21.0±0.00	38.1±0.00	107.8±0.00	-15±0.00
200 m	8.26±0.00	20.9±0.02	38.1±0.02	102.8±0.00	9.5±7.82
1000 m	8.26±0.00	20.7±0.09	38.3±0.02	99.2±0.07	17.0±20.12
Location IV					
0 m	8.07±0.00	21.72±0.04	38.4±0.02	109.4±3.4	-7±8.04
200 m	8.06±0.00	21.79±0.02	38.4±0.02	103.4±0.25	67.5±1.56
1000 m	8.06±0.01	21.83±0.32	38.4±0.02	101.8±1.07	75.5±13.64
Location V					
0 m	8.1±0.00	22.3±0.25	38.37±0.00	108.4±3.8	138±0.44
200 m	8.07±0.01	22.0±0.26	38.42±0.02	108.1±1.6	135.5±6.03
1000 m	8.14±0.00	22.1±0.71	38.62±0.11	99.55±0.20	144±1.79

Pearson correlation coefficient and Bonferroni probabilities reported that there were significant correlations between BOPA index and eleven analyzed factors. With regard to data of waste water all parameters, except pH, BOD and nitrates, showed significant and positive correlations with BOPA index; Pearson coefficients were higher in flow, conductivity and phosphates (table 4.3 and figure 4.3). Secondly, pH of seawater showed a significant and positive correlation whereas potential redox showed a significant and negative correlation; in both cases Pearson coefficients were low. Finally, pH and potential redox also showed a higher significant correlation in sediment, respectively positive and negative (table 4.3 and figure 4.3).

Table 4.3. Pearson correlation of BOPA values with studied factors. (BOD: biologic oxygen demand; COD: chemical oxygen demand) and Bonferroni probabilities (levels of significance: ns no significant, *p < 0.05, **p < 0.01 and ***p < 0.001).

	BOPA	Pearson correlation[p]
Water disposal	**pH**	0.090 ^{ns}
	Flow (m^3/month)	0.622***
	Suspended solids (mg/l)	0.381***
	BOD (mg/l)	0.156 ^{ns}
	COD (mg/l)	0.288**
	Phosphates (mg/l)	0.478***
	Nitrates (mg/l)	0.057 ^{ns}
	Conductivity	0.525***
	Turbidity (NTU)	0.266*
Sea water	**pH**	0.297**
	Temperature (ºC)	-0.095 ^{ns}
	Salinity(^{0}%)	-0.204 ^{ns}
	Pot. Redox (mV)	-0.326**
Sediment	**% Mud**	-0.060^{ns}
	% Fine Sand	0.075 ^{ns}
	% Medium sand	-0.082 ^{ns}
	% Coarse sand	0.005 ^{ns}
	% Gravel	0.013 ^{ns}
	% org mat	-0.045 ^{ns}
	Pot. Redox (mV)	-0.378***
	pH	0.200*

ECOlogical Quality Status rating of studied samples, for the entire data, showed segregation related to distance from outfall (figure 4.4). Of the total samples closest to the outfall, 52.59% of samples were classified as ‘high’, 34.07% as ‘good’, 2.96% as ‘moderate’, 8.88% as ‘poor’ and 1.48% as ‘bad’ using Dauvin and Ruellet (2007) boundaries, whereas only 34.8% of samples were classified as ‘high’ with the new thresholds (de-la-Ossa-Carretero and Dauvin, 2010), and 50.37% were established as ‘good’, 6.66% as ‘moderate’, 5.93% as ‘poor’ and 2.22 as ‘bad’. With regard to samples situated at 200 m to outfall, Dauvin and Ruellet (2007) boundaries classified as 83.33% of samples as ‘high’ and 16.66% as ‘good’, while samples situated at 1000 m to outfall 87.33% were classified as ‘high’ and 12.66% as ‘good’. The new thresholds produced more balanced classification at both distances classifying 56.66% of samples as ‘high’ and 43.33% as ‘good’.

In locations I, III, IV and V more than 64% of samples closest to the outfalls were classified as ‘high’ with Dauvin and Ruellet (2007) classification, whereas this trend to ‘high’ status were only obtained in outfall from location V using boundaries proposed in previous chapter. In location II, 56.67% of samples closest to the outfall were classified as ‘moderate’, ‘poor’ or ‘bad’ status and 16.66% as ‘high’ with Dauvin and Ruellet (2007), whereas new thresholds established 63.33% of samples as ‘moderate’, ‘poor’ or ‘bad’ and only one sample (3.3%) as ‘high’ in outfall from location II.

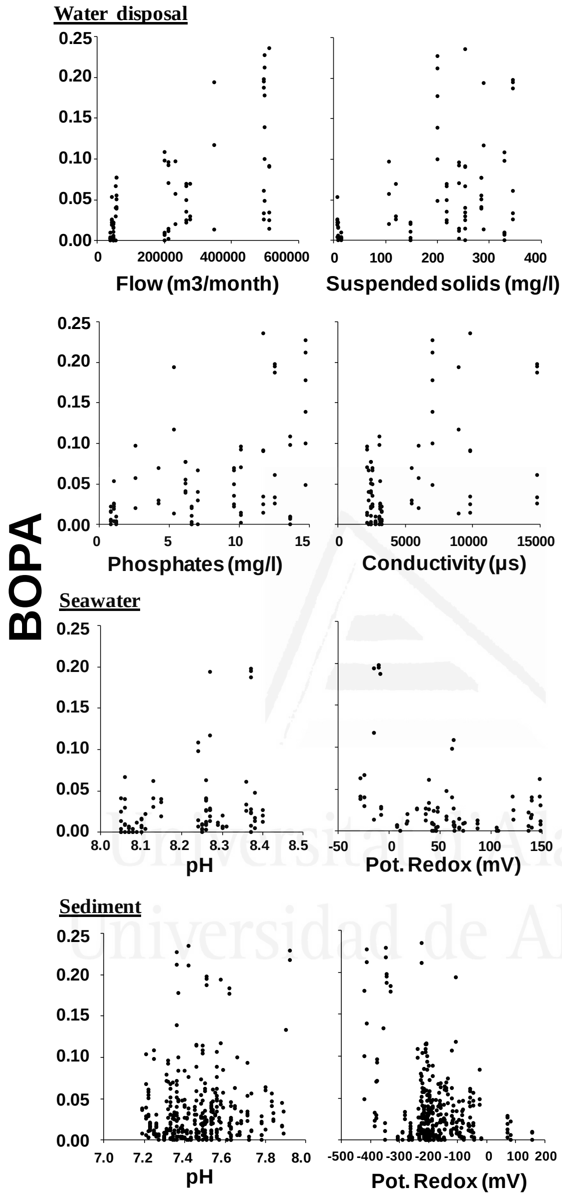


Figure 4.3. Simple scatterplots. BOPA values are plotted against analyzed factors which showed significant correlation. Water disposal measurements: flow, suspended solids, COD (chemical oxygen demand), phosphates, conductivity, and turbidity. Seawater parameters: pH and potential redox. Sediment parameters: pH and potential redox.

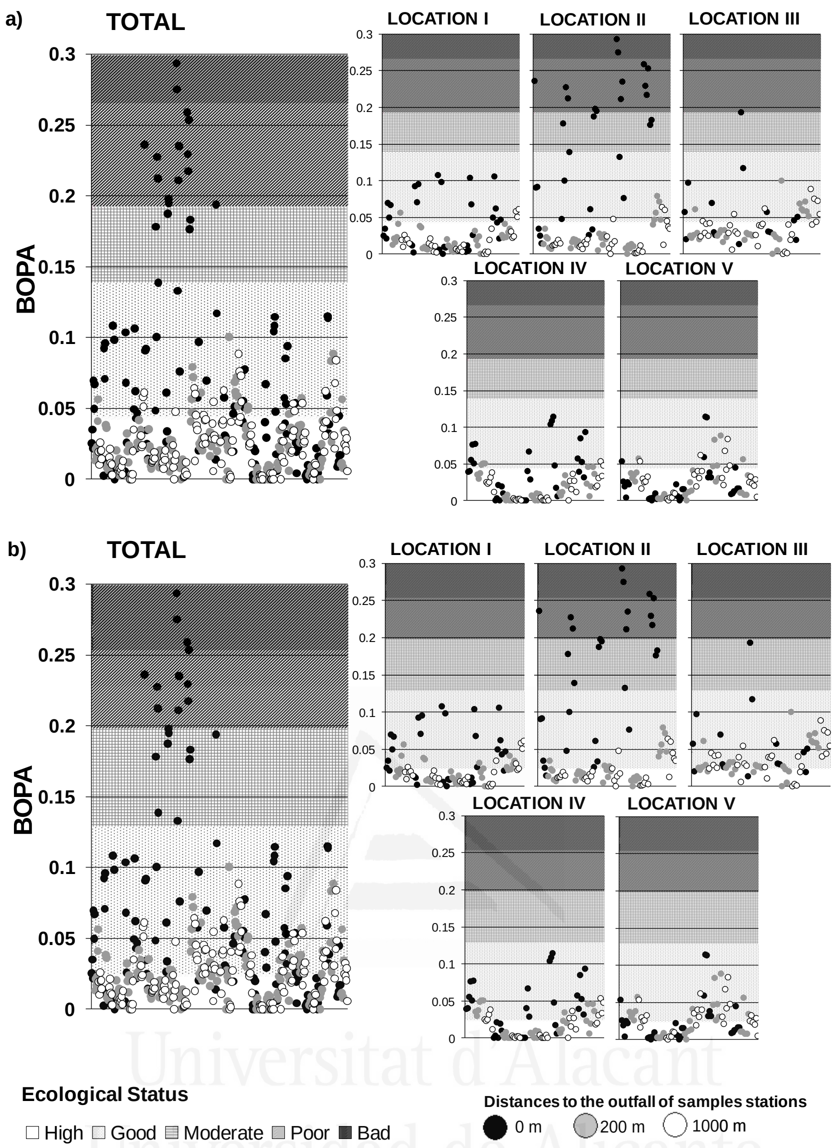


Figure 4.4. EcoQ Status for samples in each distance to outfall. White dots for 1000 m, grey dots for 200 m and black dots for 0 m. Rating was showed for total samples and for each location. Boundaries established by a) Dauvin and Ruellet (2007) and b) de-la-Ossa-Carretero and Dauvin (2010)

4.4.- Discussion.

BOPA index showed a response to the presence of pollution due to sewage outfalls. The highest values were obtained in stations close to outfalls except the location with lowest sewage flow and best waste water treatment. Therefore BOPA values appear to represent an effective tool for monitoring sewage outfall impacts.

On the one hand, this index is negatively correlated with amphipods, which are a particularly sensitive zoological group, not only to significant increases in organic matter but also to increases in other kinds of pollution including metals and hydrocarbons (Dauvin and Ruellet, 2007). The increased abundance of amphipods as we move away from the discharge, suggests that the environment was less stressed, since amphipods are more sensitive to pollution (environmental stress) than other marine species (Rand and Petrocelli, 1985; Arvai *et al.*, 2002; Cesar *et al.*, 2004; Riba *et al.*, 2004). On the other hand, polychaete group contains both sensitive and tolerant species and they are found along the whole gradient from pristine to heavily disturbed areas (Olsgard *et al.*, 2003). Some species showed significantly greater numbers of individuals at the sewage-affected site while other species densities showed no difference or in some cases even a decrease (Dauer and Conner, 1980).The presence or absence of specific polychaetes in marine sediments, therefore, provides an excellent indication of the condition or health of the benthic environment (Pocklington and Wells, 1992). Therefore the opportunistic polychaete/amphipod ratio worked as an indicator when studying the effects of sewage pollution.

Moreover, BOPA values showed significant positive correlation with waste water measurements: flow, suspended solid, phosphates, COD, conductivity and turbidity. In the locations where these factors are highest, the BOPA index increased near disposal. In location V disposal BOPA values were the lowest and values of water quality of sewage disposal (suspended solids, BOD, COD, phosphates, nitrates and turbidity) register the best values of the five disposals**.** In this way, the Urban Waste-water Treatment Directive (91/271/EEC) (UWWTD, 1991) established that there is a general need for secondary treatment of urban waste water to prevent the environment from being adversely affected. Requirements for discharges from urban waste water treatment plants which dump in sensitive areas, set by Directive 91/271, were accomplished by location V waste water (phosphates<2 mg/l, nitrates<15 mg/l, BOD<25 mg/l O_2, COD<125 mg/l $O_{2,}$ suspended solids<35mg/l).

The issue of the assessments of the physicochemical status has been addressed by several authors (Bald *et al.*, 2005; Rodriguez *et al.*, 2006; Best *et al.*, 2007; Simboura and Reizopoulou, 2008). BOPA index could be related to physicochemical status; this relation was studied analysing Pearson correlation of BOPA values with several seawater and sediment parameters which can vary according to the sewage discharges. In our study, there is only significant correlation for potential redox and pH of analysed seawater and sediment parameters. A decrease of potential redox and increase of pH produced an increase in BOPA values, indicating a relation of BOPA index with chemical quality of water and sediment especially with oxygen saturation and possible hypoxia situation. Other changeable parameters, such as organic matter content, that showed significant correlations with BOPA assessing intertidal mudflats constrained by oyster farming (Bouchet and Sauriau, 2008), did not show significant correlation. However this would not be the first time that no correlation has been found between organic matter content in the sediment and biotic indices (Borja *et al.*, 2000, Dauvin *et al.*, 2007), moreover an increase of organic matter was not detected in the proximity of studied outfalls (see chapter 2).

The principal aim of indices in the WFD is to classify EcoQ status. We classified the areas affected by sewage outfall using an indicator developed by Dauvin and Ruellet, 2007 for Atlantic communities. The results indicated that most of the stations were classified as having 'high' or 'good' status despite the presence of a disposal of waste water, only pre-treated in four of five outfalls. Only in the outfall with high flow, the impacted stations were classified as non acceptable situation ('moderate', 'poor' or 'bad' status) in 56.67% or 63.33% of samples, depending on employed classification thresholds. We could be overestimating the Ecological Quality (EcoQ) of stations close to sewage outfalls, by not differentiating sufficiently well impacted areas which could be established in 'high' or 'good' status. New thresholds proposed in previous study (de-la-Ossa-Carretero and Dauvin, 2010) decreased the percentage stations classified as 'high' status and reduce this overestimation.

Indices seem to generally overestimate the EcoQ status under different conditions. Bakalem *et al.* (2009) did not find any area with 'moderate' status in a similar zone, applying BOPA index to shallow Algeria fine sand community, due to the high diversity of amphipods in this community. Munari and Mistri (2007 and 2008), classified the majority of stations in Tyrrhenian and Adriatic coastal lagoons as 'good/high' using BOPA. They claim that amphipods associated with the common presence of macroalgae in Adriatic transitional waters are probably sufficient to increase the ecological quality of the station through the BOPA model. Pranovi *et al.* (2007) also found that BOPA showed low discrimination in the Venice lagoon, classifying all the samples in the 'high' category and, in the same way, Blanchet *et al.*

(2008) found the same problem, BOPA classified a large majority of stations of the French Coast as acceptable (‘good’ or ‘high’ status). Afli *et al.* (2008) also showed that off the Tunisian coast BOPA index appeared to be somewhat severe, classifying only two stations in moderate and poor statuses. An opposite case is the so-called `paradox of estuarine quality´ (Dauvin *et al.*, 2007), which established that transitional estuarine waters are naturally organic rich environments where stress-tolerant species are typical, so transitional environments would therefore likely be, by definition, characterised by low scores and hence low EcoQ values. In Quintino *et al.* (2006), several representative indices are shown unlikely to discriminate between changes in community structure, although they are sufficient to detect large scale differences which can be attributed to well-defined stressors (Rees *et al.*, 1990; Quintino *et al.*, 2006). Indeed the robust nature of the indicators on differing spatial spaces and under differing benthic conditions has not been rigorously assessed (Quintino *et al.*, 2006). There is a need for understanding the links between the effects of human activity and changes in populations (ICES, 2004; Munari and Mistri, 2008) for each community and even area.

Another source of variability that it is necessary to keep in mind is the differences existing among ecoregions. The Mediterranean is a characteristic sea, being an oligotrophic, euhaline and microtidal environment. In other classification systems, such as chlorophyll biomass concentration or, dissolved inorganic nitrogen, there are different boundary limits and reference condition thresholds used by different Member States and/or ecoregions (Simboura and Reizopoulou, 2008). Simboura and Reizopoulou (2008) in the Helenic area demonstrated that biotic indices perform differently on the ecoregion scale (e.g. Mediterranean, Atlantic) and on the water category scale (coastal, transitional waters).

A prerequisite for successful implementation of the European Water Framework Directive (WFD, 2000) in European waters is the intercalibration of the methods and scaling in accordance and in biological relevance to the normative definitions of WFD. The ecological quality status has become a priority issue of the directive and should be particularly tested on the upper and lower boundaries of the ‘good’ class. Among these boundaries, the ‘good/moderate’ one is essential because ‘moderate’ status sites should be restored to ‘good’ by the year 2015 (Simboura and Reizopoulou, 2008). The urgent need for assessing environmental quality within WFD using biological indices could produce erroneous conclusions. BOPA index worked differentiating sewage effect, but it tended to established low percentage of 'moderate' status in disposal stations despite the new thresholds proposed. Moreover, this problem could happen using other index as AMBI, since both indices showed high correlation and agreement for Mediterranean coast (see chapter 3). A universal index that works in all coastal areas is difficult to find because benthic communities vary widely.

For a correct assessment of a general ecological quality status of marine environments as well as for the indication of reference conditions, the natural variability of the indices on different temporal and spatial scales has to be assessed and taken into account (Reiss and Kröncke, 2005). So, it is necessary to use reference sites to assess temporal and spatial changes in the ecological quality classification, as WFD recommends. The objective of setting reference condition standards is to enable the assessment of the biological quality, against an existing 'pristine'/undisturbed site (or a site with very minor disturbance) (Muxika *et al.*, 2007). There is the need for testing and validation of boundaries and indicators in several similar areas, these being required to establish a well defined boundary and facilitate making management decisions at different scales and for each different region and community. Such partitioning would need to be carried out using big datasets containing all the ecological situations encountered in Europe and for each type of benthic community (Ruellet and Dauvin, 2007). Further validation is required especially where one indicator over-estimates the ecological status for poor areas and underestimates it for good areas.

ORDER AMPHIPODA

Orden Amphipoda

Publications.

De-la-Ossa-Carretero J.A., Dauvin J.C., Del-Pilar-Ruso Y., Giménez-Casalduero F., Sánchez-Lizaso J.L., 2010. Inventory of benthic amphipods from fine sand community of the Iberian Peninsula east coast (Spain), western Mediterranean, with new records. *Marine Biodiversity Records* **3**: e119.

De-la-Ossa-Carretero J.A., Dauvin J.C., Del-Pilar-Ruso Y., Giménez-Casalduero F., Sánchez-Lizaso J.L., 2011. Sensitivity of amphipods to sewage pollution. *Estuarine, Coastal and Shelf Science*. In press.

CHAPTER 5

Inventory of benthic amphipods from fine sand community of the Iberian Peninsula east coast, western Mediterranean, with new records

Inventario de anfípodos bentónicos de comunidades de arenas finas en la costa este de la Península Ibérica, Mediterráneo occidental, con nuevas citas.

Abstract. Recent sampling surveys (2004–2008) of the shallow (12–20 m) soft-bottom homogeneous fine-sand community have allowed the collection of 55 marine amphipod species (53 Gammaridea and 2 Caprellidea) along the 250 km of Iberian Peninsula east coast (Comunidad Valenciana, Spain). Among the species recorded, 1 recently described is new to science, 5 were collected for the first time in the Spanish Mediterranean and 14 were recorded for a second time confirming their presence. Of these 20 species; 6 are considered to be endemic to the Mediterranean Sea, 7 are also north-eastern Atlantic species, and the last 7 have a wide geographical distribution in the Indo-Pacific or Arctic and the Atlantic Oceans. Finally, multivariate analyses of species distribution showed changes among locations according to the north–south axis and depth, parameters that highly influence the benthic communities.

Resumen. Las campañas realizadas en las comunidades de fondos blandos de arenas finas han permitido la identificación de 55 especies de anfípodos (53 Gammaridea y 2 Caprellidea) a lo largo de 250 km de la costa este de la Península Ibérica (Comunidad Valenciana, España). Entre las especies encontradas, 1 fue descrita como especie nueva para la ciencia, 5 fueron identificadas por primera vez en el Mediterráneo español y 14 fueron confirmadas al ser citadas por segunda vez. De estas 20 especies, 6 son consideradas endémicas del Mediterráneo, 7 están también en el Atlántico nororiental, y las otras 7 tienen una amplia distribución geográfica en el Indo-Pacífico, Ártico y Atlántico. Un análisis multivariante de la distribución de estas especies muestra cambios entre localidades según el eje norte-sur y la profundidad, parámetros que influyen a las comunidades bentónicas.

5.1.- Introduction.

The Mediterranean Amphipoda fauna has a high richness, with more than 452 recorded species (Bellan-Santini *et al.*, 1998), and has been widely studied; however, the knowledge of this order is not uniform throughout the entire Mediterranean. In this way, knowledge of the ecology and taxonomy of amphipod species on the Mediterranean coast of the Iberian Peninsula is still fragmentary (Jimeno and Turón, 1995; Bellan-Santini *et al.*, 1998) and the central east coast has been studied relatively infrequently (Marti, 1989).

According to Jimeno (1993), there are 368 known species along the coast of the Iberian Peninsula, of which only 146 are reported from the Mediterranean side. Works on amphipods from this area have been mainly carried out on the Catalan coast (Castany *et al.*, 1982; Bibiloni, 1983; Carbonell, 1984; Jimeno, 1993; San Vicente and Sorbe, 1999; Munilla and San Vicente, 2005; Cartes *et al.*, 2007, 2009; Delgado *et al.*, 2009) and the Andalucia coast (Conradi *et al.*,1995; Conradi and López-González, 1999; Sánchez-Moyano *et al.*, 2007; González *et al.*, 2008; Guerra-García *et al.*, 2009a, b; Guerra-García and Izquierdo, 2010; Izquierdo and Guerra-García, 2010). Other studies have been produced in the Balearic Islands (Cartes *et al.*, 2003; Ortiz and Jimeno, 2003) and indeed, on the Iberian Peninsula east coast (Marti, 1989; Sanchez-Jerez *et al.*, 1999. 2000; Vázquez-Luis *et al.*, 2008, 2009), where this study was carried out (figure 5.1); however, more studies on the distribution of amphipods are still required in order to increase the knowledge of species distribution and amphipod diversity of this area.

One of the communities more frequent in shallow soft-bottom non-vegetated areas from the western Mediterranean is the medium-to-fine sand community of *Spisula subtruncata* (Cardell *et al.*, 1999). This community tends to colonize exposed or semi-exposed sublittoral habitats, from the beach environment to 30 m depth (Cardell et al., 1999; Sardá *et al.*, 1999, 2000). Although this community generally contains low numbers of individuals and low values of biomass, a high abundance and diversity of amphipods had been reported (Bakalem *et al.*, 2009).

The main objective of this study was to report the status of the knowledge of the amphipod species inhabiting this widely distributed community on the eastern Spanish

Mediterranean coast and to analyse changes in species composition among sampled locations.

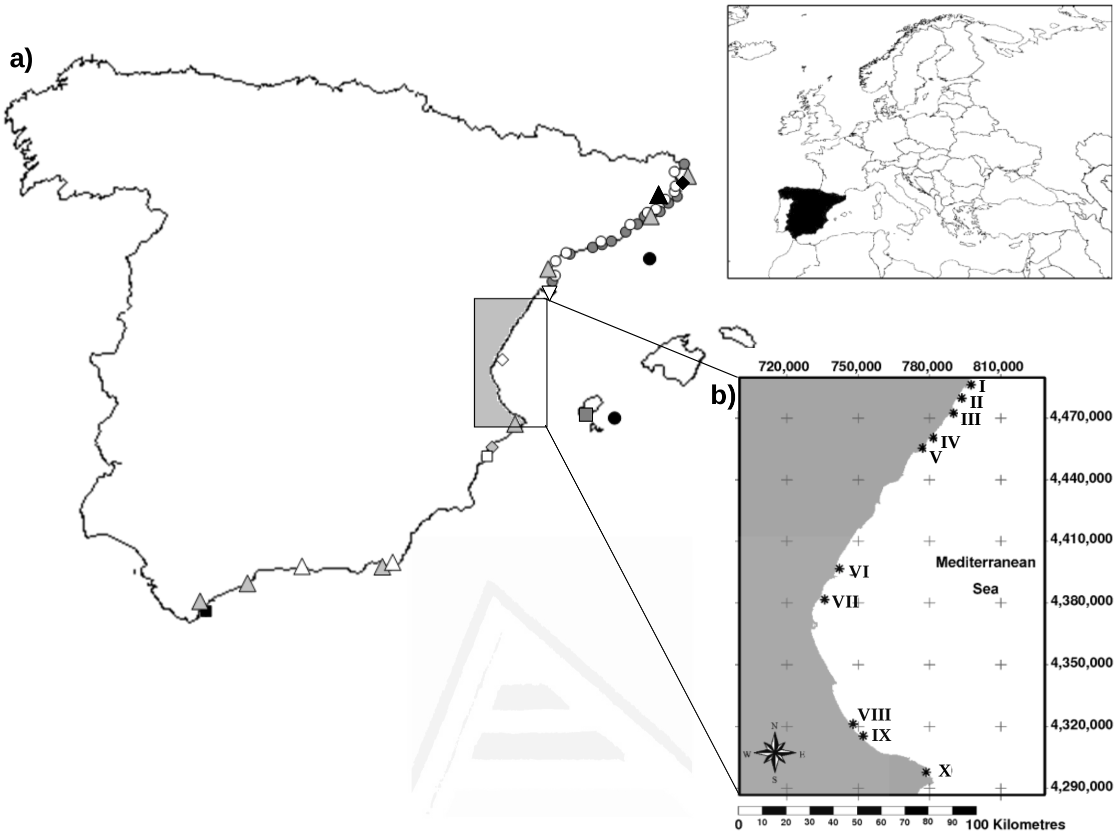


Figure 5.1. A) Locations from bibliography of amphipods checked from de Spanish Mediterranean Coast. ▲ Biblioni (1983); ♦ Carbonell, (1984); ◇ Marti (1989) ; ● Cartes and Sorbe (1993, 1999); Cartes *et al.* (2003); ● Jimeno (1993); Jimeno and Turón (1995); ■ Conradi *et al.* (1995); Conradi and López-González (1999); Sánchez-Moyano *et al.* (2007); Guerra-García *et al.* (2009a); ♦ Sanchez-Jerez *et al.* (1999, 2000); ○ San Vicente Sorbe (1999); Munilla and San Vicente (2005); ■ Ortiz and Jimeno, (2003); ▽ Cartes *et al.* (2007, 2009); Delgado *et al.* (2009); △ Gonzalez *et al.* (2008); ▲Guerra-García *et al.* (2009b), Guerra-García and Izquierdo (2010), Izquierdo and Guerra-García (2010); □ Vázquez-Luis *et al.* (2008, 2009).

B) Locations from this study I.Vinaroz, II.Benicarló, III.Peñíscola, IV. Alcossebre, V.Torreblanca, VI. Canet d'en Berenguer, VII. Puebla de Farnals, VIII. Gandia, IX. Oliva, X. Javea.

5.2.- Material and methods.

A total of 40 stations from ten different locations along approximatley 250 km of south-west coast of the Balearic Basin (Comunidad Valenciana, Iberian Peninsula east coast) were employed for this chapter (figure 5.1). Locations corresponding with Canet d'en Berenguer, Puebla de Farnals and Javea were added to locations employed in chapter 2 (figure 2.1).

All locations were characterized by homogeneous fine-sand sediment (median sediment included between 0.125 mm and 0.25 mm) with a depth-range from 12.4 to 20 m (table 5.1). For each location, four sites were sampled keeping a constant depth in each location (corresponding to stations at 200 and 1000 m to the outfalls). All samples were collected in July during five consecutive years (2004 to 2008) using the method described in chapter 2.

Table 5.1. Locations, geographical coordinates, depth (m) and physic characteristics of sediment (percentage) of the stations of each location.

Location	Latitude	Longitude	Depth	Mud	Fine sand	Medium Sand	Coarse sand	Gravel	Organic matter
I. Vinaroz	40º 28.15' N	0º 30.44' E	15.0	4.25	78.85	5.14	9.27	2.50	2.47
II. Benicarlo	40º 24.55' N	0º 27.70' E	14.0	7.56	73.55	5.68	6.79	6.43	1.61
III. Peñiscola	40º 20.79' N	0º 24.99' E	14.8	1.49	83.56	4.27	5.62	5.07	2.02
IV. Alcossebre	40º 14.45' N	0º 18.55' E	12.4	1.03	74.34	14.01	5.64	4.98	1.56
V. Torreblanca	40º 11.83' N	0º 14.59' E	13.9	1.23	86.82	5.63	3.96	2.36	1.47
VI. C. Berenguer	39º 40.96' N	0º 10.77' W	15.6	0.72	83.36	5.87	9.16	0.88	1.62
VII. P. Farnals	39º 31.97' N	0º 15.44' W	18.9	9.18	69.05	8.36	9.32	4.09	2.61
VIII. Gandia	39º 0.15' N	0º 8.31' W	16.5	1.76	88.91	4.50	3.32	1.50	2.02
IX. Oliva	38º 56.92' N	0º 5.55' W	15.6	1.79	91.94	3.33	1.54	1.39	2.46
X. Javea	38º 46.83' N	0º 12.60' E	20.0	7.77	75.71	12.55	3.09	0.88	1.61

Order Amphipoda was shorted of samples for posterior identification. Amphipods were identified using the key of Mediterranean amphipod fauna established by Bellan-Santini *et al.* (1982, 1989, 1993, 1998), except for the genus *Bathyporeia*, which was identified using d'Udekem d'Acoz and Vader (2005). The taxonomy was validated using the ERMS referential for amphipod introduced by Bellan-Santini and Costello (2001) (http://www.marbef.org/data/erms.php, consulted on 25 May 2010). New records for the Mediterranean Spanish coast were checked using published available literature (figure 5.1).

Non-parametric multivariate techniques were used to compare species composition among locations. All multivariate analyses were performed using the PRIMER v. 6 statistical package (Clarke and Warwick, 1994). Triangular similarity matrices were calculated through the Bray–Curtis similarity coefficient using abundance values that were previously square root transformed. Locations were classified into groups according to the cluster analysis of Bray–Curtis similarity coefficients. Similarities percentage analyses (SIMPER) of abundances were used to determine the species with higher percentage contribution in dissimilarity between groups.

The BEST procedure was used to determine the parameter (granulometry, organic matter %, depth and latitude) most correlated with species composition changes between sampled stations. Spearman correlation between similarity matrices of samples based on the abundances of benthic community and parameters was determined. Canonical correspondence analysis (CCA) was used to identify the relationships among the spatial distribution patterns of amphipods and environmental gradients. The CCA was conducted using the software CANOCO. The output is displayed as biplot, in which the plotted points for stations can be related to environmental gradients that are represented as arrows. The strength of the correlation of an environmental variable is reflected in the length of the arrow, and its association is reflected in the acuteness of the angle with the axis. Thus, the relationships among stations and environmental variables can be displayed on one plot.

5.3.- Results and discussion.

<u>Taxonomic composition.</u>

A total of 55 species, belonging to 38 genera and 22 families, were identified. Among them, five species were first reported from the Mediterranean Spanish coast, 14 species were recorded for the second time and this study confirms their presence along the Spanish Mediterranean coast. A new species of *Medicorophium*, *M. longisetosum* sp. nov. (Myers *et al.*, 2010) was also described from this collection.

The species list is reported in table 5.2, indicating when each species was previously cited and the bottom type where it was found. Some details on the new and second species records are given.

Table 5.2. Amphipods species identified during present study. Asterisks indicate new records (*) and second records (**) for Spanish Mediterranean Coast. Bibliography and substratum where each species was found was indicated. References: 1. Carbonell (1984); 2. Biblioni (1983); 3. Marti (1989); 4. Jimeno (1993); 5. Cartes and Sorbe (1993, 1999), Cartes *et al.* (2003); 6. Ruffo and Krapp-Shickel, personal communication in Jimeno (1993) or Conradi and López-González (1999); 7. Conradi *et al.* (1995), Conradi and López-González (1999); 8. Sanchez-Jerez *et al.* (1999, 2000); 9. San Vicente and Sorbe (1999), Munilla and San Vicente (2005); 10. Ortiz and Jimeno (2003); 11. Cartes *et al.* (2007, 2009); 12. Sánchez-Moyano *et al.* (2007); 13. González *et al.* (2008); 14. Vázquez-Luis *et al.* (2008, 2009); 15. Delgado *et al.* (2009); 16. Guerra-García *et al.* (2009a, 2009b); 17. Izquierdo and Guerra-García (2010). Substratum: A. sands, M. mud or clayey bottom, D. detrital bottom, H. hard bottom, V. among algae or seaweeds, B. among *Bugula neritina*, C. coraligene.

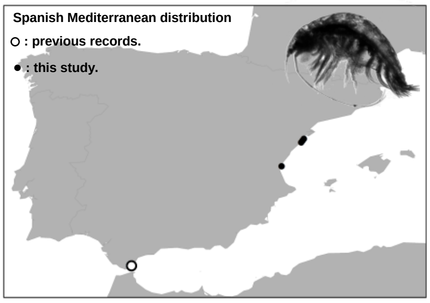
Families	**Species**	**Recorded by**	**Substratum**
Ampeliscidae	*Ampelisca brevicornis* (Costa, 1853)	3, 8, 14	A, M, D
	Ampelisca diadema (Costa, 1853)	7, 5, 14	A, D, V
	Ampelisca sarsi Chevreux, 1888 **	7	V, B
	Ampelisca spinifer Reid, 1951 *	This study	A
	Ampelisca tenuicornis Liljeborg, 1855 **	11	M
	Ampelisca typica (Bate, 1856)	7, 9	A, M, D
Amphilochidae	*Amphilochus brunneus* Della Valle, 1893	7, 11	A
Amphithoidae	*Ampithoe ramondi* Audouin, 1826	1, 4, 6, 7, 8, 9, 10,14, 17	A, V, D, C
Aoridae	*Aora spinicornis* Afonso, 1976	4, 8, 6, 12, 15	A, V
	Autonoe spiniventris Della Valle, 1893 **	14	A, V
	Microdeutopus versiculatus (Bate, 1856)	7, 12, 14	A, M, D, V
	Tethylembos viguieri (Chevreuz, 1911)	1, 7	A, M, D, V
Argissidae	*Argissa hamatipes* (Norman, 1869) *	This study	A
Atylidae	*Atylus guttatus* (Costa, 1851)	1, 4, 8, 14, 15	A, V
	Atylus massiliensis Bellan-Santini, 1975	3, 14	A, V
Bathyporeiidae	*Bathyporeia lindstromi* Stebbing, 1906*	This study	A
	Bathyporeia guilliamsoniana (Bate, 1857)	8, 7, 9, 14	A, V
	Bathyporeia borgi d'Udekem d'Acoz & Vader, 2005 *	This study	A
Caprellidae	*Pariambus typicus* (Krøyer, 1844)	3, 12, 13, 10	A, V
	Phtisica marina Slabber, 1769	1, 4, 6, 7, 8, 10, 13, 15, 16	A, V
Cheirocratidae	*Cheirocratus sundevalli* (Rathke, 1843) **	7	A, M, D, V
Corophiidae	*Leptocheirus hirsutimanus* (Bate, 1862) **	7	A, H
	*Leptocheirus pectinatus (*Norman, 1869)	7, 10	M, D, C
	Medicorophium longisetosum Myers, de-la-Ossa-Carretero & Dauvin, 2010	This study	A
	Medicorophium runcicorne (Della Valle, 1893) **	7	M, D
	Monocorophium sextonae (Crawford, 1937)	4, 6, 7, 15	A, M, V, H
	Siphonoecetes sabatieri de Rouville, 1894	3, 4, 14	A, V
Dexaminidae	*Dexamine spinosa* (Montagu, 1813)	1, 4, 9, 16, 14, 10	V, H
Eusiridae	*Apherusa alacris* Krapp-Schickel, 1969 **	7	V
Isaeidae	*Microprotopus maculatus* Norman, 1867	2, 3, 9, 14	A
Ischyroceridae	*Ericthonius punctatus* (Bate, 1857)	3, 4, 7, 8, 9, 15	A, V
Leucothoidae	*Leucothoe incisa* (Robertson, 1892)	2, 9	A
	Leucothoe oboa Karaman, 1971 **	7	A, D, H
Lysianassidae	*Hippomedon massiliensis* Bellan-Santini, 1965 **	7	A, M, D, B
	Lepidepecreum longicornis (Bate & Westwood, 1861) **	8	V
	Lysianassa costae (Milne-Edwards, 1830)	7, 14, 10	A, M, D, V, B
	Orchomenella nana (Kroyer, 1846)	5, 7	A
	Tryphosites longipes (Bate & Westwood, 1861)	7, 5, 11	A, M
Megaluropidae	*Megaluropus massiliensis* Ledoyer, 1976	7, 8, 9 , 14	A, M, V
Melitidae	*Maera grossimana* (Montagu, 1808)	1, 4, 7, 8	A, D, V
	Othomaera knudseni (Reid, 1951) **	5	V
	Elasmopus pocillimanus (Bate, 1862)	1, 4, 6, 10, 14,16, 17	V
Oedicerotidae	*Deflexilodes gibbosus (*Chevreux, 1888) **	5	M
	Perioculodes longimanus (Bate & Westwood, 1868)	3, 5, 7, 8, 9, 11	A, M, D, V
	Synchelidium haplocheles (Grube, 1864) **	9	A
	Synchelidium maculatum Stebbing, 1906	5	M
Photidae	*Gammaropsis maculata* (Johnston, 1828)	1, 4, 6, 7	A, M, D, V
	Megamphopus cornutus Norman, 1869	7	A, M, D, V, B
	Photis longicaudata (Bate & Westwood, 1862) *	This study	A
	Photis longipes (Della Valle, 1893)	3, 7	A, M, D, V
Phoxocephalidae	*Harpinia crenulata* (Boeck, 1871)	5, 11	M
	Harpinia pectinata Sars, 1891	3, 5	A
	Metaphoxus fultoni (Scott, 1890)	1, 7, 8	A, D, V
Urothoidae	*Urothoe pulchella* (Costa, 1853)	3, 7	A
	Urothoe elegans (Bate, 1857)**	5	M

Order AMPHIPODA Latreille, 1816

Suborder GAMMARIDEA Latreille, 1803

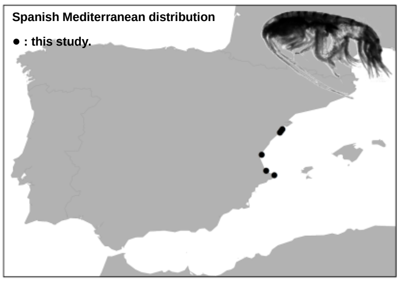
Family AMPELISCIDAE Costa, 1957

***Ampelisca sarsi* Chevreux, 1888**



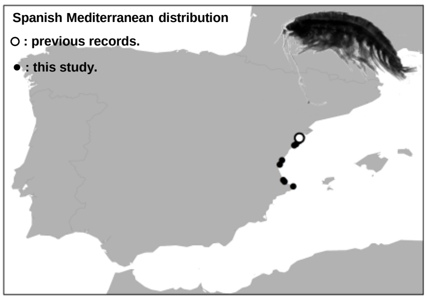
This species is common on fine-sand shallow water (10–108 m depth) of the eastern Atlantic Ocean from the western part of the Channel, where it can form abundant populations, to the African coast of Senegal, and in the Mediterranean Sea where it was present in the Marseilles Gulf and in the Adriatic Sea (Bellan-Santini *et al.*, 1982; Bellan-Santini and Dauvin, 1988). It was also present along the Algerian coast in the Bou Ismail and Algiers Bays and the Bejaia Gulf from 13 to 120 m on diverse types of sediment from mud to coarse sand (Bakalem and Dauvin, 1995). *Ampelisca sarsi* was also recorded at 383 m from the Portugal coast (Marques and Bellan Santini, 1993). From the Spanish Mediterranean coast, *A. sarsi* was previously reported as a first record by Conradi and López-Gonzalez (1999) among *Bugula neritina* and *Mesophillum* in Algeciras Bay (Andalucía coast). This species has been recorded from Vinaroz to Puebla de Farnals and always in low abundances. A total of 64 individuals have been collected from 12.4 to 15.6 m.

***Ampelisca spinifer* Reid, 1951**



It was observed in the eastern Atlantic Ocean from Ireland to the Liberia coast, and in the Mediterranean in the Marseilles Gulf, the Tyrrhenian Sea and the coast of Israel (Bellan-Santini *et al.*, 1982; Bellan-Santini and Dauvin, 1988). It was also recorded in Bou Ismail Bay in mud and muddygravel between 25 and 100 m depth (Bakalem and Dauvin, 1995). *Ampelisca spinifer* was not previously reported from the Spanish Mediterranean coast. A total of 37 specimens have been recorded in Vinaroz, Benicarlo, Peñíscola, Puebla de Farnals, Oliva and Javea, from 14 to 20 m in fine-sand bottom. Its ecology was previously established between 15 to 182 m depth on several types of sediment, from muddy to coarse sand but always in sediments with a large amount of mud (Bellan-Santini *et al.*, 1982; Bellan-Santini and Dauvin, 1988; Marques and Bellan Santini, 1993).

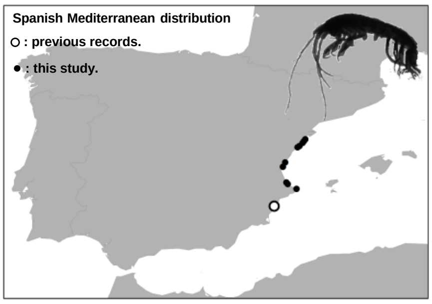
***Ampelisca tenuicornis* Liljeborg, 1855**



It was observed in the eastern Atlantic Ocean from northern Norway to the Senegal coast, from 0 to 510 m depth; where it can form abundant populations in shallow muddy fine sand community (Bellan-Santini and Dauvin, 1988). *Ampelisca tenuicornis* was previously reported from the Mediterranean Spanish coast at the continental shelf of Ebro Delta (western Mediterranean) by Cartes *et al.* (2007, 2009). We have found a total of 1084 individuals from all studied locations (from 12.4 to 20 m), obtaining the highest abundances (956 ind/m^2) in Gandia during the year 2004. This species was found in sandy and muddy bottom in shallow waters in the eastern and western part of the Mediterranean Sea including the Algerian coast (Bellan-Santini *et al.*, 1982; Bakalem and Dauvin, 1995).

Family AORIDAE Stebbing, 1899

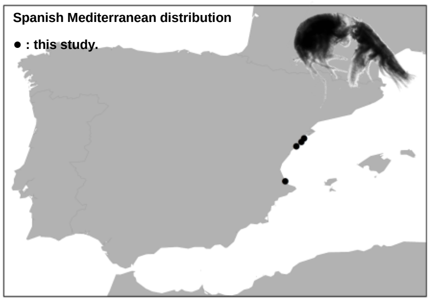
***Autonoe spiniventris* Della Valle, 1893**



Despite being previously established as Mediterranean endemic (Bellan-Santini *et al.*, 1982), *Autonoe spiniventris* has since been observed by Martínez and Adarraga (2001) on the Atlantic coast (San Sebastian, Spain). This species is common in the Bou Ismail and Algiers Bays between 15 and 100 m depth from sand to muddy coarse sand (Bakalem and Dauvin, 1995). It was reported from the Spanish Mediterranean coast among *Posidonia oceanica*, and *Cymodocea nodosa* from the Alicante coast (Vazquez-Luis *et al.*, 2009). Its depth-range was established (Bellan-Santini *et al.*, 1998) from 2 to 100 m in well-sorted sand bottoms and among photophilic algae. We have collected 5825 specimens from 12.4 to 20.0 m. It has been found in all locations, being especially abundant in northern locations (from Vinaroz to Torreblanca) and Canet d'en Berenguer, with a maximum density of 2125 ind/m^2 at Vinaroz in 2006.

Family ARGISSIDAE Walker, 1904

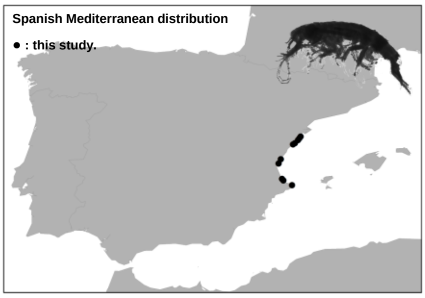
Argissa hamatipes **(Norman, 1869)**



This cosmopolitan species was previously recorded in the Atlantic Ocean, Indian Ocean and Japan Sea. In the Mediterranean Sea, it has been found from 11 to 370 m in various sediment types (Bellan-Santini *et al.*, 1982, 1998; Grimes *et al.*, 2009). From the Atlantic Iberian coast, *Argissa stebbingi* (now *A. hamatipes*) was previously reported in the Bay of Biscay (Bachelet *et al.*, 2003) and as the synonym *A. hamatipes* in Portugal (Marques and Bellan-Santini, 1993) and San Sebastian coast (Martínez and Adarraga, 2001). Eleven individuals of this first record for the Mediterranean Spanish coast have been found from 13.9 to 16.5 m water depth, in Vinaroz, Peñíscola, Torreblanca and Gandia.

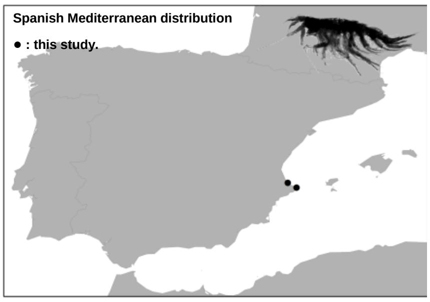
Family BATHYPOREIIDAE Bousfield & Shih, 1994

Bathyporeia borgi **d'Udekem d'Acoz & Vader, 2005**



It was described by d'Udekem d'Acoz and Vader (2005) from specimens of the Tyrrhenian and Adriatic Seas, reporting that *Bathyporeia nana* Toulmond, 1966 specimens recorded previously in the Mediterranean Sea are actually *B. borgi*. *Bathyporeia nana* was found in the western basin of the Mediterranean Sea (Bellan-Santini *et al.*, 1989) in sandy beaches from the intertidal zone to about 10 m depth. Recently, *B. borgi* was reported on muddy-sand at a depth of 10 m from the Algerian coast (Grimes *et al.*, 2009). We have found 338 specimens along all locations from 12.4 to 20 m.

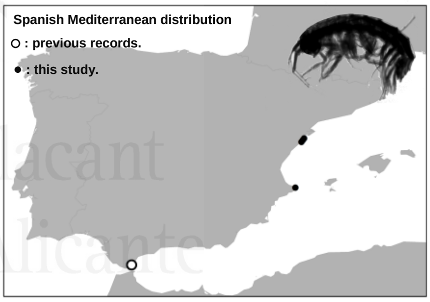
Bathyporeia lindstromi Stebbing, 1906



This endemic Mediterranean species was known on sandy bottoms, in 20 to 30 m from Italy (Tyrrhenian Sea), Sardinia, Malta and the Israeli coast (Bellan-Santini *et al.*, 1989, 1998; d'Udekem d'Acoz and Vader, 2005), and it has since been found on fine sand at a depth of 5 m from the Algerian coast (Grimes *et al.*, 2009). We have collected two individuals in Oliva and Javea at a water depth of 15.6 and 20 m.

Family CHEIROCRATIDAE Ren, 2006

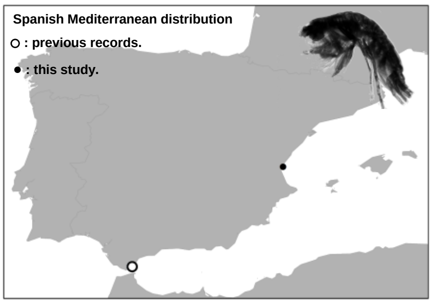
Cheirocratus sundevalli (Rathke, 1843)



This species was widely distributed in the Mediterranean Sea, the Atlantic Ocean, the Black Sea and the Arctic Ocean from 8 to 157 m, being characteristically on sandy bottoms with loose-lying algae (Bellan-Santini *et al.*, 1982, 1998; Marques and Bellan-Santini, 1993). It was also found on *Posidonia* meadows and various sediment types in the Bou Ismail Bay and Annaba Gulf from 8 to 90 m depth (Bakalem and Dauvin, 1995). On the Spanish Mediterranean coast, it was recorded for the first time by Conradi and López-Gonzalez (1999) in sand, mud and detrital bottoms as well as among *Halopteris scorparia* from the Andalusian coast in 5 to 30 m water depth. We have collected 28 individuals from Vinaroz, Benicarlo and Peñíscola in 14 to 15 m.

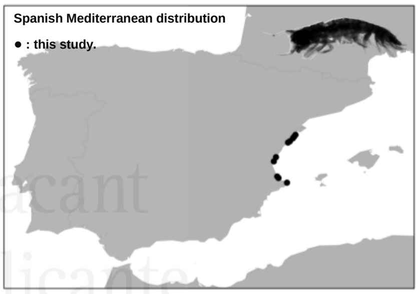
Family COROPHIIDAE Leach, 1814

***Leptocheirus hirsutimanus* (Bate, 1862)**



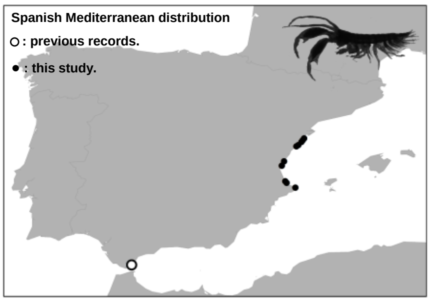
It was observed along the north-eastern Atlantic Ocean and Mediterranean Sea from 7 to 350 m (Bakalem and Dauvin, 1995; Bellan-Santini *et al.*, 1998). Its ecology was established as detritic and mud coastal bottoms and bathyal muds (Bellan-Santini *et al.*, 1982, 1998) as well as gravelly and coarse sand (Marques and Bellan-Santini, 1993). *Leptocheirus hirsutimanus* was first recorded from the Mediterranean Spanish coast by Conradi and López-Gonzalez (1999) in medium sand, biodetrital and hard bottoms from the Andalusian coast. We have found 26 individuals in Puebla de Farnals at 18.9 m water depth.

***Medicorophium longisetosum* Myers, de-la-Ossa-Carretero & Dauvin, 2010**



A total of 365 specimens were collected from all locations with a depth-range of 12.4 to 20 m. Numbers of specimens collected in the ten sampling sites varied from 211 individuals at Vinaroz to one specimen at Javea. The presence of this new species, which is habitual and relatively abundant, is the best example of the necessity for more taxonomic studies in this area. It was fully described in Myers *et al.* (2010).

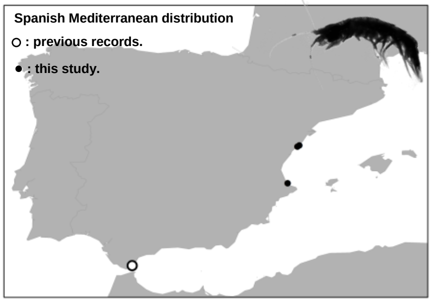
***Medicorophium runcicorne* (Della Valle, 1893)**



This species has only been reported from the Mediterranean and Black Seas in mud and mobile substrates and among algae from 15 to 105 m (Bellan-Santini *et al.*, 1982, 1998; Bakalem and Dauvin, 1995). *Medicorophium runcicorne* was recorded for the first time from the Spanish coast by Conradi and López-Gonzalez (1999) in biodetrital, mud and clayey bottoms from the Andalucia coast. A total of 1304 individuals have been collected. It has been reported in all locations from 12.4 to 20 m water depth, obtaining the highest abundances in the locations of Gandia and Oliva (325 ind/m^2).

Family EUSIRIDAE Stebbing, 1888

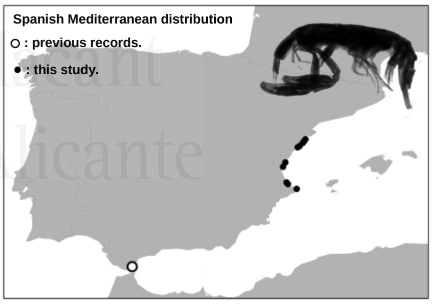
***Apherusa alacris* Krapp-Schickel, 1969**



This Mediterranean endemic species was established inhabiting among seagrasses (*Zostera*) and fine and coarse sand bottoms from 3 to 25 m (Bellan-Santini *et al.*, 1982, 1998). It was recorded for the first time in the Spanish Mediterranean coast by Conradi and López-Gonzalez (1999) among *Halopteris escorparia*. We have found 12 individuals in the locations of Alcossebre, Torreblanca and Oliva from 12.4 to 15.6 m in fine-sand bottoms.

Family LEUCOTHOIDAE Dana, 1852

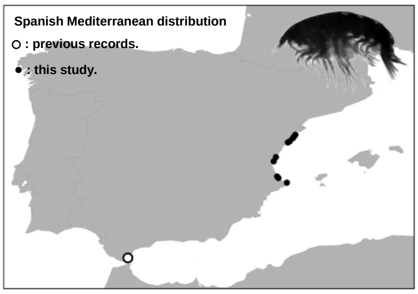
***Leucothoe oboa* Karaman, 1971**



This species was previously established as a Mediterranean endemic and its ecology was established in muddy bottoms from 14 to 400 m (Bellan-Santini *et al.*, 1989, 1998), however, it was also reported from the Portuguese coast (Marques and Bellan-Santini, 1993) on gravelly and coarse sand bottoms from 52 to 97 m water depth. It was recorded in the Oran Gulf at a depth of 80 m on sandy mud and in the Oran Harbour on mud with shells (Grimes *et al.*, 2009). *Leucothoe oboa* was first recorded from the Spanish Mediterranean coast by Conradi and López-Gonzalez (1999) in hard and sand biodetrital bottoms. We have found it in sandy bottoms with specimens of *Leucothoe incisa*. We have collected a total of 213 individuals from 12.4 to 20 m water depth. It has been found in all locations though always in low abundances (40 ind/m^2).

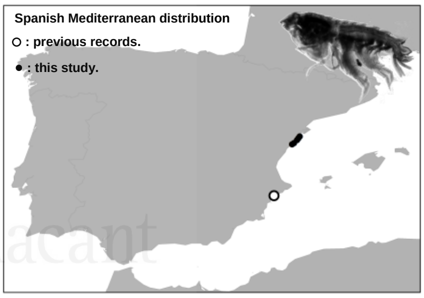
Family LYSIANASSIDAE Dana, 1849

***Hippomedon massiliensis* Bellan-Santini, 1965**



This Mediterranean endemic species was distributed on sandy and muddy bottoms from 4 to 350 m (Bellan-Santini *et al.*, 1989, 1998). It was also recorded from Bou Ismail Bay (10–88 m) from sand to muddy gravel (Bakalem and Dauvin, 1995). *Hippomedon massiliensis* was only recorded from the Spanish coast by Conradi and López-Gonzalez (1999) in sand, mud and detrital bottoms as well as among *Bugula neritina*. We have collected 228 individuals from 12.4 to 20 m depth, distributed in all locations but always in low densities (up to 40 ind/m^2).

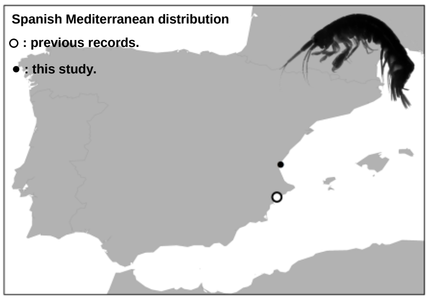
***Lepidepecreum longicornis* (Bate & Westwood, 1861)**



This species was observed in the north-eastern Atlantic Ocean and Mediterranean Sea at depths ranging from 0 to 360 m (Bellan-Santini *et al.*, 1989, 1998; Bakalem and Dauvin, 1995). Its ecology is established in gravels and sand bottoms as well as abyssal and bathyal muds. *Lepidepecreum longicornis* was observed on the Spanish Mediterranean coast among *Posidonia oceanica* meadows along the Alicante coast and the Iberian coast (Sanchez-Jerez *et al.* 2000) and it was also previously recorded in Portugal (Marques and Bellan-Santini, 1993). We have found 46 specimens in northern locations (Vinaroz to Alcossebre), from 12.5 to 15 m water depth.

Family MELITIDAE Bousfield, 1973

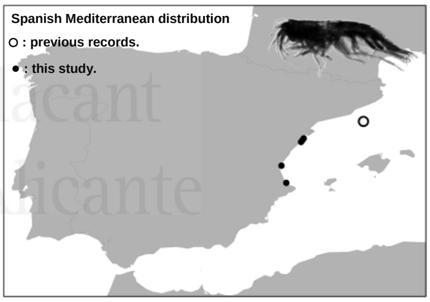
***Othomaera knudseni* (Reid, 1951)**



It is distributed in the Atlantic Ocean (West Africa) and the Mediterranean Sea (Bellan-Santini *et al.*, 1982). It was common in Bou Ismail Bay on mud and sandy-muddy gravel between 45 to 100 m depth (Bakalem and Dauvin, 1995). Its ecology was established in mud, muddy sands, coarse sands, fine gravel and detritic bottoms with a depth range from 10 to 68 m (Bellan-Santini *et al.*, 1982, 1998). *Othomaera knudseni* was previously observed in *Posidonia oceanica* meadows along the Alicante coast (Sanchez-Jerez *et al.*, 2000). We have found 18 individuals only in Puebla de Farnals at 18.9 m water depth.

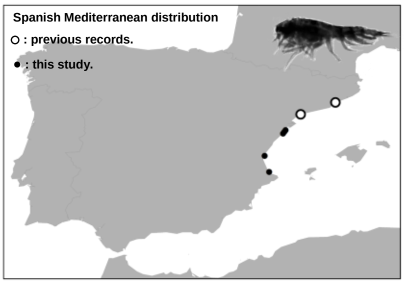
Family OEDICEROTIDAE Lilljeborg, 1865

***Deflexilodes gibbosus* (Chevreux, 1888)**



This species was previously observed in the north-eastern Atlantic Ocean and Mediterranean Sea in soft bottoms from 10 to 360 m water depth (Bellan-Santini *et al.*, 1993, 1998). It was reported in the Bejaia Gulf and Bou Ismail Bay on various sediment types ranging from mud to coarse sand and at depths from 24 to 86 m (Grimes *et al.*, 2009). *Monoculodes gibbosus*, now *Deflexilodes gibbosus*, was previously reported on the muddy bottoms of the deep slope in front of Barcelona (western Mediterranean) from 593 to 598 m water depth (Cartes and Sorbe, 1999). We have found 14 individuals from Vinaroz, Benicarlo, Peñíscola, Puebla de Farnals and Oliva in 14 to 15.6 m.

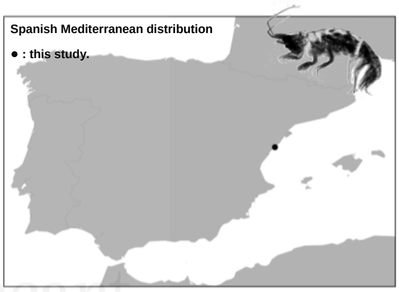
***Synchelidium haplocheles* (Grube, 1864)**



It is known from the Mediterranean Sea, Atlantic Ocean and Indo-Pacific Ocean (Bellan-Santini *et al.*, 1993, 1998; Bakalem and Dauvin, 1995), living from 3 to 100 m water depth on well sorted sand and coastal terrigenous mud bottoms. *Synchelidium haplocheles* was previously recorded on beaches from the Catalonian coast (Munilla and San Vicente, 2005). We have found 90 specimens at depths from 12.4 to 15.6 m in northern locations, Puebla de Farnals and Gandia.

Family PHOTIDAE Boeck, 1871

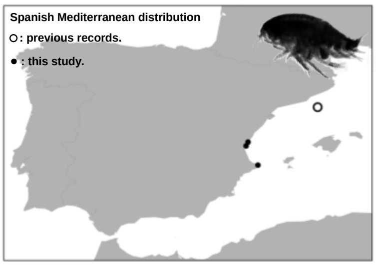
***Photis longicaudata* (Bate & Westwood, 1862)**



This cosmopolitan species was observed in the Atlantic Ocean (from Norway to West Africa), the Indian Ocean and the Mediterranean Sea. Its ecology was established from infralittoral, among algae and *Posidonia* meadows, to bathyal (400 m), among mud and detritic bottoms (Bellan-Santini *et al.*, 1989, 1998; Bakalem and Dauvin, 1995). Although it was previously recorded from the Atlantic Iberian coast (Marques and Bellan-Santini, 1993; Matínez and Adarraga, 2001), *Photis longicaudata* was not previously reported from the Spanish Mediterranean coast. Two specimens have been collected in Torreblanca at a depth of 13.9 m.

Family UROTHOIDAE Bousfield, 1979

Urothoe elegans **(Bate, 1857)**



This species was observed in the Atlantic Ocean, Indian Ocean and Mediterranean Sea (western, Tyrrhenian, Adriatic, Israel and North Africa) associated with fine sediments distributed from 2 to 644 m (Bellan-Santini *et al.*, 1989, 1998; Bakalem and Dauvin, 1995). *Urothoe elegans* was previously reported on the muddy bottoms of the deep slope in front of Barcelona (western Mediterranean) (Cartes and Sorbe, 1993, 1999). We have found 143 individuals, in locations at Canet d'en Berenguer, Puebla de Farnals and Javea, from 15.6 to 20 m water depth.

<u>Species distribution among locations.</u>

Cluster analyses based on species composition showed a segregation of stations according to the north–south axis (figure 5.2). In this way, locations from north of the studied area (Groups A and B) obtained similarities among them higher than 60% and location X (Group F), which is sited south of studied area, showed more dissimilarity than other locations.

Changes among these locations were due to a decrease in abundance of species such as *Autonoe spiniventris*, *Perioculodes longimanus* or *Siphonoecetes sabatieri* from north to south. Whereas, other species abundance increased in locations VIII and IX, such as *Ampelisca typica*, *Ampelisca tenuicornis* or *Medicorophium runcicorne*, or from locations VI to IX *Urothoe pulchella*, or from locations VII to IX *Photis longipes* (table 5.3).

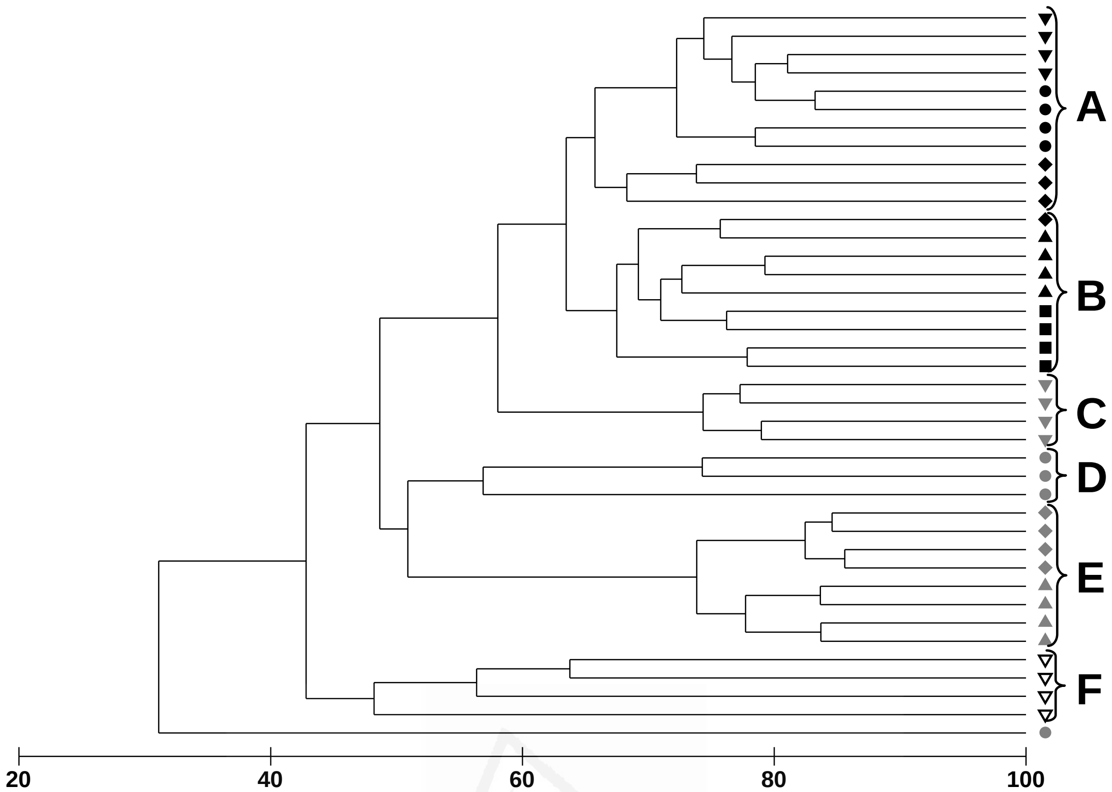


Figure 5.2. CLUSTER analysis based on amphipod assemblage of stations (indiv./m^2), indicating each location (▼: I, ●: II, ♦: III, ▲: IV, ■: V, ▼: VI, ●:VII, ♦: VIII, ▲: IX, ▽: X) and groups established (A, B, C, D, E and F).

Table 5.3. Mean abundance of species more contributing in dissimilarities among groups established in Cluster analysis.

	Average abundante (individual m^{-2})					
Species	**Group A**	**Group B**	**Group C**	**Group D**	**Group E**	**Group F**
Ampelisca tenuicornis	20.45	10.19	41.67	52.78	635.42	22.92
Ampelisca typica	140.15	54.63	143.75	194.44	277.08	35.42
Autonoe spiniventris	778.79	535.19	560.42	72.22	52.08	25
Medicorophium runcicorne	100.76	47.22	47.92	16.67	337.5	2.08
Perioculodes longimanus	220.45	187.04	89.58	16.67	63.54	16.67
Photis longipes	96.21	27.78	95.83	438.89	382.29	85.42
Siphonoecetes sabatieri	180.3	91.67	0	22.22	8.33	39.58
Urothoe pulchella	25.76	25.93	137.5	258.33	121.88	87.5

The influence of geographical situations of each location in amphipod assemblage was reflected in BEST and CCA results. Latitude together with depth obtained the highest Spearman correlation (Rho: 0.609 and 0.606) with changes in species composition. The CCA showed that both parameters were related since the southern locations are slightly deeper than northern ones (figure 5.3; table 5.1). Both latitude and depth highly influence benthic communities and were related to other abiotic parameters such as hydrodynamic conditions, temperature, dissolved oxygen, granulometry and organic content. For instance, temperature, influenced by latitude and depth, affect most biological processes and act on growth rates, maturation or reproduction cycle. Despite the fact that species contributing to differences among locations displayed a wide bathymetric distribution, some of them could be less adapted to hydrodynamic processes than others (Bellan-Santini *et al.*, 1998). The fine sand communities, such as the study area, are always influenced by high hydrodynamism which can remove the surface layer (Cardell *et al.*, 1999), in such a way that local changes could change species abundances according to their capacity to shelter.

Other parameters such as mud percentage or organic matter showed a weak correlation (Rho: 0.291 and 0.208), whereas percentages of other granulometric sizes showed the lowest correlations (Rho< 0.2) with amphipod species composition. Changes in these parameters were related to species differences among close locations. Despite the fact that sediments in the area were homogeneous and low changes in granulometry among locations were registered, grain size and organic content are factors that may be related to food availability and the ability to burrow (Oakden, 1984; Marques and Bellan-Santini, 1993); as a result light changes in sediment from close areas could produce changes in species composition.

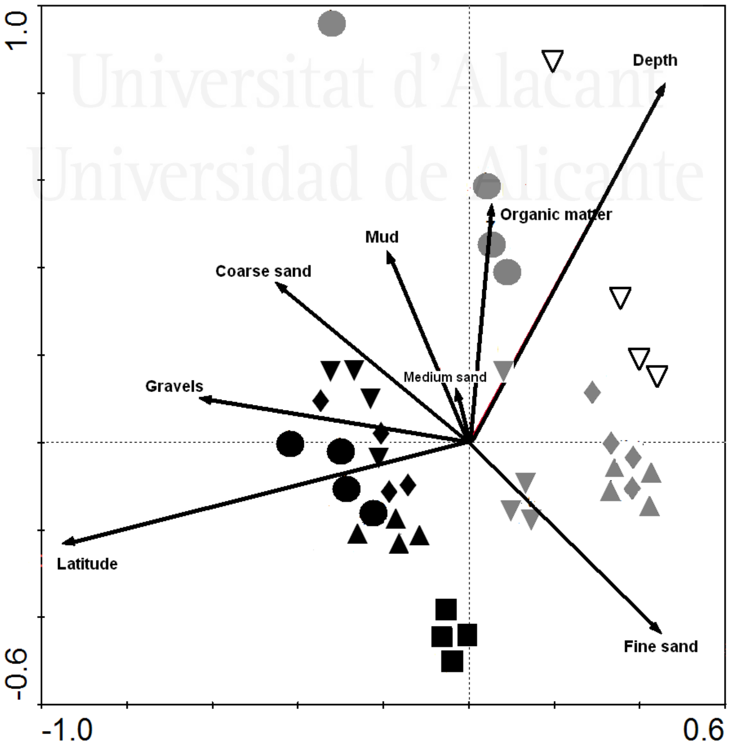


Figure 5.3. Results of canonical correspondence analysis (CCA) biplots. Points correspond to samples from locations: ▼: I, ●: II, ♦: III, ▲: IV, ■: V, ▼: VI, ●: VII, ♦: VIII, ▲: IX, ▽: X. Arrows indicate environmental variables. CCA axis I and CCA axis II had eigenvalues of 0.184 and 0.142, respectively.

Bigeographical considerations.

The present study recorded a total of 55 total species, but for a limited length of Spanish coast (250 km) and only for sandy bottoms. Among these species, 30 have a north-eastern Atlantic distribution and 17 have a wide distribution in Atlantic and Indo-Pacific or Arctic Oceans (Bakalem and Dauvin, 1995; Bellan-Santini *et al.*, 1998; Grimes *et al.*, 2009). Meanwhile, only eight species were considered Mediterranean endemics. Among these endemics, six are reported as first or second records on the Spanish Mediterranean coast; and among the north-eastern Atlantic species *Autonoe spiniventris* and *Leucothoe oboa* are known only along the Iberian coast (Marques and Bellan-Santini, 1993; Martínez and Adarraga, 2001). Among the 20 species recorded for the first or second time on the Mediterranean coast, only two species *Apherusa alacris* and the new species *Medicorophium longisetosum* were absent along the Algerian coast (Bakalem and Dauvin, 1995; Grimes *et al.*, 2009), and four species, namely the two same species for Algeria, plus the two *Bathyporeia* species, *B. borgi* and *B. lindstromi*, were absent from the French Mediterranean coast. This shows a good resemblance of the continental shelf amphipod fauna at the scale of the western part of the Mediterranean Sea. A total of six species (10% of the recorded species) was new for the Spanish coast, and among them a new species was described (Myers *et al.*, 2010). This proves the need to significantly increase the prospection of the amphipods from the Mediterranean Spanish coast. To give some comparative numbers, the French Mediterranean continental shelf accounts for 250 Gammaridea (Dauvin and Bellan-Santini, 2002). Nevertheless records depended on the sampling efforts among the regions, respectively 240 in the ‘Provence-Alpes Côte d’Azur’ region which included the Marseilles Gulf where numerous studies were made mainly through the expertise of Denise Bellan-Santini, 100 for the ‘Languedoc–Roussillon’ region at the frontiers with Spain, and only 78 species around Corsica (Dauvin and Bellan-Santini, 2002). Recent works on Algerian amphipods, including three orders, i.e. Caprellidea, Gammaridea and Hyperiidea (Bakalem and Dauvin, 1995; Grimes *et al.*, 2009), revealed that the fauna accounts for 298 species of the 451 species recorded in the mid-1990s for the whole Mediterranean fauna. Using these records, we expected that the total amphipod marine fauna (Caprellidea, Gammaridea and Hyperiidea) of the Mediterranean coast of Spain should include between 250 and 300 species. A first inventory of all species collected in this area should be completed from published works particularly those of J.M. Guerra-García on caprellids (Guerra-García *et al.*, 2000, 2001a,b, 2002, 2009a,b). Moreover, the higher percentage of endemics among first or second records indicates the need for more sampling studies in order to increase distribution knowledge of Amphipoda species from the Spanish Mediterranean coast as a requisite to facilitate amphipods employment in monitoring studies.

CHAPTER 6

Sensitivity of Order Amphipoda to sewage pollution

Sensibilidad del Orden Amphipoda al vertido de aguas residuales

Abstract. Order Amphipoda is an abundant and ecologically important component of benthic habitats. Benthic amphipods live in direct contact with sediment and are highly sensitive to polluted sediments, although certain species can be more tolerant. Moreover, crustacean amphipods are widely used for bioassay, and numerous species are usually employed in ecotoxicology tests for diverse contaminants. In this chapter, we examined the impact of sewage outfalls on amphipod assemblages from the Castellon coast (NE Spain). Sewage pollution produced a decrease in the abundance and richness of amphipods in the proximity of the outfalls. Therefore, most of the species showed high sensitivity, particularly species such as *Bathyporeia borgi*, *Perioculodes longimanus* and *Autonoe spiniventris*, whereas other species appeared to be more tolerant to the sewage input, such as *Ampelisca brevicornis*. These different responses could be related to burrowing behaviour, with fossorial species being more sensitive and domicolous species being less affected.

Resumen. El orden Amphipoda es un componente abundante y ecológicamente importante del bentos. Los anfípodos bentónicos viven en contacto directo con el sedimento y son muy sensibles a sedimentos contaminados, aunque ciertas especies pueden ser más tolerantes. Además, este grupo es ampliamente empleado en bioensayos, y un gran número de especies son utilizadas en ecotoxicología de distintos contaminantes. En este capítulo, se estudia el impacto de los vertidos de aguas residuales en los poblamientos de anfípodos de la costa de Castellón. La presencia de estos vertidos provoca un descenso en la abundancia y diversidad. La mayor parte de las especies muestran una alta sensibilidad, particularmente especies como *Bathyporeia borgi*, *Perioculodes longimanus* y *Autonoe spiniventris*, mientras que otras son más tolerantes a estos vertidos, como es el caso de *Ampelisca brevicornis*. Estas diferencias en la respuesta podrían estar relacionadas con el tipo de enterramiento, siendo la especies fosoriales más sensibles que las tubícolas.

6.1.- Introduction.

Order Amphipoda is an abundant and ecologically important component of soft-bottom marine benthic communities (Thomas, 1993). This high abundance and wide distribution suggest that amphipods could often play major roles in the ecology of these habitats (Conlan, 1994). Benthic amphipods meet several criteria that render them highly recommendable for inclusion in marine monitoring programmes and in sediment ecotoxicology tests. They are ecologically and trophically important, numerically dominant, exhibit a high degree of niche specificity, are tolerant to varying physico-chemical characteristics in sediment and water, have relatively low dispersion and mobility capabilities, live in direct contact with the sediment, have a documented sensitivity to pollutants and toxicants compared to other benthic organisms and indeed they have been considered capable of accumulating toxic substances (Reish, 1993; Thomas, 1993; Gomez Geistera and Dauvin, 2000; Dauvin and Ruellet, 2009).

Despite it being established that most amphipods are sensitive to different kinds of pollutions, see chapter 2 (Dauvin, 1987, 1998; Gomez Geistera and Dauvin, 2000; Dauvin and Ruellet, 2007), sensitivity to pollution for the same taxonomic group may differ from one species to another (Afli *et al.*, 2008). In this way, Reish and Barnard (1979) observed that some amphipod species are more tolerant than others to organic pollution, and Bellan-Santini (1980) observed changes in compositions of amphipods inhabiting rocky environments related to the degree of pollution. Nowadays, there are more than 6300 species of gammaridean amphipods (Gruner, 1993) and little is known about the ecology of most of the species. Conlan (1994) has reviewed the role of amphipods in environment disturbance and could only compile biological information for less than 3% of all the described species. The wide distribution in both marine and fresh waters, together with the high abundance in both benthic and pelagic environments, means that there is a need for knowledge regarding the specific sensitivity of different species or at least of more abundant species. The application and use of amphipods as a biological indicator is limited by a comprehensive taxonomic and natural history knowledge (Thomas, 1993) that explained the degree of sensitivity of each species.

Moreover, ecological factors must also be considered when evaluating the potential information value of various amphipod groups. The sensitivity of a benthic species is dependent on the organism's living habits, such as burrowing behaviour and feeding strategy (Simpson and King, 2005; King *et al.*, 2006). Tube-builder amphipods may exhibit different habitat requirements and dispersion capabilities than burrowers (Thomas, 1993) and different trophic groups may be influenced by different routes of exposure to contaminants. These attributes must be taken into account when considering amphipods for monitoring and biodiversity programmes.

Shallow soft-bottom non-vegetated areas of the western Mediterranean Sea are commonly inhabited by the medium-to-fine sand community of *Spisula subtruncata* (Cardell *et al.*, 1999). This community colonises exposed or semi-exposed sublittoral habitats, from the beach environment to depth of 30 metres (Sardá *et al.*, 1999). Although this community generally contains low numbers of individuals and low biomass values, a high abundance and diversity of amphipods has been reported (Bakalem *et al.*, 2009). In this study, we examined the impact of the five sewage outfall sites, with different flows and wastewater treatment processes, on amphipod populations. The main objective of this chapter is to test the effect of these outfalls on amphipod populations in order to characterise the sensitivity of different species and the relation with burrowing and feeding behaviour.

6.2.- Material and methods.

In this chapter, we analysed the amphipod assemblage of the five locations affected by sewage outfalls from Castellon coast (NE Spain) (see chapter 4). This area represents an ideal site for investigating links between macrofaunal assemblages and the effect of contaminants (de-la-Ossa-Carretero *et al.*, 2008, 2009, 2010b; Del-Pilar-Ruso *et al.*, 2010). The study area, sewage outfalls and sampling methods were previously described in chapters 2 and 4. Amphipods were identified as in chapter 5.

An analysis of variance (ANOVA), with location and distance as fixed factors and year as a random factor, was used in order to test differences in abundance, Shannon-Wiener diversity index and abundance of key species. Prior to ANOVA, the homogeneity of variance was tested using Cochran's test. Data were Ln (X+1) transformed when variances were significantly different. The SNK test (Student-Newman-Keuls) was used to determine which samples were involved in the differences.

Non-parametric multivariate techniques were used to compare the composition of species and to determine key species that are mainly affected by sewage presence. All multivariate analyses were performed using the PRIMER version 6 statistical package (Clarke and Warwick, 1994). Triangular similarity matrices were calculated through the Bray-Curtis similarity coefficient using mean annual abundance values, in order to cluster stations according to sewage effect, regardless of temporal variability. The values were previously dispersion weighted in order to reduce “noise” produced by species with an erratic distribution, and whose abundance indicates a great variance between replicates (Clarke et al., 2006). A graphical representation of multivariate patterns of amphipod assemblages was obtained by non-metric multidimensional scaling (nMDS). Similarity percentage analysis (SIMPER) of abundances was used to determine the species with a higher percentage of contribution in dissimilarity between stations.

Meta-analysis was applied in order to assess the sensitivity levels of species indicated by the SIMPER analysis. Meta-analysis is a set of methods designed to synthesise the results of disparate studies (Hedges and Olkin 1985), in this case different locations and sampling years. To carry out a meta-analysis of studies with continuous measures, such as amphipod abundance, a standardised difference between treatments is typically used (Cooper and Hedges, 1994). We used Hedges’ g statistic (Hedges and Olkin, 1985) as a measure of effect size (standardised differences in mean of sediment parameters or species’ abundance between outfall sites and sites at 1000 m from the outfall).

Species were classified according to trophic groups and behaviours. Using the classification suggested by Mearns and Word (1982), who simplify it into four main trophic groups (TG): i) TG-1 (suspension feeders), ii) TG-2 (carrion feeders), iii) TG-3 (surface deposit feeders and those species that are both suspension feeders and surface deposit feeders) and iv) TG-4 (subsurface deposit feeders that feed on sedimentary detritus and bacteria).

Regarding behaviour, species were classified into three types: domicolous (species that build tubes), fossorial (species that burrow using their periopods) and interstitial (species that live in the interstices between grains of sand). The classification for each species was obtained from the current bibliography (table 6.1). Analysis of variance (ANOVA) was used in order to test differences in abundance percentages of groups from both classifications. The appropriate transform for the analysis of this kind of data is the arc-sin of the square-root of the proportion.

6.3.- Results.

A total of 17643 specimens were collected and identified as 44 species, belonging to 38 genera and 22 families. Among these, *Autonoe spiniventris* was the most abundant species which contributed to 30.3% of total abundance, followed by *Perioculodes longimanus* (11.2%) and *Siphonoecetes sabatieri* (10.8%). Other species contributed less than 0.1%, such as *Photis longicaudata*, *Elasmopus pocillamus* or *Harpinia crenulata*; or indeed only one specimen was collected, *Ampithoe ramondi*, *Microdeutopus versiculatus* or *Dexamine spinosa*. New records from the Mediterranean Spanish coast and a new species of *Medicorophium*, *M. longisetosum* sp. nov. (Myers *et al.*, 2010) were also described from this collection (de-la-Ossa-Carretero *et al.*, 2010a).

The highest population density detected was 2833 individuals/m^2, in a station situated at 1000 m from the outfall of location I, whereas the lowest abundance was obtained in a station situated at the outfall of location III (47 individuals/m^2). The abundance of amphipods showed a decrease at 0 m from outfall stations of locations I, II, III and IV (figure 6.1). Significant differences were detected in the interaction between the three factors due to the fact that this decrease was not detected in 2007 in any location or in 2005 in location I (table 6.2). The Shannon-Wienner diversity index reached values of 1.11 to 2.66. As well as abundance, the diversity index showed a decrease in outfalls (figure 6.1), with significant differences in the interaction of the three factors. Differences in distances were detected in locations I, II, III and IV for certain years. In this way, despite annual variability, an effect of sewage presence was detected in locations I, II, III and IV.

Table 6.1. Amphipoda species collected. N. number of specimens collected. Feeding. Classification in feeding behaviour: D. deposit feeders, F. filter feeders, O. omnivorous, P. predators, S. scavengers, G. grazer); TG. trophic group assigned according to Mearns and Word (1982): TG-1 (suspension feeders), TG-2 (carrion feeders), TG-3 (species that are both suspension feeders and surface deposit feeders) and TG-4 (subsurface deposit feeders); BEHAUVIOUR. Burrowing behaviour: dom. domicolous, fos. fossorial and int. interstitial. Bibliography where classification was found was indicated. References: 1. MarBEF Data System ERMS, Bellan-Santini and Costello (2001), 2. Crawford (1937), 3. Enequist (1949), 4. Krapp-Schickel and Krapp (1975), 5. Lincoln (1979), 6. Word (1980), 7. Wildish and Peer (1981), 8. Grosse *et al*. (1986), 9. Bellan-Santini and Dauvin (1988), 10. Eleftheriou and Basford (1989), 11. Marti (1989), 12. Jimeno (1993), 13. Beare and Moore (1994), 14. Bellan-Santini *et al.* (1998), 16. Grandi *et al.* (2007) and P. Guerra-Garcia and Tierno de Figueroa (2009). EG. Ecological Group of AMBI (Borja *et al*, 2000, http://www.ambi.azti.es) and BENTIX (Simboura and Zenetos, 2002; http://www.hcmr.gr).

Families	**Species**	**N**	**FEEDING**	**TG**	**BEHAVIOUR**	**REFERENCES**	**EG** AMBI/BENTIX
Ampeliscidae	*Ampelisca brevicornis*	269	D, F	3	Dom.	1, 9, 5, 3, 14	I/II
	Ampelisca diadema	301	D, F	3	Dom.	1, 9, 5, 15, 14	II/II
	Ampelisca sarsi	56	D, F	3	Dom.	1, 9, 5, 3, 14 (as *Ampelisca* sp.)	I/II
	Ampelisca spinifer	25	D, F	3	Dom.	1, 9, 5, 3, 14 (as *Ampelisca* sp.)	I/-
	Ampelisca tenuicornis	208	D, F	3	Dom.	1, 9, 14	I/I
	Ampelisca typica	1215	D, F	3	Dom.	1, 9, 5, 3, 14	I/I
Amphilochidae	*Amphilochus brunneus*	23	D	4	Int	1, 12 (as *Amphilochus* sp.)	II/-
Amphithoidae	*Ampithoe ramondi*	1	D, G	4	Dom.	12, 4, 14	III/II
Aoridae	*Aora spinicornis*	503	D	4	Dom.	12, 14	I/I
	Autonoe spiniventris	5343	D, F	3	Dom.	1, 14 (as Aoridae)	I/I
	Microdeutopus versiculatus	1	D, F	4	Dom.	12, 14 (as Aoridae)	I/-
Argissidae	*Argissa hamatipes*	10	D	4	Int	1, 7	-/I
Atylidae	*Atylus massiliensis*	20	P, S	2	Int	1, 12 (as *Atylus* sp.)	I/II
Bathyporeiidae	*Bathyporeia borgi*	321	D, F	3	Fos	14, 9 (as O. *nana*)	I/I
	Bathyporeia guilliamsoniana	250	D	4	Fos	14, 9	I/I
Caprellidae	*Pariambus typicus*	991	D	4	Int	16, 2	III/-
	Phtisica marina	295	D	4	Int	16 , 2	I/II
Cheirocratidae	*Cheirocratus sundevalli*	28	D	4	Int	1, 3, 2	I/-
Corophiidae	*Medicorophium longisetosum*	316	D, F	3	Dom.	1, 14, 11, 2, 8 (as *Corophium* sp.)	-/I
	Medicorophium runcicorne	825	D, F	3	Dom.	1, 14, 11, 2, 8 (as *Corophium* sp.)	III/I
	Siphonoecetes sabatieri	1904	D, F	3	Dom.	1, 11	I/I
Dexaminidae	*Dexamine spinosa*	1	D	4	Int	1, 12	III/I
Eusiridae	*Apherusa alacris*	11	D, F	3	Int	9	I/I
Isaeidae	*Microprotopus maculatus*	57	O, P, S	2	Dom.	1, 2	I/I
Ischyroceridae	*Ericthonius punctatus*	30	F	1	Dom.	1, 11	I/I
Leucothoidae	*Leucothoe incisa*	143	D	4	Fos	2, 12 (as *Leucothoe* sp.)	I/I
	Leucothoe oboa	150	D	4	Fos	2, 12 (as *Leucothoe* sp.)	I/II
Lysianassidae	*Hippomedon massiliensis*	146	D	4	Int	1, 3, 12 (as Lysianasidae)	I/II
	Lepidepecreum longicornis	46	O, P, S	2	Int	1, 3, 12 (as Lysianasidae)	-/-
	Orchomenella nana	99	O, P, S	2	Int	1, 12 (as *Orchemene* sp.)	II/-
	Tryphosites longipes	6	D	4	Int	1, 3, 5, 12 (as Lysianassidae)	I/I
Megaluropidae	*Megaluropus massiliensis*	111	F	1	Fos	1, 2 (as *M. agilis*)	I/-
Melitidae	*Elasmopus pocillamus*	3	D	4	Int	12, 11	-/-
Oedicerotidae	*Deflexilodes gibbosus*	12	P	2	Fos	1, 13, 8	I/I
	Perioculodes longimanus	1981	D	4	Fos	8, 1, 3, 11	II/I
	Synchelidium haplocheles	84	D,F, G	3	Fos	1, 3, 8	I/II
Photidae	*Gammaropsis maculata*	16	D,F	3	Dom.	1, 8 (as *Photis* sp.)	I/I
	Megamphopus cornutus	217	D,F	3	Dom.	1, 8 (as *Photis* sp.)	I/-
	Photis longicaudata	2	D,F	3	Dom.	1, 8, 2, 6	I/I
	Photis longipes	894	D,F	3	Dom.	8, 1, 6	I/II
Phoxocephalidae	*Harpinia crenulata*	6	D	4	Fos.	1, 3, 8	I/I
	Harpinia pectinata	20	D	4	Fos.	1, 3, 8	I/I
	Metaphoxus fultoni	67	F	1	Fos	1, 2, 8	I/-
Urothoidae	*Urothoe pulchella*	636	D	4	Fos	14, 1, 11, 10	I/II

Table 6.2. Results of ANOVA for abundance (individuals/m^2) and Shannon-Wiener diversity index for the factors distance (0, 200, 1000 m), location (I, II, III, IV and V) and year (2004, 2005, 2006, 2007, 2008) RES = Residual, df: degrees of freedom, F of each factor = mean square factor/mean square residual because all the factors are orthogonal. Levels of significance: ns no significant difference, *p < 0.05, **p < 0.01 and ***p < 0.001.

		Abundance		Shannon-Wiener Diversity	
Source	**DF**	**F**	**P**	**F**	**P**
Distance	2	13.20	***	7.11	*
Location	4	8.77	***	0.25	ns
Year	4	74.55	***	16.88	***
Dis.xLoc.	8	3.86	***	1.11	ns
Dis.xYear	8	5.26	***	5.42	***
Loc.x Year	16	3.87	***	2.77	***
Dis.x Loc.x Year	32	1.81	**	3.37	***
RES	375				
TOT	449				

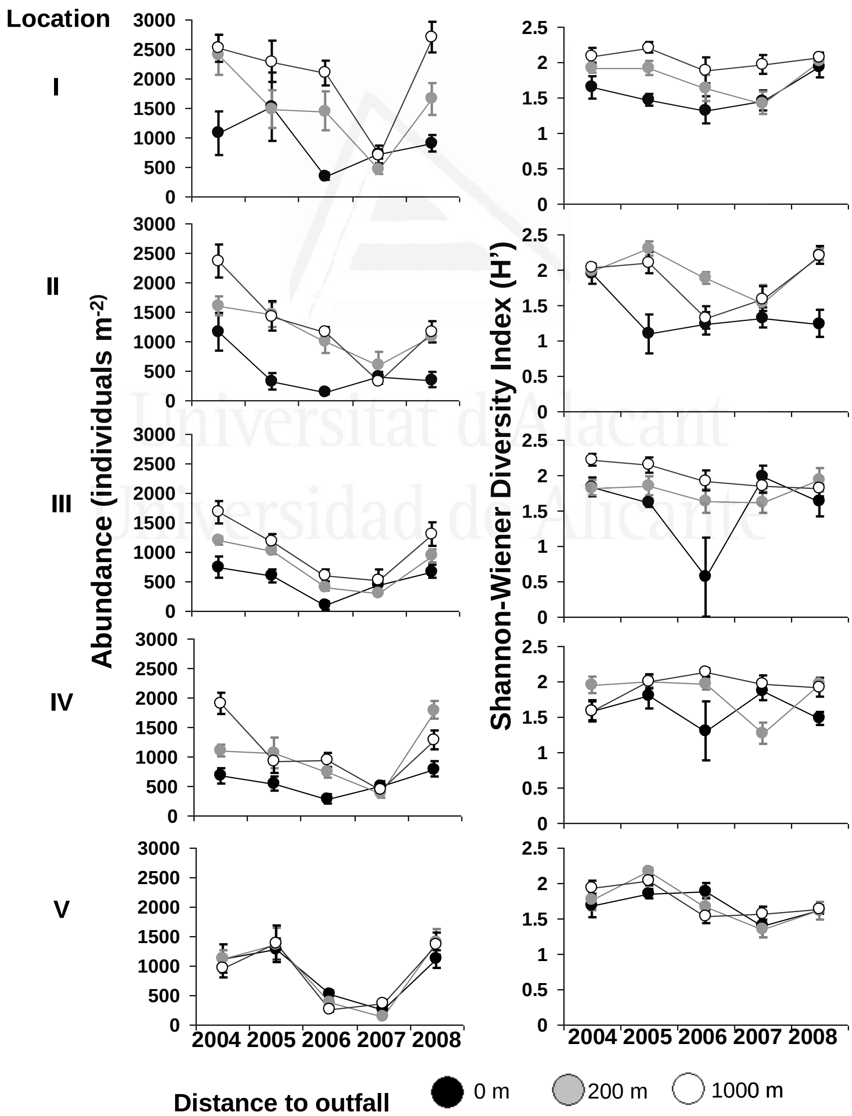


Figure 6.1. Mean and standard error of amphipod abundances and Shannon-Wiener Diversity Index at each location (I–V), year (2004-2008) and distance to the outfall (0, 200 and 1000 m).

nMDS plots of the mean annual abundances of amphipod species showed a segregation of the stations, from the sites closest to the outfalls on the right hand side to those furthest away on the left hand side (figure 6.2), except for those closest to the location V outfall which is nearest to stations situated at 200 m and 1000 m (Group C). Meanwhile, a gradient corresponding to a latitudinal pattern, from location I to location V, could be observed rising from the bottom to the top of the nMDS. Based on similarities in different stations, seven groups were established (figure 6.2). Among these groups, E, F and G corresponded with stations sited in disposals, whereas group B included the majority of stations sited at 200 and 1000 m from the outfalls. The SIMPER routine indicated that contribution to the average Bray-Curtis dissimilarity between groups of outfall stations and group B were mainly due to the following species: *Autonoe spiniventris*, *Perioculodes longimanus*, *Photis longipes*, *Bathyporeia borgi*, *Siphonoecetes sabatieri*, *Medicorophium runcicorne*, *Urothoe pulchella*, *Phtisica marina*, *Ampelisca typica*, *Aora spinicornis*, *Pariambus typicus* and *Ampelisca brevicornis*.

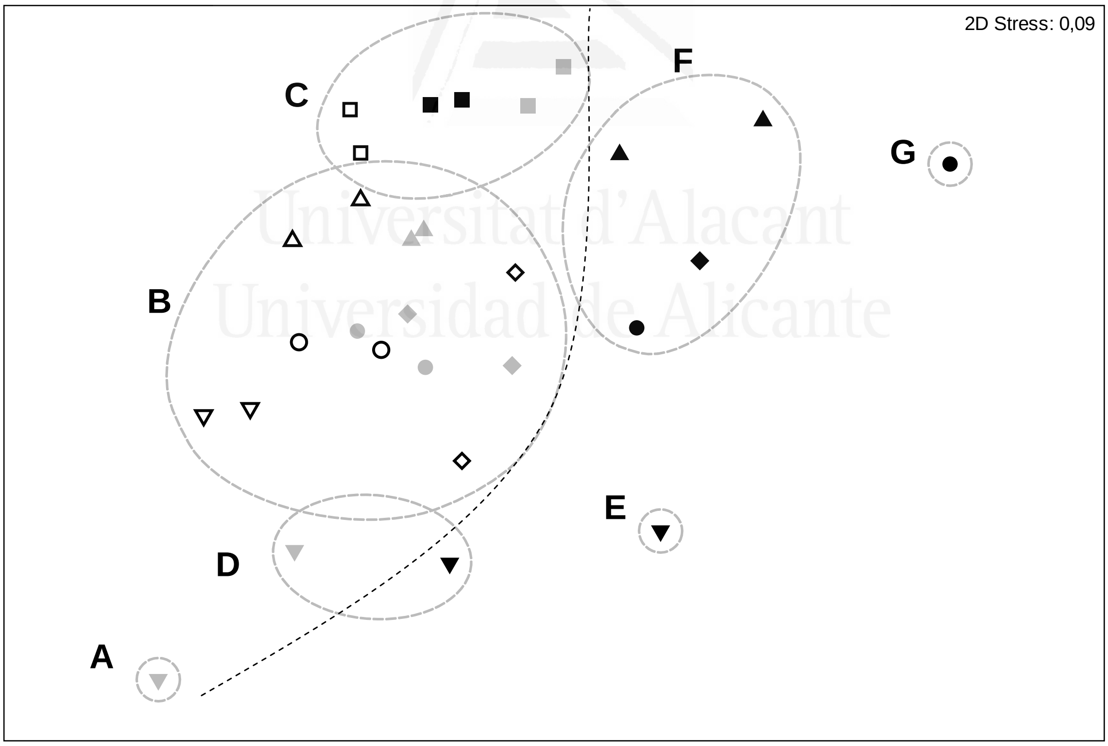


Figure 6.2. nMDS ordination of annual mean amphipod abundance (indiv./m^2) and associated stress value. Differentiating location (I: ▼, II: ●, III: ♦, IV: ▲ and V: ■) and distance to the outfall (black: 0, grey: 200 and white: 1000 m). Letters (A, B, C, D, E, F and G) indicate groups based on similarity.

A forest plot showed the differences in the abundance of these species between stations sited at 0 m and at 1000 m from the outfall (figure 6.3). The species that showed the clearest sensitivity to sewage presence were *Bathyporeia borgi*, *Perioculodes longimanus* and *Autonoe spiniventri*; whereas *Siphonoecetes sabatieri* did not show a negative response and *Ampelisca brevicornis* showed a certain positive response to sewage presence.

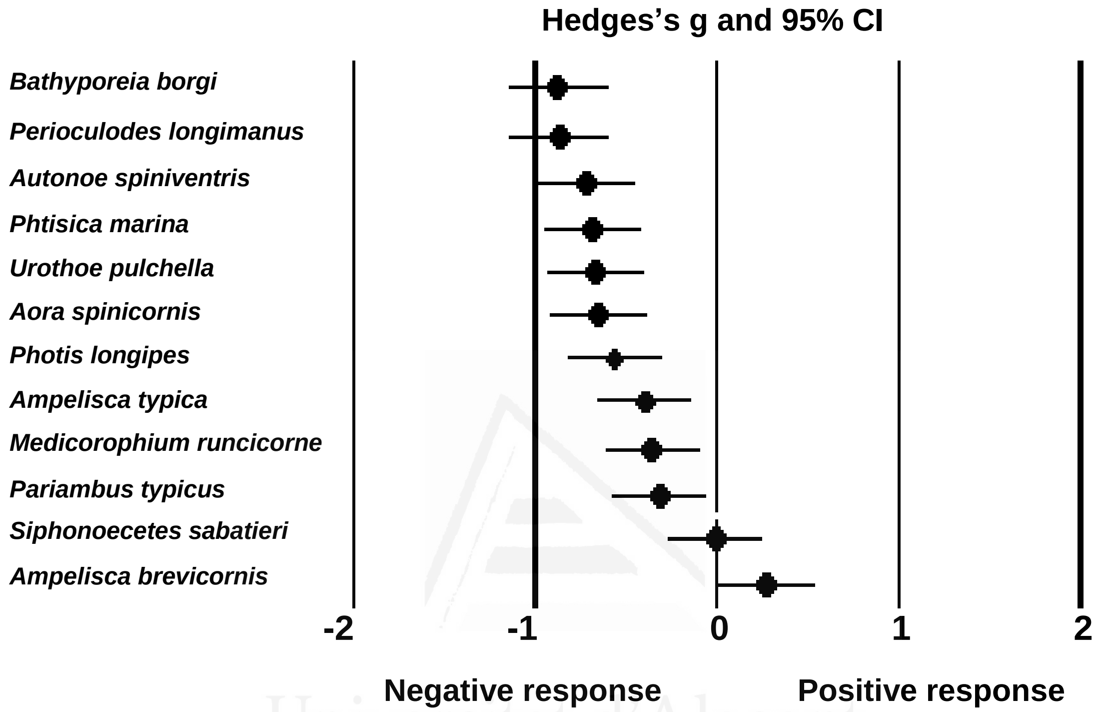


Figure 6.3. Forest plot of effect sizes for Amphipoda species abundances (standardized differences in abundances between sited at 0 m and 1000 m) based on different locations and years. The vertical line represents no difference and the error bars are equivalent to 95% confidence intervals.

Abundance tends to decrease for most of these species at sites closest to the outfall (figure 6.4). However, ANOVA results showed differences among species for the analysed factors (table 6.3). Species such as *A. spiniventris* or *P. longimanus* showed significant differences in the interaction between distance and location. This difference was due to a decrease of abundance at 0 m from the outfall in all locations except location V. In the same way, *U. pulchella* showed a significant difference for interactions between distance and location, obtaining a decrease at stations closest to the outfall of locations I, II and IV. *Bathyporeia borgi*, *P. marina* and *A. spinicornis* decrease at 0 m in all locations, showing significant differences for the factor distance. This decrease was less clear in location V. A similar pattern was observed in abundance for *P. longipes*, where significant differences were not detected every year for the factor distance. Interaction for the three factors was detected in species *A. typica* and *P. typicus*, whose abundances decrease in sites near outfalls but only in certain locations and years. The abundance of other species, such as *S. sabiateri* and *M. runcicorne*, decrease at 0 m but only in certain years, in fact an increase of *S. sabiateri* was detected at an outfall station in location I. Finally, *A. brevicornis* showed significant differences for the interaction distance x location, due to an increase of abundance in location IV at sites 0 m from the outfall.

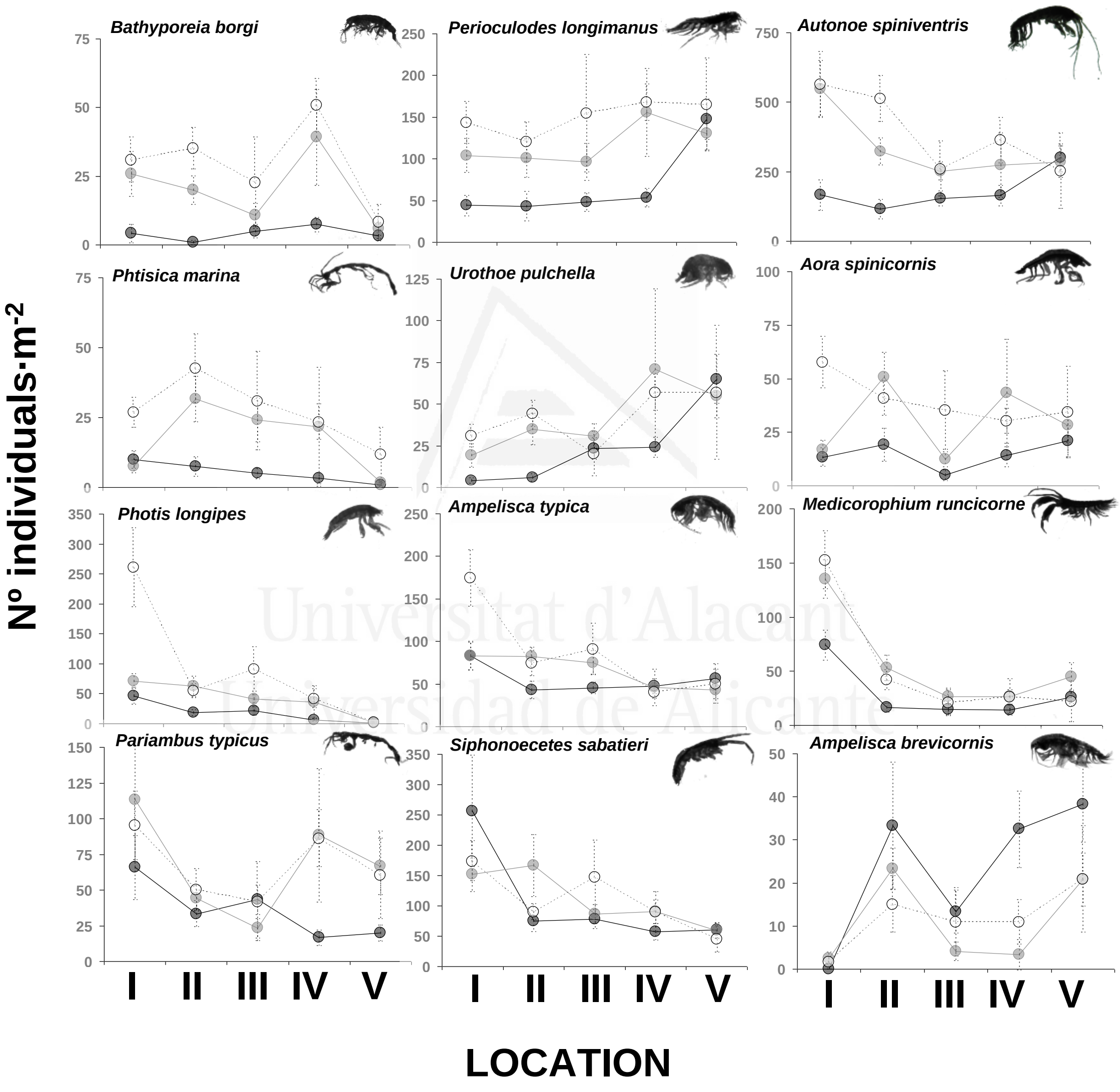


Figure 6.4. Annual mean abundances (indiv./m^2) and standard errors of more contributing Amphipoda species in each location and distance to the outfall (black: 0, grey: 200 and white: 1000 m).

Table 6.3. Results of ANOVA for abundance (individuals/m^2) of each species for the factors distance (0, 200, 1000 m), location (I, II, III, IV and V) and year (2004, 2005, 2006, 2007, 2008) RES = Residual, df: degrees of freedom, F of each factor = mean square factor/ mean square residual because all the factors are orthogonal. Levels of significance: ns no significant difference, *p < 0.05, **p < 0.01 and ***p < 0.001.

		B. borgi		*P. longimanus*		*A. spiniventris*	
Source	**DF**	**F**	**P**	**F**	**P**	**F**	**P**
Distance	2	17.73	***	39.21	***	5.40	*
Location	4	8.07	***	4.98	**	0.13	ns
Year	4	8.52	***	48.02	***	62.84	***
Dis.xLoc.	8	1.67	ns	2.30	*	5.42	***
Dis.xYear	8	2.31	*	0.88	ns	7.08	***
Loc.x Year	16	1.38	ns	2.79	***	8.55	***
Dis.x Loc.xYear	32	1.26	ns	1.32	ns	1.24	ns
RES	375						
TOT	449						
		P. marina		*U. pulchella*		*A. spinicornis*	
Source	**DF**	**F**	**P**	**F**	**P**	**F**	**P**
Distance	2	13.51	***	5.20	*	16.29	***
Location	4	2.12	ns	3.84	*	1.28	ns
Year	4	8.16	***	15.26	***	5.39	***
Dis.xLoc.	8	1.29	ns	4.27	***	1.71	ns
Dis.xYear	8	1.86	ns	1.72	ns	1.36	ns
Loc.x Year	16	3.91	***	2.31	***	2.81	***
Dis.x Loc.x Year	32	0.98	ns	0.99	ns	1.31	ns
RES	375						
TOT	449						
		P. longipes		*A. typica*		*M. runcicorne*	
Source	**DF**	**F**	**P**	**F**	**P**	**F**	**P**
Distance	2	10.46	**	1.53	ns	1.38	ns
Location	4	8.49	***	2.21	ns	6.77	***
Year	4	14.95	***	28.91	***	12.67	***
Dis.xLoc.	8	2.00	ns	0.85	ns	1.04	ns
Dis.xYear	8	2.69	**	1.16	ns	4.19	***
Loc.x Year	16	6.00	***	3.23	***	5.14	***
Dis.x Loc.xYear	32	1.24	ns	2.27	***	1.24	ns
RES	375						
TOT	449						
		P. typicus		*S. sabatieri*		*A. brevicornis*	
Source	**DF**	**F**	**P**	**F**	**P**	**F**	**P**
Distance	2	7.78	**	3.14	ns	1.96	ns
Location	4	0.45	ns	1.46	ns	5.18	**
Year	4	22.66	***	9.72	***	1.45	ns
Dis.xLoc.	8	1.74	ns	1.63	ns	2.32	*
Dis.xYear	8	1.45	ns	3.19	***	1.16	ns
Loc.x Year	16	4.76	***	4.31	***	2.83	***
Dis.x Loc.xYear	32	1.50	*	1.28	ns	0.97	ns
RES	375						
TOT	449						

Regarding trophic group percentages (figure 6.5, table 6.4), significant differences for factor distance were observed in TG-1 and TG-4 due to a decrease in stations near outfalls, and a difference close to significance was detected in TG-3 due to an increase in outfall stations. Meanwhile, differences for interaction between distance and year were detected in TG-2, showing a decrease near outfalls in 2004 and an increase in 2005 at outfall stations. Significant differences between locations were also detected.

Burrowing behaviour groups (Figure 6.6, table 6.5) showed differences in factor distance for domicolous group and in the interaction between the three factors in fossorial group. While domicolous species showed higher percentages in stations near outfalls, fossorial species percentages decreased in locations I, II and IV certain years. Differences in interaction between location and year were detected in the three classes, due to an increase in fossorial species in locations IV and V with respect to locations I, II and III, where domicolous species showed higher percentages.

Table 6.4. Results of ANOVA for percentage of abundance for each trophic group for the factors distance (0, 200, 1000 m), location (I, II, III, IV and V) and year (2004, 2005, 2006, 2007, 2008) RES = Residual, df: degrees of freedom, F of each factor = mean square factor/ mean square residual because all the factors are orthogonal. Levels of significance: ns no significant difference, *p < 0.05, **p < 0.01 and ***p < 0.001.

		TG 1		**TG 2**		**TG 3**		**TG 4**	
Source	**DF**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**
Distance	2	4.83	*	0.50	ns	3.95	ns	7.03	*
Location	4	5.33	**	0.46	ns	9.15	***	10.51	***
Year	4	1.39	ns	3.45	**	18.44	***	17.69	***
Dis.xLoc.	8	0.17	ns	0.34	ns	0.73	ns	0.77	ns
Dis.xYear	8	0.99	ns	3.07	***	1.59	ns	1.24	ns
Loc.x Year	16	0.82	ns	1.65	*	3.94	***	3.21	***
Dis.x Loc.x Year	32	1.18	ns	1.22	ns	1.32	ns	1.21	ns
RES	375								
TOT	449								

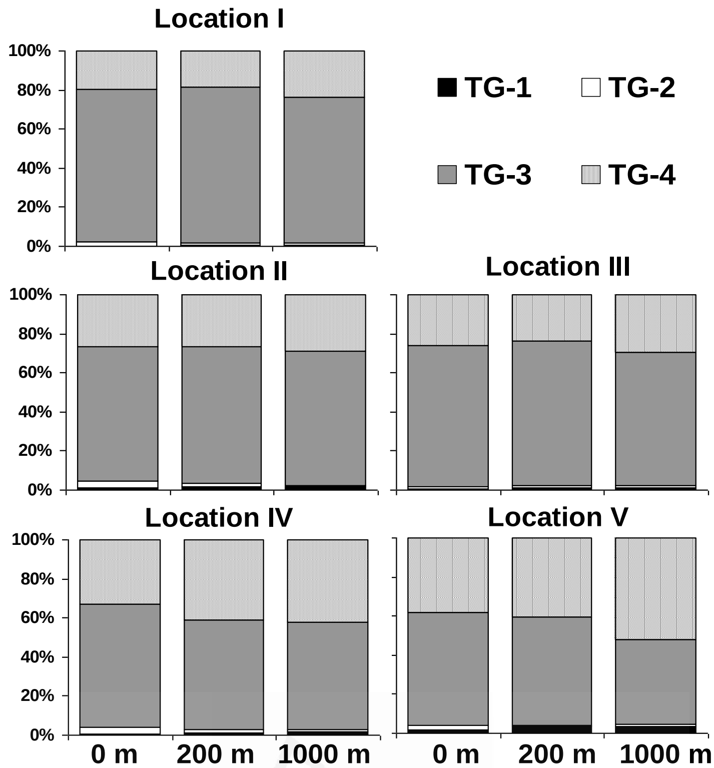


Figure 6.5. Percentage of abundance for each trophic group at each location (I–V) and distance (0, 200 and 1000 m). Classification according to Mearns and Word (1982). TG-1 (suspension feeders), TG-2 (carrion feeders), TG-3 (surface deposit feeders and those species that are both suspension feeders and surface deposit feeders) and TG-4 (subsurface deposit feeders that feed on sedimentary detritus and bacteria).

Table 6.5. Results of ANOVA for abundance (individuals/m^2) of each percentage of burrowing behaviour group for the factors distance (0, 200, 1000 m), location (I, II, III, IV and V) and year (2004, 2005, 2006, 2007, 2008) RES = Residual, df: degrees of freedom, F of each factor = mean square factor/ mean square residual because all the factors are orthogonal. Levels of significance: ns no significant difference, *p < 0.05, **p < 0.01 and ***p < 0.001.

		Domicolous		Interstitial		Fossorial	
Source	**DF**	**F**	**P**	**F**	**P**	**F**	**P**
Distance	2	4.70	*	0.83	ns	16.29	***
Location	4	8.01	***	0.49	ns	15.40	***
Year	4	15.84	***	10.49	***	6.78	***
Dis.xLoc.	8	0.77	ns	1.64	ns	1.03	ns
Dis.xYear	8	1.65	ns	1.32	ns	0.94	ns
Loc.x Year	16	4.23	***	2.72	***	2.85	***
Dis.x Loc.x Year	32	1.35	ns	1.23	ns	1.64	*
RES	375						
TOT	449						

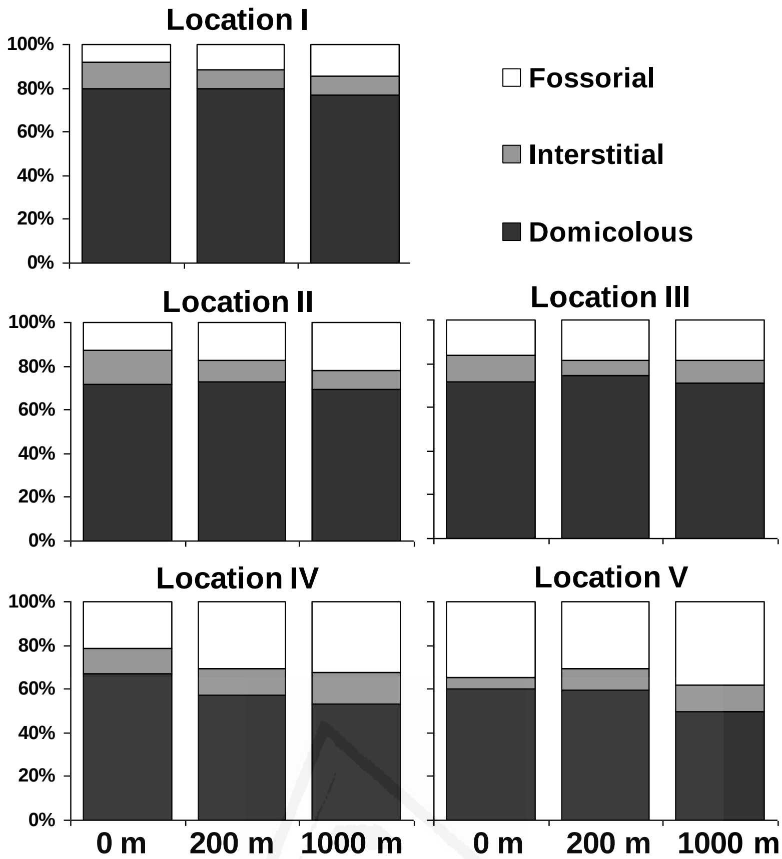


Figure 6.6. Percentage of number of individual for each burrowing behaviour group at each location (I–V) and distance (0, 200 and 1000 m).

6.4.- Discussion.

Amphipods showed a general sensitivity to sewage outfalls, decreasing their abundance and diversity near these discharges, from locations I to IV, as we observed in chapter 2. This decrease was not observed near location V, since this was the location with the lowest flow and the only one where biological treatment of activated sludge takes place (de-la-Ossa-Carretero *et al.*, 2008, 2009, 2010b; Del-Pilar-Ruso *et al.*, 2010). This sensitivity of Order Amphipoda contrasts with the response of class Polychaeta to some of these sewage outfalls (Del-Pilar-Ruso *et al.*, 2010). Polychaete diversity decreases in stations affected by pre-treated sewage, but only in locations with medium and high flow rates, not in stations with low flow such as location IV (Alcossebre) where amphipods showed a sensitive response. Moreover, an abundance of certain opportunistic polychaetes can increase close to outfalls, particularly location II (Benicarlo), where high flow and low water quality values have been reported. This inverse response of amphipods and opportunistic polychaetes has previously been employed to develop the Benthic Opportunistic Polychaetes Amphipods index

(BOPA/BO2A), in order to infer environmental status from the assessment of the state of the benthic community in the implementation of the European Water Framework Directive 2000/06/EC (WFD, 2000; Dauvin and Ruellet, 2007, 2009; de-la-Ossa-Carretero and Dauvin, 2010). The effectiveness of the BOPA index for monitoring sewage outfalls was previously analysed in chapter 4 (de-la-Ossa-Carretero *et al.*, 2009).

Therefore, published literature has established that amphipods are more sensitive to polluted sediments than to other benthic organisms (Gomez Gestiara and Dauvin, 2000; Dauvin and Ruellet, 2007, 2009), with a general decrease of amphipod abundance and diversity when pollution increases (Bellan-Santini, 1980; Conlan, 1994). Thus, its sensitivity to certain types of pollution, such as oil pollution, is clearly established (Gomez Gesteira and Dauvin 2000). Moreover, crustacean amphipods are widely used for bioassay and numerous species are usually employed in ecotoxicology tests for contaminants such as polycyclic aromatic hydrocarbons (PHAs), polychlorinated biphenyls (PCBs), organochlorine pesticides (DDT), heavy metals, ammonium or nitrite (Riba *et al.*, 2003; Anderson *et al.*, 2008; Ramos-Gómez *et al.*, 2009). However, despite this general sensitive trend of Order Amphipoda, not all species showed the same level of sensitivity. While some species such as *Bathyporeia borgi*, *Autonoe spiniventris* or *Perioculodes longimanus* showed a homogeneous negative response to disposal presence, reducing its abundance in stations sited near the outfalls, other species may show an unclear pattern or, indeed, certain tolerance to sewage presence. AMBI and BENTIX, another two biotic indices developed for WFD (Borja *et al.*, 2000; Simboura and Zenetos, 2002), cluster benthic species since this response to pollution. Though most amphipods were classified in the sensitive group, some amphipod species were recorded as tolerant (table 6.1); and we can find some discrepancies between both classifications; e.g. *Autonoe spiniventris* is classified as tolerant in BENTIX, whereas AMBI, as observed in our results, established this species as sensitive. The response of a given species is dependent on the kind of perturbation (Bustos-Baez and Frid, 2003) and classification of species along a sensitivity-tolerance continuum is thus a very difficult task and still a matter of debate (Labrune *et al.*, 2006; Grémare *et al.*, 2009). Abundance of *Grandidierella japonica* increased in Richmond harbour (Swartz *et al.*; 1994), although it has been reported as reliable in bioassays and its sensitivity in sediment toxicity test methods from areas adjacent to wastewater outfalls and indeed harbours has been proved (Nipper *et al.*, 1989). *Phtisica marina* is capable of resisting stress conditions in harbours and calm zones (Conradi *et al.*, 1997; Sánchez-Moyano and García-Gómez, 1998; Guerra-Garcia and García-Gómez, 2001), but we detected a certain sensitivity of this caprellid, whose abundance decreased in outfall stations. According to the AMBI list, this species is sensitive, whereas the BENTIX classification reported this species as tolerant. Another caprellid species, *Pariambus typicus* showed certain sensitivity to sewage presence, but it was classified with some tolerance in the AMBI and BENTIX lists.

Although the genera *Ampelisca* have been reported as well adapted to environmental stress (Lowe and Thompson, 1997; Ingole *et al.*, 2009), several species of the genera *Ampelisca* are used for sediment toxicity assessment. In fact, *Ampelisca abdita* is recommended and used in the US bioassay tests (EPA, 1990; Thomas, 1993) but it was reported as numerical dominant in polluted areas (Santos and Simon, 1980) and its highly variable seasonal abundances mean that it is not considered suitable as an assessment indicator. In our case, despite the fact that *Ampelisca brevicornis* has been reported as a good test organism in sediment toxicity analysis (Riba *et al.*, 2003, Ramos-Gómez *et al.*, 2009), we have detected a certain tolerance of this species to sewage discharge. After the high pollution of the muddy fine sand community of the Bay of Morlaix (western English Channel), and the destruction of the dominant *Ampelisca* species, *A. brevicornis* was one of those which colonised the benthic polluted community the quickest: one year after at the more polluted site (Rivière de Morlaix) and two years later in a less polluted site (Pierre Noire) (Dauvin 1998, 2000). *Ampelisca brevicornis* shows a large ecological distribution from muddy sand to gravel and from the intertidal to subtidal sediments which gives an advantage over other species of *Ampelisca.* The AMBI and BENTIX lists showed discrepancies in the classification of this species; while AMBI classified it as sensitive BENTIX classified it as tolerant.

Among the *Corophium* genera, *C. volutator* was chosen by OSPAR for use in the standard sediment test for offshore chemical products (OSPAR, 1995), and can be considered as sensitive to metal contamination (Warwick, 2001). Conversely, Norkko *et al.* (2006) described the opportunistic behaviour of this species following experimental defaunation on an intertidal location on the Swedish west coast. In fact, certain species of the Corophiidae family exhibit greater production near sewer outfalls (Lowe and

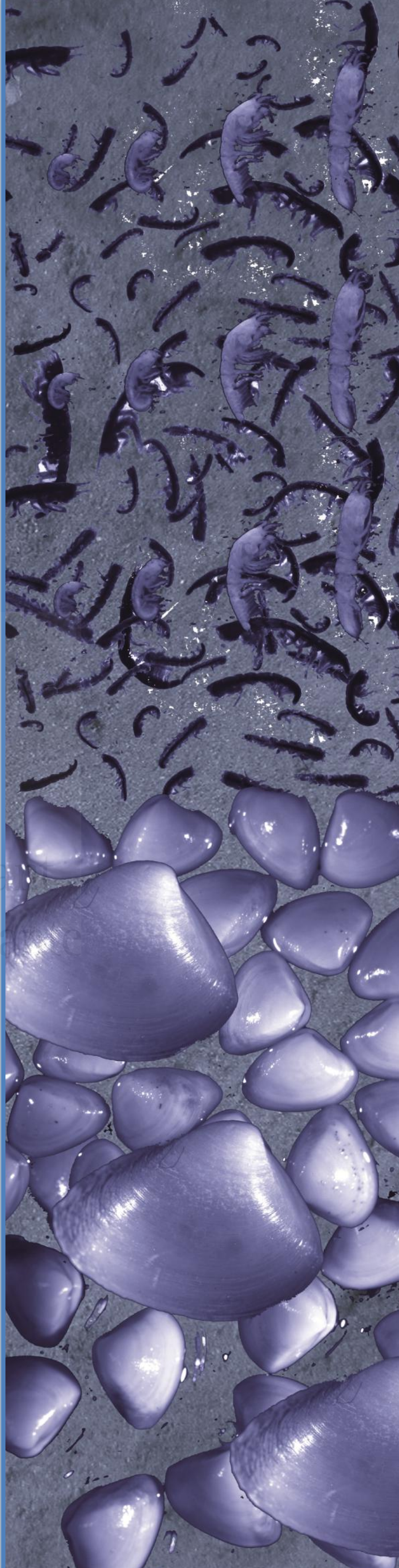
Thompson, 1997). *Corophium ellisi* productivity depends on oxygen conditions, and Grizzle (1984) found higher densities in a sewage polluted station on the east coast of Florida. *Corophium salmonis* are also somewhat pollution tolerant (Arvai *et al.*, 2002). Among the Corophiidae family found in our study, *Medicorophium runcicorne* and *Siphonoecetes sabatieri* showed a decrease in abundance at outfall stations but not for all years. An increase of *S. sabiateri* is observed at outfall stations of location I. AMBI and BENTIX lists classified the *Corophium* genera with certain tolerance, whereas *Siphonocetes* was sensitive in both indices. *Siphonoecetes* spp. are known to feature unstable population dynamics resulting in sharp peaks in abundance correlated with the nature of the sediment or interaction with other species (Cunha *et al.*, 2000; Bigot *et al.*, 2006). Therefore, sensitivity of specimens from this family could be ambiguous as they may be affected by locally environmental conditions.

Regarding feeding and burrowing behaviour, our results showed higher sensitivity to sewage presence in fossorial species than domicolous species, whereas trophic group species showed only slight differences in sensitivity with lower sensitivity in species that are both suspension and deposit feeders. Previously, King *et al.* (2006) have reported less sensitivity in tube-dwelling amphipods than in epibenthic amphipods, as well as more tolerance in filter feeding species. They recommended using epibenthic amphipods as indicators given that they are more sensitive than infaunal tube dwellers. Several reasons could explain that amphipods with distinct burrowing behaviour respond differently (Anderson *et al.*, 2008). Tube builder amphipods are more isolated from sediment contaminants than free-burrowing species and the tube construction may reduce interstitial water contact with this species. On the other hand, sewage presence may alter the biochemical composition of sediments (Cotano and Villate, 2006), producing organic enrichment, and its posterior degradation could lead to lower oxygen concentrations (Gray *et al.*, 2002). Domicolous amphipods are capable of pumping oxygenated water down into their burrows and tubes, whereas fossorial species depend on dissolved oxygen penetrating into the sediment by molecular diffusion. Moreover, Okladen *et al.* (1984) reported the possibility that burrowing amphipods actively avoided polluted sediment and chose more desirable sediment.

In summary, the Order Amphipoda is generally sensitive to sewage pollution, showing a decrease in abundance and diversity in stations close to outfalls in the studied locations, except in the location with the lowest sewage flow and the best wastewater treatment processes. However, affected species showed some differences in level of sensitivity. This varied response could be due to burrowing and feeding behaviour, in such a way that suspension and surface deposit feeders and tube builders showed less sensitivity to disposal presence than others, and are thus even able to increase in abundance, as occurs with *Ampelisca brevicornis* which showed a weak positive response.

SENTINEL SPECIES

Especies centinelas



Publications.

De-la-Ossa-Carretero J.A., Del-Pilar-Ruso Y., Giménez-Casalduero F., Sánchez-Lizaso J.L. 2010b. Sensitivity of tanaid *Apseudes latreillii* (Milne-Edwards, 1828) populations to sewage pollution. *Marine Environmental Research* **69**: 309-317.

De-la-Ossa-Carretero J.A., Del-Pilar-Ruso Y., Giménez-Casalduero F., Sánchez-Lizaso J.L. 2008. Effect of Sewage Discharge in *Spisula subtruncata* (da Costa, 1778) populations. *Archives of Environmental Contamination and Toxicology* **54**: 226-235.

CHAPTER 7

Sensitivity of tanaid *Apseudopsis latreillii* (Milne-Edwards, 1828) populations to sewage pollution

Sensibilidad del tanaidáceo *Apseudopsis latreillii* (Milne-Edwards, 1828) al vertido de aguas residuales

Abstract. *Apseudopsis latreillii* (Milne-Edwards, 1828) is a common and abundant tanaid in soft-bottom communities from waters off East Atlantic and Mediterranean coasts. Its sensitivity to pollution is not clear despite being an abundant and widely distributed crustacean, since it has been reported as both a tolerant and sensitive species. This study tests the sensitivity of *A. latreillii* to sewage discharges in fine-sand communities along the Castellon coast (W. Mediterranean). We analysed variation in tanaid populations between sites at varying distances from sewage outfalls with respect to population density, size distribution, sex ratio and their correlation with different abiotic factors of waste water and sediment. Results showed clearly that *A. latreillii* populations were affected by the presence of sewage outfalls, to such an extent that sewage disposal outlets produced a decrease in population density and changes in size spectra.

Resumen. *Apseudopsis latreillii* (Milne-Edwards, 1828) es una especie de tanaidáceo muy habitual en las comunidades de fondos blandos de las costas del Mediterráneo y del Atlántico oriental. Sin embargo, a pesar de esta amplia distribución y de presentarse en altas densidades, su sensibilidad a la contaminación no está clara, ya que ha sido considerada tanto como una especie tolerante como una especie sensible. En este capítulo se ha analizado el efecto del vertido de aguas residuales urbanas sobre poblaciones de *Apseudopsis latreillii* en comunidades de arenas finas de la costa de Castellón (Mediterráneo occidental). La densidad de población, distribución de tallas, sex ratio y presencia de huevos o embriones en hembras han sido analizados a distintas distancias del punto de vertido en cada una de las localidades. Los resultados reflejan que la población de *A. latreillii* está afectada por la presencia de estos vertidos, de modo que se detecta una descenso en la densidad y cambios en el espectro de tallas en las proximidades de los vertidos.

7.1.- Introduction.

Apseudopsis latreillii (Milne-Edwards, 1828) is an abundant and common tanaid in Mediterranean and Easter Atlantic coastal waters. It inhabits sandy and muddy bottoms between 0 and 138 m (Sanz, 1992; Guerra-García *et al.*, 2003; Le Hir and Hily, 2005; Marín-Guirao *et al.*, 2005; De Juan *et al.*, 2007; Bouchet and Sauriau, 2008; Lourido *et al.*, 2008; Moreira *et al.*, 2008; Bakalem *et al.*, 2009), though its presence has also been reported in sediments of *Posidonia oceanica* meadows (Como *et al.*, 2008) or associated within hard-bottom areas at the volcanic Nisyros island (Greece) (Conides *et al.*, 1999).

Benthic assemblages, due to their high diversity and abundance, could be essential in defining trophic guilds and play a vital role in cycling nutrients and materials between the underlying sediment and the overlying water column (Dauvin *et al.*, 2007). As well as amphipods, other peracarid crustaceans are important in the structuring of benthic assemblages, with a relevant contribution to benthic production (Mancinelli and Rossi, 2002) and represent a significant source of food for other benthic animals and fishes of commercial importance (Dauvin, 1988; Lourido *et al.*, 2008).

Crustaceans are usually more sensitive to pollution than other marine taxa (Rand and Petrocelli, 1985; Dauvin and Ruellet, 2007) and their distribution and abundance in marine sediments are influenced by a number of abiotic factors, such as sediment composition (Parker, 1984; De Grave, 1999) and organic content (Robertson *et al.*, 1989). Their abundance and species diversity may be used as indicators of environmental conditions (Marques and Bellan-Santini, 1987; Corbera and Cardell, 1995; Gomez-Gesteira and Dauvin, 2000; Conradi and López-González, 2001; Guerra-García and García-Gómez, 2005; Dauvin and Ruellet, 2007). Therefore it is important to know the extent of their sensitivity to different pollution sources in order to monitor environmental quality or use them as biological indicators.

Despite being an abundant and widely distributed crustacean species, *A. latreillii* response to pollution is not clear, since it has sometimes been reported as a tolerant species (Grall and Glémarec, 1997; Marín-Guirao *et al.*, 2005; de Juan *et al.*, 2007) and by others as a sensitive species (Sanz-Lázaro and Marín, 2006; Bouchet and Sauriau, 2008). It is necessary to clarify its response to pollution in order to avoid errors when using it as a biological indicator as sentinel species in environmental assessment.

As we reported in chapter 2, *A. latreillii* is an abundant species in fine-sand communities in the north-western Mediterranean Sea. This chapter aims to test the sensitivity of *A. latreillii* to sewage. In this study we examined the possible impact of five sewage outfall sites on the *A. latreillii* populations along the Castellon coast. We analysed changes in population dynamic parameters such as abundance, size distribution, sex ratio and their correlation with characteristics such as sediment and waste water discharged.

7.2.- Materials and methods.

In this chapter, we analysed the *A. latreillii* populations of the five locations affected by sewage outfalls from Castellon coast (NE Spain) (locations I to V, figure 2.1). This homogeneous area with an established pollution gradient represents an ideal site for investigating links between macrofaunal assemblages and the effect of contaminants (de-la-Ossa-Carretero et al., 2009). The study area, sewage outfalls and sampling methods were previously described in chapters 2 and 4. *A. latreillii* individuals were putting aside from the other taxa.

A. latreillii individuals were counted and individuals of 2004 to 2007 were measured (anteriorposterior length) using the image manager software Leica IM50 after being photographed through a Leica Mz. 9.5 stereomicroscope. Males were differentiated from females and juveniles, by observing cheliped development, which is more robust in males and which features a tooth on the inner margins of the propodus and dactylus (Sanz, 1992) (figure 7.1). Ovigerous females with presence of eggs or embryos were also counted.

Stem–Leaf Plot of length values of previously identified individual males were obtained in order to obtain a boundary length between immature and mature individuals (lower hinge in Stem–Leaf Plot was considered).

Abundances of *A. latreillii* were examined using 3-factor analyses of variance (ANOVA) with distance and location as fixed factors and year as random factor. Prior to ANOVA, the homogeneity of variance was tested using Cochran's test. Data were

√X + 1 transformed when variances were significantly different. SNK test (Student–Newman-Keuls) was used to determine which samples were implicated in the differences.

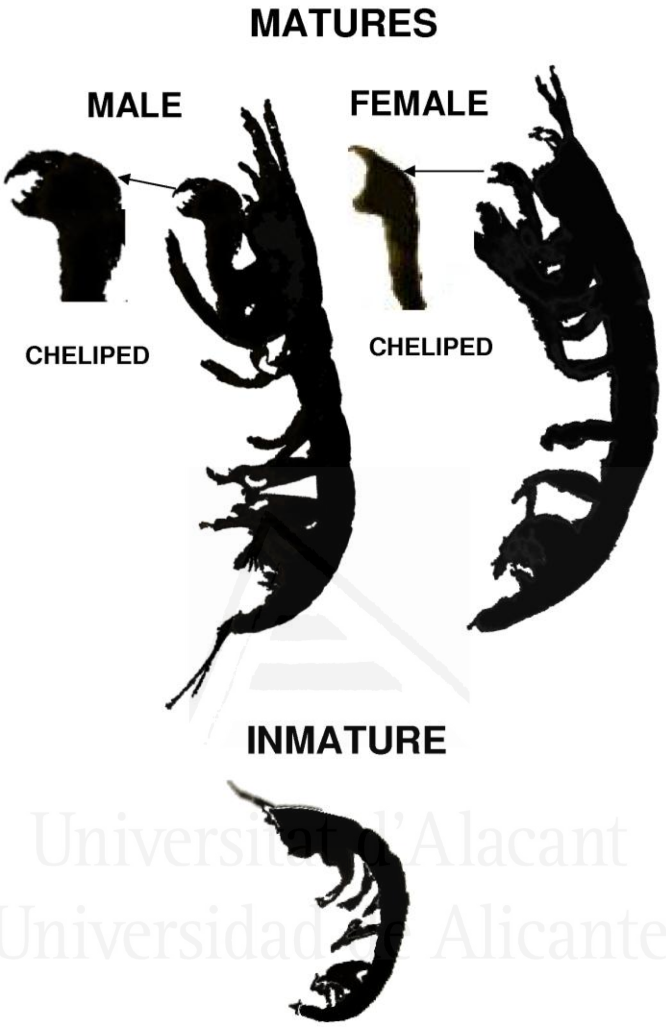


Figure 7.1. *Apseudopsis latreillii* individuals. Differentiation between male, female, and immature individuals.

Size frequency distributions were grouped and plotted into 11 size classes using a 0.75 mm total length class interval. Since it is not possible to use analysis of variance with the Tanaidacea length data because there were significant differences in sample size, we used a non-parametric Kruskall–Wallis test to compare length means between varying distances from outfall for each location and year. In order to exclude the influence of recruitment, it was decided not to consider measurements of juveniles. Kruskall–Wallis test was also used to determine differences in the percentage of males and in the presence of eggs or embryos in females between distances in each location.

Correlation between mature size, immature, mature and total abundances and waste water and sediment descriptors were determined using Pearson correlation, testing probability of each correlation coefficient with Bonferroni test. Waste water descriptors employed included flow, suspended solids, BOD, COD, phosphates, nitrates and turbidity, whilst granulometric analysis, potential redox, organic matter and pH were used as sediment descriptors.

7.3.- Results.

A total of 68890 specimens belonging to different invertebrate taxa were collected in locations I, II, III, IV and V (Amphipoda, Bivalvia, Cephalochordata, Copepoda, Cumacea, Decapoda, Echinoidea, Gasteropoda, Holoturoidea, Isopoda, Mysidacea, Nematoda, Nemertea, Ostracoda, Ophiuroidea, Polychaeta, Pycnogonida, Scaphopoda, Sipunculida, and Tanaidacea). Of these specimens, 7310 individuals were identified as *A. latreillii*; representing 10.6% of total macroinvertebrate community abundance. The highest population density detected was 2575 individuals/m^2, in a station situated at 1000 m from the outfall of location IV.

ANOVA detected significant differences in *A. latreillii* abundances for the interactions between the pairs of factors distancexlocation, distancexyear, and locationxyear (table 7.1). SNK results (table 7.2) showed that differences for interaction distancexlocation were due to decrease of abundance in stations closer to outfall at all locations except in location V (figure 7.3). These decreases produced differences between location V and the others. Differences for interaction distancexyear were due to a decrease of abundance in year 2007 (figure 7.3) which produced non-significant differences among distances during this year. Finally, despite the fact that location V usually obtained higher abundances than the others, in years 2005 and 2006 this trend was not so marked, producing significant differences for the interaction location x year.

Table 7.1. Results of ANOVA for abundance (individuals/m^2) of *Apseudopsis latreelli* for the factors distance (0, 200, 1000 m), location (I, II, III, IV and V) and year (2004, 2005, 2006, 2007, 2008) RES = Residual, df: degrees of freedom, MS= mean square, F of each factor =MS factor/MS residual because all the factors are orthogonal. Levels of significance: ns no significant difference, *p < 0.05, **p < 0.01 and ***p < 0.001.

Source	df	MS	F	P
Distance	2	6431.50	24.99	***
Location	4	1237.62	6.42	**
Year	4	1227.89	18.72	***
Distancexlocation	8	210.31	3.3	**
Distancexyear	8	257.33	3.92	***
Locationxyear	16	192.92	2.94	***
Distancexlocationxyear	32	63.82	0.97	ns
RES	375	65.59		
TOTAL	449			

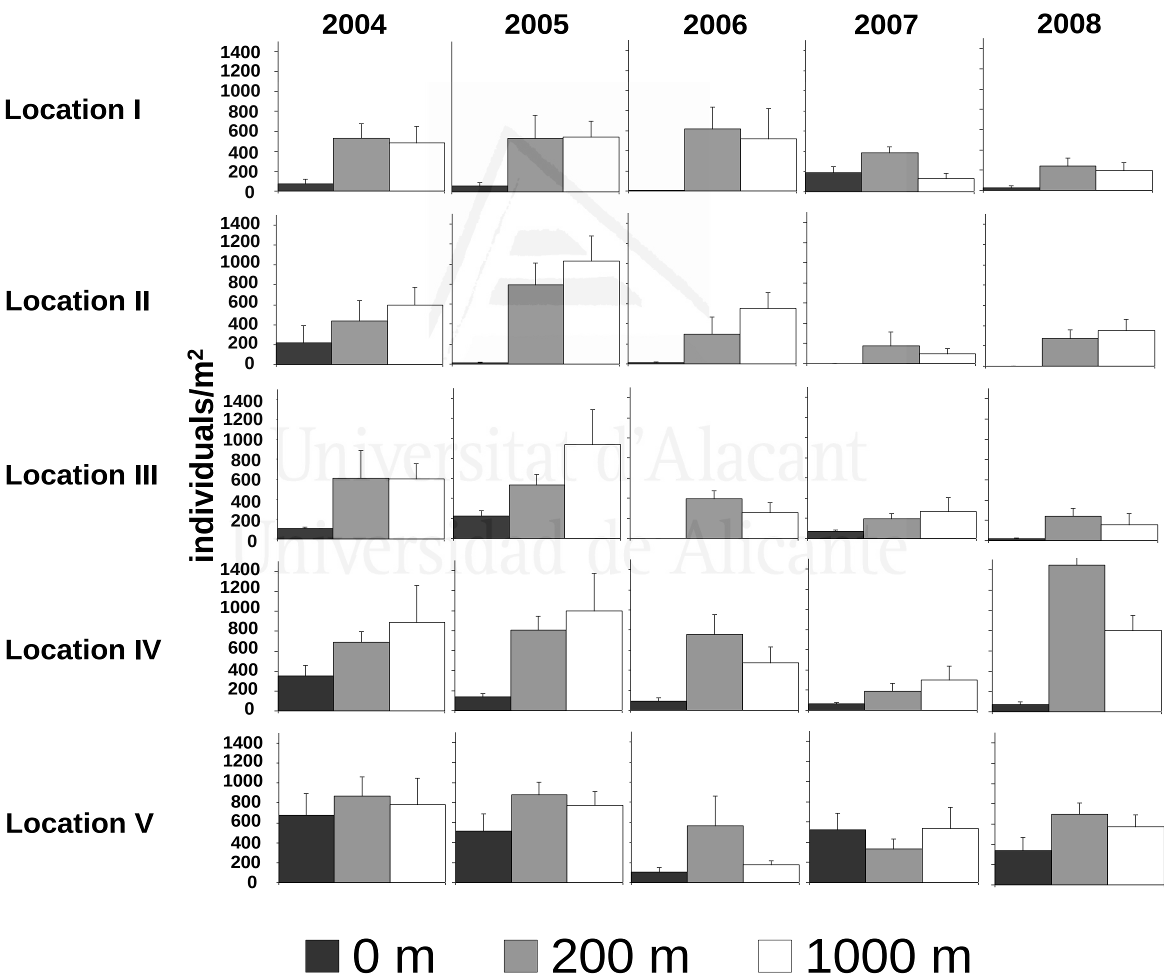


Figure 7.2. Mean and standard error of *Apseudopsis latreillii* abundances at each location (I to V), year (2004, 2005, 2006, 2007 and 2008) and distance (0, 200 and 1000 m).

Table 7.2. Summary of the results of SNK post-hoc test for the interactions distance x location, distance x year and location x year.
Factors considered distance (0, 200, 1000 m), location (I, II, III, IV and V) and year (2004, 2005, 2006, 2007).* Significant at $p < 0.05$.** $p < 0.01$.*** $p < 0.001$

Interaction	**Factors**	**Level**	**SNK**
Distancexlocation	dis(loc)	loc 1	** 0<200 m, ** 0<1000m
		loc 2	** 0<200 m, ** 0<1000m
		loc 3	** 0<200 m, ** 0<1000m
		loc 4	** 0<200 m, ** 0<1000m
	loc(dis)	0 m	** 2<5,**3<5, **4<5, **1<5, *2<4
		200 m	** 1<4,**2<4, **3<4, *1<5, * 2<5,*3<5
		1000 m	*1<4,*3<4
Distancexyear	dis(year)	2004	**0<1000, *0<200
		2005	** 0<1000, **0<200
		2006	** 0<1000, **0<200
		2008	** 0<1000, **0<200
Locationxyear	loc(year)	2004	**1<5, *1<4, *2<5,*3<5
		2005	** 1<5
		2007	** 2<5, *1>2, *3<5, *4<5
		2008	** 1<4,**2<4, **3<4, **1<5, ** 2<5,**3<5

The mean length of 5957 specimens measured (from year 2004 to 2007) was 3.58 mm, with a range between 0.8 mm and 8.9 mm. The boundary length between immature and mature specimens was established at 3.7 mm (lower hinge in Stem–Leaf Plot of values of length of previously differentiated males based on the sexual dimorphism). Based on this boundary length individuals were classified in 616 males, 1796 females and 3545 immatures.

The mean length of males was 5.22 mm and the greatest length was 8.6 mm, whereas the mean length of females was 4.84 mm and the greatest length 8.4 mm. Finally the mean length in immatures was 2.66 mm and the lowest 0.8 mm. The results of the Kruskall–Wallis test showed significant differences with distance in mean length of mature individuals. It was found that in location I, years 2004 and 2007, and in location II, year 2004, the mean length of mature individuals was lower in stations closer to the outfall (figure 7.3), where individuals over 4.5 mm in location I and over 6.5 mm in location II were absent (figure 7.4). Moreover, individuals over 5.25 mm disappeared in year 2006 in stations at 0 m from outfall in locations IV and V, though significant differences in distance in mean length of mature individuals were not detected in these cases. On the other hand, higher abundances of individuals over 5.25 mm were obtained in stations closer to the outfall in year 2007 (figure 7.4) producing a higher mean length of mature individuals. Regarding juveniles, the abundance of the smallest class size decreased or even disappeared in stations closer to the outfall in location I, for years 2005 and 2006, in location II, in location III and in location IV, for years 2005 and 2007 (figure 7.4). Finally, almost all *Apseudopsis* disappeared in stations closer to the outfall in location I, year 2006, in location II, years 2005–2007 and in location III, years 2006 and 2007 (figure 7.4).

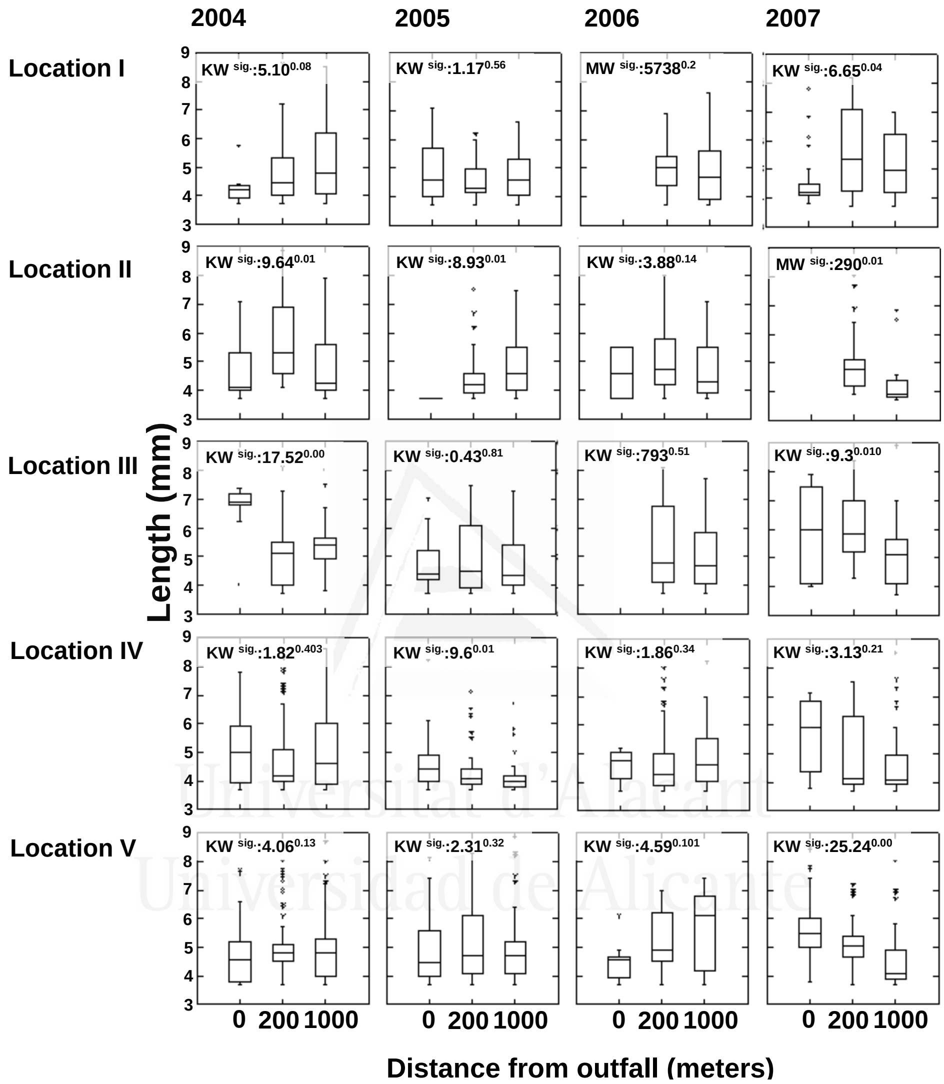


Figure 7.3. Mean length and results of Kruskal-Wallis Test for factor distance at each location (I to V) and year (2004, 2005, 2006 and 2007) of mature individuals. The boxes indicate the 25th percentile, median, and 75th percentile, whiskers extend from the 10th to the 90th percentile, while dots indicate the 5th and 95th percentile values.

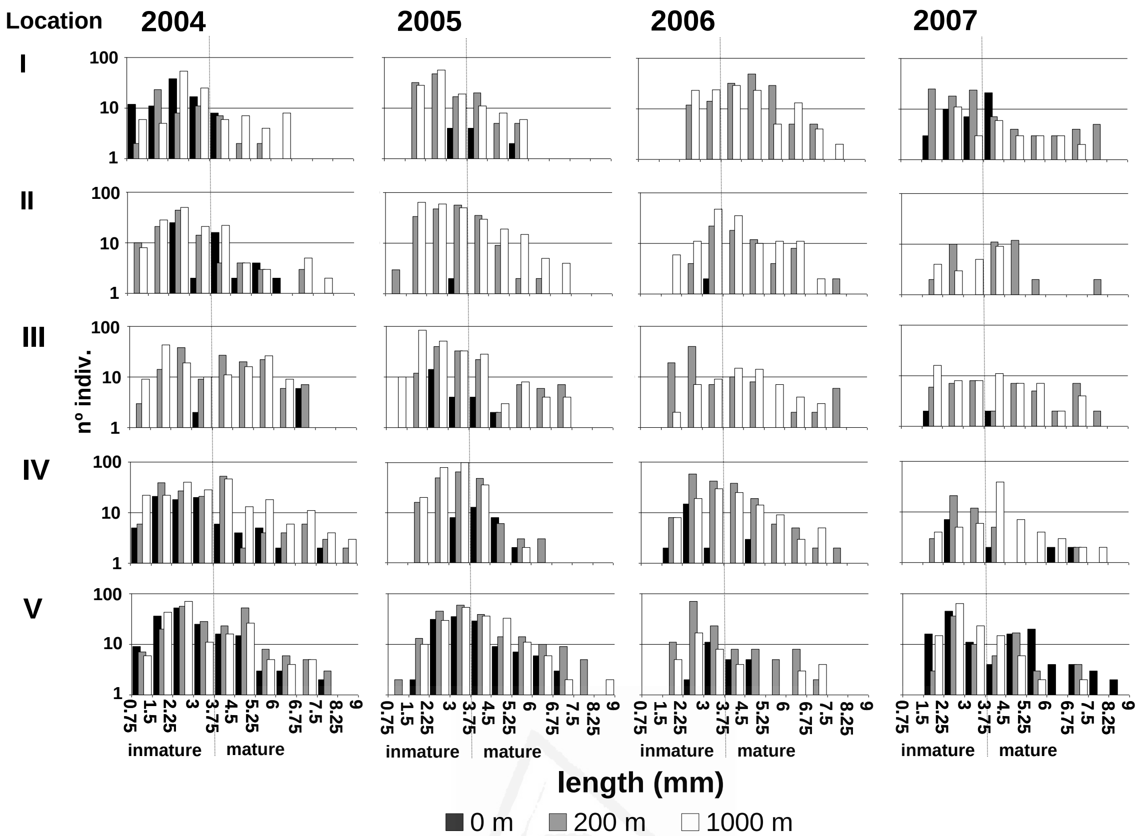


Figure 7.4. Size distribution of *Apseudopsis latreillii* for each location (I–V), year (2004–2007) and distance (0, 200 and 1000 m). All bars are plotted at the midpoint of the 0.75 mm class intervals.

The mean sex ratio (females:males) of samples obtained at different distances to the outfall was similar, being 1:0.32 at stations closer to the outfall; whereas at 200 m to outfall it was 1:0.35 and at 1000 m to outfall it was 1:0.37. However, Kruskall–Wallis test showed significant differences in the percentage of males between distances for location IV and for location II. Plot showed a decrease of male percentage approaching the outfall in both locations (figure 7.5).

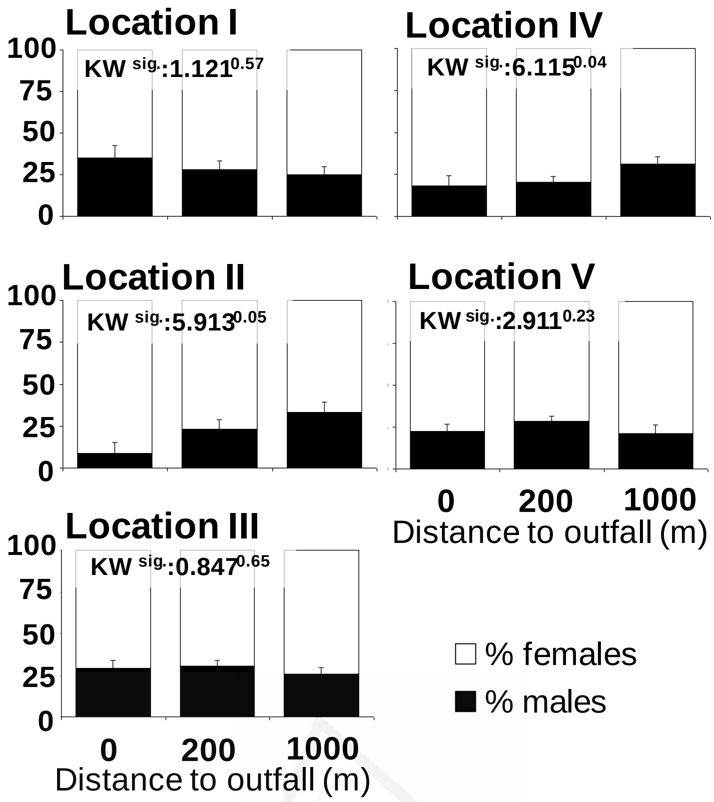


Figure 7.5. Percentage of males and females of *Apseudopsis latreillii* population at each location (I to V) and distance (0, 200 and 1000 m). Kruskal-Wallis Test Statistic and p values for factor distance were indicated.

The percentage of females with eggs or embryos did not show any changes due to the outfalls. Kruskall–Wallis test did not show significant differences between distances for any location (Table 7.3).

Table 7.3. Mean ± standard error of percentage of females of *Apseudopsis latreillii* with eggs or embryos at each location (I to V) and distance (0, 200 and 1000 m). Kruskal-Wallis Test Statistic for factor distance were indicated. Levels of significance: ns no significant difference, *p < 0.05, **p < 0.01 and ***p < 0.001.

Mean±SEM of % presence of eggs or embryos in females				
Location	**0 m**	**200 m**	**1000 m**	**K-W^p**
I	28.98±10.73	12.95±5.96	22.34±7.92	0.638^{ns}
II	14.29±9.22	33.21±8.35	12.30±5.44	4.704^{ns}
III	29.17±12.89	19.84±6.77	8.87±3.36	1.998^{ns}
IV	7.46±5.37	12.25±5.20	12.42±3.70	3.981^{ns}
V	3.34±1.31	11.98±3.76	8.67±4.89	2.466^{ns}

Pearson correlation coefficient and Bonferroni probabilities reported that there were significant correlations between abundance values and several abiotic factors; however most of the Pearson coefficients were low (table 7.4). With regard to waste water flow data, all coefficients showed significant negative correlations with total abundance values. Suspended solids obtained the highest correlation followed by phosphates, COD and flow. For immature abundances, the highest Pearson coefficient was also obtained in suspended solids, phosphates and COD whereas pH, BOD and nitrates did not show any significant correlation. Pearson coefficients for mature abundance values were higher than for immature abundances, and suspended solids obtained the highest values, although high Pearson coefficients were also obtained in nitrates and COD. Although length values showed significant negative correlations with water disposal pH; Pearson coefficient was low. On the other hand, percentage of coarse sand in sediment showed a significant and negative correlation with total and mature abundance values. Potential redox of sediment also showed a significant and positive correlation with abundance values, whereas pH values showed a negative correlation with total and immature abundance values. These Pearson coefficients proved to be weaker than with waste water data.

Table 7.4. Pearson correlation, total abundance, immature abundance, mature abundance and matures lengh values with studied factors and Bonferroni probabilities.

Levels of significance: ns no significant, *p < 0.05, **p < 0.01 and ***p < 0.001.

		Abundance			Matures
		Total	**Immatures**	**Matures**	**length**
Water disposal	**pH**	-0.235*	-0.179^{ns}	-0.283**	-0.264***
	Flow (m^3/month)	-0.300**	-0.244*	-0.329**	-0.022^{ns}
	S.S. (mg/l)	-0.377***	-0.288**	-0.453***	-0.008^{ns}
	BOD (mg/l)	-0.269*	-0.194^{ns}	-0.350***	-0.000^{ns}
	COD (mg/l)	-0.334***	-0.251**	-0.412***	-0.013^{ns}
	P (mg/l)	-0.349***	-0.275**	-0.354***	-0.087^{ns}
	Ni (mg/l)	-0.251*	-0.167^{ns}	-0.403***	-0.063^{ns}
	Turb. (NTU)	-0.295**	-0.214*	-0.380***	-0.008^{ns}
Sediment	**% Mud**	0.035^{ns}	0.070^{ns}	-0.036^{ns}	-0.016^{ns}
	% Fine Sand	0.076^{ns}	0.088^{ns}	0.025^{ns}	0.019$^{ns\,*}$
	% Medium sand	-0.059^{ns}	-0.098^{ns}	0.028^{ns}	-0.011^{ns}
	% Coarse sand	-0.126*	-0.101^{ns}	-0.113*	0.017^{ns}
	% Gravel	0.073^{ns}	0.069^{ns}	0.049^{ns}	-0.031^{ns}
	Pot. Redox (mV)	0.193***	0.163***	0.159**	0.044*
	% org mat	0.035^{ns}	0.008^{ns}	0.064^{ns}	-0.042^{ns}
	pH	-0.171**	-0.180**	0.087^{ns}	-0.036^{ns}

7.4.- Discussion.

Despite the fact that *A. latreillii* has sometimes been described as a species tolerant to disturbance (Borja *et al.*, 2000; Marín-Guirao *et al.*, 2005; Simboura and Reizopoulou, 2007), it did not respond to a source of sewage pollution keeping or increasing its densities. Our results clearly showed that the presence of sewage outfalls adversely affected *A. latreillii* populations. The presence of a sewage disposal outlet produced a decrease in population densities, indeed values as low as zero were recorded in some of the sampling sites. However, this effect was reduced if waste water was treated to a higher level prior to being discharged, as observed at the only location where biological treatment of activated sludge took place.

Apseudopsis latreillii was included in lists of tolerant species (GIII in AMBI, Borja *et al.*, 2000; GII in BENTIX Simboura and Zenetos, 2002), using biotic indices based on the classification of species (or groups of species) in several ecological groups representing specific levels of sensitivity to disturbance. It was also reported as being tolerant to metal contamination caused by old mining activities in Marín-Guirao *et al.* (2005), and was reported in high density in Ceuta harbour (Guerra-García *et al.*, 2003). However, *A. latreillii* individuals were also obtained in areas with little human activity that had been classified, using previously cited index, as having good or high ecological status, in muddy sands in S. Evvoilos coast (Aegean Sea, Greece) (Simboura and Reizopoulou, 2007) and in fine-sand communities in Bou Ismail Bay, on the Algerian coast (Bakalem *et al.*, 2009).

Moreira *et al.* (2008) and Lourido *et al.* (2008) found remarkable increases in numbers of individuals in sandy sediments in Galician rías (NW Spain), which are not apparently related to organic input. *Apseudopsis latreillii* was also reported as being a non-tolerant species in other studies. Bouchet and Sauriau (2008) studying influence of oyster culture in Pertuis Charentais (SW France) found tanaidacea individuals only in the reference station. Similarly, in Sanz-Lázaro and Marín (2006), *A. latreillii* was one of the best represented taxa in the reference station and in the impacted station following fishfarm abatement.

Regarding length distribution, whilst all size classes showed sensitivity to sewage pollution, differences between locations were detected. As such, larger mature individuals disappeared in outfalls with higher flows, whereas an increase of bigger individuals was detected the last year in the location with the lowest flow and secondary treatment. Therefore sewage inputs could limit the maximum potential size attainable in species *A. latreillii* since body size is often smaller in stressed environments (Barnes, 2005). Moreover, smaller juvenile individuals seemed to have a high sensitive pattern. *Apseudopsis latreillii* is a subsuperficial sessile deposit feeder, abundant in the first 5 cm of sediment (de Juan *et al.*, 2007). The length of an individual is an important factor in burrowing; and immature individuals normally live near the surface. If immature individuals were more exposed to sewage pollution a higher mortality would be expected.

Sex ratio showed a decrease of the percentage of males in the outfall with the highest flow. Differences in sex ratio could be attributed to the fact that crustaceans show distinct growth rates between sexes (Low, 1978) and this could produce differences in sensitivity to pollution. Another less likely option is a de-masculinisation process (Ford, 2008). Crustacea are by default female and the expression of male secondary characteristics show degrees of plasticity influenced by environmental variables such as light and temperature (Dunn *et al.*, 2005), and even diet (Zupo and Messina, 2007). To date, it is unclear whether widespread sexual disruption of crustaceans is occurring in the wild (Ford, 2008). However, Ford *et al.* (2004) observed reduced gnathopod sizes in normal male amphipods collected from a field site categorised as contaminated, then it is possible that we were detecting a non-developed male secondary character (robuster chelipeds), although we did not observe an increase in females near the outfalls.

Changes in population density showed significant positive correlation with waste water quality. This was consistent with the fact that minor changes were detected in the location with the lowest flow, and the only site where biological treatment of activated sludge takes place, and also where water quality parameters of sewage disposal showed the best values.

Parameters of sediment that showed correlations with tanaid abundances were potential redox and pH. Both could indicate that *A. latreillii* may be sensitive to hypoxia in sediment, a factor which is reflected by negative potential redox and acidification sediment. In general, hypoxia causes mortality in many invertebrates, and crustaceans are especially sensitive to this lack of oxygen (Sánchez-Moyano and García-Gómez, 1998; Gray *et al.*, 2002; Sánchez-Moyano *et al.*, 2002; Guerra-García and García-Gómez, 2006). Furthermore, oxygen availability could limit the growth of crustacean, suggesting that that size was governed by water oxygen content in the same way as it is in amphipods (Chapelle and Peck, 2004).

Another aspect that must be taken into account is sediment granulometry. *A. latreillii* is abundant in the sediments with dominance of fine sands as presented in our study area (Marín-Guirao *et al.*, 2005; Bouchet and Sauriau, 2008; Lourido *et al.*, 2008; Moreira *et al.*, 2008; Bakalem *et al.*, 2009). Despite the fact that granulometry of the sediment did not show variability related to the presence of sewage outfalls (see chapter 2) negative correlation of tanaid abundances with coarse sand percentage was detected. Sediment type defines habitats, and changes in grain sizes could produce high variations in infaunal species whose distribution is closely correlated with certain types of sediment (Snelgrove and Butman, 1994; Bishop, 2005). Moreover, peracarids display the ability to search actively for preferred sediments, a factor which may be related to food availability and the ability to burrow (Oakden, 1984; Moreira *et al.*, 2008). However since sediments only showed punctual changes and no relation is observed between grain size and sewage discharge, this correlation could reflect the variability related to natural small scale changes in sediment size and is unlikely to explain the pattern of abundance reduction near the outfalls.

Focusing on our results, *A. latreillii* responded as a sensitive species. We detected a decrease of density in stations closer to outfalls with a higher input of suspended solids, nutrients (nitrates and phosphates) and a possible trend to hypoxia in affected stations. Previous *A. latreillii* reported tolerance could be explained by the fact that organisms are not equally sensitive to all types of anthropogenic disturbance and are likely to respond differently to different types of perturbation (Washington, 1984) and its response to a pollution source could vary. This variability in responses produces a need for testing the tolerance/sensitivity of each species to different pollution sources prior to it being used as an indicator.

CHAPTER 8

Effect of sewage discharge in *Spisula subtruncata* (da Costa, 1778) populations

Efecto del vertido de aguas residuales en poblaciones de *Spisula subtruncata* (da Costa, 1778)

Abstract. *Spisula subtruncata* is a dominant species in structuring the medium to fine sand macrobenthic communities from Western Mediterranean, particularly during the spring and summer months when its abundance increase. Moreover it is generally considered a major food source for demersal fishes. This bivalve is common in the five locations affected by sewage discharge along the Castellon coast (Western Mediterranean). In this chapter, *Spisula subtruncata* (da Costa, 1778) populations were analysed in order to detect changes related to outfall presence. We detected that *Spisula* populations are affected by sewage discharges. In the stations near the outfalls, the abundance decrease and the average size of *S. subtrunc*ata is lower due to a disappearance of larger individuals. Although further research could be needed to understand the factors that produce observed trends, decreased abundance and size of this species could be used as an indicator of pollution levels

Resumen. *Spisula subtruncata* es una especie dominante en las comunidades de arenas medias a finas del Mediterráneo occidental, especialmente en primavera y verano, cuando es muy abundante. Además es considerada la mayor fuente de alimento para peces demersales que habitan en estas comunidades. Esta especie de bivalvo es muy común en las cinco localidades de la costa de Castellón (Mediterráneo occidental). En este capítulo, se estudia la población de *S. subtruncata* para detectar posibles cambios debido a la presencia de los vertidos. Se detectó un efecto de lo vertidos sobre la población de *Spisula*. En las estaciones más cercanas a los emisarios, la abundancia disminuyó y la talla media de *Spisula subtruncata* fue menor debido a la desaparición de individuos más grandes. Aunque nuevos estudios podrían ser necesarios para entender mejor estos cambios, el descenso de la abundancia y de la talla de esta especie puede ser empleado como indicador del nivel de contaminación.

8.1.- Introduction.

The molluscan epifauna has been successfully used as a bioindicator of coastal environmental conditions in Southern Spain (Sánchez-Moyano *et al.*, 2000). Filterfeeding bivalves, such as oysters (*Saccostrea commercialis*), cockles (*Anadara trapesium*), or mussels (*Mytilus edulis*), can accumulate contaminants (Ajani *et al.*, 1999), and the concentrations of these compounds in the fresh tissues of aquatic organisms are present at several orders of magnitude higher than those found in the environment (bioconcentration) (Corsi *et al.*, 1992). Concentrations are also amplified up the food web (Oliver and Niimi, 1988). This causes planktivorous siphon feeders, such as surfclams, to be especially affected by sewage outfalls.

Spisula subtruncata (da Costa, 1778) is a common shallow-burrowing bivalve that lives in coastal areas of Europe with a distribution from Norway to the Mediterranean and the Atlantic coast of Morocco (Tebble, 1966). It is generally considered a major food source for demersal fishes because of its high growth rate and numeric dominance in important fishing grounds as well as its importance as a dominant species in structuring the macrobenthic community during the spring and summer months (Fraschetti *et al.*, 1997). The habitat of this species (sandy bottoms between 2 and 30 m) represents an environment in which the concentration of suspended particulate matter (i.e., seston) may be variable in time as a result of resuspension of fine sediments during periods of high-current velocity, wind–wave activity, and storm events, especially in shallow-bottom habitats (Smith, 1994). Such habitats represent dynamic environments, with variations in temperature, seston concentration, and food quantity and quality (Rueda and Smaal, 2004). The life span of *S. subtruncata* consists in a massive spring and summer recruitment, a massive fall and winter mortality, and strong seasonal and interannual fluctuations of density and biomass (Ambrogi and Occhipinti, 1985; Fraschetti *et al.*, 1997; Albertelli *et al.*, 2001).

Several investigators have studied aspects of the biology of the genus *Spisula*, especially *S. solidissima*, an Atlantic surfclam of commercial interest. Larval settlement of surfclams depends on larval supply, the near-bottom hydrodynamics (Hongguang, 2002), and sediment grain size (Snelgrove *et al.*, 1998). The growth and survival of this bivalve depends on density (Wagner, 1984; Weinberg, 1998), temperature (Ambrose *et al.*, 1980; Cerrato and Keith, 1992; Weissberger and Grassle, 2006), salinity (Cerrato and Keith, 1992), dissolved oxygen (Wagner 1984; Weinberg and Helser, 1996), distance from shore (Jones *et al.*, 1978; Ambrose *et al.*, 1980; Wagner, 1984), latitude (Weinberg and Helser, 1996), and seston quantity and quality (Rueda and Smaal, 2002). The presence of a domestic outfall could affect these variables, thus causing changes in the bivalve population.

Medium- to fine-sand *S. subtruncata* communities characterize shallow sublittoral soft bottoms in the northwest Mediterranean Sea (Pérès and Picard, 1964; Cardell *et al.*, 1999; Sardá *et al.*, 1999, 2000), and these communities are common off the coast of Castellon (northeastern Spain). In this study, we examined the possible impact of the five sewage outfall sites on *S. subtruncata* populations along the Castellon coast.

8.2.- Materials and Methods.

In this chapter, we analysed the *Spisula subtruncata* populations of the five locations affected by sewage outfalls from Castellon coast (NE Spain) (locations I to V, figure 2.1). This homogeneous area with an established pollution gradient represents an ideal site for investigating links between macrofaunal assemblages and the effect of contaminants (de-la-Ossa-Carretero *et al.*, 2009). The study area, sewage outfalls and sampling methods were previously described in chapters 2 and 4.

S. subtruncata and the other Bivalvia individuals that were alive on sampling were shorted and counted from samples collected during the month of July from 2004 to 2008. *S. subtruncata* individuals collected in 2004 and 2005 were measured (anterior–posterior length) using the image manager software Leica IM50 after being photographed through a Leica 9.50-Mz stereomicroscope.

Non-parametric multivariate techniques were used to compare the bivalvia composition. All multivariate analyses were performed using the PRIMER version 6 statistical package (Clarke and Warwick, 1994). Triangular similarity matrices were calculated through the Bray-Curtis similarity coefficient using mean annual abundance values, in order to cluster stations according to sewage effect, regardless of temporal variability. The values were previously dispersion weighted in order to reduce “noise” produced by species with an erratic distribution, and whose abundance indicates a great variance between replicates (Clarke *et al.*, 2006). A graphical representation of multivariate patterns of bivalvia assemblages was obtained by non-metric multidimensional scaling (nMDS). Similarity percentage analysis (SIMPER) of abundances was used to determine the species with a higher percentage of dissimilarity between stations.

Abundances of *S. subtruncata* were examined using three-factor analyses of variance (ANOVA) with distance and location as fixed factors, and year as ramdom. Before ANOVA, homogeneity of variance was tested using Cochran's test (Winer, 1971). Post hoc identification of differences between means was done using Student-Newman-Keuls (SNK) tests (Snedecor and Cochran, 1989).

Because it was not possible to use ANOVA with the bivalve length data, because there were significant differences in sample size, we used a nonparametric Kruskal-Walllis test with distance as factor. Size-frequency distributions were grouped and plotted using a 0.5-mm shell length class interval.

8.3.- Results.

nMDS plots of the mean annual abundances of bivalves showed a segregation of the stations, from the sites closest to the outfalls on the right hand side to those furthest away on the left hand side (figure 8.1).

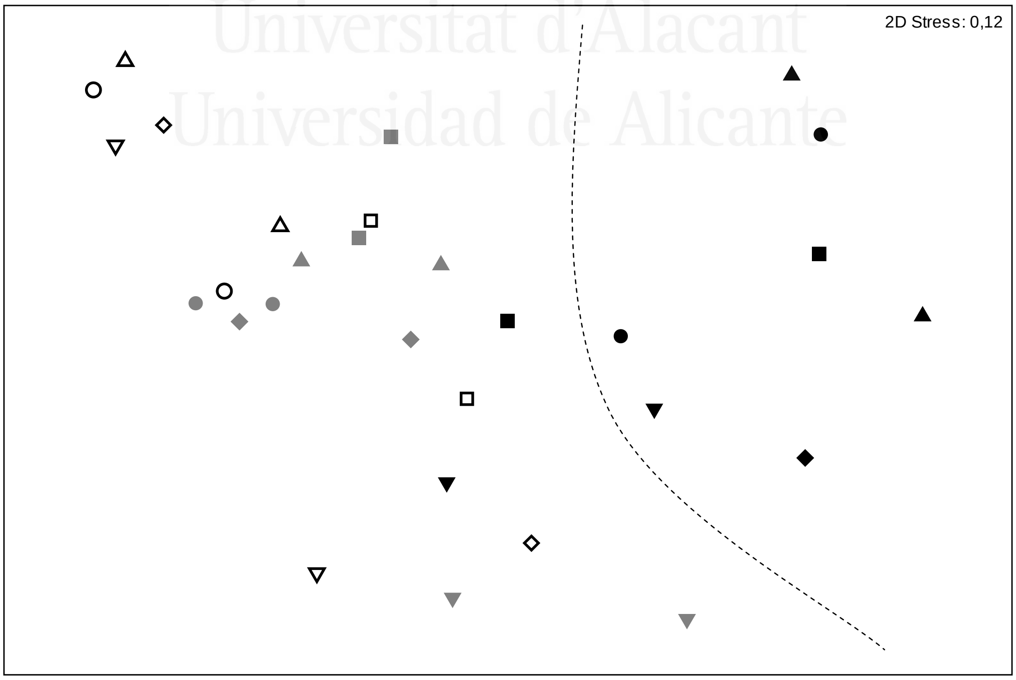


Figure 8.1. nMDS ordination of annual mean bivalvia abundance (indiv./m^2) and associated stress value. Differentiating location (I: ▼, II: ●, III: ♦, IV: ▲ and V: ■) and distance to the outfall (black: 0, grey: 200 and white: 1000 m).

The SIMPER routine indicated that contribution to the average Bray-Curtis dissimilarity between distances to outfall was mainly due to the bivalves: *Spisula subtruncata, Tellina spp, Corbula gibba* and *Loripes lacteus.* Among these taxa, *Spisula subtruncata* was highly dominant representing close to 90% of total abundance (figure 8.2).

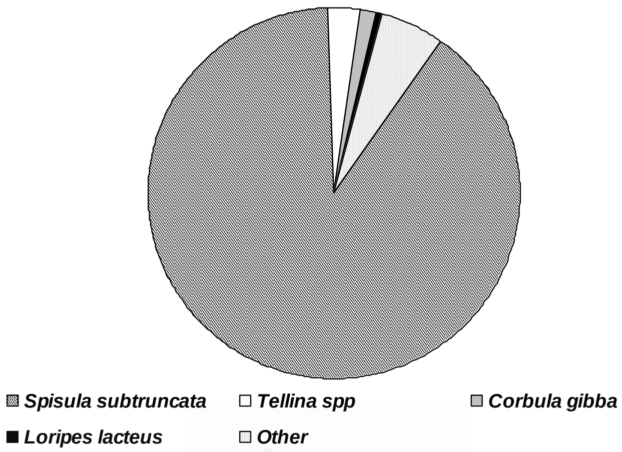


Figure 8.2. Percentage of total abundance of more abundant Bivalvia species.

The population density of *S. subtruncata* varied from 3462.5 individuals/m^2 at 1000 m to the outfall in location IV to 4.16 individuals/m^2 at 0 m to outfall of location I. ANOVA detected significant differences in *S. subtruncata* abundances for the interactions between the three factors (table 8.1). Despite the fact that lower abundances could be observed in stations close to the outfall (figure 8.3), SNK only detected significance difference for factor distance (location x year) in some cases, year 2007 in location I, II and III and year 2005 in location IV.

Table 8.1. Results of ANOVA for abundance (individuals/m^2) of *Spisula subtruncata* for the factors distance (0, 200, 1000 m), location (I, II, III, IV and V) and year (2004, 2005, 2006, 2007, 2008) RES = Residual, df: degrees of freedom, MS= mean square, F of each factor =MS factor/MS residual because all the factors are orthogonal. Levels of significance: ns no significant difference, *p < 0.05, **p < 0.01 and ***p < 0.001.

Source	Df	MS	F	P
Distance	2	18248804.2	4.47	*
Location	4	1096186.11	0.32	ns
Year	4	8051022.92	10.59	***
Distancexlocation	8	1335248.61	0.88	ns
Distancexyear	8	4078825	5.36	***
Locationxyear	16	3415617.53	4.49	***
Distancexlocationxyear	32	1514661.81	1.99	**
RES	375	760344.722		
TOTAL	449			

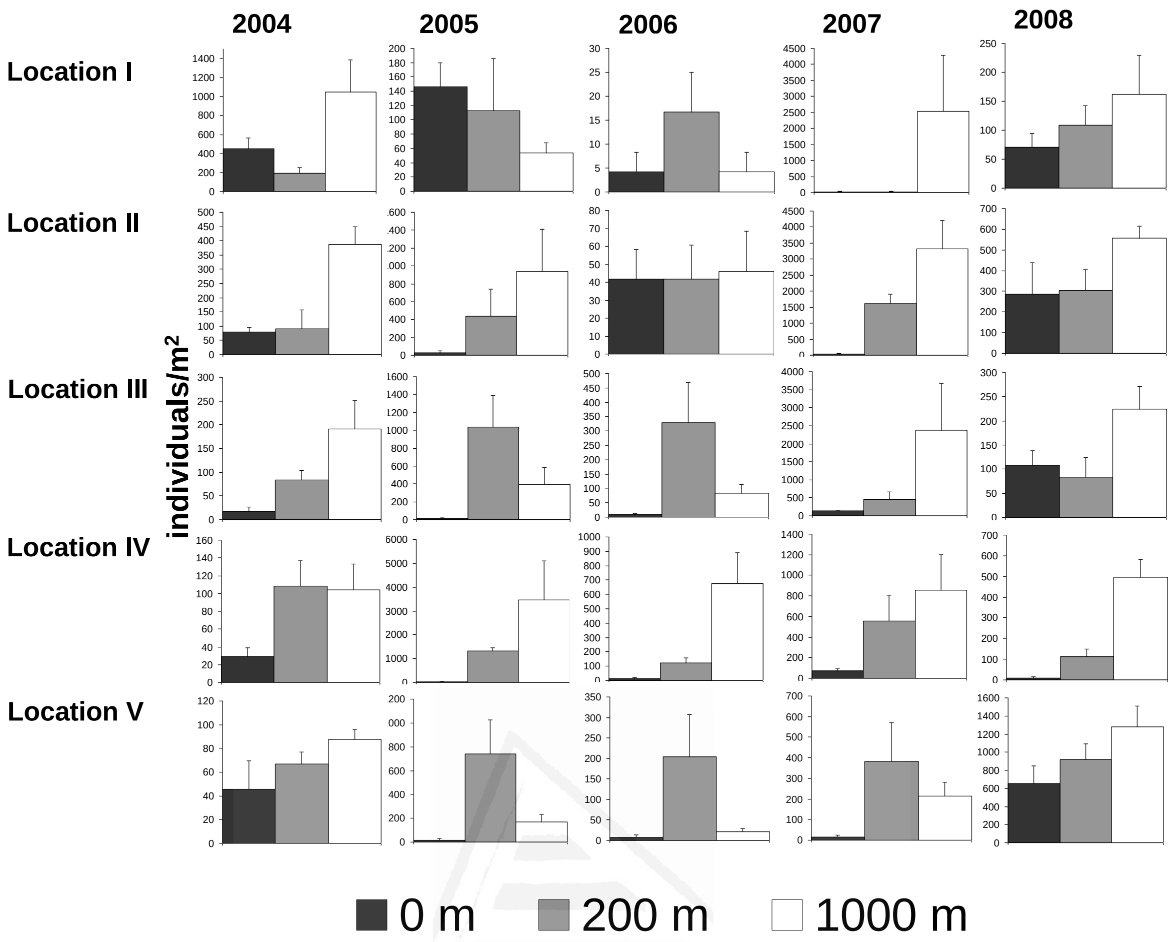


Figure 8.3. Mean and standard error of *Spisula subtruncata* abundances at each location (I to V), year (2004, 2005, 2006, 2007 and 2008) and distance (0, 200 and 1000 m).

Mean length of the 2485 specimens captured in 2004 and 2005 was 7.71 mm (range 1.04 to 13.05 mm). Kruskal-Wallis test results showed significant differences in distance at locations II (Benicarló) and III (Peñíscola) in both years, locations IV (Alcossebre) and V (Torreblanca) in 2005, and location I (Vinaroz) in 2004 (table 8.2).

In most of the cases, when significant differences appeared, smaller individuals were found near the outfall, except in the cases of locations I (Vinaroz) and III (Peñíscola) in 2004 (figure 8.4). Size distributions also reflected the absence of individuals >8 mm in the vicinity of the outfalls in all of the locations studied (figures 8.4 and 8.5).

Table 8.2. Mean and standar desviation of *Spisula subtruncata* length at each locality (I to V), year (2004 and 2005) and distance (0, 200 and 1000 m). Results of Kruskal-Wallis Test for the factor distance in each area and year. Levels of significance: ns no significant difference, *p < 0.05, **p < 0.01 and ***p < 0.001.

		Kruskal-Walli Test	
Location	**Year**	**Statistic**	**p**
I	2004	16.20	***
	2005	0.06	ns
II	2004	9.09	*
	2005	18.04	***
III	2004	25.50	***
	2005	10.70	**
IV	2004	4.18	ns
	2005	98.05	***
V	2004	1.80	ns
	2005	11.74	**

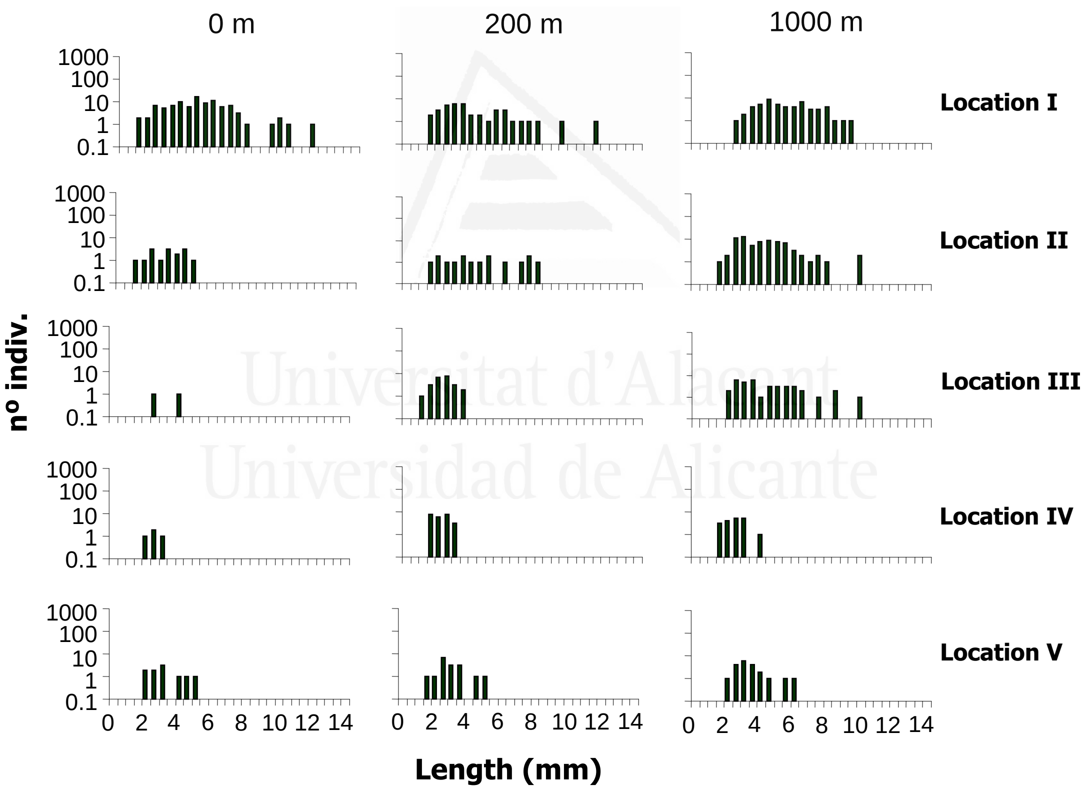


Figure 8.4. Size distribution of *Spisula subtruncata* in 2004 for each locality (I to V) and distance (0, 200 and 1000 m). All bars are plotted at the midpoint of the 0.5 mm class intervals.

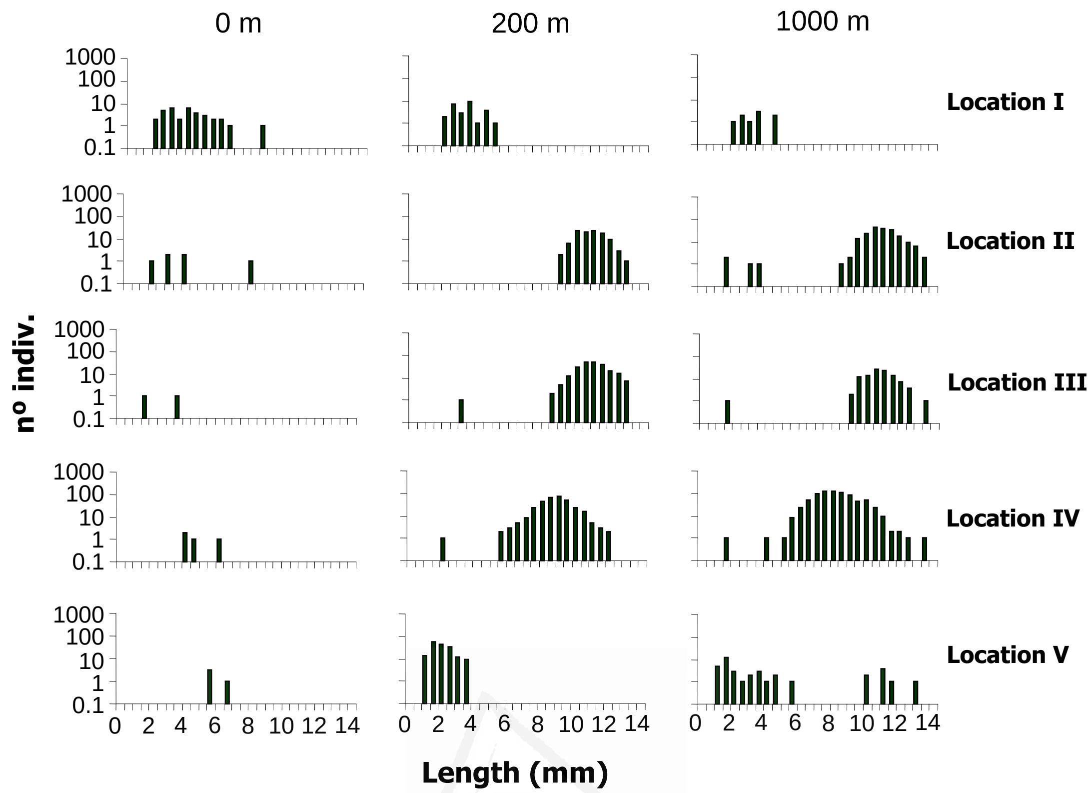


Figure 8.5. Size distribution of *Spisula subtruncata* in 2005 for each locality (I to V) and distance (0, 200 and 1000 m). All bars are plotted at the midpoint of the 0.5 mm class intervals.

8.4.- Discussion.

The presence of domestic outfalls affects *S. subtruncata* populations. Although, differences among distances were only statistically detected some years or some locations, abundance decreased and individuals were usually smaller in outfall stations. While, settlement was higher and sizes were larger at stations farther from the outfall, reflecting higher survival rates. Specimens were collected just after recruitment, which occurs in spring and summer (Albertelli *et al.*, 1994). Decrease in abundance near outfalls can be attributed to a low larval supply or a high mortality rate in settlement events. The lack of adults in stations closer to the outfall can be attributed to a higher postsettlement mortality.

The presence of sewage discharge changes environmental characteristics that may affect *Spisula* populations. Changes in grain size or percentage of organic matter in sediments influence bivalve populations; *S. solidissima* larvae appear to be capable of sediment selection. It has been observed that the highest abundances of adults occurred in sandy habitats, and low numbers occurred in muddy areas (Snelgrove *et al.*, 1998). The presence of allochthonous sources (wastewaters) may influence the distribution of organic materials in sediments (Cotano and Villate, 2006) and could produce an increase of mud near an outfall (Martínez and Adarraga, 2003). However, in the study area, these physical characteristics (e.g., granulometry, percent of organic matter, pH) of the sediment did not show variability related to the presence of sewage outfalls. Although sediment does not reflect an increase of the organic matter, sewage outfall discharge content has a high concentration of suspended POM (Smith and Shackley, 2006). This directly affects filter feeders (Grant *et al.*, 1997; Riisgard and Larsen, 2000) such as *S. subtruncata*, causing physiologic changes in filtration, absorption, and rejection rates (Rueda and Smaal, 2002).

Sewage outfall discharges also may contain toxic substances. In 1980, sewage sludge in Boston, MA, was found to contain 55 ppm silver (dry weight), approximately 1000 times higher than is found in uncontaminated fine-grained marine sediments. Silver concentrations in sewage particles are related largely to the use and discharge of silver by the photography, electronic, and electroplating industries (Bothner *et al.*, 2002). Bivalves, such as *Mytilus galloprovincialis*, accumulate contaminants such as heavy metals and hydrocarbons (Widdows and Donkin, 1992; Wade *et al.*, 1998). This exposure to concentrations of dissolved metals decreases the survival of filter-feeding bivalves (McGreer, 1979, 1982; King *et al.*, 2004). Though, it was not detected metals in stations close to outfalls (chapter 2), in addition, discharge could also contain other contaminants -such as acid-volatile sulphide, pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and sulphides- the concentrations of which could also have an adverse effect on the survival of bivalves. Thus far, we have no data related to the distribution of pollutants around the studied outfalls, and it will be necessary to ascertain if the observed decrease in *S. subtruncata* near the sewage outfall is related to an enrichment of pollutants.

In summary, *S. subtruncata* is affected by the sewage outfalls that produce decreased bivalve settlement and increased bivalve mortality. Although further research could be needed to understand the factors that produce observed trends, decreased abundance of this species could be used as an indicator of pollution levels in *S. subtruncata* communities even though interannual changes made finding a clear response difficult.

DISCUSIÓN GENERAL Y CONCLUSIONES

General discussion and conclusions

CAPÍTULO 9

Discusión general

La protección de los ambientes marinos costeros requiere una correcta evaluación de su estado ecológico, especialmente en áreas afectadas por impactos antropogénicos, como son los vertidos de aguas residuales urbanas. Esta evaluación es necesaria para valorar la integridad ecológica, comprobar si se está produciendo una degradación significativa, identificar la extensión y localización de esta degradación y determinar las causas para establecer que medidas correctoras hay que aplicar (Borja y Dauer, 2008). Para esta valoración se pueden emplear tanto indicadores fisicoquímicos como biológicos (Borja *et al.*, 2008), sin embargo debido a la complejidad de los ecosistemas acuáticos marinos, determinar su estado ecológico no resulta una tarea sencilla.

El término integridad ecológica implica que el ecosistema debe tener la capacidad de mantener y sostener una comunidad de organismos de forma balanceada, integra y adaptada, y con una composición específica, diversidad y organización funcional comparable a un ecosistema similar en buen estado de la misma región (Karr y Dudley, 1981). De modo que el análisis de parámetros abióticos resulta incompleto a la hora de evaluar el efecto del vertido de aguas residuales urbanas sobre esta integridad. Los parámetros fisicoquímicos estudiados en este trabajo no fueron afectados por la presencia del vertido, la gran cantidad de posibilidades: enriquecimiento orgánico, metales pesados, PCBs, PAHs, amonio, esteroides… complica encontrar la variable o combinación correcta de variables afectadas por este tipo de vertidos. De hecho, entre los parámetros abióticos estudiados el único que respondió a la presencia del vertido, potencial redox, depende de un proceso biológico como es la descomposición de compuestos orgánicos. De modo que el empleo de indicadores basados en la respuesta de la biota son más adecuados para evaluar el efecto de este tipo de vertidos (Hering *et al.*, 2010), siendo esencial medir la integridad biológica para valorar esta integridad ecológica (Borja y Dauer, 2008). Es necesario desarrollar diferentes indicadores biológicos que permitan una correcta descripción o clasificación del estado del ecosistema, así como determinar la extensión y el efecto de los impactos antrópicos sobre su integridad ecológica.

En este trabajo se ha analizado el empleo de las comunidades bentónicas como herramienta de evaluación de impactos generados por aguas residuales urbanas, tema que a pesar de haber sido ampliamente discutido en la literatura (Dauer y Conner, 1980; Mearns y Word, 1982; Chapman *et al.*, 1995; Ajani *et al.*, 1999; Bellan *et al.*, 1999; Cardell *et al.*, 1999; Elias *et al.*, 2005; Borja *et al.*, 2006; Smith y Shackley 2006) todavía mantiene ciertos problemas. Establecer un bioindicador adecuado que permita su uso de manera generalizada es un objetivo muy complicado o incluso imposible de alcanzar. De hecho, implantar programas estándar de seguimiento ambiental es todavía un reto, ya que los empleados hasta ahora presentan una alta variabilidad entre ellos (Hering *et al.*, 2010).

9.1. Características de un bioindicador

Hall y Grinnel (1919) fueron de los primeros autores en utilizar el concepto de indicador biológico o bioindicador, asociando especies de plantas y animales a regiones con una estructura y composición particular. Desde entonces el término bioindicador se ha utilizado en un amplio rango de situaciones, como son describir el estado del sistema (Walz, 2000), analizar cambios medioambientales (McGeoch, 1998), evaluar riesgos medioambientales (Suter, 2001) o establecer objetivos medioambientales (Van Hoey *et al.*, 2010).

Blandin (1986) define el termino bioindicador como un organismo o grupo de organismos que permiten caracterizar el estado de un ecosistema basándose en variables bioquímicas, citológicas, fisiológicas, etológicas o ecológicas. En lo que se refiere a evaluación de impactos podemos considerar que un bioindicador es todo organismo o sistema biológico utilizado para apreciar una modificación del medio o del ecosistema (Iserente y de Sloover, 1976; Lebrún, 1981). Por lo que dependiendo de la situación, un indicador puede ser una comunidad, un poblamiento, una única especie o una porción de organismo (Occhipinti-Ambrogi y Forni, 2004)

Entre los diferentes bioindicadores o indicadores biológicos utilizados para la evaluación de impactos se encuentran los taxones indicadores: especies u otros grupos taxonómicos que son considerados representativos de la biodiversidad total de un hábitat particular (McNally y Fleishman, 2002) y en los que parámetros como la densidad, tasa de supervivencia, presencia o ausencia son empleados como medidas del estado del ecosistema (Hilty y Merenlender, 2000; Goodsell *et al.*, 2009). Sin embargo, aunque el empleo de taxones indicadores se integra normalmente en estudios de monitoreo, emplear un taxón u otro puede resultar problemático, debido a la necesidad de elegir un indicador sencillo de aplicar que represente el estado del ecosistema y permita diferenciar las variaciones provocadas por el hombre de las variaciones naturales. En el desarrollo de esta tesis hemos evaluado el efecto del vertido de aguas urbanas sobre distintos componentes de la comunidad bentónica, de modo que su respuesta a este tipo de contaminación posibilita su empleo como bioindicadores en trabajos de seguimiento y control ambiental de estos vertidos.

9.2. Macroinvertebrados bentónicos: ¿son buenos bioindicadores?

A la hora de adoptar un bioindicador como adecuado es importante conocer las limitaciones de cada componente y determinar en que medida nos puede ayudar a evaluar el estado de integridad ecológica de una zona. Alfsen y Sæbø (1993) establecen que un indicador, puede simplemente proporcionar datos que nos ayuden a interpretar los cambios que se están produciendo en un ecosistema. De esta forma, cualquier componente que responda a una perturbación puede servirnos para evaluar su efecto sobre la integridad ecológica.

Aunque los macroinvertebrados bentónicos, debido a su capacidad de respuesta ante cambios en el ecosistema, son considerados como uno de los bioindicadores más adecuados para el seguimiento ambiental en el medio marino, en ocasiones no cumplen los requisitos necesarios para su utilización. El desarrollo de metodologías y herramientas adecuadas para detectar y valorar un impacto antropogénico debe de estar sujeto a una correcta validación en áreas donde se conozca la presencia de un foco de contaminación, como la estudiada en este trabajo.

Es necesario establecer una relación causal entre contaminante y bioindicador mediante estudios con un adecuado nivel de replicación espacial y temporal (Goodsell *et al.*, 2009). Es esencial comparar las estaciones afectadas con varios controles, ya que en el caso de emplear un único control la variabilidad entre estaciones no pueden atribuirse inequívocamente a un impacto (Underwood, 1992; Chapman *et al.*, 1995). Un impacto sólo puede ser demostrado cuando la diferencia observada entre las áreas supuestamente impactadas y las no impactadas es mayor que la diferencia entre distintas áreas no impactadas (Chapman *et al.*, 1995). La respuesta de un indicador puede variar a lo largo de una escala espacial y temporal, esto supone que la búsqueda de controles hay que realizarla tratando de disminuir ciertas fuentes de variabilidad. Pero, aunque se demuestre asociación entre un taxón y la presencia de contaminante, el taxon no puede emplearse como indicador de niveles de contaminación fuera de los sitios donde esta relación ha sido establecida (Goodsell *et al.*, 2009). Es necesario implantar programas de monitoreo con los suficientes muestreos que permitan estimar el error provocado por estas variaciones naturales y reducirlo (Hering *et al.*, 2010). Si existe un presión localizada y podemos establecer áreas con distinto grado de impacto, es posible diferenciar los efectos de origen antrópicos producidos en un bioindicador (Van Hoey *et al.*, 2010).

En este trabajo los distintos componentes analizados, (estructura de la comunidad bentónica, índice BOPA, poblamiento de anfípodos, población de *A. latreillii* y población de *S. subtruncata*) muestran diferencias en las estaciones situadas a 0 m que no fueron detectadas en las estaciones situadas tanto a 200 m como a 1000 m del punto de vertido. De modo que son susceptibles de ser empleados como bioindicadores para evaluar el efecto del vertido de aguas residuales urbanas en comunidades de arenas finas del Mediterráneo occidental.

Entre los distintos componentes susceptibles de ser utilizados como bioindicador, a la hora de elegir uno u otro hay que tener en cuenta distintos aspectos (Johnson *et al.*, 1993): i) considerar el esfuerzo que supone su empleo, que sea sencillo de cuantificar; ii) conocer adecuadamente su nivel de sensibilidad o tolerancia al impacto que queremos evaluar; iii) controlar la variabilidad natural espacial y temporal.

9.2.1.- Complejidad del indicador.

Un indicador tiene que ser sencillo y de fácil manejo, y no debe requerir técnicas o trabajos muy especializados. Es necesario considerar el esfuerzo de trabajo que requiere el empleo de un bioindicador, de modo que en el caso del estudio de las comunidades bentónicas es imprescindible tener en cuenta el tiempo requerido para el procesado de las muestras. Algunos grupos taxonómicos son complicados de identificar y más en la actualidad, cuando debido al desarrollo de nuevas técnicas microscópicas, genéticas y filogenéticas, la taxonomía está en un proceso dinámico en el que continuamente se describen un gran número de nuevas especies; se detectan sinonimias y se pone en duda la clasificación de algunas especies en niveles taxonómicos superiores (Dauvin, 2005).

Este problema se incrementa si no existen listas de especies para la región dónde se realiza el estudio. Es esencial realizar estudios taxonómicos exhaustivos con el fin de establecer la distribución de cada especie, y facilitar de esta manera su identificación cuando sea necesario. En este sentido, hay que resaltar el caso del orden Amphipoda. Como hemos visto, en la zona del Mediterráneo español, los estudios del orden Amphipoda en comunidades fondos blandos son escasos, y prueba de ello es el resultado del trabajo taxonómico realizado en esta tesis (capítulo 5), dónde analizando únicamente comunidades de arenas finas se identificaron varias citas nuevas e incluso una nueva especie de *Medicorophium, M. longisetosum* sp. nov. (Myers *et al.*, 2010), la cual es relativamente abundante a lo largo de toda la costa este del Mediterráneo española.

Una identificación correcta a nivel de especie requiere de taxónomos especialistas y de un tiempo que no se tiene a la hora de realizar ciertos estudios de seguimiento ambiental. Una posibilidad de reducir este esfuerzo, es focalizar el estudio en ciertas especies, denominadas centinelas, cuya abundancia o presencia sean descriptivas del estado ecológico. Aunque el empleo de estas especies requiera una identificación inicial, al tratarse de una sola especie el esfuerzo taxonómico se reduce considerablemente. Otra posible solución a las dificultades taxonómicas, que permite obtener resultados con un análisis relativamente sencillo, es el principio de Suficiencia Taxonómica (Ellis, 1985). Si la abundancia y composición al nivel taxonómico estudiado difiere entre zonas contaminadas y no contaminadas, poca información se pierde mediante la identificación a altos niveles taxonómicos (Ferraro y Cole, 1990). En nuestro caso hemos visto como incluso analizando la comunidad a niveles de Clase y Orden, el impacto del vertido de aguas residuales puede ser detectado e, incluso, se puede llegar a diferenciar entre el nivel de tratamiento y caudal. Así la sensibilidad de grupos de crustáceos como anfípodos y tanaidáceos, y la respuesta, en algunos casos oportunista, de los poliquetos permiten evaluar el impacto de un vertido empleando abundancias de grupos taxonómicos. Este análisis llega a dar resultados más concluyentes que el estudio de algunos parámetros físicos que no permiten detectar el efecto de vertidos en zonas como la estudiada. Del mismo modo, el índice BOPA muestra una respuesta correcta a la presencia de los vertidos con un bajo esfuerzo taxonómico, permitiendo evaluar su efecto al integrar de manera combinada la sensibilidad de los anfípodos y el carácter oportunista de ciertas familias de poliquetos.

Thompson *et al*. (2003) estiman una reducción en el tiempo de procesado de las muestras de un 40% para familias, 76% para orden y 88% para clase con respecto a una identificación a nivel de especie. Utilizando dicha estimación podemos calcular el nivel de esfuerzo necesario para emplear cada una de los componentes analizados en este trabajo (localidades I, II, III, IV y V) y compararlo con el esfuerzo requerido para identificar a nivel de especie el orden Amphipoda (tabla 9.1). Por un lado, aplicando el concepto de Suficiencia Taxonómica a la comunidad reducimos el nivel de esfuerzo considerablemente, casi un 90% con respecto al esfuerzo requerido para identificar a nivel de especie el orden Amphipoda. También, en el caso del índice BOPA el nivel de esfuerzo se reduce significativamente, ya que además de no necesitar una indentificación a nivel de especie se centra únicamente en dos grupos taxonómicos. Del mismo modo, el empleo de especies centinelas reduce considerablemente el nivel de esfuerzo puesto que a pesar de tener que identificar a nivel de especie, sólo se necesita conocer un único taxón.

Tabla 9.1. Evaluación del esfuerzo taxonómico para cada uno de los componentes utilizados. Esfuerzo estandarizado: valor de esfuerzo/ valor de esfuerzo máximo. Donde valor de esfuerzo: nº especímenes x nº de taxa x nivel de esfuerzo estimado por Thompson *et al.* (2003).

		Nº de especímenes	**Nº de taxa**	**Nivel del esfuerzo (Thompson *et al.*, 2003)**	**Esfuerzo estandarizado**
Comunidad bentónica (Suficiencia Taxonómica)		68890	20	Clase: 12%; Orden: 24%	11%
Índice BOPA		11897	5	Orden: 24% Familia: 60%	3%
Orden Amphipoda		17643	44	Especie:100%	100%
Especies Centinelas	*A. latreillii*	7310	1	Especie:100%	1%
	S. subtruncata	7522	1	Especie:100%	1%

Además del esfuerzo que supone el procesado de las muestras, es importante desarrollar herramientas que permitan un análisis sencillo y fácilmente interpretable. Este aspecto se complica a la hora de evaluar los cambios producidos en una comunidad o un poblamiento, donde varios grupos taxonómicos o especies son analizados en su conjunto por medio de un análisis multivariante. En estos casos el empleo del índice de similitud de Bray Curtis, es de los análisis más adecuados (Warwick y Clarke, 1991; Clarke, 1993; Johnson *et al.*, 2008). Estos métodos son muy sensibles a los cambios de la comunidad (Warwick, 1993), sin embargo, aunque los análisis multivariantes evitan perdida de información, al incorporar un gran número de variables de la población, esto puede ser un inconveniente puesto que la variabilidad procedente del impacto antropogénico a veces no es fácilmente diferenciable de otras fuente de variabilidad (Hewitt *et al.*, 2005). Además, aunque representaciones como el nMDS nos permiten una visualización de los resultados, este análisis es más difícil de interpretar que otras herramientas desarrolladas, como son los índice bióticos. Estos índices permiten establecer el estado ecológico de la zona, integrando los resultados del monitoreo de bentos en un dato fácilmente evaluable. Su empleo es importante en la gestión ambiental puesto que proporcionan un criterio para clasificar una zona en impactada o no (Rakocinski *et al.*, 1997).

9.2.2.- Nivel de tolerancia.

El empleo de comunidad bentónicas como indicadores está basado en una clasificación de los taxa en grupos ecológicos, en base a sus estrategias ecológicas adaptativas y su respuesta sensible o tolerante a un ambiente estresado (Borja y Muxika, 2008). De modo que determinar la respuesta de cada taxón a una fuente de contaminación es esencial para posibilitar su empleo como indicador biológico.

Algunos autores determinan que un indicador debe tener ciertos requerimientos que en muchos casos son difíciles de alcanzar. Goodsell *et al.* (2009) establece que es necesario una correlación fuerte y ecológicamente importante así como una relación causal, directa y predecible entre el indicador y las variables o contaminantes que represente. Sin embargo, en el caso de las comunidades bentónicas llegar a demostrar estos requisitos es complicado. Por ejemplo, el hecho de que un taxón o una comunidad respondan a la presencia de un vertido no conlleva que se obtengan correlaciones altas con las concentraciones de contaminantes de este efluente o con las alteraciones en el medio que este contaminante pueda provocar. Parte del comportamiento de cualquier especie o grupo taxonómico está relacionado con el componente natural, incluso cuando su respuesta a la presencia de un impacto es clara.

Los indicadores analizados en este trabajo tienen capacidad para evaluar el efecto producido por los vertidos de aguas residuales urbanas, pero los niveles de correlación con aspectos como el caudal o parámetros de calidad del agua vertida pueden ser bajos en ciertas ocasiones (tabla 9.2). De hecho, el mayor coeficiente de correlación alcanzado se limita a 0.622, obtenido entre el índice BOPA y el caudal vertido. Sin embargo, la ausencia de una fuerte correlación con el contaminante no invalida el empleo de este componente como bioindicador, sino que lo limita, ya que requiere un proceso de validación previo antes de ser empleado. De modo que si su empleo puede satisfacer el objetivo del programa, como es identificar un impacto y valorar su efecto, podría ser empleado como bioindicador teniendo en cuenta sus limitaciones. Cualquier componente del ecosistema que nos permita detectar cambios en esta integridad ecológica es susceptible de ser empleado como bioindicador (Carignan y Villard, 2002). En este sentido, emplear varios descriptores puede ayudar a realizar una correcta valoración del efecto de la contaminación y diferenciar los cambios provocados por el impacto de los debidos al componente natural.

Tabla 9.2. Correlaciones entre los componentes estudiados y los parámetros de los vertidos correspondientes a las localidades I a V. Comunidad bentónica y orden Amphipoda: correlación de Spearman. Índice BOPA y especies centinelas: correlación de Pearson. Valores en negrita indican que la correlación es significativa.

	Comunidad bentónica (TS)	**Índice BOPA**	**Orden Amphipoda**	**Especies centinelas**	
				A. latreillii	*S. subtruncata*
pH	**0.291**	0.090	0.069	**-0.235**	-0.100
Caudal	**0.178**	**0.622**	**0.164**	**-0.300**	0.246
S.S. (mg/l)	**0.172**	**0.381**	0.037	**-0.377**	0.11
DBO (mg/l)	0.091	0.156	0.01	**-0.269**	0.266
DQO	0.132	**0.288**	0.011	**-0.334**	0.244
Nt	**0.135**	0.057	0.05	**-0.251**	0.359
Pt	**0.213**	**0.525**	0.011	**-0.349**	0.192
Turb.	0.08	**0.366**	0.018	**-0.295**	0.288

Aunque el análisis de la comunidad bentónica a nivel taxonómico alto, así como el índice BOPA son bioindicadores que responden a la presencia de los vertidos de aguas residuales urbanas, emplear niveles taxonómicos altos puede generar ciertos problemas y no ser siempre el método más adecuado a la hora de establecer la sensibilidad o tolerancia de un grupo taxonómico a la contaminación. Cuando sea posible, es importante utilizar niveles taxonómicos bajos antes de emplear el concepto de Suficiencia Taxonómica para minimizar la posibilidad de incluir especies inapropiadas como bioindicadores (Hilty y Merenlender, 2000). Por ejemplo, la generalización de que el orden Amphipoda es sensible a la contaminación es en gran medida cierta en el caso de la comunidad de arenas finas estudiada en este trabajo, ya que la mayoría de las especies analizadas mostraron una respuesta negativa a la presencia del vertido. Sin embargo, ciertas especies de anfípodos pueden mostrar una respuesta tolerante o incluso oportunista, *Ampelisca brevicornis* se ve favorecida por la presencia de los vertidos en algunas de las localidades; otras especies pueden considerarse indiferentes al presentar una amplia valencia ecológica, como *Siphonocetes sabiateri* cuya respuesta no se relaciona con la presencia del vertido. Esta diferencia en la sensibilidad podría provocar conclusiones erróneas en la evaluación de un área, en el caso hipotético de que una de las especies fuese dominante o muy abundante. Por lo que es necesario tener en cuenta estas situaciones para evitar posibles errores.

En otras ocasiones, es complicado determinar el grado de sensibilidad de una especie a la contaminación. Una especie puede mostrar diferente tipo de respuesta dependiendo del tipo de contaminación o el hábitat que estemos estudiando (Grèmare *et al.*, 2009). Un ejemplo muy notorio de la necesidad de establecer el grado de sensibilidad de una especie para el área y el tipo de contaminación a evaluar es el de la especie de tanaidáceo *Apseudopsis latreilli*. Esta especie debido a su abundancia, es importante en la comunidad analizada. Aunque en el área estudiada mostró una alta sensibilidad a la presencia de los vertidos, esta especie ha sido considerada por algunos autores como tolerante u oportunista (Borja *et al.*, 2000; Marín-Guirao *et al.*, 2005; Simboura y Reizopoulou, 2007).

Generalizaciones erróneas en el tipo de respuesta de una especie o componente taxonómico a la presencia de un impacto puede provocar que la evaluación de este no sea la correcta, de modo que dependiendo del grado de sensibilidad el resultado de la evaluación puede variar de un bioindicador a otro. Por lo que se refiere a los distintos componentes aplicados a las zonas afectadas por el vertido de aguas residuales urbanas, observamos como todos los indicadores presentan cierta sensibilidad a los vertidos. Sin embargo, a la hora de establecer el nivel de degradación entre los distintos emisarios, existen ciertas diferencias entre las distintos componentes empleados (tabla 9.3).

Los distintos elementos estudiados establecen que las estaciones cercanas al emisario de la localidad de Benicarló son las más impactadas, el emisario de esta localidad vierte el mayor caudal de un efluente cuyo tipo de depuración se limita a un pretratamiento.

Únicamente la población de *S. subtruncata* refleja una mayor degradación en las estaciones cercanas al emisario de Alcossebre, donde a pesar de que el efluente es también únicamente pretratado, el volumen de vertido es inferior. En el caso opuesto, a la hora de valorar las estaciones cercanas al emisario de Torreblanca, todos los indicadores coinciden en que son las menos afectadas, este emisario es el único de los cinco donde se realiza tratamiento biológico.

No obstante, evaluando el impacto generado por los vertidos con valores de caudal y calidad de agua intermedios, encontramos diferencias entre los distintos bioindicadores estudiados. En el caso del emisario de Peñíscola observamos como el análisis de la comunidad bentónica o del poblamiento de anfípodos no detectan la contaminación producida por este vertido, mientras que *A. latreilli* y *S. subtruncata* fueron muy sensibles al vertido de esta localidad. Por otro lado los vertidos de las localidades de Vinaroz y Alcossebre también son evaluados de distinta manera dependiendo del indicador. Mientras que el poblamiento de anfípodos y *S. subtruncata* son menos afectados por el emisario de Vinaroz, el resto de indicadores fueron menos afectados por el emisario de Alcossebre. Entre ambos emisarios observamos diferencias en el caudal, de modo que el emisario de Alcossebre vierte un menor volumen y debido a su menor caudal de vertido Del-Pilar-Ruso *et al.* (2010) no detectaron efectos sobre el poblamiento de poliquetos en las estaciones más cercanas a este emisario.

De esta manera, cada componente de la comunidad utilizado como indicador puede responder de manera ligeramente diferente ante la presencia de un vertido. En algunos casos, el grado de conocimiento de la taxonomía, biología, distribución de las especies o de los procesos ecológicos que afectan a su demografía no es el necesario para emplearla de modo fiable como bioindicador universal (Salas *et al.*, 2001). No es posible emplear las mismas especies de organismos como indicadores en todas las costas del planeta, además de que las especies tienen una distribución geográfica y ecológica limitada (Giménez-Casalduero, 2002), su grado de sensibilidad puede variar entre regiones y su abundancia puede ser dependiente de multitud de factores naturales. Es necesario desarrollar indicadores específicos para cada localidad y hábitat, debido a la variabilidad en las características ambientales y la influencia que estas producen sobre las poblaciones o comunidades bentónicas (Hewitt *et al.*, 2005). Un indicador no es universalmente aplicable, ya que las sensibilidad de los organismos varía según el tipo de impacto antropogénicos (Buhl-Mortensen *et al.*, 2009), la región geográfica (Dauvin, 2007) y el tipo de hábitat (Tagliapietra *et al.*, 2009). Estas incertidumbres provocan que emplear un único indicador pueda generar evaluaciones medioambientales erróneas. Es importante integrar distintos componentes, con el fin de estudiar distintos aspectos de la comunidad bentónica que pueden verse afectados por los vertidos con distinto grado de sensibilidad.

Tabla 9.3. Evaluación del nivel de impacto (de 0 a 1) detectado en cada uno de los emisarios correspondientes a las localidades I, II, III, IV y V. Comunidad bentónica y orden Amphipoda: valor de R del ANOSIM entre estaciones a 0 m y 1000 m. Índice BOPA: valor del índice a 0 m frente a 1000 m del emisario. Especies centinelas: disminución de la abundancia a 0 m frente a 1000 m del emisario.

		Localidad I Vinaroz	**Localidad II Benicarló**	**Localidad III Peñíscola**	**Localidad IV Alcossebre**	**Localidad V Torreblanca**
Comunidad bentónica (Suficiencia Taxonómica)		0.65	0.8	-	0.55	-
Índice BOPA		0.61	0.88	0.37	0.61	0.00
Orden Amphipoda		0.50	0.75	-	0.60	-
Especies Centinelas	*A. latreillii*	0.81	0.91	0.81	0.79	0.24
	S. subtruncata	0.82	0.91	0.91	0.97	0.58

9.2.3.- Variabilidad en el espacio y el tiempo.

Un indicador ideal debería ser sensible a las presiones antrópicas que actúan sobre el ecosistema y presentar una sensibilidad limitada a las variaciones naturales físicas y biológicas del ambiente (Karr, 1991). Sin embargo, los invertebrados bentónicos están influidos por interacciones en procesos bióticos (competencia, depredación, reproducción, alimentación) y abióticos (granulometría, materia orgánica, profundidad, salinidad, temperatura) que ocurren a múltiples escalas tanto espaciales como temporales (Borja y Muxika, 2008). De modo que la evaluación de esta variabilidad natural y la respuesta del indicador es esencial antes de establecer un componente como bioindicador (Van Hoey *et al.*, 2010).

Estrategias como la Suficiencia Taxonómica puede reducir esta variabilidad. Algunos autores (Warwick, 1988, 1993; Ferraro y Cole, 1990; Olsgard *et al.*, 1997) establecen que cuando el objetivo es detectar un gradiente de contaminación, estudiar la comunidad a niveles taxonómicos altos puede ser más apropiado que a nivel de especie puesto que un análisis específico presenta una mayor variabilidad ante la heterogeneidad ambiental natural. Por ejemplo la profundidad o el tipo de sedimento afecta a la distribución de especies pero no a los patrones de distribución de grupos taxonómicos más altos (Warwick, 1988). En el caso del orden Amphipoda, la distribución específica depende tanto de la latitud, como de la profundidad. De modo que detectamos variaciones en la composición entre las distintas localidades a pesar de que el tipo de fondo, la comunidad y el rango batimétrico es muy similar entre ellas. En la zona estudiada la variabilidad espacial y temporal es ligeramente inferior al analizar el poblamiento de anfípodos a nivel de especie frente a analizar la comunidad a nivel taxonómico alto (tabla 9.4). A pesar de que en el análisis del orden Amphipoda se empleó el nivel taxonómico inferior, hay que tener en cuenta que sólo estamos estudiando una parte de la comunidad y que la variabilidad natural es diferente según el componente o grupos taxonómico que estudiemos. Por ejemplo, en el caso de las especies estudiadas, el tanaidáceo *A. latreillii*, al igual que ocurre con el orden Amphipoda, mostró cierta variabilidad espacial y temporal, con mayores abundancias en las localidades del sur y un descenso en la abundancia durante el año del 2007. Sin embargo, esta variabilidad es menor que en otras especies. En el caso del bivalvo *Spisula subtruncata*, su sensibilidad a la presencia de los vertidos es aceptada (de-la-Ossa-Carretero *et al.*, 2008) pero la alta variabilidad temporal y espacial de esta especie en el reclutamiento genera dificultades a la hora de comparar situaciones entre años y localidades (tabla 9.3). Especies con larvas plantónicas pueden mostrar una alta variabilidad interanual en el reclutamiento, de modo que las abundancias de un año a otro pueden no ser comparables ya que el mismo número de individuos que encontramos en el vertido durante un año puede encontrarse al año siguiente en uno de los controles más alejados. Esta alta variabilidad temporal en la abundancia de *Spisula subtruncata* provoca que en muchos años no se detecte un descenso de la abundancia en las proximidades de los emisarios, a pesar de su sensibilidad a la presencia de vertidos de aguas residuales urbanas (de-la-Ossa-Carretero *et al.*, 2008). Sin embargo, en otros grupos, como el orden Amphipoda o el tanaidáceo *Apseudopsis latreillii*, las abundancias son más estables facilitando la diferenciación del impacto generado por los vertidos de las variaciones de origen natural. Por otro lado, esta variabilidad natural, se reduce con el empleo de índices como el BOPA, cuya respuesta fue más homogénea entre años y localidades, debido que al trabajar con frecuencias no se ve tan influenciado por variaciones naturales en la abundancia.

Tabla 9.4. Variabilidad detectada entre controles para cada uno de los componentes utilizados. Variabilidad espacial: media de la variabilidad entre controles en los distintos años. Variabilidad temporal: media de la variabilidad entre años en las distintas localidades. Comunidad bentónica y orden Amphipoda: índice de dispersión multivariante. Índice BOPA y especies centinelas: coeficiente de variación

		Variabilidad espacial	Variabilidad temporal
Comunidad bentónica (Suficiencia Taxonómica)		0.90	0.94
Índice BOPA		0.80	0.81
Orden Amphipoda		0.86	0.84
Especies Centinelas	*A. latreillii*	0.97	1.01
	S. subtruncata	1.59	2.01

9.3.- ¿Qué indicador elegir?

A la vista de los resultados obtenidos en los distintos trabajos realizados en esta tesis, el empleo de las comunidades bentónicas para evaluar el grado de impacto de los emisarios submarinos es una herramienta adecuada. Sin embargo, cada una de las aproximaciones empleadas presenta una serie de limitaciones a considerar. Entre los componentes estudiados, el índice BOPA es el que menos limitaciones presenta, puesto que no requiere un nivel de esfuerzo alto, evalúa correctamente el efecto de los distintos vertidos y presenta una menor variabilidad natural. Sin embargo a la hora de clasificar las zonas afectadas con la escala propuesta para este índice (Dauvin y Ruellet, 2007), posteriormente modificada por de-la-Ossa-Carretero y Dauvin (2010), los resultados no fueron satisfactorios, puesto que estaciones afectadas por vertidos fueron clasificadas en un estado ecológico aceptable. El empleo de una escala universal para este tipo de índices es problemático, puesto que cambios naturales locales y temporales pueden modificar su valor independientemente de la presencia o ausencia de un impacto. Una única escala para una indicador que funcione universalmente no es apropiada, debido a que existen variaciones geográficas, incluso para el mismo tipo de hábitat (Van Hoey *et al.*, 2010). Este hecho no supone la no validez de este índice, o de otros como AMBI –que en este tipo de comunidades ha mostrado un comportamiento muy similar-, sino que su empleo conlleva el empleo de zonas de referencia donde no se este produciendo un impacto. De hecho, otros índices generados para la aplicación de la DMA incluyen el empleo de una zona de referencia para el cálculo y la evaluación del estado ecológico.

Además, es necesario tener en cuenta que los índices bióticos pueden simplificar o generalizar demasiado los procesos biológicos o el estado del ecosistema, de modo que aunque indicadores ecológicos como los índices pueden desarrollarse como herramientas para evaluar el estado ecológico de una zona, no pueden remplazar un estudio completo de la comunidad bentónica (Rakocinski *et al.*, 1997). Emplear un solo indicador, aunque facilita la valoración del estado del ecosistema, es una reducción demasiado drástica de la complejidad del ecosistema, si queremos proporcionar una conclusión correcta de calidad ecológica del sistema (Van Hoey *et al.* 2010). Una evaluación en la que se integren distintos bioindicadores ayudará a interpretar adecuadamente el impacto que esté generando un vertido sobre la integridad ecológica de una zona y evaluar correctamente la complejidad del ecosistema reduciendo el nivel de incertidumbre de los resultados (Dauvin, 2007).

Conclusiones.

1. El estudio de las comunidades bentónicas es una herramienta de seguimiento ambiental adecuada para evaluar el efecto del vertido de aguas residuales urbanas sobre comunidades de arenas finas del Mediterráneo occidental.

2. El análisis de esta comunidad a niveles taxonómicos altos resulta más efectivo que el análisis de ciertos parámetros físico-químicos. Permitiendo diferenciar entre el efecto producido por el vertido de aguas residuales según su caudal y nivel de depuración.

3. En el Mediterráneo, los valores del índice biótico BOPA tienen una correlación significativa y fuerte con los valores del AMBI. Con una calibración como la realizada en el capítulo 3 se obtienen resultados muy similares con ambos índices a la hora de establecer el estado de calidad ecológica.

4. El índice BOPA muestra una respuesta correcta a la presencia de los vertidos y relacionada con el caudal y nivel de depuración. Por lo tanto, es un índice adecuado para la evaluación del vertido de aguas residuales urbanas.

5. La calibración de un índice biótico de manera universal puede ser una tarea muy complicada, siendo necesario establecer límites a escalas locales adecuadas para el impacto a valorar.

6. El conocimiento taxonómico del orden Amphipoda en el Mediterráneo español debería ampliarse, ya que analizando la comunidad de arenas finas hemos encontrado un alto número de nuevas citas, así como el descubrimiento de una nueva especie para la ciencia.

7. El orden Amphipoda muestra, en líneas generales, sensibilidad a la presencia de vertidos de aguas residuales urbanas. Sin embargo existen diferencias en el nivel de sensibilidad entre las especies estudiadas.

8. Estas diferencias de sensibilidad pueden estar relacionadas con el tipo de enterramiento de la especie: especies tubícolas son menos sensibles a la presencia del vertido de aguas residuales urbanas que las fosoriales o intersticiales.

9. Establecer el grado de sensibilidad de una especie puede ser complicado: el tanaidáceo *Apseudopsis latreillii*, a pesar de ser clasificado como tolerante u oportunista por algunos autores, mostró sensibilidad alta al vertido de aguas residuales urbanas. Esta sensibilidad de *A. latreillii* se detecta en un descenso de sus abundancias, una reducción de su espectro de tallas y cambios en la proporción de sexos en las proximidades del vertido.

10. La población de *Spisula subtruncata* está afectada por la presencia de este tipo de vertido, detectándose un descenso de abundancia, así como la desaparición de individuos adultos en las estaciones más cercanas a los emisarios. Sin embargo, se detecta una alta variabilidad natural en las abundancias de este bivalvo tanto entre años como localidades.

11. De todos los componentes estudiados en esta tesis, el índice BOPA se considera más adecuado debido al bajo esfuerzo requerido para su aplicación, su capacidad de diferenciar el impacto de cada uno de los vertidos y la menor variabilidad natural detectada en las estaciones no afectadas por los vertidos. Sin embargo, integrar distintos bioindicadores ayudará a interpretar adecuadamente el impacto reduciendo el nivel de incertidumbre de los resultados.

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ACRONYMS

Acrónimos

ACRONYMS

ANOSIM	: Analysis of similarities.
ANOVA	: Analysis of variance.
AMBI	: AZTI Marine Biotic Index.
BO2A	: Benthic Opportunistic Annelida/ Amphipod index.
BOD	: Biological oxygen demand.
BOPA	: Benthic Opportunistic Polychaetes Amphipods index.
CANOCO	: Canonical Community Ordination software.
CCA	: Canonical correlations analysis.
COD	: Chemical oxygen demand.
DMA	: Directiva Marco del Agua.
DDT	: Dichloro Diphenyl Trichloroethane.
EC	: European Council
EcoQs	: Ecological Quality status.
EDAR	: Estación depuradora de aguas residuales.
EEA	: European Environment Agency.
EG	: Ecological group.
ERMS	: European Register of Marine Species.
EPA	: Environmental Protection Agency.
GIP	: Groupement d'Intérêt Public.
ITI	: Infaunal Trophic Index.
MABES	: Macrobenthos of the Bay and Estuary of Seine.
M-AMBI	: Multivariate AZTI Marine Biotic Index.
nMDS	: Non-metric Multidimensional Scaling.
Nt	: Nitrate.
OSPAR	: Oslo Paris Conviction.
PARCOM	: Paris Commission.
PCBs	: Poly-Chlorinated Biphenyls.
PHAs	: Potentially Hazardous Asteroids.
PRIMER	: Plymouth Routines In Multivariate Ecological Research.
Pt	: Phosphate.
SNK	: Student-Newman-Keuls.
SIMPER	: Similarity Percentage.
S.S.	: Suspended solids.
TG	: Trophic Group.
UNEP/MAP	: United Nations Environment Programme/ Mediterranean Action Plan
US	: United States.
UWWTD	: Urban Wastewater Treatment Directive.
WFD	: Water Framework Directive.

La defensa de la tesis doctoral realizada por D/Dª Jose Antonio de la Ossa Carretero se ha realizado en las siguientes lenguas: español y inglés, lo que unido al cumplimiento del resto de requisitos establecidos en la Normativa propia de la UA le otorga la mención de “Doctor Europeo”.

Alicante, de de

EL SECRETARIO EL PRESIDENTE

Reunido el Tribunal que suscribe en el día de la fecha acordó otorgar, por a la Tesis Doctoral de Don/Dña. Jose Antonio de la Ossa CArretero la calificación de .

Alicante de de

El Secretario,

El Presidente,

UNIVERSIDAD DE ALICANTE
CEDIP

La presente Tesis de D. Jose Antonio de la Ossa Carretero ha sido registrada con el

nº ____________ del registro de entrada correspondiente.

Alicante ___ de __________ de _____

El Encargado del Registro,

Doctoral thesis

Tesis doctoral

Jose Antonio de la Ossa Carretero
Universidad de Alicante
Departamento de Ciencias del Mar y Biología Aplicada

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