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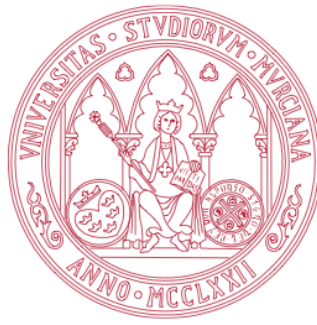
DEPARTAMENTO DE ECOLOGÍA E HIDROLOGÍA

Ecosystem responses to natural and anthropogenic stress: from biomonitoring tools to predictive ecology

Respuestas de los ecosistemas al estrés de origen natural y humano: de las herramientas de biomonitoreo a la ecología predictiva

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2014



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2014

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Resumen

Introducción general

Respuestas ecológicas y evolutivas al estrés: del individuo al ecosistema

El término estrés (*stress*) se puede definir como las condiciones abióticas o bióticas que, excediendo el rango habitual experimentado por los organismos, pueden causar daños biológicos y derivar en cambios a escala evolutiva cuando estas condiciones son persistentes (Hoffman & Parsons 1991; Badyaev 2005; Parsons 2005). Por otra parte, el término perturbación (*disturbance*) se suele reservar para describir condiciones agresivas de carácter puntual como las inundaciones, huracanes o los incendios. Por lo tanto, los ecosistemas caracterizados por estrés crónico y que son poco propicios para la gran parte del conjunto regional de especies, se pueden catalogar como ecosistemas estresados o extremos.

A lo largo de la Tierra, existen numerosos ejemplos de ecosistemas terrestres y acuáticos que están estresados de forma natural, como las tundras, desiertos, fuentes volcánicas marinas o las aguas de gran salinidad o acidez. El estrés natural es un potente catalizador de los procesos evolutivos cuando persiste a escala geológica (Vetaas & Grytnes 2002; Parsons 2005; Bradley 2008; Flowers *et al.* 2010). La tolerancia diferencial que muestran los organismos por las condiciones estresantes está relacionada con el contexto evolutivo de cada linaje, ya que el ambiente evolutivo en el que surgió el linaje determina la presencia de rasgos biológicos que permiten tolerar determinadas intensidades de estrés o incluso adaptarse más fácilmente a nuevas condiciones (Buchwalter *et al.* 2008). Por estas razones, la tolerancia a las condiciones extremas puede mostrar una señal filogenética, lo que se observa en que algunos linajes exhiben una mayor afinidad por los medios estresantes (Garbuz *et al.* 2008; Abellán *et al.* 2009; Arribas *et al.* 2014).

A pesar de su corta historia en relación a la historia geológica de la Tierra, las actividades humanas están introduciendo un amplio rango de condiciones estresantes (estrés antropogénico), modificando la manera en la que las comunidades locales se ensamblan e incrementando las tasas de extinción de especies a niveles sin precedentes en el registro fósil (Sala *et al.* 2000). Además, estos factores antropogénicos pueden tener efectos sinérgicos, lo que recientemente ha generado un gran interés de estudio (Vinebrooke *et al.* 2004; Ormerod *et al.* 2010). La modificación de los ecosistemas resulta particularmente dañina cuando las condiciones resultantes son completamente nuevas para el conjunto regional de especies (especies invasoras,

organoclorados) o aparecen a niveles sin precedente (acidez, metales pesados) (Buchwalter *et al.* 2008). Algunos estudios realizados recientemente proporcionan evidencias de cómo las funciones que realizan los ecosistemas para mantener la vida, y que tienen una gran repercusión en el bienestar humano, podrían verse alteradas o perdidas si el estrés antropogénico continúa reduciendo la biodiversidad (Sala *et al.* 2000; Hooper *et al.* 2005; 2012).

Aunque son pocos los trabajos científicos que han desarrollado marcos conceptuales generales sobre las tendencias esperadas en respuesta al estrés, algunos autores han proporcionado teorías y predicciones a nivel de organismo y ecosistema (ej. Pianka 1970; Grime 1977; Southwood 1977; Odum 1985; Taylor *et al.* 1990; Bertness & Callaway 1994), ofreciendo distinto grado de soporte empírico.

A nivel de organismo, la teoría ecológica predice que los organismos de pequeño tamaño serán más abundantes en ambientes estresantes (Odum 1985). El tamaño corporal está correlacionado con otras características biológicas (ciclo de vida, tasa reproductiva o hábitat), por lo que tiene grandes implicaciones para los organismos en términos energéticos y de interacciones tróficas, como sugiere la Teoría Ecológica Metabólica (Brown *et al.* 2004). Esta teoría predice que la tasa metabólica aumenta con la biomasa del organismo, indicando que los tamaños corporales más grandes necesitarán más energía para sobrevivir, lo que podría incrementar su sensibilidad al estrés. Sin embargo, en términos de eficiencia, la tasa metabólica por unidad de biomasa es menor en organismos grandes, lo que podría explicar su mayor longevidad en comparación con los organismos pequeños, que normalmente muestran unas tasas de crecimiento más rápidas, y se reproducen y mueren a edades tempranas. Además, la mayor tasa reproductiva de los organismos pequeños podría conferirles ventajas en respuestas a perturbaciones o estrés (Cardillo 2003). Las especies de gran tamaño muestran un gran número e intensidad de interacciones tróficas per cápita con el resto de la comunidad, lo que les hace más sensibles a cualquier cambio que ocurra a escala de ecosistema (Woodward *et al.* 2005). Los estudios realizados durante las últimas décadas indican que la extinción de ectotermos (Solan *et al.* 2004) y endotermos no marinos está influida por el tamaño corporal (Gaston & Blackburn 1995; Cardillo *et al.* 2005). Así, por ejemplo, el calentamiento global parece estar favoreciendo a los animales de pequeño tamaño en los ecosistemas acuáticos (Daufresne *et al.* 2009).

Aunque el tamaño corporal es capaz de predecir muchos de los patrones biológicos que ocurren a macroescala (por ejemplo, cuando comparamos organismos que difieren en gran medida en su tamaño corporal, como ballenas y bacterias), cuando miramos más detalladamente entre organismos de tamaño

similar, encontramos que hay otros rasgos biológicos capaces de explicar los cambios biológicos a lo largo de gradientes ambientales (Tilman *et al.* 2004). Por ejemplo, se ha observado que organismos de tamaño y morfología similar ocupan hábitats distintos, siendo los rasgos fisiológicos los que determinan este patrón (Buchwalter *et al.* 2008; Picazo *et al.* 2012; Céspedes *et al.* 2013).

Los animales que ocupan posiciones tróficas altas podrían ser más sensibles al estrés debido a la reducción del número de presas potenciales, al incremento de la toxicidad del alimento, en algunos casos (metales pesados o salinidad), y de la mayor energía requerida para hacer frente al estrés (Bradley 2008). De hecho, los depredadores son muy escasos en los ecosistemas estresantes, que están típicamente caracterizados por productores primarios y herbívoros (Menge & Sutherland 1987). Por otra parte, el estrés parece seleccionar aquellos organismos con una gran variabilidad fenotípica y un nicho ecológico fundamental amplio (MacArthur & Levins 1967; Stubbs & Wilson 2004). El generalismo se observa en aquellos individuos que muestran una actividad biológica similar (*fitness*) en un amplio rango de condiciones (Kassen 2002), debido a su amplia tolerancia abiótica (ej. Fridley *et al.* 2007) o a la diversidad de sus interacciones biológicas (Bascompte *et al.* 2003; Bolnick *et al.* 2007; Bastolla *et al.* 2009). Por el contrario, los especialistas aparecen solamente en un subconjunto reducido de los posibles ambientes, mostrando un rango de interacciones tróficas mucho menor (Poisot *et al.* 2011). El generalismo podría surgir en especies que evolucionan en hábitats ambientalmente heterogéneos (Kassen 2002) o en ausencia de competición interespecífica (Van Vallen 1965; Roughgarden 1972; Bolnick *et al.* 2010). Sin embargo, hay casos complejos como el de los organismos especialistas en ambientes estresantes, que presentan grandes diferencias entre su nicho fundamental (conjunto de condiciones ambientales en las que la especie podría aparecer en ausencia de interacciones bióticas) y realizado (conjunto de condiciones ambientales en las que la especie se distribuye). Los resultados de varios estudios indican que los organismos que se han adaptado a ambientes extremos lo hicieron a través de una expansión de su nicho fundamental más que a través de especializarse a dichas condiciones estresantes (Scoville & Pfrender 2010; Latta *et al.* 2012). Sin embargo, ese cambio en su nicho fundamental no se traduce en un comportamiento generalista, ya que los especialistas en estrés sólo aparecen en porciones muy concretas del gradiente ambiental, probablemente debido a que la adaptación a hábitats extremos conlleva un coste de oportunidad respecto a la capacidad competitiva, lo que les excluye de medios más benignos (no estresantes), donde la competencia es más intensa (Wilson & Keddy 1986; Herbst 2001). A pesar de la importancia de los generalistas para garantizar la estabilidad de los ecosistemas (Bascompte *et al.* 2003), los organismos especialistas pueden incrementar la diversidad de respuestas y funciones de los

ecosistemas (Devictor *et al.* 2008; Clavel *et al.* 2011). El cambio global podría provocar una homogeneización funcional de los ecosistemas a nivel planetario, como se ha observado en respuesta a cambios ambientales pasados y presentes (Clavel *et al.* 2011).

La ecología tiene una larga tradición en el estudio de cómo las comunidades biológicas se configuran a lo largo de los gradientes ambientales y qué propiedades derivan de las distintas configuraciones estructurales (Southwood 1977; Keddy 1992; Rosenfeld 2002; Mouillot *et al.* 2005). De manera tradicional, las comunidades locales se han considerado un subconjunto del total de especies que podrían haber colonizado el hábitat (*pool* de especies regional). El proceso de ensamblado se rige por una combinación de factores bióticos (MacArthur & Levins 1967; Stubbs & Wilson 2004), abióticos (Weiher & Keddy 1995; Maire *et al.* 2012) y neutrales (Hubbell 2001). La teoría de nicho predice que las especies se seleccionan de una manera determinista (no azarosa) a lo largo de los filtros ambientales en función de sus rasgos biológicos (Southwood 1977; Mouillot *et al.* 2005; Shipley *et al.* 2006; Weiher *et al.* 2011), mientras que la teoría neutral, que asume la equivalencia ecológica de las especies, predice que los cambios en las abundancias a nivel local y regional se deben a procesos demográficos estocásticos (azarosos) y evolutivos (Hubbell 2001). Recientemente, se ha ampliado la visión de la comunidad ecológica incluyendo también los procesos evolutivos a nivel de población, así como la interacción entre estas poblaciones a lo largo de distintas escalas espaciales y temporales que determinan los procesos regionales de especiación (Ricklefs 2008).

A nivel de ecosistema, la diversidad de especies en medios estresantes es generalmente baja, aunque la relación entre estrés y diversidad puede adquirir distintas formas y magnitudes en función del grupo y el factor estresante estudiados (ej. Petrin *et al.* 2007; Flynn *et al.* 2009; Millán *et al.* 2011). La Hipótesis del Gradiente de Estrés establece que la competencia interespecífica disminuye cuando las condiciones ambientales se vuelven extremas, mientras que los procesos de facilitación adquieren importancia con la intensificación del estrés (Bertness & Callaway 1994). Estudios en plantas (Callaway *et al.* 2002) y animales (Kawai & Tokeshi 2007) suelen confirmar esta predicción. Sin embargo, el desarrollo de marcos conceptuales más refinados subraya el papel de ciertas combinaciones de rasgos biológicos o del tipo de estrés para encontrar patrones similares a los predichos por esta teoría (Maestre *et al.* 2009).

El estudio de la relación biodiversidad-función de los ecosistemas (*Biodiversity-Ecosystem Function*) muestra una relación positiva tanto a nivel teórico (Duffy 2009), como a nivel experimental (ej. Hooper *et al.* 2005) y de campo (ej. Maestre *et al.* 2012), sugiriendo que la variedad y la magnitud de las funciones de los

ecosistemas podrían reducirse a lo largo de los gradientes de estrés (Laliberté *et al.* 2010; Woodward *et al.* 2012). Además, considerando que el estrés reduce la diversidad de especies, los procesos de producción y consumo de materia orgánica se podrían desestabilizar o volverse ineficientes (Hooper *et al.* 2005). Las condiciones estresantes pueden afectar de distinta forma a productores y consumidores, a través de una reducción en la producción o en el consumo debido a cambios en la cantidad total de materia orgánica producida o importada o en su palatabilidad. Estos procesos podrían desviar la relación producción : respiración de la unidad, convirtiendo el ecosistema en altamente autótrofico o heterótrofico (Odum 1985).

Herramientas de biomonitorio para los sistemas estresados de forma natural

Las herramientas de biomonitorio tienen como objetivo detectar síntomas de impacto humano en los ecosistemas, usando como referencia los valores de un conjunto de indicadores biológicos (medidas de diversidad, composición de la comunidad, funciones del ecosistema, etc.) bajo condiciones prístinas o cercanas a la naturalidad, que se comparan con los sitios que vamos a evaluar para estimar en qué grado se desvían de las condiciones naturales.

Para los ecosistemas que muestran condiciones benignas, las herramientas de biomonitorio que se han desarrollado parecen funcionar bien a la hora de detectar el estrés de origen humano (ej. Bonada *et al.* 2006). Sin embargo, detectar signos de degradación en ecosistemas que están estresados de forma natural es mucho más complejo, debido a que estos ambientes muestran ciertas similitudes con los que están contaminados o alterados por el ser humano (Elliott & Quintino 2007), como se menciona anteriormente. Esto es particularmente peligroso en el contexto actual de cambio global donde una gran parte de la Tierra está experimentando grandes modificaciones. Por lo tanto, es necesario desarrollar herramientas que cuantifiquen la integridad ecológica de los ecosistemas estresados de forma natural, especialmente, considerando que estos sistemas albergan una parte única de la biodiversidad, en términos de genes, taxones y funciones (Vetaas & Grytnes 2002; Flowers *et al.* 2010; Millán *et al.* 2011; Latta *et al.* 2012; Arribas *et al.* 2013; 2014).

Construyendo nuevos marcos y metodologías para predecir las respuestas de los ecosistemas al estrés

A pesar de que la ecología de comunidades ha proporcionado ideas clave para entender cómo el estrés ambiental afecta a las comunidades (Simberloff 2004),

una de las mayores críticas a esta disciplina es la ausencia de leyes generales acerca de cómo las comunidades se ensamblan o responden a lo largo de los gradientes de estrés ambiental, lo que sería de gran utilidad para predecir las respuestas biológicas al cambio global (Keddy 1992; McGill *et al.* 2006; Ricklefs 2008), en vez de evaluar el estado ecológico *a posteriori*. Sin embargo, el uso combinado de distintos tipos de información, junto al desarrollo de nuevos marcos analíticos como la descomposición de la diversidad beta (Baselga 2010) o los enfoques funcionales (McGill *et al.* 2006), ofrecen grandes posibilidades en el campo de la ecología de comunidades, permitiendo integrar los procesos históricos desde la escala local a la regional (Ricklefs 2008).

La descomposición de la diversidad beta (cambios en la composición de especies entre localidades) en los componentes de reemplazamiento (sustitución de especies) y anidamiento (desarrollo de conjuntos anidados de especies que comparten una gran parte de especies a lo largo de un gradiente de riqueza) ha sido utilizada para explicar procesos históricos que determinaron los patrones actuales de biodiversidad, en particular los gradientes de riqueza de especies a nivel regional, continental y global (ej. Hortal *et al.* 2011; Svenning *et al.* 2011; Dobrovolski *et al.* 2012; Baselga *et al.* 2013). Por ejemplo, Baselga *et al.* (2012) sugiere que los patrones latitudinales de riqueza de especies que se observan en Europa se podrían explicar parcialmente por la capacidad diferencial que tuvieron los organismos para recolonizar el continente después del último máximo glacial (hace aproximadamente 25.000 años). En este caso, las especies con una baja capacidad para dispersarse muestran un reemplazamiento a lo largo del gradiente de latitud. Sin embargo, hay un gran desconocimiento sobre qué patrones de reemplazamiento y anidamiento podrían surgir a lo largo de gradientes de estrés natural y antropogénico a pequeña escala, considerando el distinto grado con el que los conjuntos regionales de especies se han adaptado a lo largo de su historia evolutiva.

Los gradientes de estrés natural han persistido a lo largo de la historia geológica promoviendo la especiación y el desarrollo de rasgos biológicos apropiados, que han permitido que algunas especies sean capaces de desplazar a otras especies tolerantes, resultando en un mayor conjunto de especies especialistas. Esta es la idea central de la hipótesis del conjunto evolutivo de especies (Evolutionary Species Pool, en Taylor *et al.* 1990), que predice que los hábitats con una mayor persistencia y abundancia histórica deberían contener más especies especialistas en relación con otros hábitats menos persistentes o frecuentes. Por lo tanto, a lo largo de gradientes de riqueza donde los sitios difieren en su intensidad de estrés y comparten pocas especies, las diferencias en composición se deberán fundamentalmente al reemplazamiento de especies

(Fig. 1a). Estas especies mostrarán un alto grado de especificidad a lo largo del gradiente, debido a que se trata de especialistas que se han adaptado a lo largo de su evolución. Por otra parte, cuando los gradientes de riqueza se producen a través de la pérdida de especies resultando en que las comunidades más pobres son subconjuntos de aquellas más ricas, la diversidad beta se explica por anidamiento (Fig. 1b). En este caso, las comunidades comparten un alto porcentaje de especies porque las especies que habitan los sitios más pobres son generalistas capaces de tolerar el estrés y aparecer bajo un amplio rango de niveles de estrés.

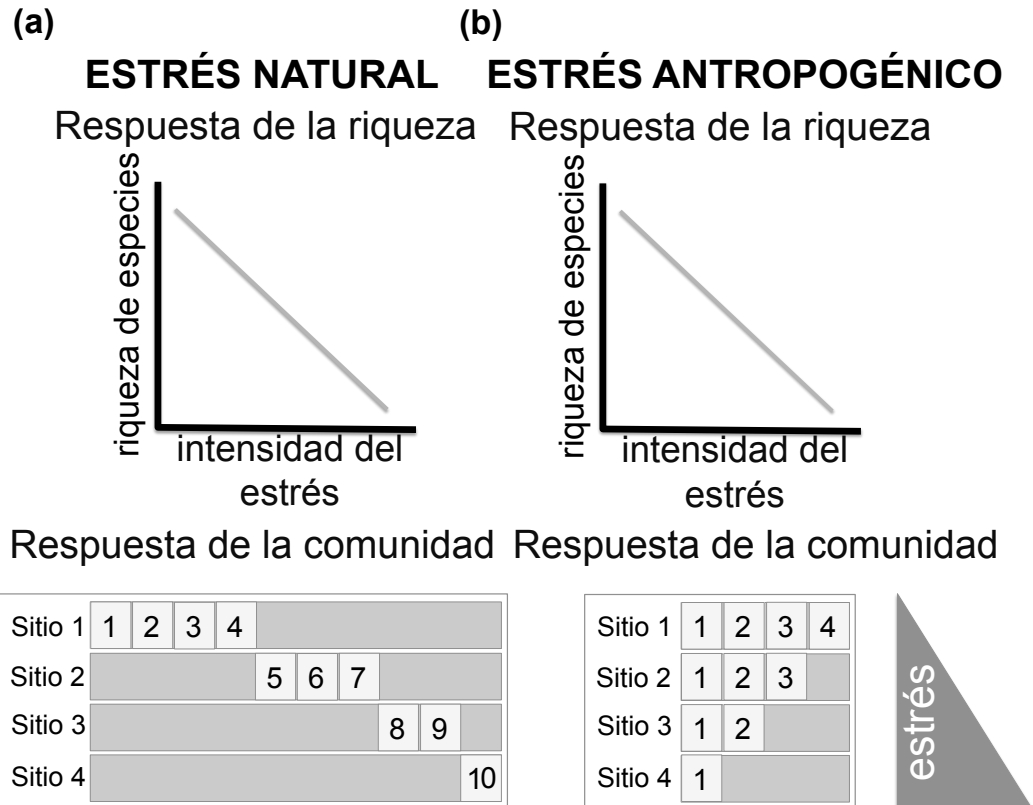


Fig. 1 Predicciones de los patrones de riqueza de especies y diversidad beta taxonómica esperados en respuesta a factores estresantes naturales (a) y antropogénicos (b).

Las aproximaciones analíticas basadas en los rasgos biológicos ofrecen grandes ventajas en comparación con los métodos convencionales basados en información taxonómica. Por ejemplo, los métodos funcionales permiten una mayor comparabilidad entre taxones o regiones (ej. McGill *et al.* 2006), una predicción mecanicista a través de los vínculos que existen entre el cambio ambiental y las funciones del ecosistema (ej. Hooper *et al.* 2005), y reflejando procesos evolutivos de adaptación a las condiciones ambientales (ej. Badyaev 2005). Recientemente, se han desarrollado un conjunto de herramientas analíticas que han implementado el uso de la información funcional para el cálculo de medidas de diversidad funcional usando los rasgos biológicos de los organismos (Villéger *et al.* 2008; Laliberte & Legendre 2010). Las propiedades

funcionales de un organismo o ecosistema se pueden describir de distintas maneras, incluyendo componentes de la diversidad funcional, como la riqueza, la equidad y la divergencia (Mason *et al.* 2005), y la redundancia funcional (Rosenfeld 2002).

Los patrones funcionales que podemos encontrar en los ecosistemas estresados de forma natural podrían ser útiles para anticipar y predecir los cambios futuros que los ecosistemas sufrirán debido al cambio global. Algunos estudios previos han observado que los rasgos que proporcionan tolerancia al estrés natural podrían ser útiles para soportar nuevos factores estresantes (Badyaev 2005; Buchwalter *et al.* 2008; Arribas *et al.* 2014). En tal caso, las especies que aparecen en ambientes estresados de forma natural y por la acción humana, deberían compartir una serie de rasgos biológicos (Southwood 1977; Odum 1985; Stanton *et al.* 2000). Esta circunstancia debería producir tendencias similares en las características funcionales de los organismos y los ecosistemas a lo largo de los gradientes de estrés, independientemente de si las especies se han adaptado y especializado a lo largo de la historia evolutiva (estrés natural *vs.* antropogénico). En este caso, el estrés actuaría como un potente filtro ambiental que configura de manera determinista la evolución de los rasgos y las características funcionales de las comunidades.

Los ríos como modelo para el estudio del efecto del estrés en los ecosistemas

Los ríos son ambientes especialmente propicios para examinar el efecto de los factores estresantes de origen natural y humano. Primero, estos hábitats están bien definidos y compartimentados, persistiendo a lo largo de la historia geológica y permitiendo procesos de especiación (Abellán *et al.* 2009; Arribas *et al.* 2014). Segundo, están caracterizados por amplios gradientes ambientales naturales (altitud, geomorfología, hidroquímica) y antropogénicos (acidificación, contaminación orgánica) (Moss *et al.* 1987; Díaz *et al.* 2008). Tercero, albergan una gran diversidad biológica, por ejemplo, entre macroinvertebrados, cuyo muestreo e identificación son relativamente sencillos (Bonada *et al.* 2006). Los macroinvertebrados bentónicos representan una importante fracción de la biodiversidad acuática y su composición y riqueza exhiben una fuerte relación con los cambios ambientales a escala de tramo y cuenca (Strayer 2006). Cuarto, a pesar de las diferencias en la historia evolutiva entre regiones, podemos encontrar los mismos grandes grupos de macroinvertebrados (a nivel de familia y género), particularmente en el Paleártico y el Hemisferio norte. Además, hay evidencias claras de que algunos

organismos acuáticos, como los insectos, responden de manera similar al estrés ambiental en distintas áreas del planeta (Iwasaki & Ormerod 2012).

En particular, entre los ríos de la Cuenca Mediterránea podemos encontrar buenos ejemplos de gradientes de estrés natural y antropogénico (Millán *et al.* 2011; Belmar *et al.* 2013; Cooper *et al.* 2013; Bruno *et al.* 2014) que muestran relaciones significativas con las comunidades de productores primarios y macroinvertebrados (Moreno *et al.* 1997; 2001; Velasco *et al.* 2006; Ros *et al.* 2009; Sánchez-Montoya *et al.* 2010; Guareschi *et al.* 2012). Entre los factores estresantes naturales, la salinidad del agua es uno de los más importantes a la hora de configurar los patrones biológicos (Finocchiaro *et al.* 2009; Ros *et al.* 2009; Moreno *et al.* 2010). La litología determina la cantidad y el tipo de las sales disueltas en las aguas (Millán *et al.* 2011; Griffith 2014), produciendo enormes gradientes de salinidad que van desde aguas prácticamente destiladas (conductividad $\approx 15 \mu\text{S cm}^{-1}$) en las cabeceras silíceas, hasta aguas altamente mineralizadas (conductividad $\approx 300,000 \mu\text{S cm}^{-1}$) en cuencas caracterizadas por sedimentos evaporíticos altamente solubles. El estrés osmótico afecta a los organismos por medio de la citotoxicidad y la deshidratación, impidiendo que el organismo pueda mantener su composición interna de sales dentro de rangos aceptables (Bradley 2008; Flowers *et al.* 2010). Este gradiente de salinidad se ha mantenido relativamente estable en la región mediterránea a lo largo de la historia evolutiva, permitiendo los procesos de adaptación y especiación (Abellán *et al.* 2007; Abellán *et al.* 2009; Arribas *et al.* 2014).

En la cuenca Mediterránea podemos encontrar ríos salinos en el este y el sur de la Península Ibérica (Millán *et al.* 2011; Arribas *et al.* 2013), Sicilia (Gerecke 1991) y el Norte de África (Margat 1961; Bennis *et al.* 2008). Estos ecosistemas contienen especies únicas con rangos de distribución muy restringidos (Sánchez-Fernández *et al.* 2008), que representan adaptaciones singulares a los ambientes estresantes (ej. Pallarés *et al.* 2012), mostrando en algunos casos una gran diversidad genética intraespecífica (Abellán *et al.* 2007). Sin embargo, a pesar de su gran valor ecológico, los programas de biomonitorio más importantes están ignorando estos ecosistemas que además están prácticamente desprotegidos por la Red Natura 2000 (Sánchez-Fernández *et al.* 2008) y la legislación ambiental marroquí (Bennis *et al.* 2008). En consecuencia, la escasez de métodos adecuados de clasificación y biomonitorio está dificultado la correcta evaluación del estado ecológico de los ríos salinos, que continúan sufriendo grandes presiones humanas (Velasco *et al.* 2006).

La intensificación en los usos del suelo es uno de los factores estresantes de origen humano de mayor importancia y con mayor impacto en los ecosistemas acuáticos a nivel mundial (Scanlon *et al.* 2007; Monteagudo *et al.* 2012). En la

Cuenca Mediterránea, la superficie dedicada agricultura y núcleos urbanos ha crecido enormemente en los últimos siglos (Cooper *et al.* 2013), produciendo grandes efectos en los ecosistemas fluviales como la contaminación orgánica (ej. Monteagudo *et al.* 2012), el incremento de los contaminantes tóxicos como pesticidas o medicamentos (e.g. Lopez-Doval *et al.* 2013) o cambios en las concentraciones iónicas debido a los drenajes procedentes de la agricultura de regadío, que puede producir estrés por dilución en ríos salinos, es decir, un descenso significativo en las concentraciones de sales (Velasco *et al.* 2006), o salinización secundaria en cuerpos de agua dulce (Cañedo-Argüelles *et al.* 2013). Ambos procesos podrían alterar la estructura y el funcionamiento de los ecosistemas acuáticos.

Objetivos

Los objetivos principales de esta tesis son (1) proporcionar herramientas de biomonitorio para detectar impactos humanos en ríos salinos (*Capítulos 1, 2 y 3*) y (2) desarrollar enfoques metodológicos que permitan predecir las respuestas de las comunidades y ecosistemas al estrés, en base a la teoría ecológica y la diferente historia evolutiva de cada factor estresante en la región estudiada (*Capítulos 4 y 5*).

Más concretamente, los objetivos específicos de esta tesis son:

1. Describir los efectos del estrés por dilución en el funcionamiento de un río salino mediterráneo. Este estudio analiza los cambios en el metabolismo del ecosistema y la biomasa de productores primarios y consumidores en condiciones de dilución y después de que se recuperaran los valores previos a la perturbación (*Capítulo 1*).
2. Cuantificar el impacto del estrés por dilución puntual y crónica en el metabolismo y la estructura trófica de un río salino mediterráneo. Este estudio analiza los cambios en la relación producción : respiración, la biomasa de productores primarios y consumidores, y la estructura trófica usando un diseño factorial entre sitios que difieren en el grado de dilución crónica (control y perturbado), y durante dos periodos que muestran claras diferencias en su salinidad media (dilución puntual) (*Capítulo 2*).
3. Evaluar diferentes procedimientos automáticos de clasificación que cubran la diversidad de ríos que aparece en condiciones naturales en el

Mediterráneo occidental y testar una serie de indicadores biológicos capaces de evaluar el estado ecológico de los ríos salinos (*Capítulo 3*).

4. Explorar qué mecanismos explican los patrones de diversidad beta a lo largo de gradientes de estrés con distinta persistencia histórica en la región (estrés natural *vs.* antropogénico). Se testan dos hipótesis en relación a los mecanismos que explican la diversidad beta: (1) el estrés natural (salinidad, altitud) causará un progresivo reemplazamiento de especies y (2) el estrés antropogénico (acidez, metales pesados e intensificación de los usos del suelo) resultará en una pérdida de los especialistas dando lugar al anidamiento de las comunidades (*Capítulo 4*).

5. Analizar los cambios en las características funcionales a lo largo de gradientes de estrés crónico que muestran diferencias en su persistencia histórica (estrés natural *vs.* antropogénico). En particular, este estudio compara las tendencias de medidas funcionales de insectos acuáticos a nivel de taxón (riqueza funcional media a escala de taxón, similitud funcional media) y comunidad (riqueza funcional a escala de comunidad, dispersión funcional y redundancia funcional) a lo largo de gradientes de estrés natural (salinidad) y antropogénico (usos del suelo) (*Capítulo 5*).*

* La lista de referencias citadas está al final de la sección *General Introduction*.

Capítulos

Capítulo 1: Efectos del estrés por dilución en el funcionamiento de un río salino mediterráneo

Este primer capítulo evalúa los efectos del estrés por dilución y la estacionalidad en el funcionamiento un río hipersalino situado en el sureste ibérico (Rambla Salada de Fortuna). El estudio se llevó a cabo en dos periodos de un año de duración, durante los cuales se produjeron importantes cambios ambientales. En el primer periodo, se redujo la concentración de sales y aumentó el caudal debido a varias entradas de agua dulce procedentes de la rotura de un gran canal de irrigación (canal del Trasvase Tajo-Segura) que atraviesa la cuenca de drenaje. Durante el segundo año, se recuperaron los niveles de salinidad y caudal previos a la dilución gracias a la reparación del canal. Para representar el funcionamiento del río, se midieron el metabolismo y la biomasa de productores primarios y consumidores, cubriendo la variabilidad estacional en cada periodo analizado. El efecto del estrés por dilución y la estacionalidad se estudió a través de modelos predictivos, en los que las variables funcionales actuaron como variables de respuesta. Las tasas de producción primaria y respiración de Rambla Salada oscilaron entre 0.07-21.05 y 0.19-17.39 g O₂ m⁻² día⁻¹, respectivamente. Los valores medios de esas variables durante el periodo de estudio fueron de 7.35 y 5.48 g O₂ m⁻² día⁻¹, respectivamente. La tasa media de metabolismo neto diario fue de 1.87 ± 0.52 g O₂ m⁻² día⁻¹, mientras que el valor medio de coeficiente producción primaria : respiración fue de 2.48 ± 1.1, reflejando un metabolismo autotrófico (producción neta de materia orgánica). La producción primaria y la respiración registraron su valores máximos durante el verano, valores intermedios durante primavera y otoño, llegando a valores mínimos en el invierno. La producción primaria y la respiración fueron explicadas satisfactoriamente por la estacionalidad (76% y 83%, respectivamente). Sin embargo, la biomasa de productores primarios y de consumidores respondieron a los cambios en caudal y salinidad producidos por la entrada de agua dulce. De hecho, estas entradas redujeron significativamente la biomasa de biofilm. Por lo tanto, los resultados de este estudio indican que la biomasa de productores primarios, en particular la biomasa de biofilm, es un indicador eficaz para evaluar el estado ecológico de los ríos salinos.

Gutiérrez-Cánovas, C.; Velasco, J. & A. Millán. 2009. Effects of dilution stress on the functioning of a saline Mediterranean stream. *Hydrobiologia*, 619: 119-132.

Capítulo 2: Impacto del estrés crónico y puntual en el metabolismo y la estructura trófica de un río salino mediterráneo

En este segundo capítulo, se estudió un río salino (Rambla Salada de Fortuna, SE ibérico) para evaluar la hipótesis general de que el estrés crónico tiene un efecto mayor que el estrés puntual en el metabolismo del ecosistema y la estructura trófica de los macroinvertebrados acuáticos. Para ello, se compararon dos tramos que diferían en la persistencia del estrés por dilución durante dos años. El *tramo estresado de forma crónica* estuvo afectado por una dilución persistente (estrés crónico), causada por la entrada difusa de aguas procedentes de la agricultura de regadío. Además, este tramo presentaba una gran abundancia de carrizo (*Phragmites australis*) en su ribera. En cambio, el *tramo de referencia* presentaba condiciones prácticamente inalteradas y vegetación halófila de bajo porte en la ribera. Durante el primer año, ambos tramos se vieron afectados por varias entradas de agua dulce causadas por la rotura de un gran canal de irrigación (Canal del Traspase Tajo-Segura) que redujeron significativamente la conductividad del agua (estrés puntual). Durante el segundo año, la conductividad recuperó los niveles previos a la perturbación. Los resultados de este estudio revelan que el estrés puntual no tuvo efectos significativos en el coeficiente producción primaria : respiración (P/R), aunque la biomasa de macroinvertebrados depredadores presentó un incremento significativo. Sin embargo, el estrés crónico redujo significativamente tanto el coeficiente P/R, como la biomasa de productores primarios y consumidores. Por otra parte, la importancia relativa de los distintos grupos tróficos de macroinvertebrados no se vio afectada por ningún tipo de estrés. Las señales isotópicas mostraron que la comunidad de macroinvertebrados se sustenta fundamentalmente a partir de los productores primarios acuáticos en ambos tramos. A pesar de la gran entrada de ramas y hojas de carrizo en el tramo estresado de forma crónica, éstas apenas entraron en la cadena trófica estudiada. Este estudio destaca la relevancia de la persistencia del estrés y la utilidad de los indicadores funcionales para predecir las consecuencias de los impactos humanos en los ecosistemas. De esta manera, mientras que el estrés puntual tuvo efectos menores en el funcionamiento del río, el estrés crónico redujo de manera drástica la biomasa, causando que el tramo impactado por estrés crónico se volviera heterotrófico

Gutiérrez-Cánovas, C.; Hernández, J.; Velasco, J. & A. Millán. 2012. Impact of chronic and pulse dilution disturbances on metabolism and trophic structure in a saline Mediterranean stream. *Hydrobiologia*, 686(1): 225-239.

Capítulo 3: Evaluación de clasificaciones automáticas de ríos mediterráneos y de métricas de biomonitoreo para los tipos de ríos salinos.

La Directiva Marco de Agua (DMA) tiene como principal objetivo el desarrollo de métodos que evalúen el estado ecológico de las masas de agua europeas. En este contexto, las clasificaciones y los métodos de biomonitoreo usados actualmente muestran claras limitaciones para implementar la DMA en los ríos de la Cuenca Mediterránea. Las clasificaciones no son capaces de representar toda la diversidad de ríos ni de tipificar automáticamente grandes bases de datos. Además, los bioindicadores más usados podrían ser inadecuados para evaluar el estado ecológico de los ríos salinos (conductividad $\geq 5,000 \mu\text{S cm}^{-1}$), que representan una parte insustituible de la biodiversidad mediterránea. Este trabajo pretende superar estas limitaciones desarrollando tres clasificaciones automáticas (basada en criterio de expertos, clúster ambiental, clúster biológico) que, además de estar validadas biológicamente, son capaces de abarcar toda la diversidad de ríos del Mediterráneo occidental. Además, se evaluó la capacidad de métricas tradicionales y alternativas para evaluar el estado ecológico de los ríos salinos. Para caracterizar y desarrollar los análisis, se compilaron bases de datos ambientales y biológicas (macroinvertebrados a nivel de familia y especie) en España, Marruecos e Italia. Los resultados indican que la clasificación basada en el criterio de expertos y el clúster biológico proporcionan siete clases de ríos con características similares, mientras que el clúster ambiental mostró discrepancias. La clasificación biológica mostró la mayor robustez para los datos españoles, mientras que la clasificación basada en el criterio de expertos mostró los valores más altos para Marruecos e Italia. Los resultados de la validación biológica indicaron que los cambios en las comunidades entre clases se debieron fundamentalmente al reemplazamiento de especies. Finalmente, a la hora de evaluar el estado ecológico de los tipos salinos, se detectó una mayor eficacia en los indicadores alternativos propuestos en este estudio respecto a los convencionales. Los resultados de este estudio ayudarán a mejorar la capacidad de los gestores ambientales para evaluar el estado ecológico de los ríos Mediterráneos, en especial en el caso de los ríos salinos.

Gutiérrez-Cánovas, C., P. Arribas; Naselli-Flores, L.; Bennis, N.; Finocchiaro, M., Millán, A. & J. Velasco. Assessing automatic classifications of the Mediterranean streams and biomonitoring metrics for saline stream types. Enviado a *Ecological Indicators*.

Capítulo 4: Los factores estresantes de origen natural y antropogénico producen patrones distintos en la diversidad beta de ecosistemas fluviales

En este trabajo se examinaron los patrones de diversidad beta que surgen en relación a factores estresantes de origen natural y humano en ecosistemas fluviales. Se testaron dos hipótesis: (1) la diversidad beta en ríos se explica por un reemplazamiento de especies que muestran una gran especificidad a lo largo de gradientes de estrés natural (persistentes en términos evolutivos), mientras que (2) el estrés antropogénico (nuevo para los organismos) produce una pérdida de las especies más sensibles, generando subconjuntos anidados a lo largo de un gradiente de riqueza taxonómica. Para ello, se usaron cinco bases de datos que contienen registros de macroinvertebrados acuáticos a lo largo de gradientes de estrés natural (altitud, Himalaya; salinidad, Península Ibérica) y antropogénico (acidez, Gran Bretaña; metales pesados, Gran Bretaña; intensificación de los usos del suelo, Península Ibérica). Las hipótesis se testaron construyendo modelos que relacionan la riqueza taxonómica y los componentes de la diversidad beta (total, reemplazamiento y anidamiento) con la intensidad de estrés. Los resultados mostraron que la intensidad de estrés explica una gran parte de la variabilidad en la riqueza taxonómica ($r^2 = 0.64-0.93$), que se reduce conforme el estrés aumenta. Las comunidades exhibieron una mayor disimilitud conforme aumenta la diferencia en el nivel de estrés al que están sometidas. Estos cambios se debieron al reemplazamiento de especies, cuando el estrés era de origen natural, y al desarrollo de subconjuntos anidados cuando el factor estresante fue antropogénico. Estos resultados apoyan la hipótesis de que el estrés natural y antropogénico generan patrones distintos de diversidad beta, lo cual, tiene distintas implicaciones en la gestión y conservación de los ecosistemas.

Gutiérrez-Cánovas, C.; Millán, A; Velasco, J.; Vaughan, I.P. & Ormerod, S.J. Contrasting effects of natural and anthropogenic stressors on beta-diversity in river organisms. *Global Ecology and Biogeography* 22(7): 796-805.

Capítulo 5: Similitud en la diferencia: cambios no aleatorios en las características funcionales a nivel de comunidad a lo largo de gradientes de estrés natural y antropogénico

El efecto del estrés en las comunidades biológicas puede variar en relación al grado de adaptación que los organismos hayan experimentado a lo largo de su evolución. En este capítulo se comparó la respuesta de las características funcionales de comunidades de insectos de ríos a lo largo de gradientes de estrés con distinta persistencia histórica. La concentración de sales en el agua y la intensificación en los usos del suelo se han usado como ejemplos de estrés natural (persistente de manera histórica) y antropogénico (nuevo para los organismos), respectivamente. Se usó una nueva herramienta para cuantificar los componentes de la diversidad funcional y la redundancia funcional en un mismo espacio multidimensional, usando métricas a nivel de taxón y de comunidad. Los resultados mostraron respuestas similares a lo largo de los gradientes de estrés natural y antropogénico. En ambos casos, la riqueza funcional media a nivel de taxón y la similitud funcional media aumentaron conforme se incrementó el nivel de estrés, mientras que tanto la riqueza funcional a nivel de comunidad y como la redundancia funcional se redujeron a lo largo de ambos gradientes. A pesar de la distinta persistencia histórica, ambos factores estresantes actúan como potentes filtros no aleatorios, produciendo respuestas funcionales convergentes. Los resultados de este estudio contribuyen a mejorar nuestra capacidad para predecir las respuestas de los ecosistemas frente nuevos factores estresantes a escala ecológica y evolutiva.

Gutiérrez-Cánovas, C.; Sánchez-Fernández, D.; Velasco, J.; Millán, A. & N. Bonada. Similarity in the difference: non-random changes in community functional features along natural and anthropogenic stress gradients. Enviado a *Ecology*.

Conclusiones generales

Capítulo 1

1. Las variables climáticas explicaron las tasas metabólicas, que presentaron los valores más altos durante primavera y verano.
2. La conductividad y el caudal explicaron los cambios en la biomasa de los productores primarios y consumidores, que alcanzaron valores máximos durante el periodo de dilución.
3. La biomasa del biofilm fue la única variable que mostró diferencias significativas entre el periodo de dilución y el de recuperación, siendo un buen indicador del estrés por dilución puntual en ríos salinos.

Capítulo 2

4. La dilución puntual incrementó la biomasa de los macroinvertebrados depredadores pero no tuvo un efecto significativo sobre los coeficientes de producción : respiración (P/R).
5. La dilución crónica produjo un descenso significativo de los valores del P/R así como de la biomasa de consumidores y productores primarios.
6. La importancia relativa de los distintos grupos funcionales no se vio afectada por ninguno de los dos tipos de dilución.
7. Los análisis isotópicos revelaron que la comunidad de macroinvertebrados está basada en los productores primarios acuáticos en ambos tramos, a pesar de la gran entrada de carrizo en el *tramo perturbado crónicamente*.
8. La perturbación puntual tuvo efectos mínimos en el funcionamiento del ecosistema, mientras que la perturbación crónica produjo una gran reducción de la biomasa y un cambio en el ecosistema, que pasó de ser un sistema autotrófico a uno heterotrófico.

9. El coeficiente P/R y la biomasa de productores primarios y consumidores son buenos indicadores del estrés por dilución crónica en ríos salinos.

Capítulo 3

10. Las clasificaciones basadas en el criterio de expertos y en el clúster biológico produjeron tipificaciones similares, mientras que la clasificación basada en el clúster ambiental mostró grandes discrepancias.
11. La clasificación biológica presentó los valores más altos de robustez para los datos de ríos españoles, mientras que la clasificación basada en el criterio de expertos mostró los valores más altos de robustez para los datos marroquíes e italianos. La clasificación ambiental exhibió los valores de robustez más bajos en casi todos los casos.
12. Los bioindicadores propuestos en este estudio para evaluar el estado ecológico de los ríos salinos (e.g. *fams.deg*) funcionaron mucho mejor que los indicadores convencionales (e.g. IBMWP, ICM-11a, EPT, *family richness*) a la hora de detectar la integridad ecológica o el impacto antropogénico.
13. Los resultados de este estudio ayudarán a mejorar la capacidad de los gestores ambientales para evaluar el estado ecológico de los ríos mediterráneos, en especial en el caso de los ríos salinos.

Capítulo 4

14. La intensidad del estrés explicó la mayor parte de la variabilidad en la riqueza de especies, que se redujo conforme aumentó el estrés.
15. La disimilitud en la composición de la comunidad entre localidades se incrementó con la diferencia en intensidad de estrés en todos los casos. Para los factores estresantes naturales, los cambios en diversidad beta reflejaron principalmente reemplazamiento de especies, mientras que en el caso de los factores estresantes antropogénicos, las diferencias en las comunidades se debieron principalmente al anidamiento.

16. Nuestros resultados apoyan la hipótesis de que los factores estresantes naturales y antropogénicos generan patrones distintos de diversidad beta a través de diferentes mecanismos.

Capítulo 5

17. Se encontraron respuestas funcionales similares a lo largo de gradientes de estrés de origen natural y humano
18. En ambos gradientes, la riqueza funcional media a escala de taxón y la similitud funcional media aumentaron con el estrés, mientras que la riqueza funcional de la comunidad y la redundancia funcional disminuyeron.
19. Nuestros resultados sugieren que las comunidades podrían presentar cambios funcionales predecibles en respuesta a nuevos factores estresantes a los que los ecosistemas se enfrentarán en el futuro, lo cual podría incrementar nuestra capacidad para predecir las respuestas biológicas al cambio global.

General introduction

Ecological and evolutionary responses to stress: from individuals to ecosystems

Stress can be defined as abiotic or biotic environmental conditions exceeding the normal range experienced by organisms, which cause potential injurious changes to biological systems, having potential to drive evolutionary processes over geological time when severe conditions persist (Hoffman & Parsons 1991; Badyaev 2005; Parsons 2005). On the contrary, the term disturbance is often used to describe temporary, or pulse, harsh environmental conditions, such as flash floods, hurricanes or fires. Consequently, those ecosystems subject to chronic conditions that are unsuitable for the vast majority of the regional pool of species can be considered stressed or extreme ecosystems.

Well-known examples of naturally stressed ecosystems are those found in tundra, deserts, volcanic springs, and acid or saline waters, among many others. Natural stress that is persistent over time is recognised as a powerful driver of evolution and speciation (Vetaas & Grytnes 2002; Parsons 2005; Bradley 2008; Flowers *et al.* 2010). Differential tolerance of organisms to stress may be explained by the contrasting evolutionary contexts of each lineage. Lineages evolving under particular stressful conditions acquire some traits that may facilitate their adaptation to novel environmental challenges (Buchwalter *et al.* 2008). For these reasons, tolerance to stressful conditions show phylogenetic signal, causing most lineages to show preference for stressful environments (Garbuz *et al.* 2008; Abellán *et al.* 2009; Arribas *et al.* 2014).

Despite its short historical persistence, human activity has introduced additional stressful conditions for life globally, modifying the way in which local communities are assembled and increasing extinction rates to amounts that exceed the observed background fossil records (Sala *et al.* 2000). Moreover, given the increasing number of anthropogenic stressors, there is a growing interest in studying the effect of multiple stressors on ecosystems (Vinebrooke *et al.* 2004; Ormerod *et al.* 2010). Anthropogenic modification of ecosystems can be particularly dangerous when stressors generate environmental conditions that are completely novel for the regional pool (i.e. invasive species, organochlorines) or occur at unprecedented levels (e.g. acidity, trace metals) (Buchwalter *et al.* 2008). Recent studies have yielded dramatic predictions and evidence of how the essential functions that nature performs to maintain life and human welfare can be modified or lost, as increasing anthropogenic stress reduces biodiversity (Sala *et al.* 2000; Hooper *et al.* 2005; Hooper *et al.* 2012).

Although there is a limited body of literature devoted to developing general conceptual frameworks concerning the trends expected under stressful conditions, some authors have provided seminal theories and predictions at the individual and ecosystem level (e.g. Pianka 1970; Grime 1977; Southwood 1977; Odum 1985; Taylor *et al.* 1990; Bertness & Callaway 1994).

At the individual level, organisms with small body sizes are expected to be more abundant at stressful conditions (Odum 1985). Body size is correlated with many other biological features (i.e. life cycle, reproductive rate and habitat occupancy), having great implications for organisms in terms of energetics and trophic interactions, as predicted by the Metabolic Theory of Ecology (Brown *et al.* 2004). Metabolic rate increases with biomass, indicating that larger body sizes will need higher amounts of energy to survive, which would increase sensitivity to increased stress. However, in terms of efficiency, the metabolic rate per biomass for larger organisms is lower, which could explain their prolonged lifespans in comparison with small-sized body forms, which tend to breed early, grow quickly and die young. Also, it has been hypothesised that the higher reproductive output of smaller species may confer advantages in response to disturbances or stressful habitats (Cardillo 2003). Moreover, larger species have stronger trophic interactions per capita in comparison with the other species, and are sensitive to a wide range of ecosystem-level changes (Woodward *et al.* 2005). Empirical evidence supports a size-biased extinction risk for ectotherms (Solan *et al.* 2004) and non-marine endotherm animals (Gaston & Blackburn 1995; Cardillo *et al.* 2005). Thus, for example, global warming benefits the presence of small-sized animals in aquatic ecosystems (Daufresne *et al.* 2009).

Although body size is able to predict many biological patterns at the macro scale (i.e. when comparing organisms that largely differ in their body size, such as bacteria and whales), when we look more carefully at organisms of similar body sizes, other traits appear to be more relevant when explaining biological changes along environmental gradients (Tilman *et al.* 2004). For example, it has been reported that organisms showing similar size and morphology occupy different habitats due to differences in physiological traits (Buchwalter *et al.* 2008; Picazo *et al.* 2012; Céspedes *et al.* 2013). Furthermore, we can expect that higher trophic positions would be more sensitive to stress as a result of the reduction in the number of potential food resources under stressful conditions, increased food toxicity and the higher energy demands needed to cope with stress (Bradley 2008). In fact, predators are frequently absent from stressful habitats, which are characterised by simple food webs typically composed of primary producers and herbivores (Menge & Sutherland 1987). Also, stress is

expected to select those organisms with higher phenotypic variability and wider fundamental ecological niches (MacArthur & Levins 1967; Stubbs & Wilson 2004). Generalism is observed when organisms demonstrate an almost constant performance across a wide range of environmental conditions (Kassen 2002), due to wide abiotic tolerance (e.g. Fridley *et al.* 2007) or diverse biotic interactions (e.g. Bascompte *et al.* 2003; Bolnick *et al.* 2007; Bastolla *et al.* 2009). In contrast, specialists occur only in a subset of their potential environments, showing narrower ranges of habitat preference or trophic interactions (Poisot *et al.* 2011). Generalism is thought to arise in species that have evolved in environmentally heterogeneous habitats (Kassen 2002) or in the absence of interspecific competition (Van Vallen 1965; Roughgarden 1972; Bolnick *et al.* 2010), although more complex cases exist, such as stress specialist species, which show a combination of features and display wide differences between their fundamental and realised niches. Thus, empirical evidence demonstrates that organisms adapting to extreme environments expand their fundamental niche rather than specialise in the more stressful environments (Scoville & Pfrender 2010; Latta *et al.* 2012). However, field data reveal that stress specialists are confined to stressful habitats, probably due to a trade-off between competitive ability and stress-tolerance (Wilson & Keddy 1986; Herbst 2001), despite their wide fundamental niche (Pallarés *et al.* 2012; Céspedes *et al.* 2013). Despite the importance of generalists for ecosystem stability (Bascompte *et al.* 2003), specialist species may render biological diversity that increases the diversity of responses and functions at the ecosystem level (Devictor *et al.* 2008; Clavel *et al.* 2011). Global change may lead to a worldwide functional homogenisation of ecosystems, as empirical evidence reveals that past and current environmental changes have produced specialist decline (Clavel *et al.* 2011).

Ecology has a long tradition of studying species assembly along environmental axes and the derived ecosystem properties (Southwood 1977; Keddy 1992; Rosenfeld 2002; Mouillot *et al.* 2005). Traditionally, local communities were considered as a subset of the pool of potential colonisers (i.e. regional species pool). The process of assembly is driven by a combination of biotic (MacArthur & Levins 1967; Stubbs & Wilson 2004), abiotic (Weiher & Keddy 1995; Maire *et al.* 2012) and neutral factors (Hubbell 2001). Niche theory predicts a non-random species sorting along environmental filters determined by species traits (Southwood 1977; Mouillot *et al.* 2005; Shipley *et al.* 2006; Weiher *et al.* 2011), while neutral theory, which assumes the ecological equivalence of species, predicts that changes in local and regional abundances are governed by stochastic demographical and evolutionary processes (Hubbell 2001). More recent views have extended the concept of community by including population

and evolutionary processes, as well as interaction among populations over spatial and temporal scales at the regional level that result in speciation (Ricklefs 2008).

At the ecosystem level, one of the most acknowledged properties expected to emerge along stress gradients is reduced biological diversity. Although the relationship between diversity and stress intensity probably differs in shape and magnitude depending on the stressor type and the taxonomical group studied, empirical evidence often finds that high intensities of stress reduce biological diversity (e.g. Petrin *et al.* 2007; Flynn *et al.* 2009; Millán *et al.* 2011). Furthermore, the Stress Gradient Hypothesis predicts that interspecific competition will decline as environmental conditions become extreme, while facilitative processes will gain importance along stress gradients (Bertness & Callaway 1994). Studies performed on plants (Callaway *et al.* 2002) and animals (Kawai & Tokeshi 2007) generally support this prediction, but more refined conceptual frameworks have highlighted that the Stress Gradient Hypothesis will apply more frequently for certain combinations of life-history traits and stressor types (Maestre *et al.* 2009).

The positive relationship between species richness and ecosystem multifunctionality (Biodiversity-Ecosystem Function) has been supported theoretically (Duffy 2009), and empirically in experimental (e.g. Hooper *et al.* 2005) and field studies (e.g. Maestre *et al.* 2012), suggesting that the variety and magnitude of ecosystem functions can decrease along stress gradients (Laliberté *et al.* 2010; Woodward *et al.* 2012). Furthermore, as stress reduces species diversity, the processes of production and consumption of organic matter can be also affected, becoming more unstable or inefficient (Hooper *et al.* 2005). Stressful conditions could affect producers and consumers differently, possibly resulting in reduced production or consumption rates due to changes in the total amount of organic matter produced or imported, in its palatability, thereby unbalancing the ratio between primary production : respiration (P/R). Thus, stressed ecosystems are expected to show unbalanced P/R, being either highly autotrophic or heterotrophic (Odum 1985).

Bioassessment tools for naturally-stressed ecosystems

Biomonitoring tools aim to detect signs of anthropogenic stress in ecosystems by using the values of a set of stress-sensitive biological indicators (e.g. diversity measures, community composition, ecosystem functions) under pristine conditions as a reference to be compared with test sites to quantify the degree to which the focal site departs from unmodified conditions.

For naturally benign ecosystems, biomonitoring tools can adequately detect human-induced stress (e.g. Bonada *et al.* 2006). However, detecting signs of degradation in naturally-stressed ecosystems is much more complicated, because these spots share some similarities with anthropogenically-stressed ecosystems (Elliott & Quintino 2007), as mentioned previously. This is particularly dangerous given the current context of global change where a great extent of the Earth is experiencing sudden changes. Therefore, we need tools to assess the ecological integrity of ecosystems, especially considering that naturally-stressed ecosystems are acknowledged for holding a unique fraction of genetic, taxonomic and functional biodiversity (Vetaas & Grytnes 2002; Flowers *et al.* 2010; Millán *et al.* 2011; Latta *et al.* 2012; Arribas *et al.* 2013; 2014).

Building new frameworks and methodologies to predict ecosystem responses to increased stress

Despite the great insights that community ecology has provided for understanding how environmental stress affects local communities (Simberloff 2004), one major criticism is the absence of general laws of assembly or response along stress gradients, which will be useful in predicting biological responses to ongoing global change (Keddy 1992; McGill *et al.* 2006; Ricklefs 2008), rather than performing *a posteriori* bioassessments. However, recent advances that combine the use of different types of information and new analytic frameworks, such as beta-diversity partitioning (Baselga 2010) or functional approaches (McGill *et al.* 2006), offer promising advances in this task by allowing the integration of historical processes from local to regional scales (Ricklefs 2008).

The partitioning of beta-diversity (i.e. changes in species composition among locations) into turnover (i.e. species replacement) and nestedness components (i.e. nested subset of species along a richness gradient) has been used to explain the historical processes that have determined current biodiversity patterns, especially the spatial gradients of species richness at regional, continental and global scales (e.g. Hortal *et al.* 2011; Svenning *et al.* 2011; Dobrovolski *et al.* 2012; Baselga *et al.* 2013). For example, Baselga *et al.* (2012) suggests that the latitudinal richness gradient of species observed in Europe can be partially explained by their varying capacities to recolonise after the Last Glacial Maximum (around 25000 years ago), where poor dispersers showed a high species turnover rate latitudinally. However, it is still unclear which turnover and nestedness patterns would emerge along different natural and anthropogenic stress gradients at smaller scales, considering the varying degree to which regional species pools have adapted over evolutionary time.

Natural stress gradients that have persisted over evolutionary time are expected to promote speciation through the development of specialised biological traits, which allow some species to outperform other tolerant taxa and result in a richer pool of specialists. This is a core idea of the Evolutionary Species Pool hypothesis (Taylor *et al.* 1990), which predicts that historically more persistent and abundant habitats should contain more native species in comparison with rare or less persistent habitats. Therefore, along richness gradients where sites differ in stress intensity and share a low proportion of species, assemblage dissimilarities will arise due to species turnover (Fig. 1a); species will exhibit a high degree of specificity along the gradient and occur as specialists that are adapted over geological time (i.e. specialists to natural stress). Alternatively, where richness gradients are a consequence of communities being reduced to nested subsets of those at richer sites, beta-diversity gradients are more likely to arise from nestedness (Fig. 1b); in this case, sites share a higher percentage of species because taxa occurring at poorer sites are stress-tolerant generalists.

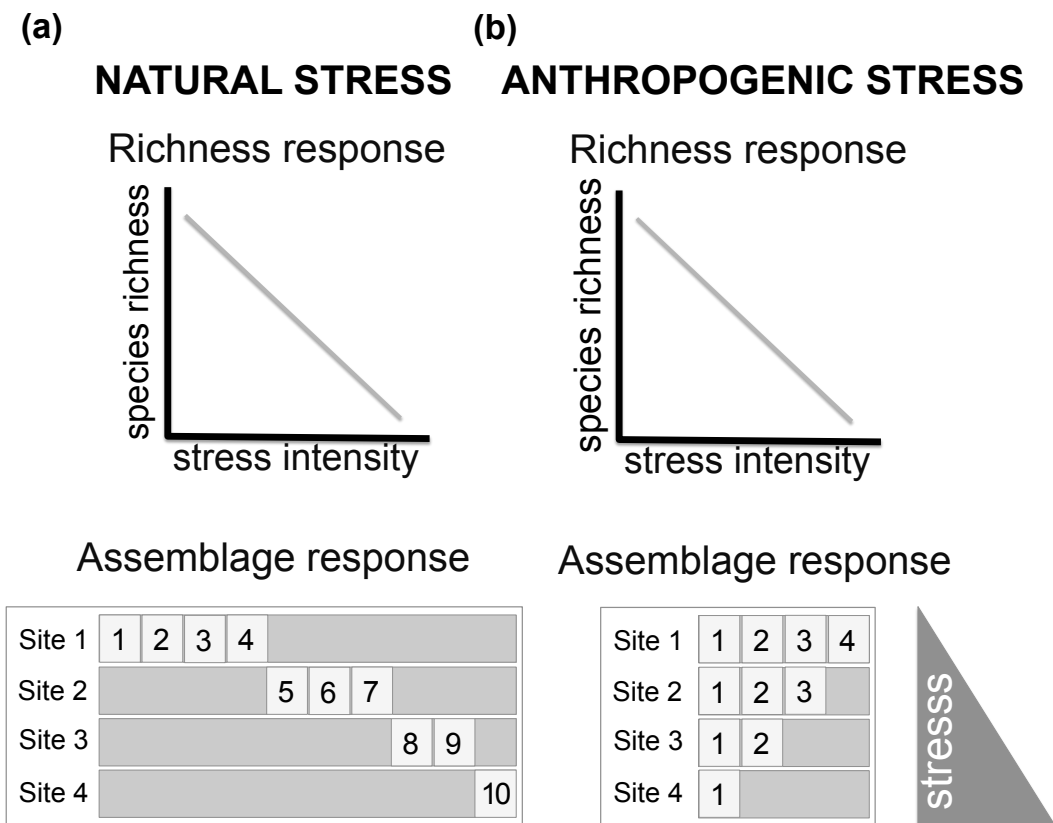


Fig. 1 Predicted patterns of taxonomic richness and beta-diversity along natural (a) and anthropogenic (b) stress gradients.

On the other hand, trait-based approaches show clear advantages over conventional taxonomic methods for predicting biological changes in response

to environmental conditions, such as better inter-taxon and inter-region comparability (e.g. McGill *et al.* 2006), predicting mechanistic links between environmental change and ecosystem function (e.g. Hooper *et al.* 2005), and reflecting evolutionary processes of adaptation to environmental conditions (e.g. Badyaev 2005). Recently developed analytical tools have improved the methodological feasibility of functional approaches to allow the estimation of functional diversity components from a trait-based approach (Villéger *et al.* 2008; Laliberte & Legendre 2010). Functional properties can be described in different ways, including functional diversity components such as functional richness, evenness, divergence (Mason *et al.* 2005), and functional redundancy (Rosenfeld 2002).

Functional patterns found in naturally-stressed ecosystems can be useful for anticipating and predicting future changes in natural ecosystems experiencing anthropogenic stress. Previous studies have documented that traits providing natural stress tolerance can also grant a species the ability to tolerate novel stressors (Badyaev 2005; Buchwalter *et al.* 2008; Arribas *et al.* 2014). In such a case, a subset of biological features will be shared among the different organisms that inhabit naturally and anthropogenically stressed environments (Southwood 1977; Odum 1985; Stanton *et al.* 2000). These commonalities should result in similar trends of functional features along stress gradients, irrespective of the degree to which species have adapted and specialised over evolutionary time (i.e. natural or anthropogenic stressor). In this case, stress would act as a strong, non-random environmental filter that configures trait evolution and community functional properties.

Rivers as model habitats to study the effects of stress on ecosystems

Rivers are particularly effective environments in which to examine the effect of natural and anthropogenic stress gradients. First, they are well-defined habitats that have persisted over geological time-scales, thus allowing long-term speciation (Abellán *et al.* 2009; Arribas *et al.* 2014). Second, they are characterised by marked natural (e.g. elevation, geomorphological longitudinal variation, water chemistry) or anthropogenic (e.g. acidification, pollution) gradients (Moss *et al.* 1987; Díaz *et al.* 2008). Third, they are characterised by great biological diversity; for example, among macroinvertebrates that can be readily sampled and identified (Bonada *et al.* 2006). Benthic macroinvertebrates also represent an important fraction of stream biodiversity and their assemblage composition and richness exhibit a strong correlation with environmental change at reach and basin levels (Strayer 2006). Fourth, despite

some contrasts in evolutionary history among regions, broadly similar groups of aquatic invertebrates (e.g. at family and genus levels) are present – particularly across the Palaearctic and Northern Hemispheric regions. There is clear evidence that organisms such as aquatic insects respond in similar ways to major anthropogenic stressors throughout large areas of the world (Iwasaki & Ormerod 2012).

In particular, the streams and rivers of the Mediterranean basin display well-documented examples of natural and anthropogenic stress gradients (Millán *et al.* 2011; Belmar *et al.* 2013; Cooper *et al.* 2013; Bruno *et al.* 2014), showing significant relationships with aquatic primary producers and macroinvertebrates (Moreno *et al.* 1997; 2001; Velasco *et al.* 2006; Ros *et al.* 2009; Sánchez-Montoya *et al.* 2010; Guareschi *et al.* 2012). Among natural stressors of fluvial ecosystems, natural water salinity is one of the most important drivers of biological variability (Finocchiaro *et al.* 2009; Ros *et al.* 2009; Moreno *et al.* 2010). Lithology determines the amount and type of dissolved salts in the waterbodies (Millán *et al.* 2011; Griffith 2014), resulting in a wide gradient of water salinity that ranges from almost distilled water (conductivity $\approx 15 \mu\text{S cm}^{-1}$) in siliceous catchments, to highly mineralised waters (conductivity $\approx 300,000 \mu\text{S cm}^{-1}$) as a result of water-draining evaporitic basins. Osmotic stress causes cytotoxicity and dehydration when organisms are not able to keep internal salt concentrations within a suitable range via osmoregulation (Bradley 2008; Flowers *et al.* 2010). This gradient was relatively stable over geological time, promoting species adaptation and radiation (Abellán *et al.* 2007; 2009; Arribas *et al.* 2014).

In the Mediterranean basin, we can find many naturally saline running waters distributed throughout the eastern and southern Iberian Peninsula (Millán *et al.* 2011; Arribas *et al.* 2013), Sicily (Gerecke 1991) and North Africa (Margat 1961; Bennis *et al.* 2008). These ecosystems contain unique species with narrow distributions (Sánchez-Fernández *et al.* 2008), that represent singular adaptations to stressful environments (e.g. Pallarés *et al.* 2012; Céspedes *et al.* 2013), showing, in some cases, high intraspecific genetic variability (Abellán *et al.* 2007). However, despite their high conservation interest, the most important biomonitoring programs are ignoring these valuable spots, which are also underrepresented by the Natura 2000 network (Sánchez-Fernández *et al.* 2008) and Moroccan legislation (Bennis *et al.* 2008). As a result, the paucity of classification and biomonitoring methods is constraining the appropriate bioassessment of these unique ecosystems, which are experiencing impacts from human activities (Velasco *et al.* 2006).

Among anthropogenic stressors, land-use intensification is one of the most significant drivers of global change affecting aquatic ecosystems worldwide (Scanlon *et al.* 2007; Monteagudo *et al.* 2012). In the Mediterranean basin, agriculture and urbanisation have been developed historically, closely associated with increasing human populations (Cooper *et al.* 2013). This has produced direct effects on fluvial ecosystems, such as organic enrichment (e.g. Monteagudo *et al.* 2012), increased toxic pollutants like pesticides and/or drugs (e.g. Lopez-Doval *et al.* 2013) and changes in natural ionic concentrations due to irrigation surplus draining from agriculture. This can produce either dilution stress in saline streams with significant decreases in natural salt concentrations (Velasco *et al.* 2006) or secondary salinisation in freshwater waterbodies (Cañedo-Argüelles *et al.* 2013). Both processes could alter the structure and function of aquatic ecosystems.

Objectives

The two main goals of this study are: (1) to provide biomonitoring tools to detect impacts in naturally saline streams caused by anthropogenic pressures (*Chapters 1, 2 and 3*) and (2) to develop frameworks aiming to predict community and ecosystem responses to stress, considering some theoretical trends expected under stressful conditions and the different evolutionary histories of stressors in the focal region (*Chapters 4 and 5*).

More specifically, the aims of this thesis are to:

1. Describe the effects of dilution stress on the functioning of a saline stream. This study analysed changes in ecosystem metabolism, and the biomass of primary producers and consumers between diluted and recovered periods (*Chapter 1*).
2. Quantify the impact of chronic and pulse dilution stress on metabolism and trophic structure in a saline Mediterranean stream. This study analysed shifts in the production : respiration ratio, the biomass of primary producers and consumers, and trophic structure using a factorial design between a control and chronically disturbed site (chronic dilution) during two periods of contrasting mean salinities (pulse dilution) (*Chapter 2*).
3. Test different stream classification procedures that address the range of natural stream diversity found in the western Mediterranean basin and

to propose a set of biological indicators able to detect the anthropogenic impacts in the saline stream types defined (*Chapter 3*).

4. Explore which mechanisms explain beta diversity patterns along stress gradients with contrasting historical persistence (i.e. natural vs. anthropogenic stress). The hypothesis tested was that mechanisms underlying beta diversity in rivers would differ between gradients where (1) natural stressors (salinity, altitude) result in progressive species turnover with high specificity and (2) anthropogenic stressors (acidity, metals, land-use) result in the loss of specialist taxa, thus giving rise to nestedness (*Chapter 4*).
5. Analyse changes in community functional features along chronic stress gradients with contrasting historical persistence (i.e. natural vs. anthropogenic stress). In particular, this study compared trends in the changes of taxon (mean taxon functional richness and mean functional similarity) and the whole community's (functional richness, dispersion and redundancy) functional measures of stream insect communities along natural (salinity) and anthropogenic (land-use intensification) stress gradients (*Chapter 5*).

References

- Abellán, P., Millán, A. & Ribera, I. (2009). Parallel habitat-driven differences in the phylogeographical structure of two independent lineages of Mediterranean saline water beetles. *Molecular Ecology*, 18, 3885-3902.
- Abellán, P., Sánchez-Fernández, D., Velasco, J. & Millán, A. (2007). Effectiveness of protected area networks in representing freshwater biodiversity: the case of a Mediterranean river basin (south-eastern Spain). *Aquatic Conservation: Marine and Freshwater ecosystems*, 361-374.
- Arribas, P., Andújar, C., Abellán, P., Velasco, J., Millán, A. & Ribera, I. (2014). Tempo and mode of the multiple origins of salinity tolerance in a water beetle lineage. *Molecular Ecology*, 23, 360-373.
- Arribas, P., Andújar, C., Sánchez-Fernández, D., Abellán, P. & Millán, A. (2013). Integrative taxonomy and conservation of cryptic beetles in the Mediterranean region (Hydrophilidae). *Zoologica Scripta*, 42, 182-200.
- Badyaev, A.V. (2005). Stress-induced variation in evolution: from behavioural plasticity to genetic assimilation. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, 272, 877-886.
- Bascompte, J., Jordano, P., Melian, C.J. & Olesen, J.M. (2003). The nested assembly of plant-animal mutualistic networks. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 9383-9387.
- Baselga, A. (2010). Partitioning the turnover and nestedness components of beta diversity. *Global Ecology and Biogeography*, 19, 134-143.
- Baselga, A., Fujisawa, T., Crampton-Platt, A., Bergsten, J., Foster, P.G., Monaghan, M.T. *et al.* (2013). Whole-community DNA barcoding reveals a spatio-temporal continuum of biodiversity at species and genetic levels. *Nature Communications*, 4, 1892.
- Baselga, A., Lobo, J.M., Svenning, J.C., Aragón, P. & Araújo, M.S. (2012). Dispersal ability modulates the strength of the latitudinal richness gradient in European beetles. *Global Ecology and Biogeography*, 21, 1106-1113.
- Bastolla, U., Fortuna, M.A., Pascual-García, A., Ferrera, A., Luque, B. & Bascompte, J. (2009). The architecture of mutualistic networks minimizes competition and increases biodiversity. *Nature*, 458, 1018-U1091.

- Belmar, O., Bruno, D., Martínez-Capel, F., Barquín, J. & Velasco, J. (2013). Effects of flow regime alteration on fluvial habitats and riparian quality in a semiarid Mediterranean basin. *Ecological Indicators*, 30, 52-64.
- Bennas, N., Abellán, P., Sánchez-Fernández, D. & Millán, A. (2008). *Ochthebius (Ochthebius) Lanarotis* Ferro, 1985 (Coleoptera, hydraenidae), un coleoptere endemique marocain specifique des milieux aquatiques hypersalin. *Boletín de la Sociedad Entomológica Aragonesa*, 43, 361-366.
- Bertness, M.D. & Callaway, R. (1994). Positive interactions in communities. *Trends in Ecology & Evolution*, 9, 191-193.
- Bolnick, D.I., Ingram, T., Stutz, W.E., Snowberg, L.K., Lau, O.L. & Paull, J.S. (2010). Ecological release from interspecific competition leads to decoupled changes in population and individual niche width. *Proceedings of the Royal Society B-Biological Sciences*, 277, 1789-1797.
- Bolnick, D.I., Svanback, R., Araujo, M.S. & Persson, L. (2007). Comparative support for the niche variation hypothesis that more generalized populations also are more heterogeneous. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 10075-10079.
- Bonada, N., Prat, N., Resh, V.H. & Statzner, B. (2006). Developments in aquatic insect biomonitoring: A comparative analysis of recent approaches. *Annual Review of Entomology*, 51, 495-523.
- Bradley, T.J. (2008). Saline-water Insects: Ecology, Physiology and Evolution. In: *Aquatic insects: challenges to populations* (eds. Lancaster, J & Briers, RA). CAB International Oxfordshire OX10 8DE, UK, pp. 20-35.
- Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. & West, G.B. (2004). Toward a metabolic theory of ecology. *Ecology*, 85, 1771-1789.
- Bruno, D., Belmar, O., Sánchez-Fernández, D., Guareschi, S., Millán, A. & Velasco, J. (2014). Responses of Mediterranean aquatic and riparian communities to human pressures at different spatial scales. *Ecological Indicators*, 45, 456-464.
- Buchwalter, D.B., Cain, D.J., Martin, C.A., Xie, L., Luoma, S.N. & Garland, T. (2008). Aquatic insect ecophysiological traits reveal phylogenetically based differences in dissolved cadmium susceptibility. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 8321-8326.
- Callaway, R.M., Brooker, R.W., Choler, P., Kikvidze, Z., Lortie, C.J., Michalet, R. *et al.* (2002). Positive interactions among alpine plants increase with stress. *Nature*, 417, 844-848.

- Cañedo-Argüelles, M., Kefford, B.J., Piscart, C., Prat, N., Schaefer, R.B. & Schulz, C.-J. (2013). Salinisation of rivers: An urgent ecological issue. *Environmental Pollution*, 173, 157-167.
- Cardillo, M. (2003). Biological determinants of extinction risk: why are smaller species less vulnerable? *Animal Conservation*, 6, 63-69.
- Cardillo, M., Mace, G.M., Jones, K.E., Bielby, J., Bininda-Emonds, O.R.P., Sechrest, W. *et al.* (2005). Multiple causes of high extinction risk in large mammal species. *Science*, 309, 1239-1241.
- Céspedes, V., Pallarés, S., Arribas, P., Millán, A. & Velasco, J. (2013). Water beetle tolerance to salinity and anionic composition and its relationship to habitat occupancy. *Journal of Insect Physiology*, 59, 1076-1084.
- Clavel, J., Julliard, R. & Devictor, V. (2011). Worldwide decline of specialist species: toward a global functional homogenization? *Frontiers in Ecology and the Environment*, 9, 222-228.
- Cooper, S.D., Lake, P.S., Sabater, S., Melack, J.M. & Sabo, J.L. (2013). The effects of land use changes on streams and rivers in mediterranean climates. *Hydrobiologia*, 719, 383-425.
- Daufresne, M., Lengfellner, K. & Sommer, U. (2009). Global warming benefits the small in aquatic ecosystems. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 12788-12793.
- Devictor, V., Julliard, R., Clavel, J., Jiguet, F., Lee, A. & Couvet, D. (2008). Functional biotic homogenization of bird communities in disturbed landscapes. *Global Ecology and Biogeography*, 17, 252-261.
- Díaz, A.M., Alonso, M.L.S. & Gutiérrez, M.R.V.A. (2008). Biological traits of stream macroinvertebrates from a semi-arid catchment: patterns along complex environmental gradients. *Freshwater Biology*, 53, 1-21.
- Dobrovolski, R., Melo, A.S., Cassemiro, F.A.S. & Felizola Diniz-Filho, J.A. (2012). Climatic history and dispersal ability explain the relative importance of turnover and nestedness components of beta diversity. *Global Ecology and Biogeography*, 21, 191-197.
- Duffy, J.E. (2009). Why biodiversity is important to the functioning of real-world ecosystems. *Frontiers in Ecology and the Environment*, 7, 437-444.
- Elliott, M. & Quintino, V. (2007). The estuarine quality paradox, environmental homeostasis and the difficulty of detecting anthropogenic stress in naturally stressed areas. *Marine Pollution Bulletin*, 54, 640-645.

- Finocchiaro, M., Torrisi, M. & Ferlito, A. (2009). Caratterizzazione delle comunità di diatomee bentoniche del bacino idrografico del fiume Simeto (Sicilia orientale) mediante applicazione dell'Indice Diatomico di Eutrofizzazione/Polluzione (EPI-D). *Biologia Ambientale*, 23, 53-66.
- Flowers, T.J., Galal, H.K. & Bromham, L. (2010). Evolution of halophytes: multiple origins of salt tolerance in land plants. *Functional Plant Biology*, 37, 604-612.
- Flynn, D.F.B., Gogol-Prokurat, M., Nogeire, T., Molinari, N., Richers, B.T., Lin, B.B. *et al.* (2009). Loss of functional diversity under land use intensification across multiple taxa. *Ecology Letters*, 12, 22-33.
- Fridley, J.D., Vandermast, D.B., Kuppinger, D.M., Manthey, M. & Peet, R.K. (2007). Co-occurrence based assessment of habitat generalists and specialists: a new approach for the measurement of niche width. *Journal of Ecology*, 95, 707-722.
- Garbuz, D.G., Zatssepina, O.G., Przhiboro, A.A., Yushenova, I., Guzhova, I.V. & Evgenev, M.B. (2008). Larvae of related Diptera species from thermally contrasting habitats exhibit continuous up-regulation of heat shock proteins and high thermotolerance. *Molecular Ecology*, 17, 4763-4777.
- Gaston, K.J. & Blackburn, T.M. (1995). Birds, body-size and the threat of extinction. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, 347, 205-212.
- Gerecke, R. (1991). Taxonomische, faunistische und ökologische Untersuchungen an Wassermilben aus Sizilien, unter Berücksichtigung anderer aquatischer Invertebraten. *Lauterbornia*, 7, 1-303.
- Griffith, M.B. (2014). Natural variation and current reference for specific conductivity and major ions in wadeable streams of the conterminous USA. *Freshwater Science*, 33, 1-17.
- Grime, J.P. (1977). Evidence for existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist*, 111, 1169-1194.
- Guareschi, S., Gutiérrez-Cánovas, C., Picazo, F., Sánchez-Fernández, D., Abellán, P., Velasco, J. *et al.* (2012). Aquatic macroinvertebrate biodiversity: patterns and surrogates in mountainous Spanish national parks. *Aquatic Conservation-Marine and Freshwater Ecosystems*, 22, 598-615.
- Herbst, D.B. (2001). Gradients of salinity stress, environmental stability and water chemistry as a templet for defining habitat types and physiological strategies in inland salt waters. *Hydrobiologia*, 466, 209-219.

- Hoffman, A.A. & Parsons, P.A. (1991). *Evolutionary genetics and environmental stress*. Oxford University Press, Oxford.
- Hooper, D.U., Adair, E.C., Cardinale, B.J., Byrnes, J.E.K., Hungate, B.A., Matulich, K.L. *et al.* (2012). A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature*, 486, 105-129.
- Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S. *et al.* (2005). Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecological Monographs*, 75, 3-35.
- Hortal, J., Diniz-Filho, J.A.F., Bini, L.M., Angel Rodriguez, M., Baselga, A., Noguez-Bravo, D. *et al.* (2011). Ice age climate, evolutionary constraints and diversity patterns of European dung beetles. *Ecology Letters*, 14, 741-748.
- Hubbell, S.P. (2001). *The Unified Neutral Theory of Biodiversity and Biogeography*. Princeton University Press, New Jersey.
- Iwasaki, Y. & Ormerod, S.J. (2012). Estimating safe concentrations of heavy metals from inter-continental field data on river macroinvertebrates. *Environmental Pollution*, 166, 182-186.
- Kassen, R. (2002). The experimental evolution of specialists, generalists, and the maintenance of diversity. *Journal of Evolutionary Biology*, 15, 173-190.
- Kawai, T. & Tokeshi, M. (2007). Testing the facilitation-competition paradigm under the stress-gradient hypothesis: decoupling multiple stress factors. *Proceedings of the Royal Society B-Biological Sciences*, 274, 2503-2508.
- Keddy, P.A. (1992). Assembly and response rules - 2 goals for predictive community ecology. *Journal of Vegetation Science*, 3, 157-164.
- Laliberte, E. & Legendre, P. (2010). A distance-based framework for measuring functional diversity from multiple traits. *Ecology*, 91, 299-305.
- Laliberté, E., Wells, J.A., DeClerck, F., Metcalfe, D.J., Catterall, C.P., Queiroz, C. *et al.* (2010). Land-use intensification reduces functional redundancy and response diversity in plant communities. *Ecology Letters*, 13, 76-86.
- Latta, L.C., Weider, L.J., Colbourne, J.K. & Pfrender, M.E. (2012). The evolution of salinity tolerance in *Daphnia*: a functional genomics approach. *Ecology Letters*, 15, 794-802.

- Lopez-Doval, J.C., Ginebreda, A., Caquet, T., Dahm, C.N., Petrovic, M., Barcelo, D. *et al.* (2013). Pollution in mediterranean-climate rivers. *Hydrobiologia*, 719, 427-450.
- MacArthur, R. & Levins, R. (1967). Limiting similarity convergence and divergence of coexisting species. *American Naturalist*, 101, 377-385.
- Maestre, F.T., Callaway, R.M., Valladares, F. & Lortie, C.J. (2009). Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *Journal of Ecology*, 97, 199-205.
- Maestre, F.T., Quero, J.L., Gotelli, N.J., Escudero, A., Ochoa, V., Delgado-Baquerizo, M. *et al.* (2012). Plant species richness and ecosystem multifunctionality in global drylands. *Science*, 335, 214-218.
- Maire, V., Gross, N., Boerger, L., Proulx, R., Wirth, C., Pontes, L.d.S. *et al.* (2012). Habitat filtering and niche differentiation jointly explain species relative abundance within grassland communities along fertility and disturbance gradients. *New Phytologist*, 196, 497-509.
- Margat, J. (1961). *Les eaux salées au Maroc, Hydrogéologie et Hydrochimie. Note et mémoire du service géologique.* Edition du service géologique du Maroc.
- Mason, N.W.H., Mouillot, D., Lee, W.G. & Wilson, J.B. (2005). Functional richness, functional evenness and functional divergence: the primary components of functional diversity. *Oikos*, 111, 112-118.
- McGill, B.J., Enquist, B.J., Weiher, E. & Westoby, M. (2006). Rebuilding community ecology from functional traits. *Trends in Ecology & Evolution*, 21, 178-185.
- Menge, B.A. & Sutherland, J.P. (1987). Community regulation - variation in disturbance, competition, and predation in relation to environmental-stress and recruitment. *American Naturalist*, 130, 730-757.
- Millán, A., Velasco, J., Gutiérrez-Cánovas, C., Arribas, P., Picazo, F., Sánchez-Fernández, D. *et al.* (2011). Mediterranean saline streams in southeast Spain: What do we know? *Journal of Arid Environments*, 75, 1352-1359.
- Monteagudo, L., Moreno, J.L. & Picazo, F. (2012). River eutrophication: Irrigated vs. non-irrigated agriculture through different spatial scales. *Water Research*, 46, 2759-2771.
- Moreno, J.L., Aboal, M., Vidal-Abarca, M.R. & Suárez, M.L. (2001). Macroalgae and submerged macrophytes from fresh and saline waterbodies of ephemeral streams ('ramblas') in semiarid south-eastern Spain. *Marine and Freshwater Research*, 52, 891-905.

- Moreno, J.L., Angeler, D.G. & De las Heras, J. (2010). Seasonal dynamics of macroinvertebrate communities in a semiarid saline spring stream with contrasting environmental conditions. *Aquatic Ecology*, 44, 177-193.
- Moreno, J.L., Millán, A., Suárez, M.L., Vidal-Abarca, M.R. & Velasco, J. (1997). Aquatic coleoptera and heteroptera assemblages in waterbodies from ephemeral coastal streams ("ramblas") of south-eastern Spain. *Archiv Fur Hydrobiologie*, 141, 93-107.
- Moss, D., Furse, M.T., Wright, J.F. & Armitage, P.D. (1987). The prediction of the macroinvertebrate fauna of unpolluted running-water sites in Great-Britain using environmental data. *Freshwater Biology*, 17, 41-52.
- Mouillot, D., Stubbs, W., Faure, M., Dumay, O., Tomasini, J.A., Wilson, J.B. *et al.* (2005). Niche overlap estimates based on quantitative functional traits: a new family of non-parametric indices. *Oecologia*, 145, 345-353.
- Odum, E.P. (1985). Trends expected in stressed ecosystems. *Bioscience*, 35, 419-422.
- Ormerod, S.J., Dobson, M., Hildrew, A.G. & Townsend, C.R. (2010). Multiple stressors in freshwater ecosystems. *Freshwater Biology*, 55, 1-4.
- Pallarés, S., Arribas, P., Cespedes, V., Millán, A. & Velasco, J. (2012). Lethal and sublethal behavioural responses of saline water beetles to acute heat and osmotic stress. *Ecological Entomology*, 37, 508-520.
- Parsons, P.A. (2005). Environments and evolution: interactions between stress, resource inadequacy and energetic efficiency. *Biological Reviews*, 80, 589-610.
- Petrin, Z., Laudon, H. & Malmqvist, B. (2007). Does freshwater macroinvertebrate diversity along a pH-gradient reflect adaptation to low pH? *Freshwater Biology*, 52, 2172-2183.
- Pianka, E.R. (1970). r-selection and K-selection. *American Naturalist*, 104, 592-&.
- Picazo, F., Millán, A. & Dolédec, S. (2012). Are patterns in the taxonomic, biological and ecological traits of water beetles congruent in Mediterranean ecosystems? *Freshwater Biology*, 57, 2192-2210.
- Poisot, T., Bever, J.D., Nemri, A., Thrall, P.H. & Hochberg, M.E. (2011). A conceptual framework for the evolution of ecological specialisation. *Ecology Letters*, 14, 841-851.

- Ricklefs, R.E. (2008). Disintegration of the Ecological Community. *American Naturalist*, 172, 741-750.
- Ros, M.D., Marín-Murcia, J.P. & Aboal, M. (2009). Biodiversity of diatom assemblages in a Mediterranean semiarid stream: implications for conservation. *Marine and Freshwater Research*, 60, 14-24.
- Rosenfeld, J.S. (2002). Functional redundancy in ecology and conservation. *Oikos*, 98, 156-162.
- Roughgarden, J. (1972). Evolution of the niche width. *American Naturalist*, 106, 683-781.
- Sala, O.E., Chapin, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R. *et al.* (2000). Biodiversity - Global biodiversity scenarios for the year 2100. *Science*, 287, 1770-1774.
- Sánchez-Fernández, D., Bilton, D.T., Abellán, P., Ribera, I., Velasco, J. & Millán, A. (2008). Are the endemic water beetles of the Iberian Peninsula and the Balearic Islands effectively protected? *Biological Conservation*, 141, 1612-1627.
- Sánchez-Montoya, M.M., Vidal-Abarca, M.R. & Suárez, M.L. (2010). Comparing the sensitivity of diverse macroinvertebrate metrics to a multiple stressor gradient in Mediterranean streams and its influence on the assessment of ecological status. *Ecological Indicators*, 10, 896-904.
- Scanlon, B.R., Jolly, I., Sophocleous, M. & Zhang, L. (2007). Global impacts of conversions from natural to agricultural ecosystems on water resources: Quantity versus quality. *Water Resources Research*, 43.
- Scoville, A.G. & Pfrender, M.E. (2010). Phenotypic plasticity facilitates recurrent rapid adaptation to introduced predators. *Proceedings of the National Academy of Sciences of the United States of America*, 107, 4260-4263.
- Shipley, B., Vile, D. & Garnier, E. (2006). From plant traits to plant communities: A statistical mechanistic approach to biodiversity. *Science*, 314, 812-814.
- Simberloff, D. (2004). Community ecology: Is it time to move on? *American Naturalist*, 163, 787-799.
- Solan, M., Cardinale, B.J., Downing, A.L., Engelhardt, K.A.M., Ruesink, J.L. & Srivastava, D.S. (2004). Extinction and ecosystem function in the Marine benthos. *Science*, 306, 1177-1180.

- Southwood, T.R.E. (1977). Habitat, templet for ecological strategies - Presidential-address to British-Ecological-Society, 5 January 1977. *Journal of Animal Ecology*, 46, 337-365.
- Stanton, M.L., Roy, B.A. & Thiede, D.A. (2000). Evolution in stressful environments. I. Phenotypic variability, phenotypic selection, and response to selection in five distinct environmental stresses. *Evolution*, 54, 93-111.
- Strayer, D.L. (2006). Challenges for freshwater invertebrate conservation. *Journal of the North American Benthological Society*, 2, 271-287.
- Stubbs, W.J. & Wilson, J.B. (2004). Evidence for limiting similarity in a sand dune community. *Journal of Ecology*, 92, 557-567.
- Svenning, J.-C., Flojgaard, C. & Baselga, A. (2011). Climate, history and neutrality as drivers of mammal beta diversity in Europe: insights from multiscale deconstruction. *Journal of Animal Ecology*, 80, 393-402.
- Taylor, D.R., Aarssen, L.W. & Loehle, C. (1990). On the relationship between r/K selection and environmental carrying-capacity - a new habitat templet for plant life-history strategies. *Oikos*, 58, 239-250.
- Tilman, D., Hillerislambers, J., Harpole, S., Dybzinski, R., Fargione, J., Clark, C. *et al.* (2004). Does metabolic theory apply to community ecology? It's a matter of scale. *Ecology*, 85, 1797-1799.
- Van Vallen, L. (1965). Morphological variation and width of ecological niche. *American Naturalist*, 99, 377-389.
- Velasco, J., Millán, A., Hernández, J., Gutiérrez-Cánovas, C., Abellán, P., Sánchez-Fernández, D. *et al.* (2006). Response of biotic communities to salinity changes in a Mediterranean hypersaline stream. *Saline Systems*, 2, 12.
- Vetaas, O.R. & Grytnes, J.A. (2002). Distribution of vascular plant species richness and endemic richness along the Himalayan elevation gradient in Nepal. *Global Ecology and Biogeography*, 11, 291-301.
- Villéger, S., Mason, N.W.H. & Mouillot, D. (2008). New multidimensional functional diversity indices for a multifaceted framework in functional ecology. *Ecology*, 89, 2290-2301.
- Vinebrooke, R.D., Cottingham, K.L., Norberg, J., Scheffer, M., Dodson, S.I., Maberly, S.C. *et al.* (2004). Impacts of multiple stressors on biodiversity and ecosystem functioning: the role of species co-tolerance. *Oikos*, 104, 451-457.

- Weiher, E., Freund, D., Bunton, T., Stefanski, A., Lee, T. & Bentivenga, S. (2011). Advances, challenges and a developing synthesis of ecological community assembly theory. *Philosophical Transactions of the Royal Society B-Biological Sciences*, 366, 2403-2413.
- Weiher, E. & Keddy, P.A. (1995). Assembly rules, null models, and trait dispersion - new questions front old patterns. *Oikos*, 74, 159-164.
- Wilson, S.D. & Keddy, P.A. (1986). Species competitive ability and position along a natural stress disturbance gradient. *Ecology*, 67, 1236-1242.
- Woodward, G., Ebenman, B., Emmerson, M., Montoya, J.M., Olesen, J.M., Valido, A. *et al.* (2005). Body size in ecological networks. *Trends in Ecology & Evolution*, 20, 402-409.
- Woodward, G., Gessner, M.O., Giller, P.S., Gulis, V., Hladyz, S., Lecerf, A. *et al.* (2012). Continental-scale effects of nutrient pollution on stream ecosystem functioning. *Science*, 336, 1438-1440.

Chapter 1:

Effects of dilution stress on the functioning of a saline Mediterranean stream

Published in Hydrobiologia:

Gutiérrez-Cánovas, C.; Velasco, J. & A. Millán. 2009. Effects of dilution stress on the functioning of a saline Mediterranean stream. *Hydrobiologia*, 619: 119-132.

Effects of dilution stress on the functioning of a saline Mediterranean stream

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Abstract

The effects of seasonality and dilution stress on the functioning of Rambla Salada, a hypersaline Mediterranean stream in SE Spain were evaluated. The stream is subject to diffuse freshwater inputs from the drainage of intensively irrigated agriculture in the catchment and periodic losses of water through an irrigation channel. Metabolic rates and the biomass of primary producers and consumers were estimated over a two-year period. During the first year several dilution events occurred, while during the second year the salinity recovery and reached pre-disturbance levels. Functional indicators were compared between the disturbance and recovery salinity periods. Primary production and respiration rates in the Rambla Salada ranged between 0.07-21.05 and 0.19-17.39 g O₂ m⁻² day⁻¹, respectively. The mean values for these variables were 7.35 and 5.48 g O₂ m⁻² day⁻¹, respectively. Mean NDM rate was 1.87 ± 0.52 g O₂ m⁻² day⁻¹ and mean P/R was 2.48 ± 1.1, reflecting autotrophic metabolism. The metabolic rates showed the typical seasonal pattern of Mediterranean open canopy streams. Therefore, GPP and ER registered maximum values in summer, intermediate values in spring and autumn and minimum values in winter. The metabolic rates and biomass of consumers were greater in the disturbance period than in the recovery period. However, they did not show significant differences between periods due to their important dependence on seasonal cycle. Seasonality accounted for much of the temporal variability in GPP and ER (76% and 83% in the multiregression models, respectively). Light availability seems to be the most important factor for GPP and ER in the Rambla Salada. Autotrophic biomass responded more to variations in discharge and conductivity than to seasonal variations. In fact, it was severely affected by freshwater inputs after which the epipellic biomass decreased significantly and *Cladophora glomerata* proliferated rapidly. Epipellic algal biomass was the most sensitive parameter to dilution disturbance.

Introduction

During last years, the interest in ecosystem health has been growing. Evaluating ecosystem health implies integrating human values with biophysical processes (Rapport et al., 1998). Thus, a healthy ecosystem is defined as being stable and sustainable, maintaining its organization and autonomy over time and its resilience to stress (Costanza, 1992). Traditionally, physicochemical and biological parameters of community structure have been used to assess stream health. Recently, functional indicators of ecosystem processes (metabolic rates, organic matter decomposition, nutrient processes, etc.) have received more attention and are now considered fundamental to determine the health of stream and river ecosystems (Bunn et al., 1999; Bunn & Davies, 2000; Gessner & Chauvet, 2002; Young et al., 2004; Udy et al., 2006). Ecosystem metabolism, the combination of primary production (GPP) and ecosystem respiration (ER), provides a measure of the amount of organic carbon produced and consumed within the system, respectively. Both rates often show seasonal patterns as a consequence of their dependence on environmental factors (light, temperature, nutrients), but are also highly sensitive to many human-induced environmental stressors making them good stream health indicators (Gessner & Chauvet, 2002; Young et al., 2004).

Mediterranean streams exhibit a wide range of natural and anthropogenic stressors that can affect instream processes. Streams in Mediterranean climate regions are influenced by a sequence of regular and often extreme flooding and drying (Gasith & Resh, 1999). These hydrological extremes have great influence on the dynamics of metabolic rates (Acuña et al., 2004; 2005). On the other hand, intensive agriculture and industrial and urban sewage are the main human stressors in the Mediterranean region that increase nutrient concentrations in the streams stimulating both primary production and ecosystem respiration (Guasch et al., 1995). Some Mediterranean streams in arid and semiarid areas are natural saline systems due to presence of evaporites in the basin. During last decades, changes in agricultural practices, such as the expansion of irrigated agriculture in the watersheds, are the main cause of salinity reduction in saline streams.

Several studies have determined the factors controlling metabolic rates in Mediterranean streams (Mollá et al., 1994; Guasch & Sabater, 1995; Guasch et al., 1995; Mollá et al., 1996; Velasco et al., 2003; Acuña et al., 2004; 2005), but the effects of salinity on stream functioning have not been studied.

Saline streams in semiarid zones are characterised by high light availability because of the absence of riparian vegetation. In such cases, temperature is a

good predictor of GPP and ER, although flood disturbance has a considerable influence on the biomass and production of primary producers (Velasco et al., 2003). These systems have autotrophic metabolism throughout the year, with high primary production rates that, in turn, support high secondary production rates (Perán et al., 1999; Barahona et al., 2005). Freshwater inputs (agricultural drainage, diversion channel losses) lower salinity of saline streams, affecting natural physicochemical conditions and the composition and structure of the biological community. Thus, a change from the domination of epipelagic algae towards the domination of filamentous algae and a noticeable increase in the richness of macroinvertebrate taxa have been observed to accompany decreasing salinity (Velasco et al., 2006).

The aim of this study was to describe the effect of a drop in salinity on the metabolism and on the biomass of primary producers and consumers of a hypersaline stream and to outline seasonal and anthropogenic changes. Our hypothesis was that decreasing salinity reduces salt stress, increasing the biomass of primary producers (especially filamentous algae) and the biomass of consumers, leading to an increase in GPP and ER rates.

Materials and methods

Study area

The Rambla Salada is a Mediterranean hypersaline stream located in the sedimentary Fortuna basin, which belongs to the Segura River basin in the most arid area of the Region of Murcia on the southeast of the Iberian Peninsula (Fig. 1). The climate is characterised by mean annual precipitation below 300 mm (mainly concentrated in spring and autumn) and a mean annual temperature of 18 °C, with long warm, dry summers and mild winters. Its 44.7 km² catchment is drained by a permanent main channel and short ephemeral tributaries, which flow only during rainy periods. The main 11.6 km long channel is a third order stream with intermittent flow at its head. The natural cover is open Mediterranean scrub, although much of the area is dedicated to citrus and horticultural crops (intensive agriculture). The high salinity of the Rambla Salada is principally due to Miocene gypsiferous marls and the ionic composition of the water is dominated by Na⁺ and Cl⁻ ions, followed by SO₄²⁻ and Ca²⁺ (Ramírez-Díaz et al., 1995).

Since the 1980s the basin's land uses have changed because of the increasing availability of water resources provided by the Tagus-Segura river diversion, which has increased agricultural activity. Indeed, both agricultural drainage water and diversion channel losses have led to a fall in salinity from mean

values close to 100 g L^{-1} (Vidal-Abarca, 1985) to a mean of 35.5 g L^{-1} . During this study, the diversion channel that crosses the stream was emptied for repairs in October 2003 adding substantial amounts of freshwater to the stream. During this time the stream reached the lowest salinity ever recorded.

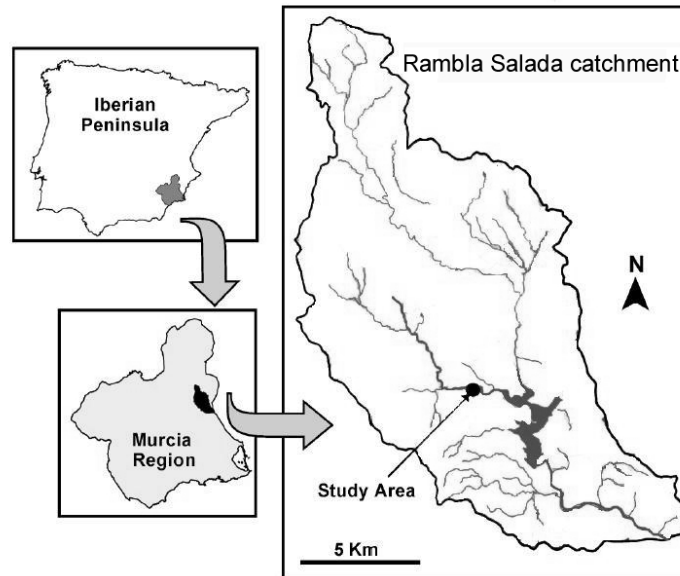


Fig. 1 Geographic location of the study area in Rambla Salada stream and main courses of its catchment.

Sampling and processing of the samples

In this contribution, we describe the dynamics of metabolic rates and algal and macroinvertebrate biomass over two years that included two periods: the first was disturbed by large inputs of freshwater (from 9 April 2003 to 11 December 2003), whilst during the second period (from 24 March 2004 to 13 January 2005) salinity recovered. Sampling was performed with a nearly bimonthly frequency, resulting in ten sampling dates (5 for each period).

The study was carried out in the middle section of the stream, close to an abandoned salt works (lat. $38^{\circ}7'28''\text{N}$, long. $1^{\circ}6'46''\text{W}$). Pools and runs constitute the principal aquatic habitats in the reach. Shallow pools ($\leq 60 \text{ cm}$), which occupy more than 80 % of the channel surface, are characterised by large deposits of silt covered by a biofilm composed by diatoms and cyanobacteria. Runs ($<10 \text{ cm}$ deep and maximum water velocity of 0.37 m.s^{-1}) are dominated by gravel and sand. This habitat was principally covered by the filamentous algae *Cladophora glomerata*.

Stream metabolism rates were measured using an open-system, single-station approach (Odum, 1956). On each date, water temperature and dissolved oxygen

were measured *in situ* at 15 minute intervals over 24 hours using a multi-parameter recorder (WTW, MultiLine P4). Reaeration coefficient (K_s) and ecosystem respiration (ER) were calculated following the night-time regression method (Thyssen & Kelly, 1985) using River Metabolism Estimator v. 1.2, an MS Excel spreadsheet available at <http://www.cawthron.org.nz> (Young & Knight, 2005), which also estimates gross primary production (GPP), net daily metabolism (NDM) and production/respiration ratio (P/R). In this method the reaeration coefficient is obtained from the slope of the linear regression between the night-time rate of change of stream dissolved oxygen versus the saturation deficit. Thus, low diel differences in oxygen saturation reduce linearity of this relation constraining the accuracy of the metabolic rates measures. Low diel differences could be produced by diurnal cloudiness or due to naturally very low gross primary production.

Precipitation for the ten days preceding sampling and radiation on each date were obtained from a regional climatic data base (SIAM, 2008). Conductivity was measured in the morning with an ECmeter (TetraConR 325) that automatically calculates salinity. Discharge was estimated from measurements of stream section and current velocity along a cross-section of the run.

In the run, three water samples were taken to measure suspended solids, chlorophyll *a* and dissolved nutrients (ammonium, nitrate and nitrite concentrations). The samples were kept cool until their arrival to the laboratory, where they were filtered onto pre-combusted and pre-weighed GF/F glass-fiber filters. The filters were oven-dried at 60 °C to constant dry weight and then combusted at 450 °C for 4h to estimate total suspended solids and suspended particulate organic matter. Chlorophyll *a* concentration was determined by spectrophotometry, following extraction in 90% acetone. Ammonium ($\text{NH}_4\text{-N}$) was converted to ammonia by adding 10 M NaOH solution and measured with an ammonia electrode connected to a pH/mV meter. The rest of the dissolved inorganic nutrients were determined according to standard methods (APHA, 2002): nitrate by the cadmium reduction method and nitrite by sulfanylic acid colorimetry.

Five benthic organic matter samples of sediments per habitat (pools/runs) were collected using cores of 5.3 cm diameter and 24.5 cm length. Sediment samples were oven-dried at 60 °C to constant dry weight and then combusted at 450 °C for 4h to estimate the content of ash free dry weight (AFDW). The biomass of primary producers was estimated from ten replicate samples (5 for each habitat) using similar cores as for sediments. For epipellic algae, we used a minicore of 2 cm diameter and 2 cm length. Samples were stored on ice and frozen until processing. Sixty milligrams of fresh macrophyte were taken from each sample

to determine chlorophyll *a* concentration (Chl *a*) by spectrophotometry, following extraction in acetone. The rest of the macrophyte was oven-dried at 60 °C to constant dry weight (DW). Epipellic samples were filtered onto Whatman A filters to determine chlorophyll *a* concentration and AFDW. The biomass of each primary producer in the studied reach was estimated by multiplying cover percentage (visually estimated) by content of chlorophyll *a* per area.

In both habitats, benthic macroinvertebrates were collected from the bottom using a 0.014 m² core. Substrate was stirred by hand and a 250 µm mesh sieve was passed across the bottom and through the water column until no individuals were found. Five replicate cores per habitat were taken randomly on each sampling date and preserved and stored in 75% ethanol. In the laboratory, macroinvertebrate were identified, counted and measured (body length). In addition, macroinvertebrates were classified as primary consumers (larvae from the families *Chironomidae*, *Baetidae*, *Dolichopodidae*, *Hydrobiidae*, *Simuliidae*, *Stratiomyidae* and adults from the genus *Berosus* spp., *Enochrus* spp. and *Ochthebius* spp.) and secondary consumers (larvae of the families *Aeshnidae*, *Ceratopogonidae*, *Ephydriidae* and the genus *Berosus* spp., *Enochrus* spp. and all the individuals of the genus *Nebrioporus* spp. and *Sigara* spp.). The mean density of each taxon was multiplied by the relative habitat area in order to obtain reach macroinvertebrate abundance. Benthic macroinvertebrate biomass was obtained from length-mass equations available for the same or nearest taxon from saline streams (Moreno, 2002; Barahona et al., 2005) or from the general equations for macroinvertebrate families (Smock, 1980; Benke et al., 1999). Macroinvertebrate biomass was calculated for primary, secondary and total consumers.

Data analyses

To characterise and summarise the environmental variations occurring during the study period, a principal components analysis (PCA) was performed using climatic and physicochemical data. Data were transformed to normalise distribution and equalise variance and were z-standardised (mean=0, SD=1) before being included in this analysis to avoid distortions due to the effect of different transformations and magnitudes. The meaning of each axis was interpreted on the basis of Pearson coefficient between PCA axes scores and environmental variables. Ordination analysis was carried out using PRIMER 6.0 (Clarke & Warwick, 1994).

The correlation between variables (including the two first PCA axes) was studied through Pearson coefficients. To compare periods *t*-tests were performed for each study variable.

Finally, PCA axes 1 and 2, which condense abiotic variables, were used as independent variables in multiple linear regression models to assess the contribution of seasonality and dilution perturbation to the variation of functional variables. We used a $P < 0.05$ as criterion for inclusion into the model. In addition, an analysis of collinearity was performed for all the variables included in the regression model and the autocorrelation residuals were studied, using a Durbin-Watson test. When multiple regression residuals showed 1st-order autocorrelation or the Durbin-Watson test value was less than 1.5 or greater than 2.5, a Prais-Winsten autoregression analysis was applied. This analysis was conducted in SPSS 13.0 program (SPSS, 2004).

NDM, P/R and *Enteromorpha intestinalis* biomass were removed from analyses due to neither transformation was able to normalise their distribution.

Results

Physicochemical changes

The disturbance period was characterised by a salinity drop due to upstream freshwater inputs from losses in the Tagus-Segura diversion channel (Fig. 2). The lowest conductivity (6.4 mS cm^{-1}) and highest discharge (235.8 L s^{-1}) were recorded in October 2003 when the diversion channel was emptied to be repaired. After the repairs had been completed, conductivity increased, reaching the highest value in October 2004 (99 mS cm^{-1}). Both periods had similar temperature and radiation conditions, although precipitation was higher in the disturbance period due to maximum precipitation registered in December 2003 (Table 1). Discharge and precipitation were not correlated. Maximum water temperatures were registered in both periods in July (around $35 \text{ }^\circ\text{C}$) whereas the lowest temperatures were found in December 2003 and January 2005 ($10.7 \text{ }^\circ\text{C}$ and $5.2 \text{ }^\circ\text{C}$, respectively). Besides significant differences in discharge and conductivity between periods, levels of oxygen saturation, and $\text{NO}_2\text{-N}$ and $\text{NH}_4\text{-N}$ concentration were significantly lower in the disturbance period than in the recovery period (Table 1).

Results of the PCA analysis are displayed in Fig. 3 and Table 3. The first axis explained 32.0% of the variation in environmental conditions and it was closely related to seasonal variables. This axis was negatively correlated with precipitation, suspended organic matter and total suspended solids and

positively with radiation and temperature (Table 3). The second axis accounted for 30.6% of the variation and can be interpreted as a descriptor of dilution disturbance. This axis was positively correlated with discharge and negatively with conductivity, nitrite, ammonium and oxygen saturation (Table 3).

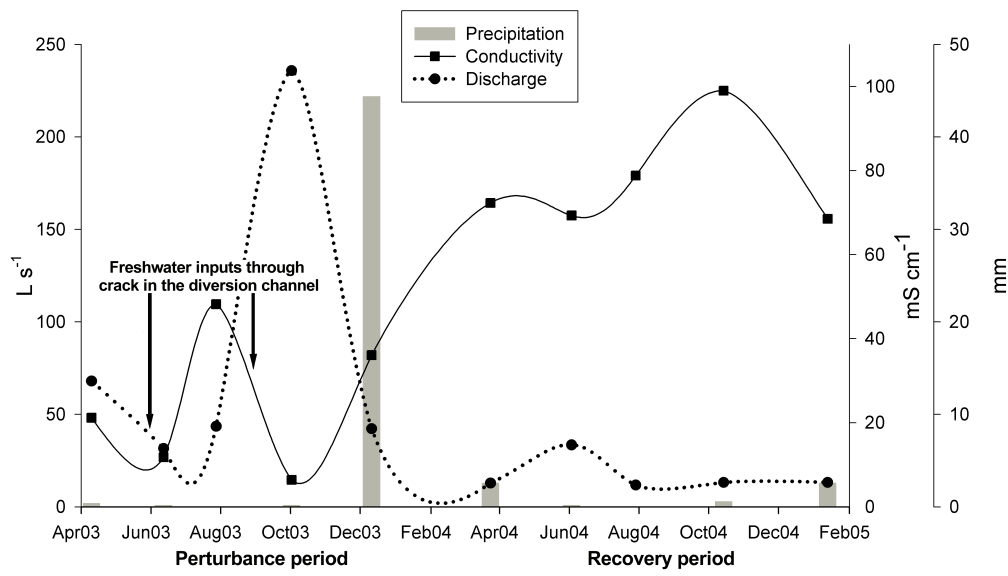


Fig. 2 Variation of precipitation, conductivity and discharge in disturbance and recovery periods.

Table 1 Mean and standard error of climatic and environmental variables during and after dilution disturbance in Rambla Salada (± 1 SE). Disturbed period: April 2003 to December 2003, $n = 5$. Recovered period: March 2004 to January 2005, $n = 5$. n.s.: non-significant, $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$.

Variable	Abbreviation	Disturbed period	Recovered period	t-test
Total daily radiation ($W m^{-2}$)	RAD	216.39 ± 38.76	211.78 ± 39.78	n.s.
Last ten days precipitation (mm)	PR10	9.04 ± 8.84	1.20 ± 0.58	n.s.
Discharge ($L s^{-1}$)	Q	84.22 ± 38.36	16.91 ± 4.15	**
Daily mean water temperature ($^{\circ}C$)	TEMP	20.82 ± 3.10	19.56 ± 3.18	n.s.
Daily mean dissolved oxygen ($mg L^{-1}$)	DO	8.59 ± 0.44	8.69 ± 0.69	n.s.
Daily mean oxygen saturation (%)	SAT	107.15 ± 5.01	133.37 ± 7.20	*
Conductivity ($mS cm^{-1}$)	COND	24.74 ± 7.73	77.58 ± 5.65	**
Benthic organic matter ($g m^{-2}$)	BOM	516.10 ± 69.14	598.76 ± 68.58	n.s.
Total suspended solids ($mg DW L^{-1}$)	TSS	33.59 ± 21.38	55.62 ± 16.02	n.s.
Suspended organic matter ($mg AFDW L^{-1}$)	SOM	12.28 ± 6.48	7.91 ± 1.69	n.s.
Chlorophyll <i>a</i> ($mg L^{-1}$)	Chl <i>a</i>	1.43 ± 0.03	1.49 ± 0.06	n.s.
Nitrate ($mg L^{-1}$)	NO_3-N	2.27 ± 1.03	1.18 ± 0.32	n.s.
Nitrite ($\mu g L^{-1}$)	NO_2-N	53.65 ± 21.52	639.15 ± 425.99	**
Ammonium ($mg L^{-1}$)	NH_4-N	0.31 ± 0.10	1.59 ± 0.47	*

Table 2 Mean and standard error of metabolic rates, producers and consumers biomass during and after dilution disturbance in Rambla Salada (± 1 SE). Disturbed period: April 2003 to December 2003, n = 5. Recovered period: March 2004 to January 2005, n = 5. -: non-analysed; n.s.: non-significant, $P > 0.05$; *: $P < 0.05$.

Variable	Abbreviation	Disturbed period	Recovered period	t-test
Gross primary production (g O ₂ m ⁻² day ⁻¹)	GPP	8.27 \pm 3.54	6.43 \pm 0.84	n.s.
Ecosystem respiration (g O ₂ m ⁻² day ⁻¹)	ER	7.05 \pm 2.85	3.91 \pm 1.26	n.s.
Net daily metabolism (g O ₂ m ⁻² day ⁻¹)	NDM	1.22 \pm 0.81	2.52 \pm 0.61	-.
Production/Respiration	P/R	1.00 \pm 0.18	3.96 \pm 2.21	-
<i>Cladophora glomerata</i> biomass (g Chl <i>a</i> m ⁻²)	CLA	115.30 \pm 85.13	6.36 \pm 4.95	n.s.
<i>Enteromorpha intestinalis</i> biomass (g Chl <i>a</i> m ⁻²)	ENT	0.09 \pm 0.09	0.03 \pm 0.02	-
Epipellic algae biomass (g Chl <i>a</i> m ⁻²)	EPI	7.42 \pm 3.08	20.29 \pm 2.41	*
<i>Ruppia maritima</i> biomass (g Chl <i>a</i> m ⁻²)	RUP	37.39 \pm 25.25	6.05 \pm 3.30	n.s.
Primary producer biomass (g Chl <i>a</i> m ⁻²)	PPB	160.19 \pm 86.86	32.74 \pm 6.65	n.s.
Primary consumer biomass (g AFDW m ⁻²)	PCB	1.22 \pm 0.34	0.85 \pm 0.17	n.s.
Secondary consumer biomass (g AFDW m ⁻²)	SCB	5.25 \pm 2.43	1.02 \pm 0.51	n.s.
Total consumers biomass (g AFDW m ⁻²)	TCB	6.57 \pm 2.58	2.29 \pm 1.05	n.s.

Autotrophic biomass

The disturbance period showed higher primary biomass values, mainly due to the abundance of *Cladophora glomerata* followed from *Ruppia maritima*, than in the recovery period (Fig. 4a, Table 2), although differences were not significant, except for epipellic algae, which were significantly higher in the recovery period than in the disturbance period (Fig. 4a, Table 2). Epipellic algal biomass was positively correlated with conductivity, nitrates and ammonium and negatively with discharge and PCA axis 2 (Table 4).

Cladophora glomerata biomass reached maximum values in April 2003 and October 2003 but was absent or showed low levels in the recovery period. *C. glomerata* biomass was positively correlated with discharge and the second PCA axis, and negatively with conductivity and TSS (Table 4). This filamentous alga was the principal contributor to total autotrophic biomass (Fig. 4a). *Ruppia maritima* occurred on all the dates with the exception of April 2003 and January 2005 and had a maximum of biomass in October 2003. This macrophyte was negatively correlated with ammonium and epipellic algae biomass (Table 4). *Enteromorpha intestinalis* biomass was scarce during the study with a maximum in October 2003.

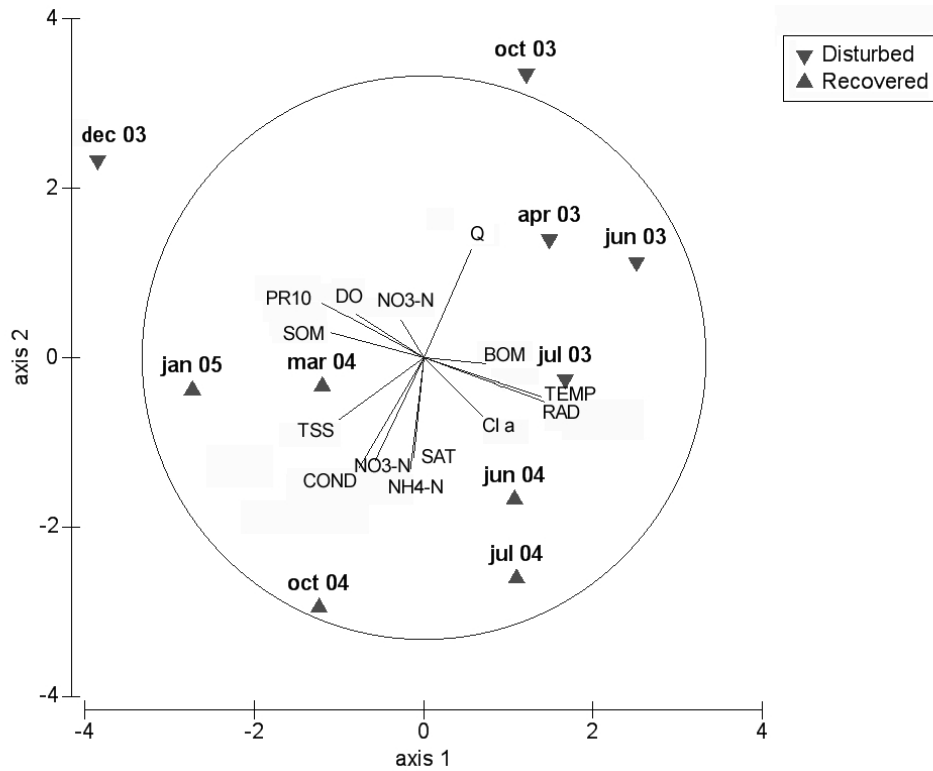


Fig. 3 PCA ordination plot of axes 1 and 2 for environmental variables. See Tables 1 and 2 to see the correspondence between variables' full names and abbreviations.

Table 3 Pearson coefficients calculated between environmental variables and PCA axes scores. See Tables 1 and 2 to see the correspondence between variables' full names and abbreviations. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

Variable	Axis 1	Axis 2
RAD	0,92***	-0,33
PR10	-0,77*	0,41
Q	0,36	0,80*
TEMP	0,88**	-0,29
DO	-0,61	0,38
SAT	-0,08	-0,74*
COND	-0,42	-0,87**
BOM	0,46	-0,04
TSS	-0,64*	-0,46
SOM	-0,70*	0,18
Chl <i>a</i>	0,44	-0,43
NO ₃ -N	-0,17	0,28
NO ₂ -N	-0,49	-0,82**
NH ₄ -N	-0,10	-0,82**

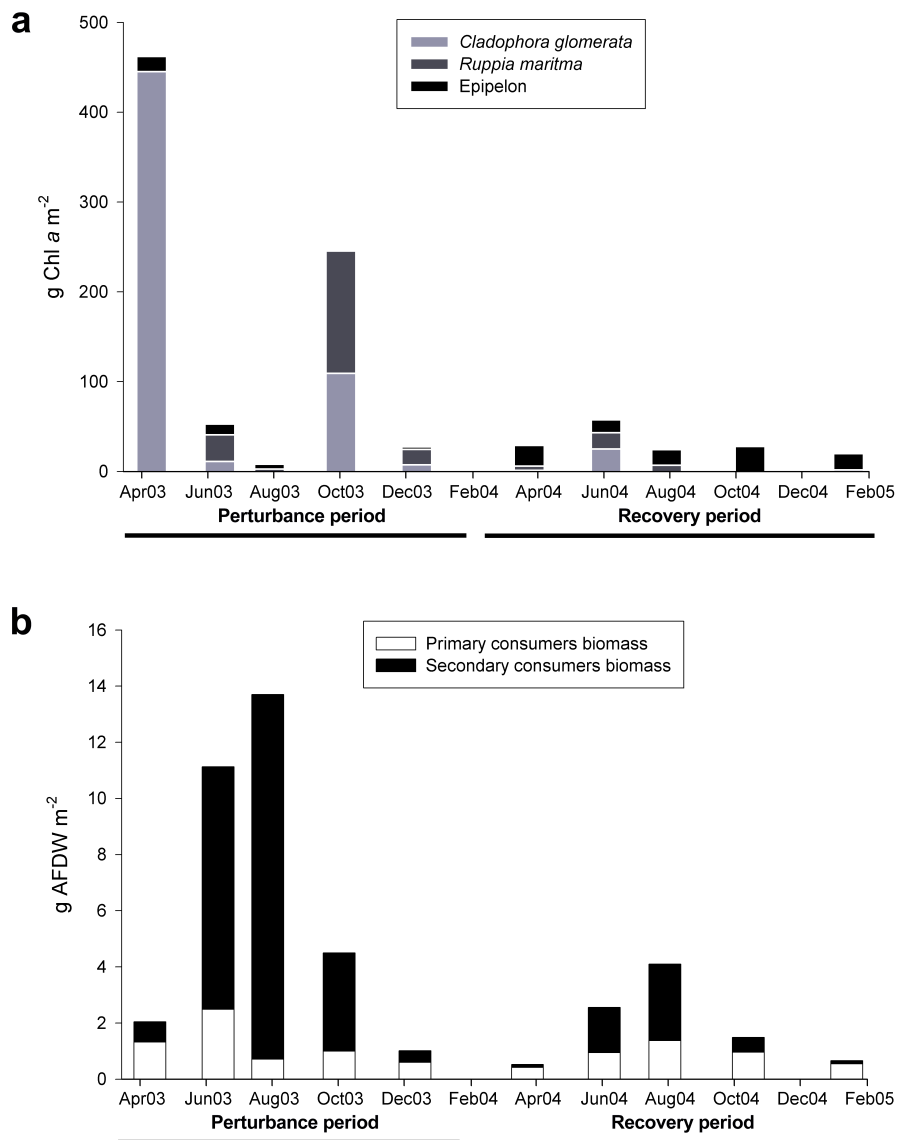


Fig. 4 Variation of autotrophic (*Cladophora glomerata*, *Ruppia maritima* and epipellic algae) (a) and consumers (primary and secondary) biomass (b).

Consumer biomass

Total consumer biomass was strongly influenced by climate conditions, showing a positive correlation with temperature and radiation, and negative correlation with precipitation (Table 4), reaching the maximum biomass in summer, in both periods (Fig. 4b). Primary, secondary and total consumer biomass were positively correlated with the first PCA axis (Table 4). In addition, primary consumer biomass was positively correlated with radiation. Although the higher consumer biomass values were reached in the disturbance period, no significant differences were observed between periods (Table 2).

Table 4 Pearson coefficients calculated between functional variables and environmental variables and first and second PCA axes. See Tables 1 and 2 to see the correspondence between variables' full names and abbreviations. *: $P < 0.05$; **: $P < 0.01$.

Variables	RAD	PR10	Q	TEMP	DO	SAT	COND	BOM	TSS	SOM	Chl <i>a</i>	NO ₃ -N	NO ₂ -N	NH ₄ -N	Axis 1	Axis 2
GPP	0,73*	-0,54	0,07	0,66*	-0,21	0,24	-0,34	0,51	-0,64*	-0,86**	0,23	-0,37	-0,39	0,04	0,79**	-0,08
ER	0,79**	-0,52	0,27	0,72*	-0,46	-0,13	-0,51	0,40	-0,63	-0,74*	0,38	-0,28	-0,56	-0,30	0,89**	0,12
CLA	0,08	0,25	0,73*	-0,07	0,16	-0,52	-0,70*	0,10	-0,65*	-0,23	-0,05	-0,24	-0,56	-0,42	0,29	0,64*
EPI	0,16	-0,06	-0,82**	-0,08	-0,20	0,44	0,70*	0,01	0,16	-0,36	0,30	-0,59	0,70*	0,71*	-0,09	-0,77*
RUP	0,15	-0,02	0,57	0,33	0,05	-0,17	-0,60	0,03	-0,15	-0,01	0,03	0,26	-0,60	-0,70*	0,30	0,50
PPB	0,14	0,13	0,66*	0,02	0,03	-0,46	-0,65*	0,30	-0,60	-0,29	-0,09	-0,42	-0,47	-0,31	0,35	0,52
PCB	0,64*	-0,40	0,20	0,60	-0,34	-0,06	-0,47	0,70*	-0,43	-0,58	0,03	-0,61	-0,35	-0,05	0,71*	0,03
SCB	0,69	-0,70*	0,41	0,84**	-0,47	-0,06	-0,45	0,37	-0,35	-0,34	0,20	0,18	-0,52	-0,26	0,78**	0,12
TCB	0,72*	-0,76*	0,32	0,85**	-0,53	-0,06	-0,40	0,45	-0,32	-0,34	0,19	0,13	-0,48	-0,18	0,80**	0,05

Dissolved oxygen patterns and metabolic rates

Dissolved oxygen concentrations showed a typical daily pattern, displaying maximum values around noon and low values at night (example for a typical early spring day, Fig. 5a). Strong daily changes occurred, values ranging from super-saturation during the day to under-saturation at night due to high respiration rates and reaeration coefficients. In some cases, such as April 2003, cloudiness decreased light availability at noon and deviations from the typical daily pattern were observed (Fig. 5b) affecting measures of production rate and NDM.

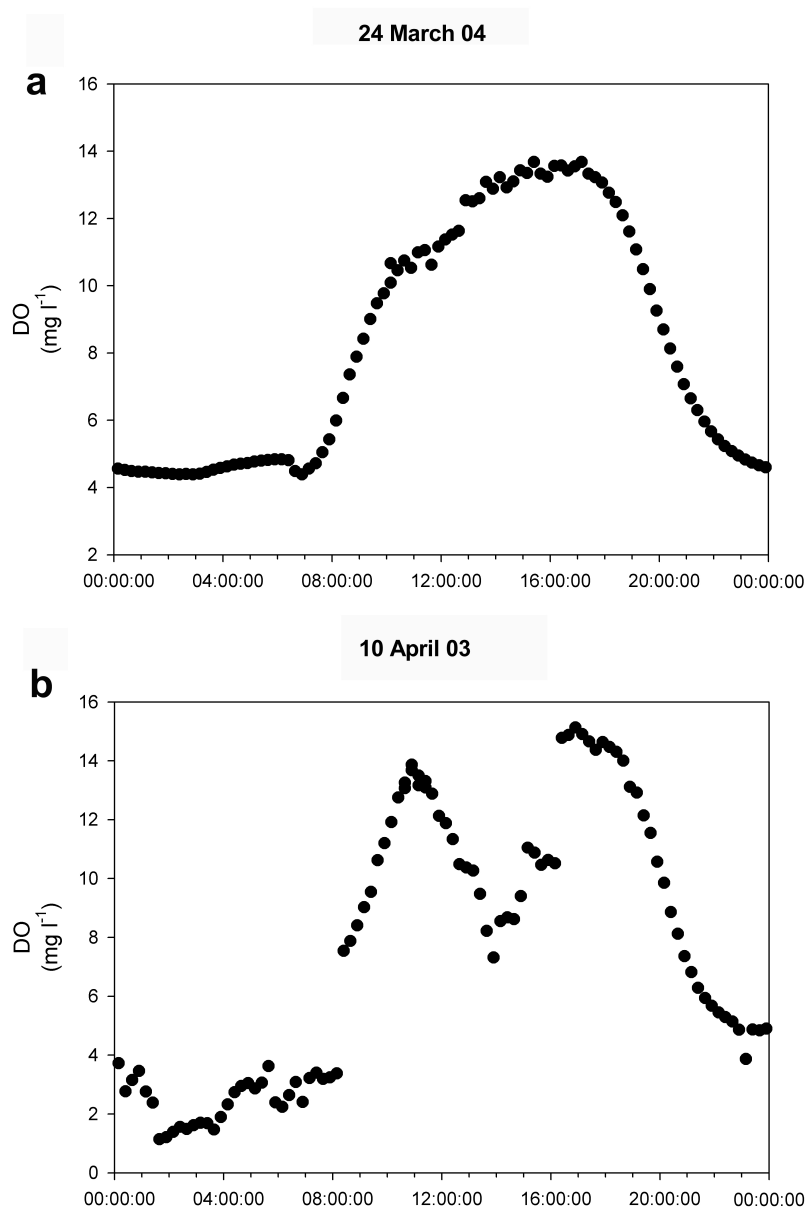


Fig. 5 Diel profiles of dissolved oxygen (DO) in Rambla Salada during a sunny day in 24 March 2004 (a) and a partly cloudy day in 10 April 2003 (b).

GPP and ER rates showed a clear seasonal pattern in both periods, with highest values in summer, followed by late spring and early autumn dates and lowest values in winter (Fig. 6). December 2003, after heavy rains, registered lowest GPP and ER rates (0.07 and 0.19 $\text{gO}_2 \text{ m}^{-2} \text{ day}^{-1}$, respectively) and June 2003 had the maximum values (21.05 and 17.39 $\text{gO}_2 \text{ m}^{-2} \text{ day}^{-1}$, respectively). GPP and ER were highly correlated ($r=0.89$, $P \leq 0.01$), in addition, both rates showed a positive correlation with total radiation and temperature and negative with SOM. Moreover, GPP and ER were positively correlated with the first PCA axis (Table 4). Respiration rate was correlated positively with primary, secondary and total consumer biomass ($r=0.74$, $P \leq 0.05$; $r=0.65$, $P \leq 0.05$ and $r=0.66$, $P \leq 0.05$, respectively) while GPP was only positively correlated with primary consumer biomass ($r=0.67$, $P \leq 0.05$).

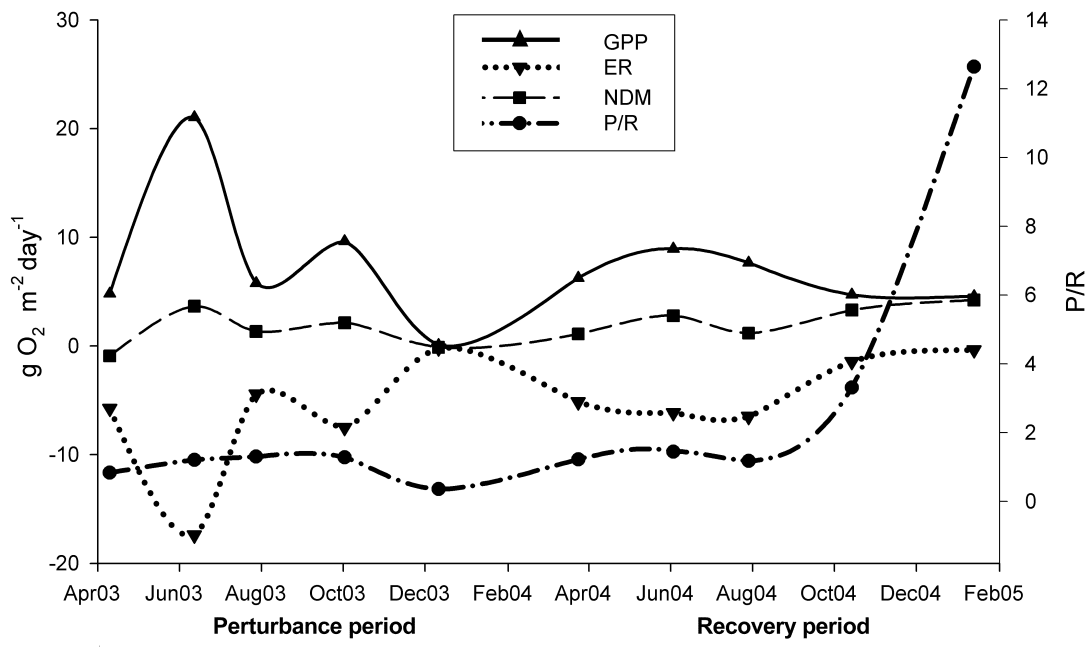


Fig. 6 Variation of metabolic indicators during study (GPP: Gross Primary Production; ER: Ecosystem Respiration; NDM: Net Daily Metabolism; P/R: Gross Primary Production : Ecosystem Respiration).

The mean GPP and ER rates were greater in the disturbance period (8.27 ± 3.54 $\text{gO}_2 \text{ m}^{-2} \text{ day}^{-1}$ and 7.05 ± 2.85 $\text{gO}_2 \text{ m}^{-2} \text{ day}^{-1}$, respectively) than in the recovery period (6.43 ± 0.84 $\text{gO}_2 \text{ m}^{-2} \text{ day}^{-1}$ and 3.91 ± 1.26 $\text{gO}_2 \text{ m}^{-2} \text{ day}^{-1}$, respectively), although these differences were not statistically significant (Table 2). Rambla Salada had positive values of NDM and $P/R > 1$, except in April 2003 and December 2003, when respiration exceeded production (Fig. 6).

Contribution of seasonality and salinity drop to the variation of functional variables

GPP and ER linear multiregression models had Durbin-Watson test values far from 2 implying some degree of residuals autocorrelation. Therefore, autoregression models were performed for GPP and ER to account for autocorrelation of model residuals. GPP and ER were predicted by first PCA axis (seasonal variation) in the autoregression model, accounting for 76% and 83% of their variation, respectively. Primary, secondary and total consumers biomass were also closely related with first PCA axis that accounted for 45%, 56% and 59% of their variation, respectively. The second PCA axis (salinity drop) was the best predictor of *C. glomerata* (33% variability) and epipellic algal biomass (54% variability).

Discussion

An important topic in bioassessment is to distinguish between the effect of natural and anthropogenic stress on ecosystem structure or functioning (Linke et al., 1999; Sheldon, 2005; Bonada et al., 2006). To achieve this objective it is essential to choose a suitable set of ecosystem health indicators (Bonada et al., 2006; Fellows et al., 2006). In this study, seasonality and dilution disturbance affected metabolism, although seasonal changes accounted for much of the temporal variability in GPP and ER (76% and 83%, respectively).

Seasonal variation of metabolic rates

Light and temperature are subjected to strong seasonal variations in Mediterranean streams and are the most important factors governing metabolic processes, although the pattern of variation depends on riparian vegetation cover, which determines light availability. In open canopy streams, maximum values of GPP and ER occur in spring and summer when temperature and radiation are high (Velasco et al., 2003). However, in closed canopy streams, GPP is maximum in early spring when light incidence on the stream surface increases, while the maximum ER occurs in autumn, when organic matter accumulations are highest (Acuña et al., 2004).

During the study, stream production predominated over stream consumption reflecting that Rambla Salada is an autotrophic ecosystem with a positive mean MDN and a mean P/R higher than 1. Other studies also found autotrophy under arid conditions in Mediterranean streams (Gasith & Resh, 1999; Velasco et al., 2003), because the autochthonous production may increase in importance

when aridity avoids the development of riparian vegetation and environmental conditions allow algal growth.

Primary production and respiration rates in Rambla Salada ranged from 0.07 to 21.05 and 0.19 to 17.39 g O₂ m⁻² day⁻¹, respectively. The mean values for these variables were 7.35 and 5.48 g O₂ m⁻² day⁻¹, respectively. These values are closer to the results obtained for desert streams, but lower than the annual average registered in Chícamo (hyposaline Mediterranean stream) and Rattlesnake (cool desert stream) streams, the two most productive streams reported in the literature (Appendix 1.1 in Supporting Information). However, our values were higher than those obtained in temperate and Mediterranean freshwater streams with closed riparian canopies. Only the mean metabolic rates of a Swiss channelised stream with open canopy, registered similar values (Uehlinger, 2006).

In Rambla Salada, metabolic rates showed the typical seasonal pattern of Mediterranean open canopy streams. GPP and ER were correlated positively with radiation and mean water temperature, registering maximum values in summer, intermediate values in spring and autumn and minimum values in winter. GPP and ER were positively correlated, indicating the importance of instream energy sources on the ecosystem functioning. GPP was negatively related to suspended solids, probably because it controls light availability for primary producers. However, no relationship was found between GPP and primary producer biomass or nutrients.

Seasonality (summarised in the first PCA axis) accounted for much of the temporal variability in metabolic rates and total consumer biomass in Rambla Salada. Similar results were found in Chícamo stream, where around 70% of the GPP and ER variation was explained by air temperature. Suspended solids concentration was the only predictor of epilithon production and respiration (Velasco et al., 2003). Mediterranean streams are characterised by highly seasonal precipitation and discharge. Around 65-80% of annual rainfall is concentrated in a few storms that can produce floods (Gasith & Resh, 1999). These floods are very important for benthic primary producers in open streams in terms of habitat suitability, biomass and distribution (Biggs, 1996). Floods reduce algal biomass and macroinvertebrate density, in addition to carrying high concentrations of suspended solids that reduce both primary production and ecosystem respiration (Acuña et al., 2004; Uehlinger, 2006; Roberts et al., 2007). In Rambla Salada, the intense rains occurring in December 2003 produced a moderate flood that caused a strong reduction in GPP and ER rates (99 and 97%, respectively), helped by the low temperature and radiation.

Effects of salinity drop on stream functioning

Much of the variation in discharge during the study was due to anthropogenic causes (freshwater inputs from the diversion channel). The amount and the persistence of the freshwater inputs during the disturbance period produced highest discharge than rainfall events. In fact, no correlation was observed between precipitation and discharge. The disturbance period was characterised by higher discharge, a strong reduction in conductivity and lower NO₂-N and NH₄-N concentrations.

A fall in salinity could change some aspects of stream functioning as might be expected. There were marked changes in the primary producer community: epipelic algae biomass decreased and *C. glomerata* proliferated rapidly. These changes could lead to higher primary production, supporting in turn, greater secondary production, as seen in the increase in macroinvertebrate biomass and ecosystem respiration. However, the intensity of the response of the functional indicators varied. Metabolic rates did not respond clearly to dilution disturbance due to its close dependence of temperature and radiation. Although no significant differences were found for metabolic rates between periods, mean values were higher in dilution conditions as we hypothesised. The high inter-seasonal variability of the metabolic rates might have limited our capacity to detect clearly the effect of dilution on GPP and ER. Changes observed in autotrophic biomass due to freshwater inputs were stronger than the changes observed in metabolic rates. Autotrophic biomass, especially epipelic algae biomass, responded more to variations in discharge or conductivity than to seasonal variations of temperature and radiation. Epipelic algae biomass was the most sensitive functional indicator of dilution disturbance, showing significant differences between periods. Disturbance PCA axis (axis 2) was the best predictor for epipelic algae biomass, with a negative slope. In contrast, biomass of *C. glomerata* increased when discharge was high and conductivity low. Thus, disturbance PCA axis was included in the regression model, with a positive slope, as the best predictor for *C. glomerata* biomass, whose biomass increased in the disturbance period favoured by low concentrations of ammonium and conductivity. The response of *R. maritima* to changes in salinity is in concordance with other studies. High concentrations of salts (over 40-50 g L⁻¹) cause a marked physiological stress in *R. maritima* due to the greater amount of energy spent by osmoregulation mechanisms (Jagels & Barnabas, 1989; Murphy et al., 2003) and its biomass may decrease until it disappears at salinity levels higher than 50 g L⁻¹ (Velasco et al., 2006). Furthermore, anaerobic sediments and water turbidity are factors that may negatively affect *R. maritima* (Kantrud, 1991). The proliferation of these

macrophytes is also associated with a major decline in water quality (Bunn et al., 1998; Bunn et al., 1999).

Others studies related to desalinization found changes in some features of stream community, such as those made by Busse et al. (1999) and Ziemann et al. (2001) in German streams after the cessation of salt-loaded effluents from the potash industry. They found marked shifts in composition and the structure of diatom assemblages, increasing species richness and a replacement of halobiontic species by oligo-halobic-indifferent forms, besides the vigorous growth of *C. glomerata* and *E. intestinalis*. Furthermore, Gell et al. (2002) demonstrated the importance of salinity influence on the diatom community and several conductivity-diatoms model have been developed for saline lakes to obtain quantitative estimates of past salinity.

In conclusion, the variation of metabolic rates and consumer biomass were related primarily to climatic conditions while the variation of primary producers biomass was associated to changes in salinity and discharge. Our results suggest that epipellic biomass could be a good health indicator for saline streams but further research is needed to ensure this question.

Acknowledgements

We thank Pedro Abellán, David Sánchez, José Barahona, Juan Hernández, Mar Ruiz and Rocío Alcántara for their assistance in field sampling and sample processing and Arturo Elosegui for useful suggestions. Thanks also to Miguel Ángel Núñez of the Centro de Interpretación de Rambla Salada for their generous support and assistance in the field and to J. Faustino Martínez, Director of the Protected Area of Humedal de Ajauque and Rambla Salada for the facilitation of sampling in the study area. This research was supported by the research project BOS2002-00702 (Spanish Investigation, Development and Innovation Program) and partially by a predoctoral grant from the Séneca Foundation, Science and Technology Agency of Murcia Region.

References

- Acuña, V., A. Giorgi, I. Munoz, U. Uehlinger & S. Sabater, 2004. Flow extremes and benthic organic matter shape the metabolism of a headwater Mediterranean stream, *Freshwater Biology* 49: 960-971.
- Acuña, V., I. Munoz, A. Giorgi, M. Omella, F. Sabater & S. Sabater, 2005. Drought and postdrought recovery cycles in an intermittent Mediterranean

stream: structural and functional aspects, *Journal of the North American Benthological Society* 24: 919-933.

APHA, 2002. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, Washington, D.C..

Barahona, J., A. Millán & J. Velasco, 2005. Population dynamics, growth and production of *Sigara selecta* (Fieber, 1848) (Hemiptera, Corixidae) in a Mediterranean hypersaline stream, *Freshwater Biology* 50: 2101-2113.

Benke, A. C., A. D. Huryn, L. A. Smock & J. B. Wallace, 1999. Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States, *Journal of the North American Benthological Society* 18: 308-343.

Biggs, B. J. F., 1996. Patterns in Benthic Algae of Streams. In Stevenson, R. J., M. L. Bothwell & R. L. Lowe (eds), *Algal Ecology*. Academic Press, San Diego: 31-56.

Bonada, N., N. Prat, V. H. Resh & B. Statzner, 2006. Developments in aquatic insect biomonitoring: A comparative analysis of recent approaches, *Annual Review of Entomology* 51: 495-523.

Bunn, S. E. & P. M. Davies, 2000. Biological processes in running waters and their implications for the assessment of ecological integrity, *Hydrobiologia* 422: 61-70.

Bunn, S. E., P. M. Davies, D. M. Kellaway & I. P. Prosser, 1998. Influence of invasive macrophytes on channel morphology and hydrology in an open tropical lowland stream, and potential control by riparian shading, *Freshwater Biology* 39: 171-178.

Bunn, S. E., P. M. Davies & T. D. Mosisch, 1999. Ecosystem measures of river health and their response to riparian and catchment degradation, *Freshwater Biology* 41: 333-345.

Busch, D. E. & S. G. Fisher, 1981. Metabolism of a Desert Stream, *Freshwater Biology* 11: 301-307.

Busse, S., R. Jahn & C. J. Schulz, 1999. Benthic diatom Communities: a comparative field study on responses to decreasing salinities, *Limnologica* 29: 465-474.

Clarke, K. R. & R. M. Warwick, 1994. *Changes in Marine Communities: an Approach to Statistical Analysis and Interpretation*, 1st edn. Plymouth Marine Laboratory, Plymouth.

Costanza, R., 1992. Toward an operational definition of ecosystem health. In Costanza, R., B. G. Norton & B. D. Haskell (eds), *Ecosystem Health: New Goals for Environmental Management*. Island Press, 239-256.

Cushing, C. E. & E. G. Wolf, 1984. Primary Production in Rattlesnake Springs, A Cold Desert Spring-Stream, *Hydrobiologia* 114: 229-236.

Fellows, C. S., J. E. Clapcott, J. W. Udy, S. E. Bunn, B. D. Harch, M. J. Smith & P. M. Davies, 2006. Benthic metabolism as an indicator of stream ecosystem health, *Hydrobiologia* 572: 71-87.

Gasith, A. & V. H. Resh, 1999. Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events, *Annual Review of Ecology and Systematics* 30: 51-81.

Gell, P. A., I. R. K. Sluiter & J. Fluin, 2002. Seasonal and inter-annual variations in diatom assemblages in Murray River-connected wetlands in northwest Victoria, Australia, *Marine and Freshwater Research* 53: 981-992.

Gessner, M. O. & E. Chauvet, 2002. A case for using litter breakdown to assess functional stream integrity, *Ecological Applications* 12: 498-510.

Guasch, H., E. Martí & S. Sabater, 1995. Nutrient Enrichment Effects on Biofilm Metabolism in A Mediterranean Stream, *Freshwater Biology* 33: 373-383.

Guasch, H. & S. Sabater, 1994. Primary production of epilithic communities in undisturbed Mediterranean streams, *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie*, 25: 1761-1764.

Guasch, H. & S. Sabater, 1995. Seasonal-Variations in Photosynthesis-Irradiance Responses by Biofilms in Mediterranean Streams, *Journal of Phycology* 31: 727-735.

Jagels, R. & A. Barnabas, 1989. Variation in Leaf Ultrastructure of *Ruppia-Maritima* I Along A Salinity Gradient, *Aquatic Botany* 33: 207-221.

Kantrud, H. A., 1991. *Wigeongrass (Ruppia maritima L.): A literature Review*. U.S. Fish and Wildlife Service.

Linke, S., R. C. Bailey & J. Schwindt, 1999. Temporal variability of stream bioassessments using benthic macroinvertebrates, *Freshwater Biology* 42: 575-584.

Mollá, S., L. Maltchik & C. Casado, 1994. Primeros datos sobre el metabolismo de un arroyo temporal mediterráneo de Sierra Morena (Córdoba), *Limnetica* 10: 59-67.

Mollá, S., L. Maltchik, C. Casado & C. Montes, 1996. Particulate organic matter and ecosystem metabolism dynamics in a temporary Mediterranean stream, *Archiv fur Hydrobiologie* 137: 59-76.

Moreno, J. L., 2002. Hábitats, recursos tróficos y estructura de la comunidad de macroinvertebrados bentónicos en un arroyo salino del Sureste Ibérico (Rambla del Reventón). University of Murcia, Murcia, Spain.

Mulholland, P. J., C. S. Fellows, J. L. Tank, N. B. Grimm, J. R. Webster, S. K. Hamilton, E. Marti, L. Ashkenas, W. B. Bowden, W. K. Dodds, W. H. McDowell, M. J. Paul & B. J. Peterson, 2001. Inter-biome comparison of factors controlling stream metabolism, *Freshwater Biology* 46: 1503-1517.

Murphy, L. R., S. T. Kinsey & M. J. Durako, 2003. Physiological effects of short-term salinity changes on *Ruppia maritima*, *Aquatic Botany* 75: 293-309.

Naiman, R. J., 1976. Primary Production, Standing Stock, and Export of Organic-Matter in A Mohave-Desert Thermal Stream, *Limnology and Oceanography* 21: 60-73.

Odum, H. T., 1956. Primary Production in Flowing Waters, *Limnology and Oceanography* 1: 102-117.

Perán, A., J. Velasco & A. Millán, 1999. Life cycle and secondary production of *Caenis luctuosa* (Ephemeroptera) in a semiarid stream (Southeast Spain), *Hydrobiologia* 400: 187-194.

Ramírez-Díaz, L., M. R. Vidal-Abarca, J. F. Calvo, M. L. Suarez, J. Palazón, M. A. Esteve, R. Gomez, A. Jiménez, J. A. Pujol, J. A. Sánchez, M. Pardo & J. Contreras, 1995. Bases ecológicas para la delimitación, ordenación y gestión del Paisaje Protegido de las Ramblas de Ajauque y Salada. Consejería de Medio Ambiente, Comunidad Autónoma de la Región de Murcia, Murcia.

Rapport, D. J., R. Costanza & A. J. McMichael, 1998. Assessing ecosystem health, *Trends in Ecology & Evolution* 13: 397-402.

Roberts, B. J., P. J. Mulholland & W. R. Hill, 2007. Multiple scales of temporal variability in ecosystem metabolism rates: Results from 2 years of continuous monitoring in a forested headwater stream, *Ecosystems* 10: 588-606.

Sheldon, F., 2005. Incorporating natural variability into the assessment of ecological health in Australian dryland rivers, *Hydrobiologia* 552: 45-56.

SIAM, 2008. Servicio de información agraria de Murcia.

Smock, L. A., 1980. Relationships Between Body Size and Biomass of Aquatic Insects, *Freshwater Biology* 10: 375-383.

SPSS, 2004. SPSS for Windows. Chicago, IL.

Thyssen, N. & M. G. Kelly, 1985. Water-Air Exchange of Carbon-Dioxide and Oxygen in A River - Measurement and Comparison of Rates, *Archiv fur Hydrobiologie* 105: 219-228.

Udy, J. W., C. S. Fellows, M. E. Bartkow, S. E. Bunn, J. E. Clapcott & B. D. Harch, 2006. Measures of nutrient processes as indicators of stream ecosystem health, *Hydrobiologia* 572: 89-102.

Uehlinger, U., 2006. Annual cycle and inter-annual variability of gross primary production and ecosystem respiration in a floodprone river during a 15-year period, *Freshwater Biology* 51: 938-950.

Velasco, J., A. Millán, J. Hernández, C. Gutiérrez-Cánovas, P. Abellan, D. Sanchez-Fernandez & M. Ruiz, 2006. Response of biotic communities to salinity changes in a Mediterranean hypersaline stream, *Saline Systems* 2: 12.

Velasco, J., A. Millán, M. R. Vidal-Abarca, M. L. Suárez, C. Guerrero & M. Ortega, 2003. Macrophytic, epipelic and epilithic primary production in a semiarid Mediterranean stream, *Freshwater Biology* 48: 1408-1420.

Vidal-Abarca, M. R., 1985. Las aguas superficiales de la cuenca del río Segura (SE. De España). Caracterización físico-química en relación al medio físico y humano. University of Murcia, Murcia, Spain.

Young, R. G. & B. R. Knight, 2005. River Metabolism Estimator. Minister of Environment SMF Project 2208., New Zealand.

Young, R. G., C. R. Townsend & C. D. Matthaei, 2004. Functional indicators of river ecosystem health an interim guide for use in New Zealand. Cawthron Institute, New Zealand: 1-54.

Ziemann, H., L. Kies & C. J. Schulz, 2001. Changes in the structure of diatom assemblages caused by a decreasing salt load and changing ion spectra in the River Wipper (Thuringia, Germany), *Limnologica* 31: 257-280.

Supporting information

Appendix 1.1 Mean values and variation range of daily gross primary production (GPP in g O₂ m⁻² d⁻¹) and ecosystem respiration (ER in g O₂ m⁻² d⁻¹) in streams from different biomes.

Appendix 1.1 Mean values and variation range of daily gross primary production (GPP in g O₂ m⁻² d⁻¹) and ecosystem respiration (ER in g O₂ m⁻² d⁻¹) in streams from different biomes.

Study area	GPP	ER	Period	Riparian canopy	Method	Reference
<i>Temperate streams</i>						
Walker Branch. TE. U.S.A.	1.37 (0.01-10.80)	4.02 (0.99-16.01)	Two years	Closed	One-station diurnal oxygen change method	(Roberts et al., 2007)
Thur. Switzerland	5.0 (3.9-6.1)	6.2 (4.1-8.8)	15 years	Open	One-station diurnal oxygen change method	(Uehlinger, 2006)
<i>Freshwater Mediterranean streams</i>						
Arroyo de la Montesina. Spain	2.30 (0.45-4.72)	2.23 (0.81-3.74)	Annual	Open	Two-station diurnal oxygen change method	(Mollá et al., 1994)
La Solana. Spain	0.35 (0.02-0.20)	0.25 (0.04-0.14)	Annual	Open	Chambers	(Guasch & Sabater, 1994)
Riera Major. Spain	0.87 (0.02-0.07)	0.74 (0.02-0.07)	Annual	Closed	Chambers	(Guasch & Sabater, 1994)
Furiosos. Spain	0.75 (0.05-1.9)	4.63 (0.4-32)	Annual	Closed	One-station diurnal oxygen change method	(Acuña et al., 2004)
<i>Saline Mediterranean streams</i>						
Río Chicamo. Spain	36.58 (13.74-70.50)	18.40 (7.16-26.20)	Annual	Open	Chambers	(Velasco et al., 2003)
Rambla Salada. Spain	7.35 (0.07-21.05)	5.48 (0.19-17.39)	Two years	Open	One-station diurnal oxygen change method	Present study
<i>Desert streams</i>						
Sycamore Creek. AZ. U.S.A.	15	8.2	Spring	Open	Two-station diurnal oxygen change method	(Mulholland et al., 2001)
Sycamore Creek. AZ. U.S.A.	8.5	5.1	Summer	Open	Chambers	(Busch & Fisher, 1981)
Tecopa Bore. AZ. U.S.A.	(5.74-14.21)		Annual	Open	¹⁴ C Uptake	(Naiman, 1976)
Rattlesnake Creek. WA. U.S.A.	24.8	21.6	Annual	Open	Two-station diurnal oxygen change method	(Cushing & Wolf, 1984)

Chapter 2:

Impact of chronic and pulse dilution stresses on metabolism and trophic structure in a saline Mediterranean stream

Published in Hydrobiologia:

Gutiérrez-Cánovas, C.; Hernández, J.; Velasco, J. & A. Millán. 2012. Impact of chronic and pulse dilution disturbances on metabolism and trophic structure in a saline Mediterranean stream. *Hydrobiologia*, 686(1): 225-239.

Impact of chronic and pulse dilution stresses on metabolism and trophic structure in a saline Mediterranean stream

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Abstract

Predicting the consequences of global change involves investigating the mechanisms by which anthropogenic stress modifies ecosystem function. In this sense, naturally stressed ecosystems provide a new framework to test hypotheses in such a context. Here, we use a saline stream (Rambla Salada, SE Spain) as a study case to test the general hypothesis that chronic stress has stronger impacts on metabolism and trophic structure than pulse disturbances. We compared two reaches differing in the persistence of dilution stress over the course of two years: the *chronically disturbed reach* was subject to persistent dilution (chronic stress) by freshwater inputs from surrounding irrigated crops and presented a dense stand of the common reed *Phragmites australis*; and the *reference reach*, which presented pristine conditions. During the first study year, both reaches were affected by several freshwater inputs due to a diversion channel break (pulse disturbance) that significantly decreased conductivity. During the second year, conductivity recovered to mean pre-pulse disturbance levels. Pulse dilution disturbance had no significant effect on production : respiration ratios (P/R), although predator biomasses increased. However, chronic dilution significantly decreased P/R values as well as consumer and producer biomasses. Dilution disturbances had no significant effects on the relative importance of the different functional feeding groups. Isotopic signatures revealed that macroinvertebrates in both reaches relied upon aquatic autotrophs, despite the common reed inputs at the *chronically disturbed reach*. In summary, this study highlights the relevance of stress persistence and the usefulness of functional measures when aiming to predict disturbance effects. Thus, while pulse disturbances had minor effects on ecosystem function, chronic dilution produced biomass depletion and a change from an autotrophic to a heterotrophic ecosystem.

Introduction

There is a growing interest in studying the consequences of global change on ecosystem structure and function as human pressure on natural resources increases (Naeem *et al.* 1994; Smith *et al.* 2009; Laliberte *et al.* 2010). The severity of such impacts may depend on their intensity and frequency since, for example, organisms often exhibit varying tolerances to stressors or different recolonisation capacities (e.g. McCabe & Gotelli 2000; Kowalik *et al.* 2007). At the community level, stress often reduce organism abundance or taxonomic richness (Resh *et al.* 1988), while ecosystem functioning is also affected when some key processes are substantially modified, as occurs with litter decomposition (e.g. Gessner & Chauvet 2002) or stream metabolism (e.g. von Schiller *et al.* 2008). Nonetheless, both responses rely on how predisturbed community and processes were organised in terms of species interactions and their equilibrium with previous environmental conditions (Bender *et al.* 1984; Schmitz 1997). For instance, in naturally stressed ecosystems one might expect to find higher richness and organism abundance after a disturbance that removed the natural stress to some degree, contrasting with the generally observed pattern (Resh *et al.* 1988). Hence, naturally stressed ecosystems may provide a new framework to study the response of ecosystem structure and function to anthropogenic disturbances. Here, we present a saline stream as a study case to assess the effect of pulse and chronic dilution stresses on stream metabolism, functional feeding groups and food webs. Pulse disturbances are temporal alterations which disrupt the community from its initial equilibrium during a short time (Bender *et al.* 1984). In this case, when the disrupting forces cease, community often recover the pre-disturbance features. On the other hand, chronic stress (also called press disturbances) involves events which permanently modify the ecosystem, reducing species number and abundance and leading community to a new equilibrium as a result of maintained stress (Bender *et al.* 1984). We have focused upon these functional parameters because (a) the structural response to dilution impacts is well-documented (Velasco *et al.* 2006; Millán *et al.* 2011), (b) functional response provides a complementary perspective in relation with these taxonomic approaches (Young *et al.* 2008) and (c) their integrative and mechanistic nature allow one to formulate more straightforward predictions that link the measured factors with environmental change (Gessner & Chauvet 2002; Young *et al.* 2008).

Osmotic stress reduction in natural saline streams (i.e., those with water salinity $\geq 3 \text{ g L}^{-1}$ or conductivity $\geq 5 \text{ mS cm}^{-1}$) due to short-term anthropogenic freshwater inputs result in higher taxonomic richness (Velasco *et al.* 2006; Gutiérrez-Cánovas *et al.* 2009) and imply the loss of halophilic species (Millán *et al.* 2011). Furthermore, under diluted circumstances, many organisms may

expend more energy growing and reproducing instead of on osmotic regulation (Potts 1954; Kefford *et al.* 2006). Hence, dilution stress can favour higher biomasses, as has been observed with macrophyte biomasses that peak after short-term salinity drops (Gutiérrez-Cánovas *et al.* 2009). However, biofilm shows higher biomasses at high salinities (Ros *et al.* 2009), likely due to the fact that diatoms and cyanobacteria are salt-tolerant, and more radiation would reach the streambed as macrophyte cover declined.

On the other hand, continuous diffuse inputs of freshwater from nearby crops on salt marshes and streams are often accompanied by a replacement of natural halophilic vegetation by the common reed *Phragmites australis* (Bart & Hartman 2003; Weis & Weis 2003; Silliman & Bertness 2004). Under these conditions, *P. australis* can grow even in places with high saline superficial water if its roots and rhizomes have access to interstitial freshwater (Adams & Bate 1999; Gómez *et al.* 2001), while halophilic vegetation grows better at moderate and high salinities, displaying less photosynthetic activity in non-saline conditions (Redondo-Gómez *et al.* 2006; Redondo-Gómez *et al.* 2010). This replacement could have important functional instream consequences. First, common reed detritus may result in a new resource for aquatic consumers. Second, *P. australis* proliferation can increase nutrient uptake and evapotranspiration, reduce current velocity and promote the accumulation of detritus in the streambed, which would enhance respiration rates and decrease primary production.

In this study, we tested the following predictions: (1) pulse dilution should not produce significant changes in production : respiration ratios (P/R) while chronic dilution should decrease P/R. (2) Pulse dilution disturbance should increase the biomass of macrophytes and invertebrates and decrease the biofilm biomass, while chronic dilution should decrease producer and consumer biomasses. Finally, (3) we expected a significant increase of the relative importance of shredder and gathering collector feeding groups in response to chronic dilution, while pulse dilution would not significantly change the trophic structure.

Materials and methods

Study area

The study was conducted in the Rambla Salada, a hypersaline (i.e., historical salinity records higher than 40 g L⁻¹) Mediterranean stream located in the sedimentary Fortuna basin (Murcia), in the semiarid southeast of the Iberian Peninsula (Fig. 1). The climate is characterised by mean annual precipitation

below 300 mm (mainly concentrated in spring and autumn) and a mean annual temperature of 18°C, with long, warm, dry summers and mild winters. The high salinity of the Rambla Salada is principally due to Miocene gypsiferous marls and the ionic composition of the water is dominated by Na⁺ and Cl⁻ ions, followed by SO₄²⁻ and Ca²⁺ (Ramírez-Díaz *et al.* 1995). The study area is protected by environmental legislation of the Region of Murcia and, furthermore, is included in the European Natura 2000 protection network (Habitats Directive, EU Council Directive 92/43/EEC). However, the basin's land uses have changed since the 1980s due to the increasing availability of water resources provided by the Tagus-Segura River diversion, which has led to increased agricultural activity. Indeed, both agricultural drainage water and diversion channel losses have led to substantial variations in discharge (increasing from mean values spanning 8-12 L s⁻¹ in the early 1980s to a mean of 52.3 L s⁻¹ during the period 2003-05) and salinity levels (decreasing from mean values close to 100 g L⁻¹ to a mean of 35.5 g L⁻¹ in the same periods) (Vidal-Abarca 1985; Velasco *et al.* 2006). Rainfall events during the study period were scarce, and were unrelated to the observed discharge peaks ($r=0.01$; $p>0.05$) and conductivity ($r=-0.06$; $p>0.05$) (Gutiérrez-Cánovas *et al.* 2009).

Sampling design

The Rambla Salada stream was selected due to the occurrence of pulse and chronic dilution disturbances in different reaches and periods, which allowed studying their effect on physical, biological and functional stream features. The varying agriculture use across the Rambla Salada basin determines the magnitude and frequency of diffuse freshwater inputs that results in reaches that range from nearly pristine to those with chronically dilute conditions. One advantage of comparing reaches belonging to the same stream is that it minimises the interference of other environmental variables distinct from the anthropogenic dilution disturbances. To assess the effects of chronic dilution, we compare two 100-m length reaches with contrasting reach-scale agricultural intensification (for location see Fig.1; for pictures see Fig. 2). The *chronically disturbed reach*, located upstream (38.1266° N, 1.1274° W), is persistently disturbed by diffuse freshwater inputs from surrounding irrigated crops (Fig. 2a). A dense stand of *P. australis*, associated to these inputs, occupies the entire stream section and the banks about 2 km upstream from the sampling point, where the presence of common reeds is constrained to the banks. This has diminished current velocity and promoted pool-habitat conditions and sediment deposition in the studied reach. Dead *P. australis* leaves and stems that enter the stream cover about 10-15% of the channel. Downstream, chronic dilution disturbance effects disappear and the physicochemical features of the water recover as downstream land uses are natural vegetation or drylands, and

several hypersaline springs (higher than 120 mS cm^{-1}), which flow into the stream and increase salinity. Thus, we selected a downstream reach (38.1944° N , 1.2278° W ; hereafter *reference reach*) that crosses a saline steppe whose halophilic vegetation reflects a low freshwater influx from surrounding crops (Fig. 2b). In fact, riparian vegetation is scarce at this reach, where only small halophilic plants occur (e.g. *Sarcocornia fruticosa* and *Arthrocnemum macrostachyum*). This reach is more heterogeneous, with pool and run habitats.

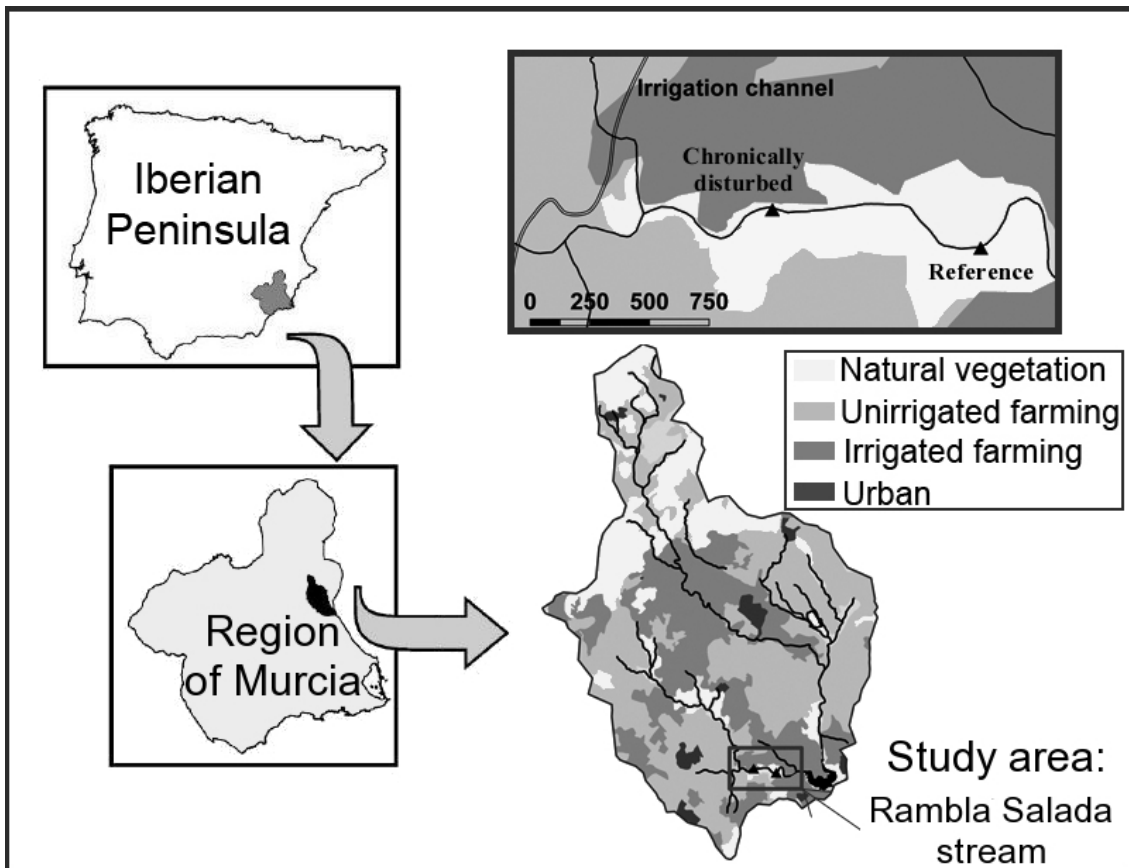


Fig. 1 Geographical location of the studied stream reaches in Rambla Salada stream and main land uses in the basin and surrounding areas.

To assess the effect of pulse dilution disturbance, we compared both reaches during disturbed and recovered periods. The pulse disturbed period (from 9 April 2003 to 11 December 2003) was characterised by several irregular inputs of freshwater due to an accidental break of the Tagus-Segura diversion channel located upstream from both reaches (see Fig. 1 and Fig. 3). The most extreme dilution event occurred on 2 October 2003, when a massive water input (around 400 L s^{-1}) entered the stream during the irrigation channel's reparation. During the recovered period (from 24 March 2004 to 13 January 2005), after the accidental inputs ceased, salinity and other habitat features recovered to pre-pulse disturbance levels (Fig. 3). At both reaches, samples were collected

bimonthly for two years during the two periods, resulting in 10 sampling dates (five for each period).

(a) Chronically disturbed reach



(b) Reference reach



Fig. 2 Reach-scale pictures of the (a) chronically disturbed and (b) reference reaches, taken during the study period.

Habitat feature measurements

Salinity and conductivity were measured *in situ* with an ECmeter (TetraConR 325), and water temperature and dissolved oxygen were measured at 15 minute intervals over a 24 hour period using a multiparameter recorder (WTW, MultiLine P4). Mean depth and current velocity were estimated by averaging ten measures across two different transects per reach. Mean channel width was calculated by averaging three width measures per reach. Discharge was estimated by multiplying mean current velocity and mean channel section (the product of mean depth and mean width) for each reach.

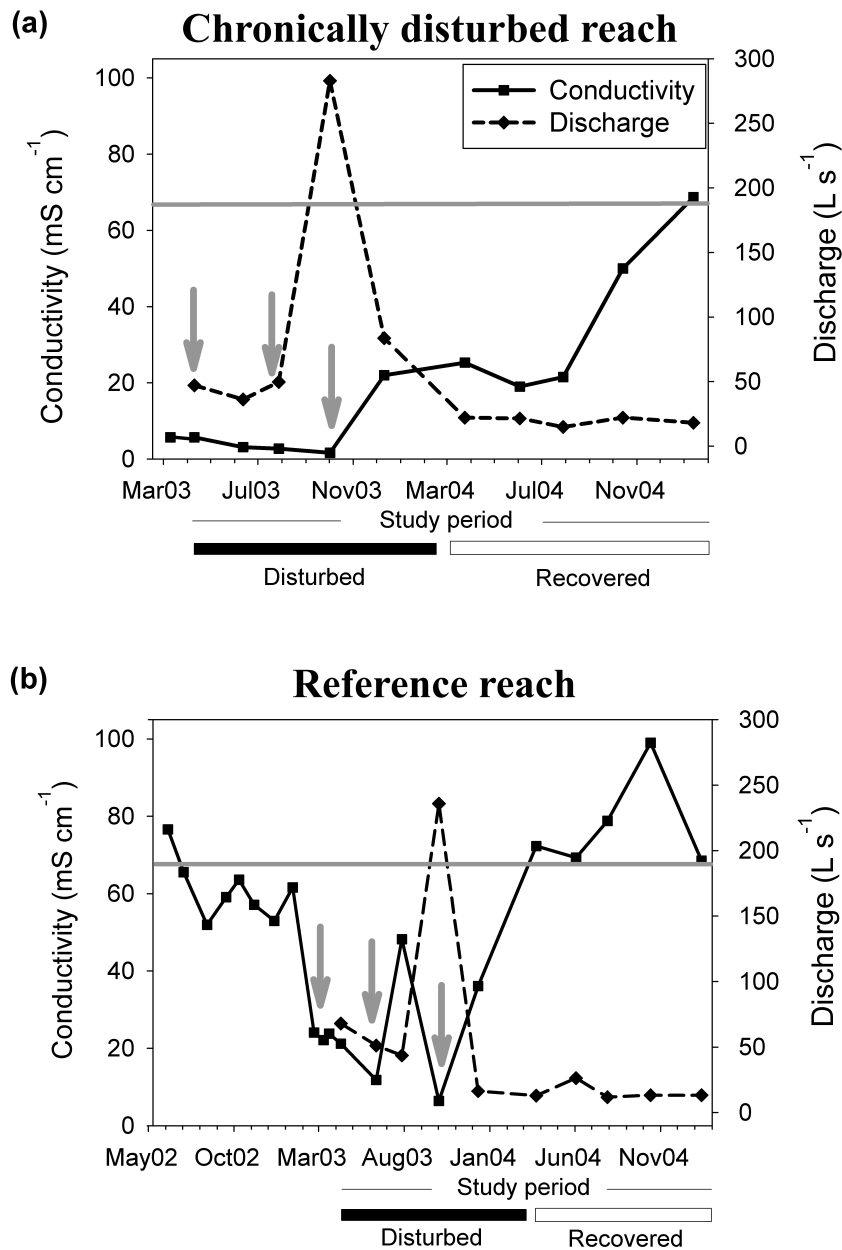


Fig. 3 Conductivity and discharge variation during pre-disturbed, pulse disturbed and recovered periods at the chronically disturbed reach (a) and reference reach (b). The arrows indicate the different dilution events. The grey solid line indicates the conductivity average of pre-disturbed and recovery periods at the reference reach (67.4 mS cm⁻¹) in both plots.

Metabolism measurements

At both reaches, stream metabolism rates were measured using an open-system, single-station approach (Odum 1956) on each sampling date. Sunny or minimally cloudy days were chosen to minimise biases in metabolic rate estimation. The mass transfer coefficient ($f_{20^{\circ}\text{C}}$) was calculated using the surface renewal method (SRM, equation 1 sensu Owens *et al.* 1964), from which reaeration coefficients ($K_{20^{\circ}\text{C}}$) were also estimated (Equation 2):

$$f_{(20^{\circ}\text{C})} = 50.8 \times V^{0.67} \times H^{-0.85} \times D^{-1} \text{ (Equation 1)}$$

$$K_{(20^{\circ}\text{C})} = f_{(20^{\circ}\text{C})} \times H^{-1} \text{ (Equation 2)}$$

where V is the current velocity and H is the mean depth. Despite the critical relevance of the method to estimate K in open-channel approaches, there is a trade-off between method accuracy (e.g. injection of volatile gas tracers) and applicability (e.g. empirical equations) (Genereux & Hemond 1992). Despite assuming some degree of bias in the metabolism measurements, the goal was to quantify the degree of autotrophy for each reach. The reaeration coefficient together with the recorded values of water temperature, oxygen concentration and saturation were used to calculate the oxygen change rate for each 15 minute interval. Net daily metabolism was calculated as the average of the corrected oxygen reaeration change rates during the photoperiod. Respiration rate was calculated by extrapolating the average of the corrected oxygen change rates during night-time to the entire 24 h. Gross primary production was calculated by adding the absolute values of respiration and net daily metabolism. Finally, P/R was calculated by dividing gross primary production and respiration rates.

Potential basal resource measurements

On each sampling day, one 2 L sample of water was taken from each reach and filtered through precombusted and preweighed GF/F glass-fibre filters to estimate total suspended particulate organic matter (seston). The filters were oven-dried at 60 °C to a constant dry weight (DW) and then combusted at 450 °C for four hours. To estimate benthic organic matter and the biomass of primary producers ten samples were randomly taken from the *reference reach* to characterise the pool and run habitats (five per habitat), and five samples were taken from the *chronically disturbed reach*, which was composed of only a large pool. Sediment samples were collected using cores of 5.3 cm diameter and 24.5 cm length. Afterwards, they were dried at 60 °C to a constant weight and then combusted at 450°C for four hours to estimate the benthic organic matter as ash-free dry weight (AFDW). Macrophyte samples were collected using similar

cores as were used for sediments, while for biofilm, a minicore of 2 cm diameter and 2 cm length was used. Sixty mg of fresh macrophytes were taken from each sample to determine their chlorophyll *a* concentrations (Chl *a*) by spectrophotometry, following extraction in acetone. The rest of the plant material was dried at 60 °C to a constant dry weight. Epipellic samples were filtered onto Whatman A filters to determine chlorophyll *a* concentration and AFDW. The biomass of each primary producer in the studied reaches was estimated by multiplying cover percentage (by visually estimating the streambed cover percentage of each primary producer along each reach) by chlorophyll *a* content per area. The cover percentage of *P. australis* leaf and stem debris was also surveyed visually in the *chronically disturbed reach*, as this plant occurred exclusively there.

Functional feeding group biomass measurements

Benthic macroinvertebrates were collected at both reaches using a 0.014-m² core sampler. Substrate was stirred by hand and a 250-µm mesh sieve was passed across the bottom and through the water column until no more individuals were found. Five replicate cores at the *chronically disturbed reach* and ten at the *reference reach* (five in the pool and five in the run habitats) were taken randomly on each sampling date and preserved and stored in 75% ethanol. In the laboratory, macroinvertebrates were identified, counted and measured (body length). The mean density of each taxon was multiplied by the relative habitat area in order to obtain reach macroinvertebrate abundance. Benthic macroinvertebrate biomass was obtained from length-mass equations available for the same or nearest taxon from saline streams (Moreno 2002; Barahona *et al.* 2005) or from the general equations for macroinvertebrate families (Smock 1980; Benke *et al.* 1999). The use of ethanol as an invertebrate preservative may result in a reduction of organism body length, causing an underestimation of total biomass, when length-biomass equations are used (Johnston & Cunjak 1999; von Schiller & Solimini 2005). However, we consider that this bias may be evenly distributed among treatments as more abundant organisms (Chironomids and *Sigara selecta*) and even most of the other taxa occurred in both reaches and periods (see Appendixes 2.1 and 2.2). Furthermore, macroinvertebrates were classified into five functional feeding groups (shredders, grazers, gathering collectors, filtering collectors and predators) (Merritt & Cummins 1996; Tachet *et al.* 2000; Monakov 2003).

Collection and treatment of samples for isotopic signature characterisation

Basal resources and macroinvertebrates were collected on 3 June 2004 for carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopic analysis. Isotopic signatures can give

time-integrated data about consumer's diets and reveal the relative use of the different resources on the aquatic food web. To determine the isotopic signals of seston, three samples were collected at each reach on this date. Macrophytes and epipellic biofilm were collected and treated to remove attached organisms, detritus and inorganic matter following standard methods (Carman & Fry 2002). *Cladophora glomerata* epiphytes were obtained by sonication for one minute and then centrifuged. Macroinvertebrates used for isotopic analysis were collected using a kick-net (500 µm mesh size) at both reaches. The 12 most abundant taxa were selected to ensure a representative picture of a saline stream macroinvertebrate community. Several individuals (3 to 10) of each taxon were isolated in vials filled with filtered stream water and transported live to the laboratory, where they were allowed to void their digestive tracts for 24 hours. All animals were then washed with distilled water and frozen in airtight containers. The gastropod *Potamopyrgus antipodarum* and the Chironomid larvae were treated with acid 1N HCl to prevent possible contamination from non-dietary carbonates.

All samples were oven-dried at 60 °C before being hand-ground to a fine powder. The dried, ground samples were oxidised at 1000 °C. The resultant CO₂ and N₂ were analysed for their contribution to the sample biomass, and stable isotope ratios using a continuous-flow isotope-ratio mass spectrometer (Finnigan MAT Delta Plus) in the stable isotope laboratory of the Universidad de A Coruña (Spain). Stable isotope ratios (¹³C / ¹²C and ¹⁵N / ¹⁴N) were calculated as parts-per-million relative to standards (Pee Dee belemnite, and atmospheric N₂). Measurement precision was approximately 0.1‰ and 0.3‰, for ¹³C / ¹²C and ¹⁵N / ¹⁴N, respectively. C : N of each of the primary sources were also calculated to provide nutritional value index (Russell-Hunter 1970; Sheldon & Walker 1998).

Data analyses

Before analysis, the following transformations were necessary to reduce the skewness in the variable values and improve their lineal relationships: an arcsine of the square root transformation of the variables expressed as percentages, a square root transformation of conductivity and biofilm biomasses and a log-transformation of the remaining variables. First, Generalised Linear Models (GLM) were employed to assess the seasonality influence on the environmental variables, P/R, potential food sources, macroinvertebrate biomass and trophic structure. To do this, we use season (*winter, spring, summer and autumn*) as categorical predictor, assuming a residual Gaussian error distribution. To remove seasonality influence in subsequent analysis, we use the residuals of these models as response variable

in the case of finding a significant relationship with seasonality. Otherwise, we use raw values as response variable. Second, we use two-way ANOVA tests to assess the effect of chronic dilution (categories: *chronically disturbed*, *reference*), pulse dilution (categories: *disturbed*, *recovered*) and their interaction on the response variables. This procedure allows considering the samples taken during each period at each reach as independent replicates to assess the putative effect of both types of dilution on the response variables.

The R-package SIAR (Parnell *et al.* 2010) was used to quantify the relative importance of basal sources supporting the food webs. The main advantage of this Bayesian mixing model is that it allows the inclusion of uncertain sources resulting from the variability of isotopic signatures within the target consumers and sources, instead of using mean values, as do traditional methods (e.g. Isosource c.f. Phillips & Gregg 2003). Individual $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were used for consumers that were classified into grazers, gathering-collectors and predators. Filtering-collectors were discarded due to their low abundance and biomass at both reaches during the study period (see Appenixes 2.1 and 2.2). These values were corrected using the mean trophic fractionation values for aquatic animals as reported by McCutchan *et al.* (2003) ($\Delta^{13}\text{C} = +0.4 \pm 1.61$; $\Delta^{15}\text{N} = +2.3 \pm 1.20$) multiplied by their trophic level. Prior to analyses, some basal sources were aggregated to reduce the number of potential sources, which can improve the final results. At both reaches, biofilm and seston signatures were averaged, as they are made up essentially of the same components (i.e. diatoms, cyanobacteria, detritus) and their signatures were very similar. At the *reference reach*, the signatures of the macrophytes *C. glomerata* and their epiphytes were also aggregated because consumers most likely feed on them as a whole. Mean (\pm SD) isotopic values of the available organic instream basal sources entered the model for each reach as potential food sources. At the *chronically disturbed reach*, the isotopic signatures of *P. australis* leaves were included to assess the contribution of allochthonous organic matter to the food web. All statistical analyses were carried out using the statistical computing software R (R-Development-Core-Team 2011) (see Appendix 2.3)

Results

Effects of seasonality on the studied variables

In general, seasonality did not have a significant effect on the studied variables, with the exception of daily mean water temperature ($F=12.609$; $P<0.001$), seston ($F=7.3057$; $P=0.003$) and the percentage of predator biomass ($F=6.8466$; $P=0.004$).

Shifts in habitat features

The residuals of the seasonality model for daily mean water temperature were significantly lower in the *chronically disturbed reach* in comparison with the *reference reach* (Table 1). Pulse dilutions significantly increased discharge at both reaches, while conductivity was clearly decreased by chronic and pulse dilution disturbances. Discharge mean values (\pm SE) significantly decreased from the pulse dilution to the recovered period, from $99.9 \pm 46.4 \text{ L s}^{-1}$ in the *chronically disturbed reach* and $83.0 \pm 39.2 \text{ L s}^{-1}$ in the *reference reach* to 19.8 ± 1.5 and $15.5 \pm 2.8 \text{ L s}^{-1}$, respectively (Table 1, Fig. 3). However, no significant differences in discharge values were observed in relation to chronic dilution. Conductivity mean values were higher at the *reference reach* during the disturbed ($24.8 \pm 7.8 \text{ mS cm}^{-1}$) and recovered periods ($77.6 \pm 5.7 \text{ mS cm}^{-1}$) in comparison with the conductivity that the *chronically disturbed reach* displayed during the same periods (7.1 ± 3.7 and $36.9 \pm 9.7 \text{ mS cm}^{-1}$, respectively). Pulse dilutions caused several drops in conductivity at both reaches, especially in October 2003, when water from the irrigation channel emptied into the stream (Fig. 3). Conductivity was significantly higher during the recovered period, showing 5-fold and 3-fold increases at the *chronically disturbed* and *reference reaches*, respectively. Pulse dilution did not affect current velocity, while chronic dilution significantly decreased it (*chronically disturbed reach*: $0.03 \pm 0.01 \text{ m s}^{-1}$; *reference reach*: $0.14 \pm 0.03 \text{ m s}^{-1}$). Dissolved oxygen mean values were also significantly lower at the *chronically disturbed reach* ($6.4 \pm 0.7 \text{ mg L}^{-1}$), in comparison to *reference reach* ($8.4 \pm 0.4 \text{ mg L}^{-1}$). However, no differences in dissolved oxygen were found between pulse dilution during disturbed and recovered periods at both reaches.

Shifts in metabolism and potential food sources

During the study, P/R values lower than 1 were usual at the *chronically disturbed reach* (mean value 0.81 ± 0.18 , Fig. 4a) while the *reference reach* showed values higher than 1 (mean value 2.97 ± 1.60 , Fig. 4b). Chronic dilution disturbance significantly decreased P/R values while no significant differences were found due to pulse dilution effects. BOM standing stock was significantly higher at the *chronically disturbed reach* ($1127.4 \pm 144.6 \text{ g m}^{-2}$) than at the *reference reach* ($827.6 \pm 136.0 \text{ g m}^{-2}$), a difference that can be explained partially by chronic dilution effects. Although BOM was made up of fine detritus at both reaches, the cover of large common reed debris in the chronically disturbed channel was 10 to 15%, whereas neither *P. australis* nor any other terrestrial plant debris was found at the *reference reach*. In contrast, similar values of the seasonality model residuals for suspended particulate organic matter were observed between periods and reaches. Total primary producer biomass was, on average, much higher at the *reference reach* ($96.5 \pm 65.4 \text{ g Chl } a \text{ m}^{-2}$) than at the *chronically*

Chapter 2: Impact of chronic and pulse dilutions on ecosystem functioning

Table 1 Mean values (\pm SE) of environmental variables, net daily metabolism and basal resources during disturbed (April 2003 to December 2003) and recovered periods (March 2004 to January 2005) for the two studied reaches, as well as two-way ANOVA results for each variable.

	Chronically disturbed reach		Reference reach		ANOVA		
	Disturbed (n=5)	Recovered (n=5)	Disturbed (n=5)	Recovered (n=5)	Chronic	Pulse	Chronic x Pulse
Habitat features and food sources	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE			
Daily mean water temperature ($^{\circ}$ C) [¶]	0.01 \pm 0.02	-0.05 \pm 0.02	0.03 \pm 0.02	0.02 \pm 0.03	0.042	n.s.	n.s.
Discharge (L s ⁻¹)	99.9 \pm 46.4	19.8 \pm 1.5	83.0 \pm 39.2	15.5 \pm 2.8	n.s.	<0.001	n.s.
Current velocity (m s ⁻¹)	0.05 \pm 0.01	0.03 \pm 0.01	0.17 \pm 0.04	0.12 \pm 0.02	<0.001	n.s.	n.s.
Conductivity (mS cm ⁻¹)	7.1 \pm 3.7	36.9 \pm 9.7	24.8 \pm 7.8	77.6 \pm 5.7	<0.001	<0.001	n.s.
Dissolved oxygen (mg L ⁻¹)	6.6 \pm 0.3	6.3 \pm 1.6	8.6 \pm 0.5	8.7 \pm 0.7	0.023	n.s.	n.s.
Production : Respiration ratio	0.65 \pm 0.02	0.97 \pm 0.25	1.79 \pm 0.20	4.16 \pm 2.25	<0.001	n.s.	n.s.
Benthic organic matter (g m ⁻²)	1,190.5 \pm 160.2	1,064.2 \pm 139.4	791.0 \pm 131.9	864.1 \pm 153.4	0.045	n.s.	n.s.
Seston (mg L ⁻¹) ¹	0.05 \pm 0.04	-0.08 \pm 0.12	0.03 \pm 0.12	0.01 \pm 0.11	n.s.	n.s.	n.s.
<i>Cladophora glomerata</i> (g Chl <i>a</i> m ⁻²)	0.0 \pm 0.0	0.0 \pm 0.0	115.4 \pm 85.2	6.4 \pm 4.9	0.002	n.s.	n.s.
<i>Enteromorpha intestinalis</i> (g Chl <i>a</i> m ⁻²)	0.0 \pm 0.0	0.0 \pm 0.0	0.1 \pm 0.1	0.1 \pm 0.1	n.s.	n.s.	n.s.
Biofilm (g Chl <i>a</i> m ⁻²)	6.5 \pm 2.1	3.8 \pm 0.7	7.5 \pm 3.1	20.3 \pm 2.5	0.010	n.s.	0.015
<i>Ruppia maritima</i> (g Chl <i>a</i> m ⁻²)	0.0 \pm 0.0	0.0 \pm 0.0	37.4 \pm 25.3	6.1 \pm 3.4	0.001	n.s.	n.s.
Total primary producer biomass	6.5 \pm 2.1	3.8 \pm 0.7	160.2 \pm 86.9	32.8 \pm 6.7	<0.001	n.s.	n.s.

[¶]: residuals of the seasonality model; n.s.: non significant (P -value>0.05).

disturbed reach (5.1 ± 1.6 g Chl *a* m⁻²). Macrophytes were either absent (*Cladophora glomerata* and *Ruppia maritima*) or rare (*Enteromorpha intestinalis*) in the *chronically disturbed reach* during the study period (Figs. 4a and 4b). However, epipellic biofilm occurred in both reaches, and both pulse and chronic dilution disturbances had effects on its biomass. Chronic dilution significantly decreased mean biofilm biomass (from 13.9 ± 2.8 g Chl *a* m⁻² in the *reference reach* to 5.1 ± 1.2 g Chl *a* m⁻² in the *chronically disturbed reach*), while pulse dilution caused different effects at each reach. Interaction term significance revealed that biofilm biomass increased during the pulse dilution period at the *chronically disturbed reach* (Fig. 4c) while it decreased at the *reference reach* (Fig. 4d).

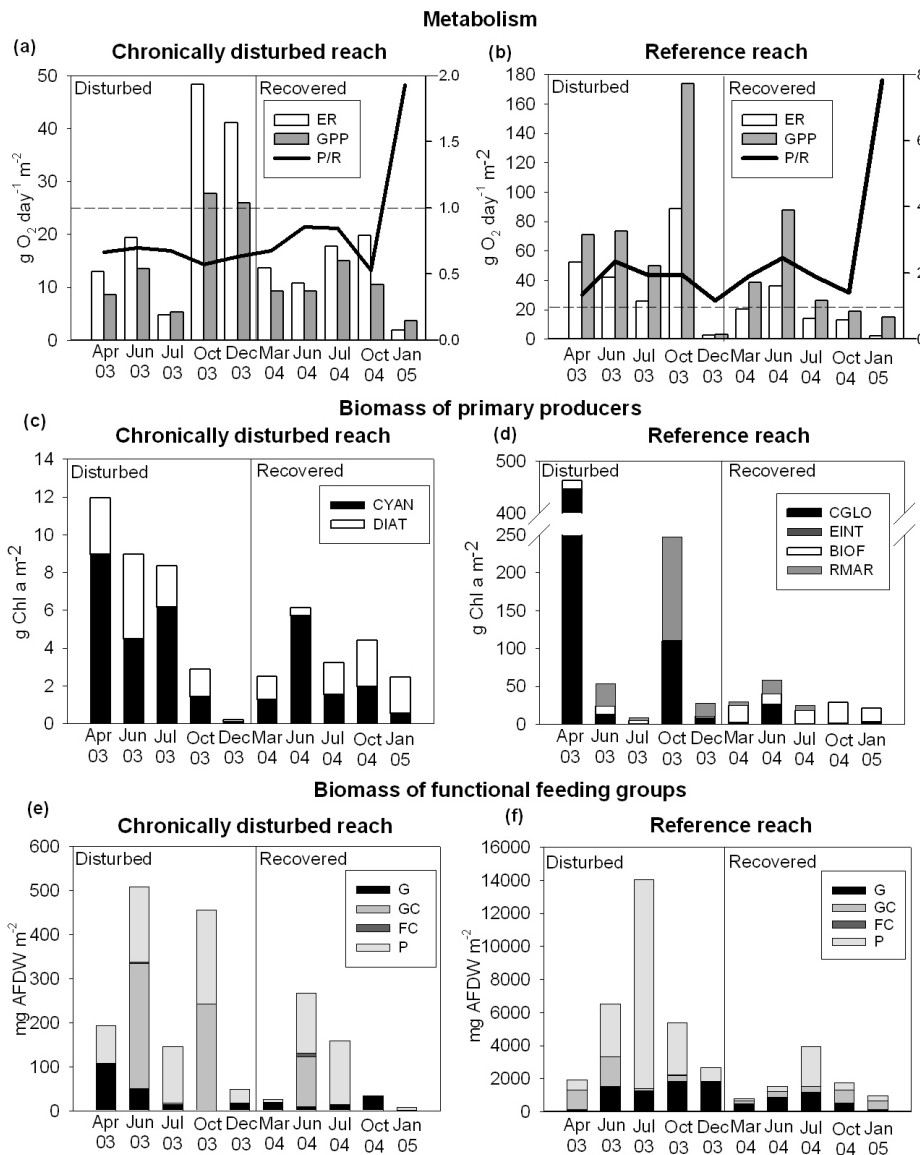


Fig. 4 Metabolism, primary producers and functional feeding group biomass variation during pulse dilution and recovered periods in the chronically disturbed (a, c, e) and reference reaches (b, d, f). ER: ecosystem respiration, GPP: gross primary production, P/R: production : respiration ratio, CYAN: cyanobacteria, DIAT: diatoms, CGLO: *Cladophora glomerata*, EINT: *Enteromorpha intestinalis*, BIOF: biofilm, RMAR: *Ruppia maritima*, G: grazers, GC: gathering collectors, FC: filtering collectors, P: predators. Dotted horizontal line represents P/R=1 (a, b).

Shifts in functional feeding group biomass

Predators were the most important group in terms of biomass, followed by grazers and gathering-collectors at both reaches (Table 2, see Appendix 2.1 and 2.2 in Supporting Information). In contrast, the biomass of filtering-collectors was lower and shredders were absent in both reaches during the study period. The effects of the chronic dilution disturbance significantly decreased the different FFG biomasses, with the exception of filtering collectors (Figs. 4e and 4f). However, the pulse dilution disturbance only had a significant effect on predator and total consumer biomasses, which increased. Finally, the percentages of total biomass of each functional feeding group were affected neither by disturbances nor seasonality, remaining relatively constant among dates, periods and reaches.

Contribution of basal resources to the food webs

At the *chronically disturbed reach*, biofilm showed the lowest C : N ratio (3.9), followed by *E. intestinalis* (12.2) and *P. australis* (16.8). The results of mixing models (see Appendix 2.3) revealed that the aggregated biofilm-seston was the most important basal source for grazers (median=0.46), gathering-collectors (median=0.45) and predators (median=0.92) (Fig. 5a). The macrophyte *E. intestinalis* also had an important contribution for grazers and gathering-collectors (medians were 0.31 and 0.28, respectively), yet this was almost negligible for predators (median=0.04). The importance of *P. australis* detritus seems to be minor for grazer (median=0.14) and gathering-collector (median=0.22) diets, and especially insignificant for predators (median=0.03). At the *reference reach*, biofilm also had the lowest C : N ratio (3.3), followed by *R. maritima* (11.8), *C. glomerata* (15.7), *E. intestinalis* (18.1) and the *C. glomerata* epiphytes (29.2). The outcomes of mixing models showed that macrophytes *R. maritima* and *E. intestinalis* were the most important basal sources for grazers (medians were 0.32 and 0.39, respectively), gathering-collectors (0.42 and 0.27) and predators (0.44 and 0.48) (Fig. 5b). The aggregated biofilm-seston seemed to have certain relevance only for gathering-collectors (median=0.25), while the macrophyte *C. glomerata* was, generally, unimportant.

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Table 2 Mean biomass and relative importance (\pm SE) of each functional feeding group during pulse dilution (April 2003 to December 2003) and recovered periods (March 2004 to January 2005) for the two studied reaches, as well as two-way ANOVA results for each variable.

Functional feeding group	Chronically disturbed reach		Reference reach		ANOVA		
	Disturbed (n=5)	Recovered (n=5)	Disturbed (n=5)	Recovered (n=5)	Chronic	Pulse	Chronic x Pulse
Grazer biomass	41.5 \pm 27.9	9.3 \pm 2.8	631.6 \pm 174.6	398.7 \pm 173	<0.001	n.s.	n.s.
Gathering collector biomass	105.9 \pm 64.4	23.3 \pm 22.5	713.3 \pm 335.4	426.8 \pm 95.7	<0.001	n.s.	n.s.
Filtering collector biomass	0.8 \pm 0.8	1.7 \pm 1.7	5.7 \pm 3.3	0.0 \pm 0.0	n.s.	n.s.	n.s.
Predator biomass	126.0 \pm 31.6	59.0 \pm 33.1	4,067.1 \pm 2,202.2	727.8 \pm 422.7	<0.001	0.013	n.s.
Total biomass	274.2 \pm 87.9	93.3 \pm 51.7	5417.7 \pm 2262.3	1553.3 \pm 562.2	<0.001	0.010	n.s.
% Grazers	19.5 \pm 11.3	30.2 \pm 16.9	15.7 \pm 5.2	26.0 \pm 7.5	n.s.	n.s.	n.s.
% Gathering collectors	22.6 \pm 13.3	12.1 \pm 7.7	21.4 \pm 11.4	36.7 \pm 8.6	n.s.	n.s.	n.s.
% Filtering collectors	0.2 \pm 0.2	0.6 \pm 0.6	0.1 \pm 0.1	0.0 \pm 0.0	n.s.	n.s.	n.s.
% Predators [†]	0.04 \pm 0.03	0.04 \pm 0.11	0.10 \pm 0.09	-0.18 \pm 0.05	n.s.	n.s.	n.s.

[†]: residuals of the seasonality model; n.s.: non significant (P -value>0.05).

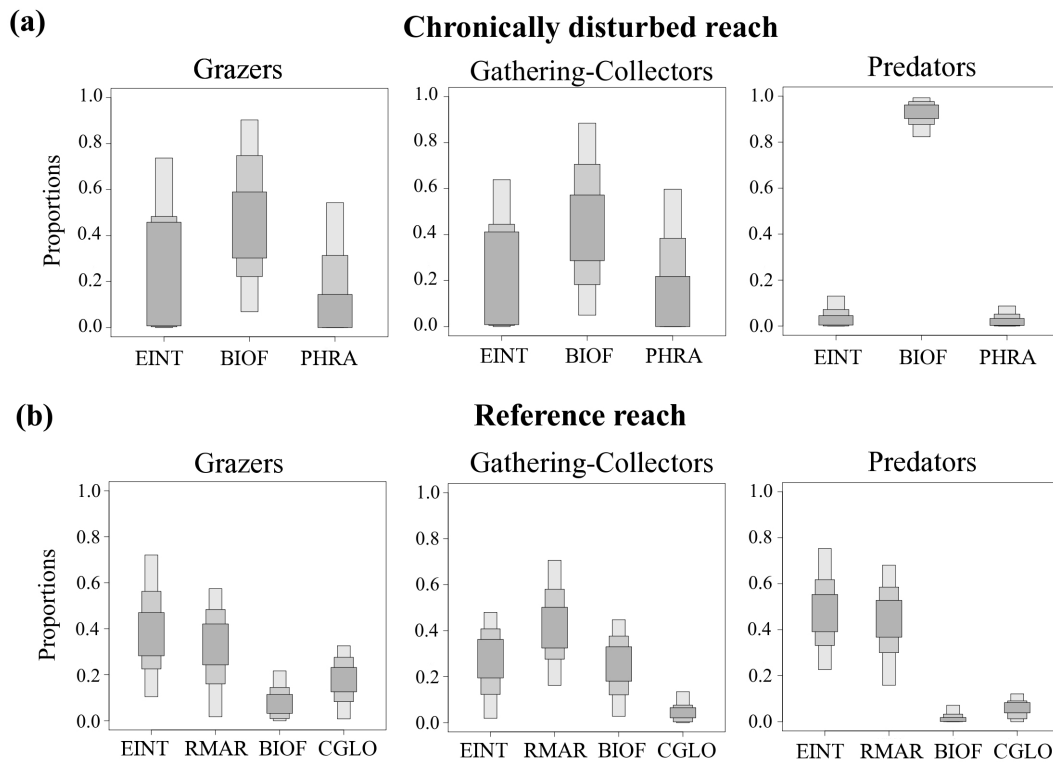


Fig. 5 Plots showing the relative importance of basal sources for each functional feeding group at the chronically disturbed (a) and reference reaches (b), expressed as 50, 75 and 95% probability intervals, which are represented by the broader central, intermediate and thinner grey rectangles, respectively. BIOF: biofilm + seston, CGLO: *Cladophora glomerata* + epiphytes, EINT: *Enteromorpha intestinalis*, PHRA: *Phragmites australis*, RMAR: *Ruppia maritima*.

Discussion

Effects of disturbance dilutions on metabolism

Our results revealed that chronic dilution disturbances produced a more severe impact on ecosystem functioning than pulse dilutions, thereby highlighting the role of persistence when attempting to predict disturbance effects on an ecosystem. In addition, functional measures are adequate indicators of the environmental change. The most remarkable effect of chronic dilution on ecosystem function was the change from an autotrophic to a heterotrophic ecosystem metabolism (i.e. from a net organic matter producer and exporter system, $P/R > 1$ (Fig. 6a) to a net consumer system, $P/R < 1$ (Fig. 6b). Although $P/R < 1$ values are common in unproductive, closed canopy ecosystems (von Schiller *et al.* 2008; Young *et al.* 2008), highly productive systems tend to be autotrophic, exhibiting $P/R > 1$ (Duarte & Agusti 1998). In fact, saline streams are autotrophic and very productive ecosystems, commonly exhibiting $P/R > 1$

values (Velasco *et al.* 2003; Gutiérrez-Cánovas *et al.* 2009), and acting as CO₂ sinks and organic matter sources. As instream production exceeds consumption, organic matter is often stored as BOM, which is partially transported downstream by floods (Vidal-Abarca *et al.* 2004). Therefore, the transformation from an autotrophic system to a heterotrophic system might have substantial consequences on associated terrestrial or aquatic ecosystems that would rely on carbon subsidies coming from saline streams as, for instance, many of the EU-protected birds that feed on such systems (Perez-Hurtado *et al.* 1997).

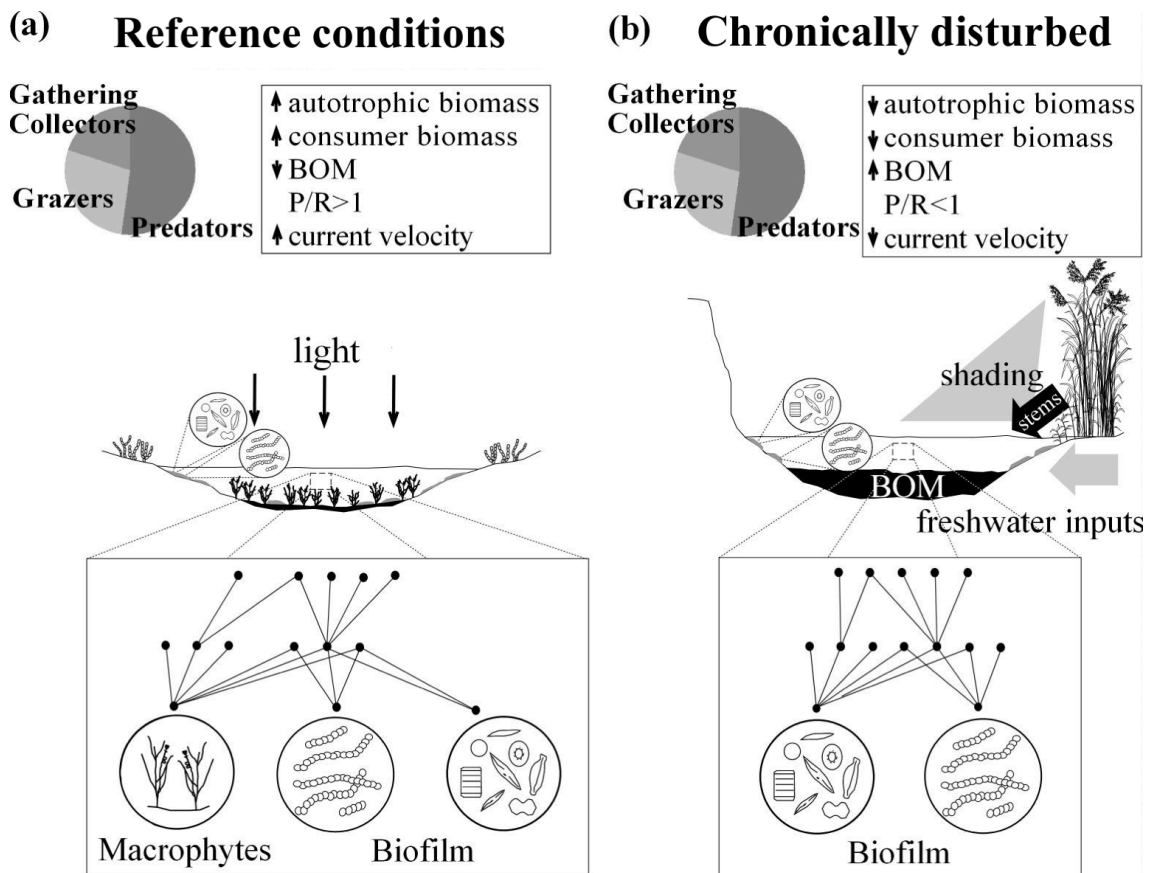


Fig. 6 Schematic picture summarising the metabolism and trophic structure under reference (a) and chronically disturbed conditions (b) in the Rambla Salada stream.

Effects of disturbance dilutions on producer and consumer biomasses

Although organism biomass generally tends to decline after disturbances (Odum 1985; Resh *et al.* 1988; Death 1996), the opposite pattern was found after pulse dilutions in the saline reference reach, as expected. The higher producer and consumer biomasses observed after short-term salinity drops may have been related to decreased osmotic stress, and, consequently, a reduction in the

amount of energy that organisms would have expended in osmotic regulation (Potts 1954; Kefford *et al.* 2006). In fact, different experiments carried out on halophile plants that inhabit terrestrial (Redondo-Gómez *et al.* 2006; Redondo-Gómez *et al.* 2010), brackish (La Peyre & Rowe 2003) and marine habitats (Koch *et al.* 2007) or for stream halotolerant invertebrates (Kefford *et al.* 2006) reported that growth rates often peak at low-moderate salinities (17-35 mS cm⁻¹), such as those recorded during the pulse dilution period. Moreover, this increase in macrophyte cover and biomass after the pulse dilutions was accompanied by a reduction in biofilm biomass, matching our initial predictions, as well as the results of other studies (Moreno *et al.* 2001; Gutiérrez-Cánovas *et al.* 2009; Ros *et al.* 2009). One possible explanation could be that under such conditions, macrophyte cover is higher, likely reducing the amount of light that would reach the streambed where biofilm is attached. However, contrary to expectations, the biofilm biomass increased with pulse dilution disturbances at the *chronic disturbed reach*. One hypothetical explanation could be that the increase of freshwater flow removed and oxygenated the upper layer of sediments, thereby favouring mineralisation processes and biofilm development.

In contrast, chronic dilution conditions appeared to be more stressful for organisms than pulse disturbances, reflecting that various underlying mechanisms were occurring. The results of the present study agree with the general response across ecosystems to chronic disturbances, which is a reduction of the abundance and biomass of organisms (Kaiser *et al.* 2000; Nystrom *et al.* 2000; Hirst *et al.* 2002; Niyogi *et al.* 2002). These studies emphasised that chronic disturbances may essentially reduce habitat complexity or disrupt organism performance as the concentration of toxic compounds increases. Thus, while pulse dilution reduced osmotic stress, chronic dilution resulted in unsuitable habitat conditions, especially for macrophytes and invertebrates, as a result of the habitat changes that the presence of *P. australis* and their inputs induced. For instance, *R. maritima* is very sensitive to anoxic sediments (Kantrud 1991) and, probably, the decreased light and temperature may also affect, as well as other autotrophs. On the other hand, invertebrates had to cope with habitat homogeneity, food depletion and low dissolved oxygen values, which could explain their low biomasses.

Effect of disturbance dilutions on food webs and trophic structure

Pulse dilution did not significantly change the trophic structure. This may have occurred due to the abundance of autochthonous basal resources in naturally saline conditions, allowing consumers to have enough food during short-term disturbed conditions. However, contrary to our predictions, chronic dilution

disturbance did not significantly change the relative importance of each functional feeding group, despite the input of the common reed debris. These results suggest that, given the absence of shredders, *P. australis* detritus can only enter the food web as a minor resource for gathering-collectors. This could have occurred because this reach has a relevant standing stock of biofilm with a very high nutritional value, while *P. australis* exhibited the highest C : N value at this reach. Furthermore, its detritus are predominantly composed of lignocellulose, which is broken down primarily by bacteria and fungi (Gessner 2001; Dinka *et al.* 2004), given that shredder invertebrates were absent. Lloret & Marín (2009) found similar results in a coastal lagoon where anthropogenic terrestrial inputs (i.e. particulate organic matter) did not fuel the benthic food web, as invertebrates had more nutritive basal sources, such as macrophytes or biofilm.

Conclusions and future research

In conclusion, this study demonstrates the importance of disturbance persistence in natural stressed ecosystems. Thus, while pulse dilution disturbances had minor effects on ecosystem function, chronic dilution disturbance leads to substantial ecosystem changes from an autotrophic to a heterotrophic metabolism and biomass depletion. Despite streambed abundance of common reed detritus, the food web was still based on autochthonous primary sources, and these inputs did not produce significant changes in the food web. The differences in metabolism, producer and consumer biomasses observed between the studied reaches and periods can be undoubtedly linked to the putative effects of chronic and pulse dilutions as seasonality had an insignificant effect for the majority of the studied variables.

Future research should be focussed upon the fate of non-consumed organic matter exported by saline streams, as well as the interrelationships between saline streams and their terrestrial surrounding environment, in order to predict the effects of the transformation of some saline stream reaches in heterotrophic environments on the terrestrial ecosystems and carbon balances. In addition, production : respiration ratios should be tested as ecosystem health indicators in a broader context for autotrophic ecosystems subject to anthropogenic organic matter or nutrient inputs.

Acknowledgements

We would like to thank Pedro Abellán, David Sánchez-Fernández, José Barahona, Mar Ruiz and Rocío Alcántara for their assistance in field sampling

and sample processing. We are grateful to Melissa Crim and Javier Lloret for correcting the English version of the manuscript and for their useful comments and suggestions. We wish to thank to María Dolores Belando for designing Figure 6. Thanks also to Miguel Ángel Núñez of the Centro de Interpretación de Rambla Salada for his generous support and assistance in the field. We also greatly appreciate the comments and suggestions made by Núria Bonada, Koen Martens and the anonymous referees that have significantly improved this paper. This research was supported by research projects BOS2002-00702 and CGL2006-04159 (Spanish Investigation, Development and Innovation Program). C. Gutiérrez-Cánovas was supported by a predoctoral grant from the Fundación SÉNECA, Agencia Regional de Ciencia y Tecnología, Región de Murcia.

References

- Adams, J.B. & Bate, G.C. (1999). Growth and photosynthetic performance of *Phragmites australis* in estuarine waters: a field and experimental evaluation. *Aquatic Botany*, 64, 359-367.
- Barahona, J., Millán, A. & Velasco, J. (2005). Population dynamics, growth and production of *Sigara selecta* (Fieber, 1848) (Hemiptera, Corixidae) in a Mediterranean hypersaline stream. *Freshwater Biology*, 50, 2101-2113.
- Bart, D. & Hartman, J.M. (2003). The role of large rhizome dispersal and low salinity windows in the establishment of common reed, *Phragmites australis*, in salt marshes: New links to human activities. *Estuaries*, 26, 436-443.
- Bender, E.A., Case, T.J. & Gilpin, M.E. (1984). Perturbation experiments in community ecology - theory and practice. *Ecology*, 65, 1-13.
- Benke, A.C., Huryn, A.D., Smock, L.A. & Wallace, J.B. (1999). Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. *Journal of the North American Benthological Society*, 18, 308-343.
- Carman, K.R. & Fry, B. (2002). Small-sample methods for delta C-13 and delta N-15 analysis of the diets of marsh meiofaunal species using natural-abundance and tracer-addition isotope techniques. *Marine Ecology-Progress Series*, 240, 85-92.
- Death, R.G. (1996). The effect of patch disturbance on stream invertebrate community structure: The influence of disturbance history. *Oecologia*, 108, 567-576.

- Dinka, M., Agoston-Szabo, E. & Toth, I. (2004). Changes in nutrient and fibre content of decomposing *Phragmites australis* litter. *International Review of Hydrobiology*, 89, 519-535.
- Duarte, C.M. & Agusti, S. (1998). The CO₂ balance of unproductive aquatic ecosystems. *Science*, 281, 234-236.
- Genereux, D.P. & Hemond, H.F. (1992). Determination of gas-exchange rate constants for a small stream on Walker Branch watershed, Tennessee. *Water Resources Research*, 28, 2365-2374.
- Gessner, M.O. (2001). Mass loss, fungal colonisation and nutrient dynamics of *Phragmites australis* leaves during senescence and early aerial decay. *Aquatic Botany*, 69, 325-339.
- Gessner, M.O. & Chauvet, E. (2002). A case for using litter breakdown to assess functional stream integrity. *Ecological Applications*, 12, 498-510.
- Gómez, R., Vidal-Abarca, M.R. & Suárez, M.L. (2001). Importance of the subsurface-surface water interaction in the wetland structure and dynamic in arid and semiarid areas. In: *Groundwater ecology: A tool fore management of water resources* (eds. Griebler, C, Danielopol, DL, Gibert, J, Nachebel, HP & Notenboom, J). European Communities Luxembourg, pp. 317-322.
- Gutiérrez-Cánovas, C., Velasco, J. & Millán, A. (2009). Effects of dilution stress on the functioning of a saline Mediterranean stream. *Hydrobiologia*, 619, 119-132.
- Hirst, H., Juttner, I. & Ormerod, S.J. (2002). Comparing the responses of diatoms and macroinvertebrates to metals in upland streams of Wales and Cornwall. *Freshwater Biology*, 47, 1752-1765.
- Johnston, T.A. & Cunjak, R.A. (1999). Dry mass-length relationships for benthic insects: a review with new data from Catamaran Brook, New Brunswick, Canada. *Freshwater Biology*, 41, 653-674.
- Kaiser, M.J., Ramsay, K., Richardson, C.A., Spence, F.E. & Brand, A.R. (2000). Chronic fishing disturbance has changed shelf sea benthic community structure. *Journal of Animal Ecology*, 69, 494-503.
- Kantrud, H.A. (1991). *Wigeongrass (Ruppia maritima L.): A literature Review*. U.S. Fish and Wildlife Service, Fish and Wildlife Research 10. Jamestown, ND: Northern Prairie Wildlife Research Center Online. <http://www.npwrc.usgs.gov/resource/plants/ruppia/index.htm> (Version 16JUL97).

- Kefford, B.J., Zalizniak, L. & Nugegoda, D. (2006). Growth of the damselfly *Ischnura heterosticta* is better in saline water than freshwater. *Environmental Pollution*, 141, 409-419.
- Koch, M.S., Schopmeyer, S.A., Kyhn-Hansen, C., Madden, C.J. & Peters, J.S. (2007). Tropical seagrass species tolerance to hypersalinity stress. *Aquatic Botany*, 86, 13.
- Kowalik, R.A., Cooper, D.M., Evans, C.D. & Ormerod, S.J. (2007). Acidic episodes retard the biological recovery of upland British streams from chronic acidification. *Global Change Biology*, 13, 2439-+.
- La Peyre, M.K. & Rowe, S. (2003). Effects of salinity changes on growth of *Ruppia maritima* L. *Aquatic Botany*, 77, 235-241.
- Laliberte, E., Wells, J.A., DeClerck, F., Metcalfe, D.J., Catterall, C.P., Queiroz, C. *et al.* (2010). Land-use intensification reduces functional redundancy and response diversity in plant communities. *Ecology Letters*, 13, 76-86.
- Lloret, J. & Marin, A. (2009). The role of benthic macrophytes and their associated macroinvertebrate community in coastal lagoon resistance to eutrophication. *Marine Pollution Bulletin*, 58, 1827-1834.
- McCabe, D.J. & Gotelli, N.J. (2000). Effects of disturbance frequency, intensity, and area on assemblages of stream macroinvertebrates. *Oecologia*, 124, 270-279.
- McCutchan, J.H., Lewis, W.M., Kendall, C. & McGrath, C.C. (2003). Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, 102, 378-390.
- Merritt, R.W. & Cummins, K.W. (1996). *An Introduction to the Aquatic Insects of North America*. Kendall Hunt Publishing, Iowa, USA.
- Millán, A., Velasco, J., Gutiérrez-Cánovas, C., Arribas, P., Picazo, F., Sánchez-Fernández, D. *et al.* (2011). Mediterranean saline streams in southeast Spain: What do we know? *Journal of the Arid Environments*, 75, 1352-1359.
- Monakov, A.V. (2003). *Feeding of freshwater invertebrates*. Kenobi Productions, Ghent, Belgium.
- Moreno, J.L. (2002). Hábitats, recursos tróficos y estructura de la comunidad de macroinvertebrados bentónicos en un arroyo salino del Sureste Ibérico (Rambla del Reventón). PhD dissertation. University of Murcia Murcia, Spain.
- Moreno, J.L., Aboal, M., Vidal-Abarca, M.R. & Suarez, M.L. (2001). Macroalgae and submerged macrophytes from fresh and saline waterbodies of

- ephemeral streams ('ramblas') in semiarid south-eastern Spain. *Marine and Freshwater Research*, 52, 891-905.
- Naeem, S., Thompson, L.J., Lawler, S.P., Lawton, J.H. & Woodfin, R.M. (1994). Declining biodiversity can alter the performance of ecosystems. *Nature*, 368, 734-737.
- Niyogi, D.K., Lewis, W.M. & McKnight, D.M. (2002). Effects of stress from mine drainage on diversity, biomass, and function of primary producers in mountain streams. *Ecosystems*, 5, 554-567.
- Nystrom, M., Folke, C. & Moberg, F. (2000). Coral reef disturbance and resilience in a human-dominated environment. *Trends in Ecology & Evolution*, 15, 413-417.
- Odum, E.P. (1985). Trends expected in stressed ecosystems. *Bioscience*, 35, 419-422.
- Odum, H.T. (1956). Primary production in flowing waters. *Limnology and Oceanography*, 1, 102-117.
- Owens, M., Edwards, R.W. & Gibbs, J.W. (1964). Some reaeration studies in streams. *International Journal of Air and Water Pollution*, 469-486.
- Parnell, A.C., Inger, R., Bearhop, S. & Jackson, A.L. (2010). Source partitioning using stable isotopes: coping with too much variation. *Plos One*, 5.
- Perez-Hurtado, A., Goss-Custard, J.D. & Garcia, F. (1997). The diet of wintering waders in Cadiz Bay, southwest Spain. *Bird Study*, 44, 45-52.
- Phillips, D.L. & Gregg, J.W. (2003). Source partitioning using stable isotopes: coping with too many sources. *Oecologia*, 136, 261-269.
- Potts, W.T.W. (1954). The energetics of osmotic regulation in brackish-water and fresh-water animals. *Journal of Experimental Biology*, 31, 618-630.
- R-Development-Core-Team (2011). R: a language and environment for statistical computing. R Foundation for Statistical Computing Vienna, Austria.
- Ramírez-Díaz, L., Vidal-Abarca, M.R., Calvo, J.F., Suarez, M.L., Palazón, J., Esteve, M.A. *et al.* (1995). *Bases ecológicas para la delimitación, ordenación y gestión del Paisaje Protegido de las Ramblas de Ajauque y Salada*. Consejería de Medio Ambiente, Comunidad Autónoma de la Región de Murcia, Murcia, Spain.

- Redondo-Gómez, S., Mateos-Naranjo, E., Figueroa, M.E. & Davy, A.J. (2010). Salt stimulation of growth and photosynthesis in an extreme halophyte, *Arthrocnemum macrostachyum* *Plant Biology*, 12, 79-87.
- Redondo-Gómez, S., Wharmby, C., Castillo, J.M., Mateos-Naranjo, E., Luque, C.J., De Cires, A. *et al.* (2006). Growth and photosynthetic responses to salinity in an extreme halophyte, *Sarcocornia fruticosa* *Physiologia Plantarum*, 128, 116-124.
- Resh, V.H., Brown, A.V., Covich, A.P., Gurtz, M.E., Li, H.W., Minshall, G.W. *et al.* (1988). The role of disturbance in stream ecology. *Journal of the North American Benthological Society*, 7, 433-455.
- Ros, M.D., Marín-Murcia, J.P. & Aboal, M. (2009). Biodiversity of diatom assemblages in a Mediterranean semiarid stream: implications for conservation. *Marine and Freshwater Research*, 60, 14-24.
- Russell-Hunter, D.M. (1970). *Aquatic productivity: an introduction to some basic aspects of biological oceanography and limnology*. Macmillan, New York.
- Schmitz, O.J. (1997). Press perturbations and the predictability of ecological interactions in a food web. *Ecology*, 78, 55-69.
- Sheldon, F. & Walker, K.F. (1998). Spatial distribution of littoral invertebrates in the lower Murray-Darling River system, Australia. *Marine and Freshwater Research*, 49, 171-182.
- Silliman, B.R. & Bertness, M.D. (2004). Shoreline development drives invasion of *Phragmites australis* and the loss of plant diversity on New England salt marshes. *Conservation Biology*, 18, 1424-1434.
- Smith, M.D., Knapp, A.K. & Collins, S.L. (2009). A framework for assessing ecosystem dynamics in response to chronic resource alterations induced by global change. *Ecology*, 90, 3279-3289.
- Smock, L.A. (1980). Relationships between body size and biomass of aquatic insects. *Freshwater Biology*, 10, 375-383.
- Tachet, H., Richoux, P., Bournaud, M. & Usseglio-Polatera, P. (2000). *Invertébrés d'eau douce. Systematique, biologie, écologie*. CNRS éditions, Paris, France.
- Velasco, J., Millán, A., Hernández, J., Gutiérrez-Cánovas, C., Abellán, P., Sánchez-Fernández, D. *et al.* (2006). Response of biotic communities to salinity changes in a Mediterranean hypersaline stream. *Saline Systems*, 2, 12.

- Velasco, J., Millán, A., Vidal-Abarca, M.R., Suárez, M.L., Guerrero, C. & Ortega, M. (2003). Macrophytic, epipelic and epilithic primary production in a semiarid Mediterranean stream. *Freshwater Biology*, 48, 1408-1420.
- Vidal-Abarca, M.R. (1985). *Las aguas superficiales de la Cuenca del Río Segura (SE de España). Caracterización físico-química en relación al medio físico y humano*. PhD dissertation. University of Murcia, Murcia, Spain.
- Vidal-Abarca, M.R., Suárez, M.L., Gómez, R., Guerrero, C., Sánchez-Montoya, M.M. & Velasco, J. (2004). Intra-annual variation in benthic organic matter in a saline, semi-arid stream of southeast Spain (Chícamo stream). *Hydrobiologia*, 523, 199-215.
- von Schiller, D., Marti, E., Riera, J.L., Ribot, M., Marks, J.C. & Sabater, F. (2008). Influence of land use on stream ecosystem function in a Mediterranean catchment. *Freshwater Biology*, 53, 2600-2612.
- von Schiller, D. & Solimini, A.G. (2005). Differential effects of preservation on the estimation of biomass of two common mayfly species. *Archiv für Hydrobiologie*, 164, 325-334.
- Weis, J.S. & Weis, P. (2003). Is the invasion of the common reed *Phragmites australis* into tidal marshes of the eastern US an ecological disaster? *Marine Pollution Bulletin*, 46, 816-820.
- Young, R.G., Matthaei, C.D. & Townsend, C.R. (2008). Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health. *Journal of the North American Benthological Society*, 27, 605-625.

Supporting Information

Appendix 2.1 Table showing mean abundance (\pm SE) of taxa collected in each reach during each period.

Appendix 2.2 Table showing mean biomass (\pm SE) of taxa collected in each reach during each period.

Appendix 2.3 Mixing polygons and consumer corrected signatures for the *chronically disturbed reach* (a) and *reference reach* (b).

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Appendix 2.1 Table showing mean abundance (\pm SE) of taxa collected in each reach during each period. A: adult, L: larvae; COL: Coleoptera, DIP: Diptera, EPH: Ephemeroptera, HEM: Hemiptera, MOL: Mollusca, OLI: Oligochaeta. FFG: functional feeding group, FC: filtering-collector, GC: gathering-collector, G: grazer, P: predator.

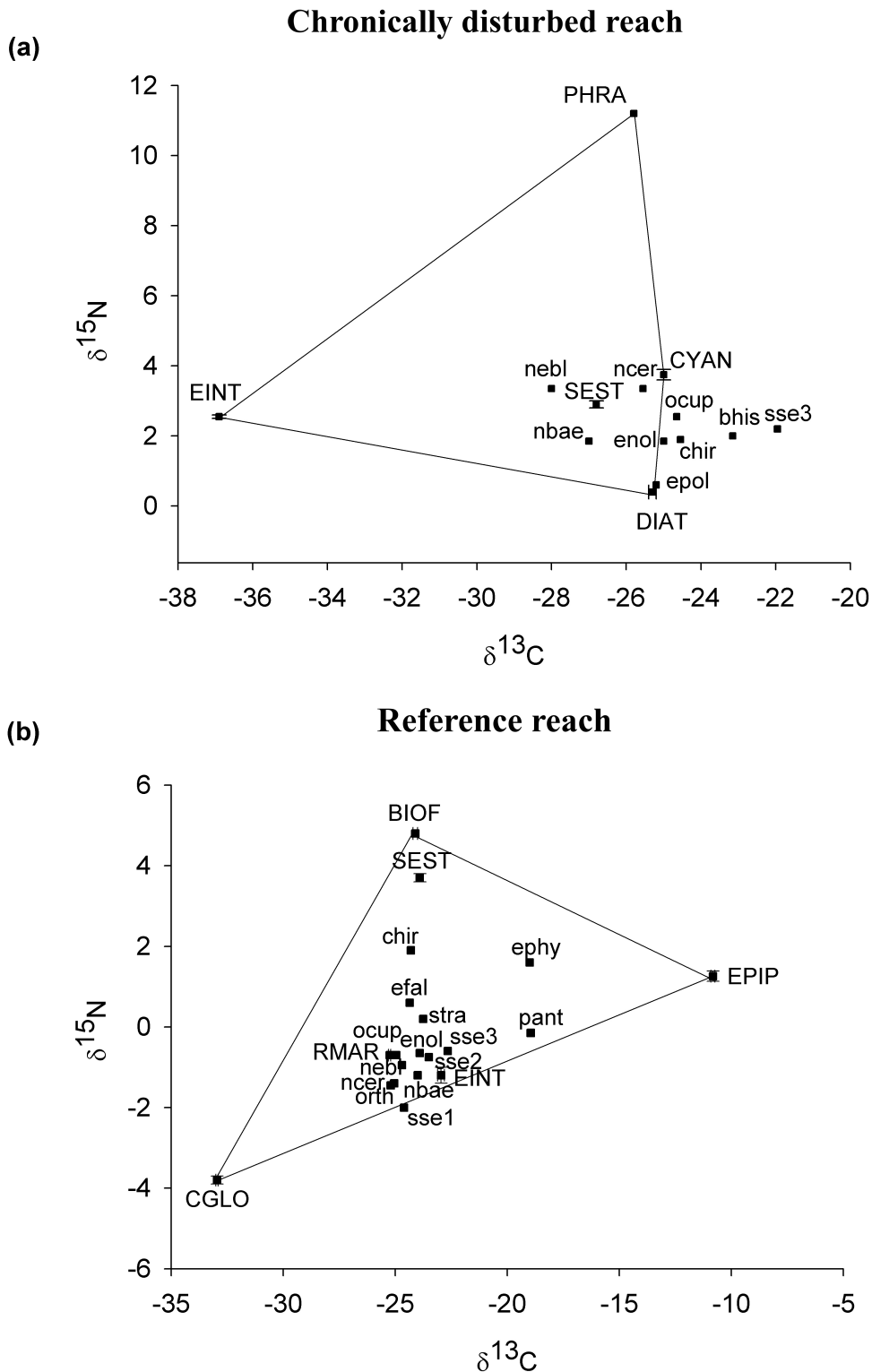
Taxon, author, year	Stage	Order	FFG	Abundance (individuals m ⁻²)			
				Chronically disturbed reach		Reference reach	
				Disturbed	Recovered	Disturbed	Recovered
				Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE
Culicidae	L	DIP	FC	2.8 \pm 2.8	5.6 \pm 5.6	0	0
Simuliidae	L	DIP	FC	0	0	29.3 \pm 19.7	0
<i>Berosus hispanicus</i> Küster 1847	A	COL	G	0 \pm 0	0 \pm 0	31.4 \pm 19.3	0 \pm 0
<i>Enochrus falcarius</i> Hebauer 1991	A	COL	G	0 \pm 0	0 \pm 0	52.5 \pm 23.2	44.4 \pm 24.8
<i>Enochrus politus</i> (Küster 1849)	A	COL	G	0 \pm 0	0 \pm 0	5.4 \pm 5.4	0 \pm 0
<i>Mercuria emiliana</i> (Paladilhe 1869)	A	MOL	G	0 \pm 0	0 \pm 0	1.2 \pm 0.9	0.2 \pm 0.2
<i>Ochthebius corrugatus</i> Rosenhauer 1856	A	COL	G	9.2 \pm 5.4	4.6 \pm 3.1	0.6 \pm 0.6	12.5 \pm 7.7
<i>Ochthebius cuprescens</i> Guillenbau 1893	A	COL	G	3.6 \pm 1.9	1.3 \pm 1.3	9.5 \pm 2.2	13.9 \pm 5.6
<i>Ochthebius</i> sp.	L	COL	G	0.7 \pm 0.7	0.2 \pm 0.1	0.3 \pm 0.3	0.2 \pm 0.1
<i>Potamopyrgus antipodarum</i> Gray 1843	A	MOL	G	0.7 \pm 0.7	1 \pm 1	243.5 \pm 81.9	110.2 \pm 35
<i>Sigara selecta</i> (Fieber 1848)	L	HEM	G	8.4 \pm 5.6	5.6 \pm 3.4	1,107.5 \pm 679.7	525.8 \pm 326.7
Ceratopogonidae	L	DIP	GC	16.8 \pm 13.5	8.4 \pm 8.4	29.1 \pm 25.1	34.8 \pm 34.8
Chironomini sp 1	L	DIP	GC	723.8 \pm 580.3	226.4 \pm 209.0	6,717.1 \pm 5,706.9	3,406.3 \pm 2,000.5
Chironomini sp 2	L	DIP	GC	44.7 \pm 35.2	0	1,128.3 \pm 1,085.6	248.4 \pm 144.4
<i>Cloeon schoenemundi</i> Bengtsson 1936	L	EPH	GC	0	0	208.9 \pm 97.5	49.9 \pm 21.7
Orthoclaadiinae	L	DIP	GC	55.9 \pm 45.7	511.4 \pm 504.4	638.1 \pm 241.6	1,978.1 \pm 758.7
Stratiomyidae	L	DIP	GC	2.8 \pm 2.8	0	2.4 \pm 2.4	10.5 \pm 8.5
Tubificidae	A	OLI	GC	0	0	21.2 \pm 11.8	62.0 \pm 62.0
<i>Anax</i> sp.	L	ODO	P	0	0	2.4 \pm 2.4	0
<i>Berosus</i> sp.	L	COL	P	2.8 \pm 2.8	2.8 \pm 2.8	67.3 \pm 55.9	26.5 \pm 11.8
Dolichopodidae	L	DIP	P	5.6 \pm 5.6	0	6.0 \pm 6.0	73.5 \pm 45.0
<i>Enochrus</i> sp.	L	COL	P	0	2.8 \pm 2.8	54.9 \pm 19.0	54.6 \pm 23.8
<i>Ephydra flavipes</i> (Macquart 1843)	L	DIP	P	114.6 \pm 88.0	128.5 \pm 104.8	335.6 \pm 290.0	1,624.9 \pm 1,374.9
Ephydridae	L	DIP	P	22.4 \pm 22.4	0	65.4 \pm 40.3	364.3 \pm 361.6
<i>Nebrioporus baeticus</i> (Schaum 1864)	A	COL	P	2.8 \pm 2.8	0	18.2 \pm 7.4	1.7 \pm 1.2
<i>Nebrioporus ceresyi</i> (Aube 1838)	A	COL	P	2.8 \pm 2.8	0	29.8 \pm 17.9	0.4 \pm 0.4
<i>Nebrioporus</i> sp.	L	COL	P	5.6 \pm 5.6	5.6 \pm 3.4	252.5 \pm 95.5	58.1 \pm 35.6
<i>Sigara selecta</i> (Fieber 1848)	A	HEM	P	39.1 \pm 19.0	25.2 \pm 21.8	1,767.5 \pm 1,194.0	183.3 \pm 138.3
Taniponidae	L	DIP	P	0	8.4 \pm 5.6	199.8 \pm 89.1	52.3 \pm 52.3

Chapter 2: Impact of chronic and pulse dilutions on ecosystem functioning

Appendix 2.2 Table showing mean biomass (\pm SE) of taxa collected in each reach during each period. A: adult, L: larvae; COL: Coleoptera, DIP: Diptera, EPH: Ephemeroptera, HEM: Hemiptera, MOL: Mollusca, OLI: Oligochaeta. FFG: functional feeding group, FC: filtering-collector, GC: gathering-collector, G: grazer, P: predator.

Taxon, author, year	Stage	Order	FFG	Biomass (mg AFDW m ⁻²)			
				Chronically disturbed reach		Reference reach	
				Disturbed	Recovered	Disturbed	Recovered
Culicidae	L	DIP	FC	0.8 \pm 0.8	1.7 \pm 1.7	0	0
Simuliidae	L	DIP	FC	0	0	5.7 \pm 3.3	0
<i>Berosus hispanicus</i> Küster 1847	A	COL	G	0 \pm 0	0 \pm 0	14.3 \pm 8.7	0 \pm 0
<i>Enochrus falcarius</i> Hebauer 1991	A	COL	G	0 \pm 0	0 \pm 0	22.2 \pm 9.6	21.2 \pm 13.1
<i>Enochrus politus</i> (Küster 1849)	A	COL	G	0 \pm 0	0 \pm 0	2.4 \pm 2.4	0 \pm 0
<i>Mercuria emiliana</i> (Paladilhe 1869)	A	MOL	G	0 \pm 0	0 \pm 0	3.2 \pm 2.3	0.4 \pm 0.4
<i>Ochthebius corrugatus</i> Rosenhauer 1856	A	COL	G	39.1 \pm 22.3	22.4 \pm 15.7	2.4 \pm 2.4	62.5 \pm 38.5
<i>Ochthebius cuprescens</i> Guillenbau 1893	A	COL	G	14 \pm 7.7	5.6 \pm 5.6	36 \pm 7.5	56.9 \pm 23
<i>Ochthebius</i> sp.	L	COL	G	27.9 \pm 27.9	8.4 \pm 5.6	12.3 \pm 9.6	8.1 \pm 5.3
<i>Potamopyrgus antipodarum</i> Gray 1843	A	MOL	G	2.8 \pm 2.8	2.8 \pm 2.8	966.1 \pm 328.5	360.1 \pm 87.5
<i>Sigara selecta</i> (Fieber 1848)	L	HEM	G	1.6 \pm 1	2.1 \pm 1.4	243.3 \pm 121	206.5 \pm 143.9
Ceratopogonidae	L	DIP	GC	0	0	0.8 \pm 2.1	15.5 \pm 15.5
Chironomini sp 1	L	DIP	GC	0.5 \pm 0.4	0.2 \pm 0.2	296.2 \pm 0.7	0.9 \pm 0.9
Chironomini sp 2	L	DIP	GC	32.2 \pm 25.5	7.1 \pm 6.5	225.6 \pm 250.7	103.9 \pm 56.4
<i>Cloeon schoenemundi</i> Bengtsson 1936	L	EPH	GC	0	0	124.5 \pm 64	15.4 \pm 6.9
Orthoclaadiinae	L	DIP	GC	30.3 \pm 27.4	0	28.0 \pm 210.2	119.0 \pm 86.6
Stratiomyidae	L	DIP	GC	2.5 \pm 2	16.0 \pm 15.7	34.5 \pm 10.7	67.6 \pm 23.4
Tubificidae	A	OLI	GC	40.6 \pm 40.6	0	3.8 \pm 34.5	104.4 \pm 78.7
<i>Anax</i> sp.	L	ODO	P	0	0	502.2 \pm 502.2	0
<i>Berosus</i> sp.	L	COL	P	0.8 \pm 0.8	2.7 \pm 2.7	20.7 \pm 16.4	38.2 \pm 25.6
Dolichopodidae	L	DIP	P	1.1 \pm 1.1	0	1.3 \pm 1.3	13.0 \pm 8.0
<i>Enochrus</i> sp.	L	COL	P	0	1.2 \pm 1.2	9.3 \pm 2.9	11.4 \pm 3.6
<i>Ephydra flavipes</i> (Macquart 1843)	L	DIP	P	27.0 \pm 20.8	21.8 \pm 17.2	143.6 \pm 132.6	323.3 \pm 264.9
Ephydridae	L	DIP	P	5.9 \pm 5.9	0	16.6 \pm 10.3	87.3 \pm 86.6
<i>Nebrioporus baeticus</i> (Schauum 1864)	A	COL	P	5.6 \pm 5.6	0	34.8 \pm 14	3.4 \pm 2.6
<i>Nebrioporus ceresyi</i> (Aube 1838)	A	COL	P	5.3 \pm 5.3	0	58 \pm 34.8	0.8 \pm 0.8
<i>Nebrioporus</i> sp.	L	COL	P	2.3 \pm 2.3	2.8 \pm 2.2	90.4 \pm 32.8	23.5 \pm 16.0
<i>Sigara selecta</i> (Fieber 1848)	A	HEM	P	71.5 \pm 35.4	29.9 \pm 25.6	3,171 \pm 2,208.7	221.6 \pm 161.7
Taniponidae	L	DIP	P	0	0.8 \pm 0.5	19.3 \pm 9.6	5.3 \pm 5.3

Appendix 2.3 Mixing polygons and consumer corrected signatures for the *chronically disturbed reach* (a) and *reference reach* (b). BIOF: biofilm, CGLO: *Cladophora glomerata*, CYAN: cyanobacteria biofilm, DIAT: diatom biofilm, EINT: *Enteromorpha intestinalis*, EPIP: *Cladophora glomerata* epiphytes, PHRAG: *Phragmites australis*, RMAR: *Ruppia maritima*, SEST: seston, behi: *Berosus hispanicus*, chir: Chironominae, enol: *Enochrus* sp. larvae, ephy: Ephydridae, epol: *Enochrus politus*, nbae: *Nebrioporus baeticus*, ncer: *Nebrioporus ceresyi*, nebl: *Nebrioporus* sp. larvae, ocup: *Ochthebius cuprescens*, orth: Orthoclaadiinae, pant: *Potamopyrgus antipodarum*, sse1: *Sigara selecta* nymph stage 1, sse2: *Sigara selecta* nymph stage 2, sse3: *Sigara selecta* adult and stra: Stratiomyidae.



Chapter 3:

Assessing automatic classifications of the Mediterranean streams and biomonitoring metrics for saline stream types

Submitted to Ecological Indicators:

Gutiérrez-Cánovas, C., P. Arribas; Naselli-Flores, L.; Bennas, N.; Finocchiaro, M., Millán, A. & J. Velasco. Assessing automatic classifications of the Mediterranean streams and biomonitoring metrics for saline stream types.

Assessing automatic classifications of the Mediterranean streams and biomonitoring metrics for saline stream types

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Abstract

Water Framework Directive aims to develop methods to assess the ecological status of European waterbodies. In such a context, current classification and biomonitoring indicators show critical limitations to implement WFD in the Mediterranean basin streams. Classifications are unable to represent the diversity of Mediterranean streams and to typify automatically large datasets of watercourses, requiring large efforts. Furthermore, widely-used biological indicators can be unsuitable to assess the ecological status of the naturally saline streams (conductivity $\geq 5000 \mu\text{S cm}^{-1}$), which represent a unique part of the Mediterranean basin biodiversity. Here, we address these limitations by developing three biologically-validated classifications (expert-based, environmental and biological clusters), covering the full environmental and biological variability of the Mediterranean basin streams. We also tested the capacity of conventional and novel biological metrics to assess the ecological status of saline streams. To characterise the streams and perform the analyses, we gathered different datasets of environmental data and aquatic macroinvertebrates at family and species level in Spain, Morocco and Italy. Our results showed that the expert based and biological classification yielded seven comparable stream classes, whereas environmental classification displayed substantial discrepancies. Biological classification showed the highest classification strength for Spanish data, while expert based displays highest values for Italian and Moroccan data. The

biological validation revealed also that community differences arose mainly due to species turnover. Finally, we found that the novel biological indicators suggested here for saline streams types worked much better to assess the ecological status than the conventional metrics. The results of this study will help environmental managers to perform a better assessment of the ecological status of Mediterranean streams, especially in saline streams.

Introduction

Water Framework Directive (WFD, Directive 2000/60/EC) is the largest European initiative aiming to implement biomonitoring and restoration strategies for inland and coastal aquatic ecosystems. Most of the biomonitoring tools developed in this context (i.e. all multimetric approaches and some predictive tools) require *a priori* classifications to summarise the variability occurring within the water bodies under natural conditions. Although classifications may result in some information loss (Linke *et al.* 2005; Olden *et al.* 2006), this approach is useful and intuitive for managers, in order to establish different reference conditions and biomonitoring thresholds for each river class (Bailey *et al.* 2007). Effective ecological classifications should show desirable characteristics as to 1) be based in objective procedures and data widely available to allow for an easy and automatic typification of new datasets (e.g. Wright *et al.* 1998; Snelder *et al.* 2012), 2) represent and predict the biological diversity that should occur under natural conditions for each type (e.g. Hawkins & Vinson 2000; Heino & Mykra 2006) and 3) consider the whole environmental variability occurring within the focal ecosystems (e.g. Hawkins & Vinson 2000; Belmar *et al.* 2011).

However, in the case of a recognised hotspot like the Mediterranean region, classifications developed to implement WFD in streams and rivers do not meet most of these desirable properties, constraining the capacity of the current biomonitoring programs to assess their ecological status and to preserve their biodiversity. In fact, there is no procedure that permits an automatic, cost-effective classification of large datasets of new test streams for a further application and extension of the WFD to the whole Mediterranean area. Such automatic typification may be especially relevant in this region, allowing the assessment of an important part of the mainly unstudied but potentially hyperdiverse streams of North Africa and Near East.

Moreover, despite some recent Mediterranean stream classifications have been validated and corrected using biological data (Sánchez-Montoya *et al.* 2007;

Belmar *et al.* 2013b), such classifications do not account correctly for the underlying processes that determine the changes in species composition, which can be explained, isolated or in combination, by turnover (i.e. species replacement) and nestedness (i.e. nested subsets of species developing along a richness gradient) (Baselga 2010). The contribution of each process is critical to assess the biological representativeness the stream classification and so, a further validation of classifications should be considered in this way.

The main bias of current Mediterranean stream classifications is caused by their partial focus respect to their environmental representativeness. Streams occurring at the Mediterranean basin show a high environmental diversity, including key natural environmental gradients, such as water salinity or altitude, which drive most of the changes in diversity and composition of stream communities (Bonada *et al.* 2005; Díaz *et al.* 2008; Finocchiaro *et al.* 2009; Ros *et al.* 2009). However, available classification procedures account only partly for this environmental variability, being focused mainly on freshwater streams flowing across high and mid mountain calcareous or siliceous basins (Sánchez-Montoya *et al.* 2007). Such situation has promoted the disregard in the most important biomonitoring programs of an important part of the Mediterranean streams, which drain evaporitic catchments resulting in naturally high mineralized waters (water electrical conductivity > 5000 $\mu\text{S cm}^{-1}$, hereafter, saline streams) (Margat 1961; Gerecke 1991; Bennis *et al.* 2008; Arribas *et al.* 2009; Millán *et al.* 2011).

Saline streams are singular ecosystems particularly exposed to anthropogenic pressures, which merit to be preserved for multiple reasons. Salinity is an important driver of speciation over geological time (Abellán *et al.* 2009; Arribas *et al.* 2014). In fact, despite saline streams show natural patterns similar to those produced by anthropogenic pollution (Odum 1985) as low richness, unbalanced Production : Respiration ratios or a higher proportion of *r* strategists (Millán *et al.* 2011), species show a high degree of specialisation and replacement along the salinity gradient (Moreno *et al.* 2001), reflecting that saline streams of this region are composed of a unique species pool (Gutiérrez-Cánovas *et al.* 2013). Most of the species that occupy saline species have narrow spatial distributions (Sánchez-Fernández *et al.* 2008; Arribas *et al.* 2013). Furthermore, some water beetles specialized in saline streams show a remarkable intraspecific phylogenetic diversity (Abellán *et al.* 2007; Sánchez-Fernández *et al.* 2011). Therefore, in spite of some preliminar classifications and evaluations of saline Mediterranean waters (Gutiérrez-Cánovas *et al.* 2008; Arribas *et al.* 2009; Millán *et al.* 2011), a further-global classification and development of ecological status

indicators is fundamental if we are to preserve this important part of the Mediterranean biodiversity.

Here, we use different datasets of macroinvertebrates collected in streams of the Spain, Morocco and Italy along gradients of altitude, climate, lithology and hydrology, covering the natural variability in the western Mediterranean to accomplish the following objectives: 1) to develop three procedures (expert-based, environmental and biological clusters) that automatically classify streams covering the full environmental and biological variability occurring in the western Mediterranean streams; 2) to assess the performance of these classifications using biological information at family and species level 3) to suggest a set of biological indicators of ecological status for saline stream types.

Methods

Study area

The study was conducted across the western Mediterranean, including streams from the eastern and southern Iberian Peninsula and the Balearic Islands (Spanish data), the Rif to the Sahara Desert, comprising the Rif and Moroccan Atlantic basins (Moroccan data) and central and south Sicily (Italian data). These regions were selected because they are the main Mediterranean areas where saline and freshwater streams occur simultaneously. The study area falls within the Mediterranean climate domain, which is characterised by hot, dry summers and cool, wet winters (Gasith & Resh 1999). The selected sites embrace wide environmental (climate, altitude, lithology and hydrology) and anthropogenic gradients (agricultural and urban intensity). We excluded large watercourses (stream order ≥ 4 and mean surface drainage area 3490 ± 1675 km² sensu Sánchez-Montoya *et al.* (2007) from this study because of the paucity of reference sites (Sánchez-Montoya *et al.* 2009).

Data collection

Aquatic macroinvertebrates are good candidates to be used as biological indicators for biomonitoring purposes, because they are well-studied in the study region and represent an important fraction of stream biodiversity (Bonada *et al.* 2006). Macroinvertebrate community composition, abundance and richness exhibit a strong correlation with environmental change at reach and basin levels (Larsen & Ormerod 2010; Bruno *et al.* 2014b).

We gathered two datasets of macroinvertebrates at family and species level to characterise the aquatic communities. First, we compiled a dataset containing semiquantitative data of macroinvertebrate families. In this dataset, macroinvertebrates were identified at family level, counted and classified into four abundance classes (1: 1-4, 2: 5-10, 3: 11-100 and 4: >100 individuals, respectively). Second, we build a database comprising count data of aquatic Coleoptera and Hemiptera genera and species. Both datasets were collected from extensive surveys across the study area from 1999-2009. Most sites were surveyed several times and, at least, during spring. This season appears to be the most representative of the annual pooled composition in Mediterranean streams (Bonada *et al.* 2007a). These datasets were composed by own data, except for family data from Spanish freshwater and lowly-mineralised sites, which were taken from the Guadalmed Project database (Prat 2002). All macroinvertebrate samples were taken following a multi-habitat semiquantitative kick-sample, as described in Jáimez-Cuéllar *et al.* (2002).

Climatic, morphologic, lithologic and land use variables at basin and reach scales (Appendix A) were obtained from digital layers after delineating stream basins. Conductivity was measured in situ on each sampling occasion with an ECmeter (TetraConR 325). For each site, hydrology was classified as perennial, intermittent or ephemeral flow from available hydrological information or field evidence. We classified streams as *pristine* or *minimally disturbed*, when they fulfilled at least 16 of the 20 Mediterranean Reference Criteria proposed by Sánchez-Montoya *et al.* (2009). Otherwise, the stream was considered *disturbed*. See Appendix A for further details about the variables gathered for this study and their labelling.

Data analysis

Stream classification

The Spanish data contained the most complete biological and environmental inventory due to the larger number of stations, area and environmental variability covered (see Appendix A). For this reason, we decided to use the Spanish dataset to develop the three automatic classification procedures (i.e. expert-based, environmental and biological classifications) aiming to represent the stream variability occurring in the western Mediterranean. For that, we selected *pristine* and *minimally disturbed* sites of the Spanish dataset.

The expert-based classification is grounded on the criteria suggested by the ECOSTAT intercalibration group for the Mediterranean rivers using the System

A of the WFD (Med-GIG, see Buffagni *et al.* 2005; European_Commission 2005), which included basin area, hydrology, site altitude and basin lithology (Table 1). We have modified this classification to allow for the discrimination of saline streams by (1) increasing altitude upper limit for R-M1 type to 2000 m a.s.l. (previously 800 m a.s.l.), (2) relaxing lithology criteria to define R-M4 to <25% siliceous (previously, non-siliceous) and (3) including mean site conductivity. Besides the five freshwater stream types proposed by Med-GIG (R-M1, R-M2, R-M3, R-M4, and R-M5), we added other three saline stream classes to cover the full environmental variability (R-M6, R-M7 and R-M8), using salinity thresholds obtained in previous classifications (Gutiérrez-Cánovas *et al.* 2008; Arribas *et al.* 2009; Millán *et al.* 2011).

The environmental classification was carried out in two steps. First, we perform a Principal Component Analysis (PCA) on a group of key environmental variables (Sánchez-Montoya *et al.* 2007; Millán *et al.* 2011). These variables were hydrology, basin mean temperature, percentage of calcareous lithology in the basin, log-mean conductivity, site altitude and log-basin area as the main environmental features. For this analysis, Hydrology was transformed into a quantitative variable (1: ephemeral flow, 2: intermittent flow, 3: perennial flow). Then, we perform a Ward's method cluster on the Euclidean distance matrix of the first two principal components, selected using the Broken Stick Rule. This methodology is similar to those used in Sánchez-Montoya *et al.* (2007) to produce a environmental classification of Spanish Mediterranean streams.

Table 1 Description of the stream classes proposed for the expert based classification. Classes R-M1 to R-M5 were defined in ECOSTAT (2004) and modified as specified in Methods and Results, whereas classes from R-M6 to R-M8 were defined according with the thresholds to classify saline streams reported in Arribas *et al.* (2009) and Millán *et al.* (2011).

Code	Description	Basin area (km ²)	Altitude (m)	Lithology	Hydrology	Mean conductivity (μS/cm)
R-M1	Small high-mid altitude streams	10-100	200-2000	Mixed	Highly seasonal	<5,000
R-M2	Small / medium lowland streams	100-1000	<600	Mixed	Highly seasonal	<5,000
R-M3	Large lowland streams	1000-10000	<600	Mixed	Highly seasonal	<5,000
R-M4	Small / medium mountain streams	10-1000	400-1,500	< 25% Siliceous	Highly seasonal	<5,000
R-M5	Small, lowland, temporary streams	10-100	<300	Mixed	Temporary	<5,000
R-M6	Small medium-lowland hyposaline streams	<400	<1,200	Calcareous and evaporitic	Highly seasonal / Temporary	5,000-32,000
R-M7	Small medium-lowland mesosaline streams	<100	<1,200	Calcareous and evaporitic	Highly seasonal / Temporary	32,000-130,000
R-M8	Small medium-lowland hypersaline streams	<100	<1,200	Calcareous and evaporitic	Highly seasonal / Temporary	>130,000

Finally, the biological classification was also conducted using a Ward's method clustering, but based on the Bray-Curtis dissimilarity matrix, calculated using semi-quantitative family data of the macroinvertebrates collected in each sample.

To allow for comparisons, we pruned environmental and biological clusters to produce seven river types, as found for the expert based classification. In order to provide automatic and cost-effective procedures of running waters classification, for the expert based classification we wrote an automatic procedure to assign a class to each stream in relation with the environmental features of each site. For the environmental and biological classifications, we employed a Random Forest modelling using the full-set of environmental variables of each stream sample as predictors (Snelder *et al.* 2012). Random Forest is a recursive partitioning tool widely used for nonparametric regression and classification due to this capacity to deal with large datasets of predictors of heterogeneous nature, even in the presence of complex interactions (Breiman 2001; Cutler *et al.* 2007). Predictors can be considered informative and important when their variable importance is higher than the absolute value of the lowest-scoring variable, since unimportant variables vary randomly around zero (Strobl *et al.* 2009). We report the percentages of correct classification for each class, overall classification accuracy and kappa-coefficients for the two predictive classifications models. Finally, such automatic procedures were applied for the classification of the reference streams of Italy, Morocco and Spain to compare classification performances in subsequent analyses.

Biological validation of stream classifications

For the Spanish, Moroccan and Italian datasets, the total beta-diversity and turnover dissimilarities of families of stream invertebrates and beetle species (e.g. Sánchez-Fernández *et al.* 2006) were used for the biological validation of the stream types previously defined by the three classification methods (expert-based, environmental and biological classifications). For Spanish data, we used the observed and predicted classes for the environmental and biological classifications, while for Morocco and Italy we used only the predicted classes. We employed betapart (Baselga 2010; Baselga & Orme 2012) to decompose changes in community composition into turnover and nestedness components. To apply this method, it requires the calculation for each dataset of three different dissimilarity matrices based on species composition matrix as follows: (1) the total pairwise beta-diversity is considered as the Sørensen dissimilarity between two communities due to differences in class membership (β_{sor}); (2) species turnover can be measured using the Simpson dissimilarity index (β_{sim})

and (3) species nestedness-resultant dissimilarity (β_{nes}) can be estimated by subtracting the turnover effect from the total beta-diversity ($\beta_{nes} = \beta_{sor} - \beta_{sim}$). The strength of each classification type (CS) was quantified as the difference between the within-class mean similarity (W) and between-classes mean similarity (B) of the total beta-diversity and turnover similarities (CS=W-B) for family and species data of the three regions. To estimate CS, dissimilarity matrices were converted to similarity matrices.

We related beta-diversity and turnover dissimilarities with the Euclidean distance matrices based on the differences in stream class (stream class as a semi-quantitative predictor) to estimate the contribution of turnover to total-beta diversity, and to quantify the capacity of stream class to predict beta-diversity and turnover changes. These analyses were calculated for each classification method and dataset using family and species level data. To do this, we used multiple regression models for distance matrices (Lichstein 2007). This method is conceptually similar to traditional multiple regression but with all variables being distance matrices instead of raw data and *P*-values being calculated through permutation tests (1000 runs).

Indicators of ecological status for saline stream types

To find adequate indicators to assess the ecological status of the saline streams types, we compared family and genus level based metrics between reference (*pristine* and *minimally disturbed*) and *disturbed* streams of the Spanish dataset, due to the greatest availability of historical data of land cover and salinity for this region. We assign each stream to its potential stream type employing the predictive biological classification using environmental descriptors as predictors (the more accurate method for the Spanish dataset, after the evaluation of CS, see results). For conductivity and hydrology, we used pre-disturbed mean values due to its potential alteration by human activities. We tested the response of different type of metrics for running-waters including conventional widely used metrics and tools to implement WFD in Mediterranean streams of Spain (Munné & Prat 2009; Sánchez-Montoya *et al.* 2010) and new metrics proposed in this study (see Appendix B for further details about conventional and novel metrics). The novel metrics are based on previous works studying the response of community structure of saline streams to anthropogenic pressures (Velasco *et al.* 2006; Gutiérrez-Cánovas *et al.* 2008; Millán *et al.* 2011). Some conventional (i.e. family richness, EPT, IASPT, sel EPTCD, IBMWP, ICM-11a; see Appendix B to see further details about the expected responses) and novel metrics (sel.col, per.col, sel.col.pa, per.col.pa) are expected to decrease with anthropogenic stress, as high values reflect good ecological status

(Gutiérrez-Cánovas *et al.* 2008; Munné & Prat 2009; Sánchez-Montoya *et al.* 2010), while others novel metrics indicating degradation (i.e. fams.deg, per.fams.deg, deg.col, deg.col.pa, per.deg.col, pa.per.deg.col, deg.hem, per.deg.hem, deg.hem.pa, pa.per.deg.hem), are expected to increase with intensified anthropogenic pressures (agriculture and urban land uses). To test the linear response of each metric to increasing degradation intensity, for each class, we estimate the Pearson correlation coefficient between biological indicators and the indicators of natural conditions (MRC) and anthropogenic pressure (the percentages of irrigated agriculture and urbanizations in the stream basin).

All statistical analyses were carried out using the statistical computing software R (R-Development-Core-Team 2013). We provide the R functions (Appendixes C and D) necessary to perform the aforementioned analyses.

Results

Stream classification

The expert based classification (Table 1, Fig. 1) showed important differences with the environmental classification (Fig. 2) and great similarities with the biological classification (Fig. 3). In general, a decreasing gradient of altitude and increasing gradients of aridity and salinity are observed across classifications from type 1 to type 8. All classifications converged in detecting the most extreme types, which were small high-mountain siliceous streams with perennial flow (type 1) and small lowland hypersaline streams draining evaporitic catchments and intermittent flow regime (type 8). Environmental classification detected three types of perennial freshwater streams with calcareous catchments differing in basin area and altitude (medium mid-mountain in type 2, small mid mountain in type 4 and small mid-high mountain in type 5), while the other classifications detected just two types (medium mid-mountain streams in type 2 and small high-mountain streams in type 4). Expert based and biological classifications detected a group of small mid-mountain temporary freshwater streams with calcareous catchments (type 5), which was merged with hyposaline streams in the environmental based classification (type 6). Expert based and biological cluster classifications detected other two types of saline streams: small lowland hyposaline streams (type 6) and small lowland mesosaline streams (type 7). These types drained evaporitic-calcareous catchments showing a high percentage of courses showing temporary flows.

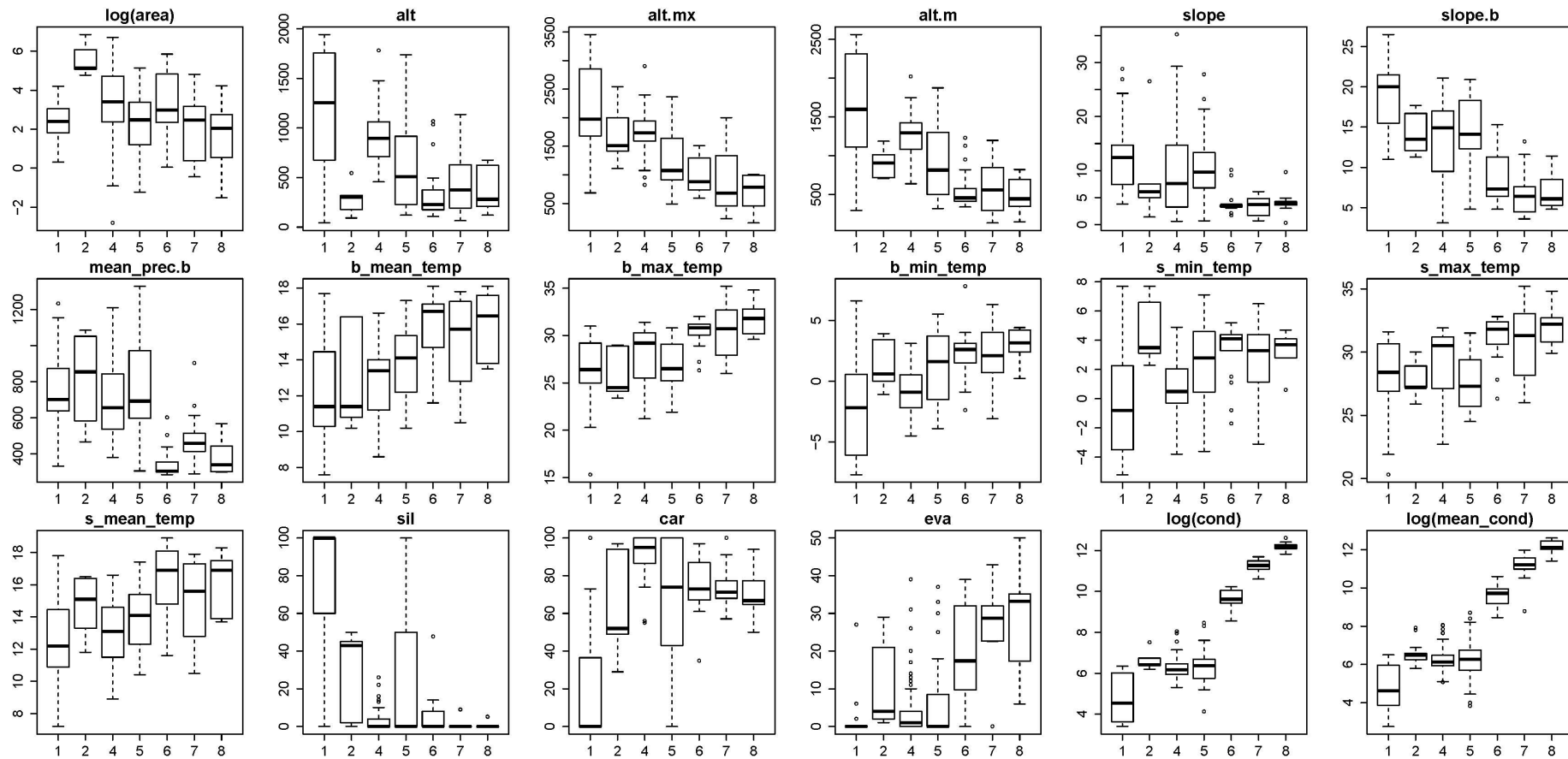


Fig. 1 Boxplot of the environmental features of the main stream types for the Spanish dataset using the expert based classification procedure. See Appendix A for further details and labels about environmental features. Hydrology was excluded because it was a categorical variable.

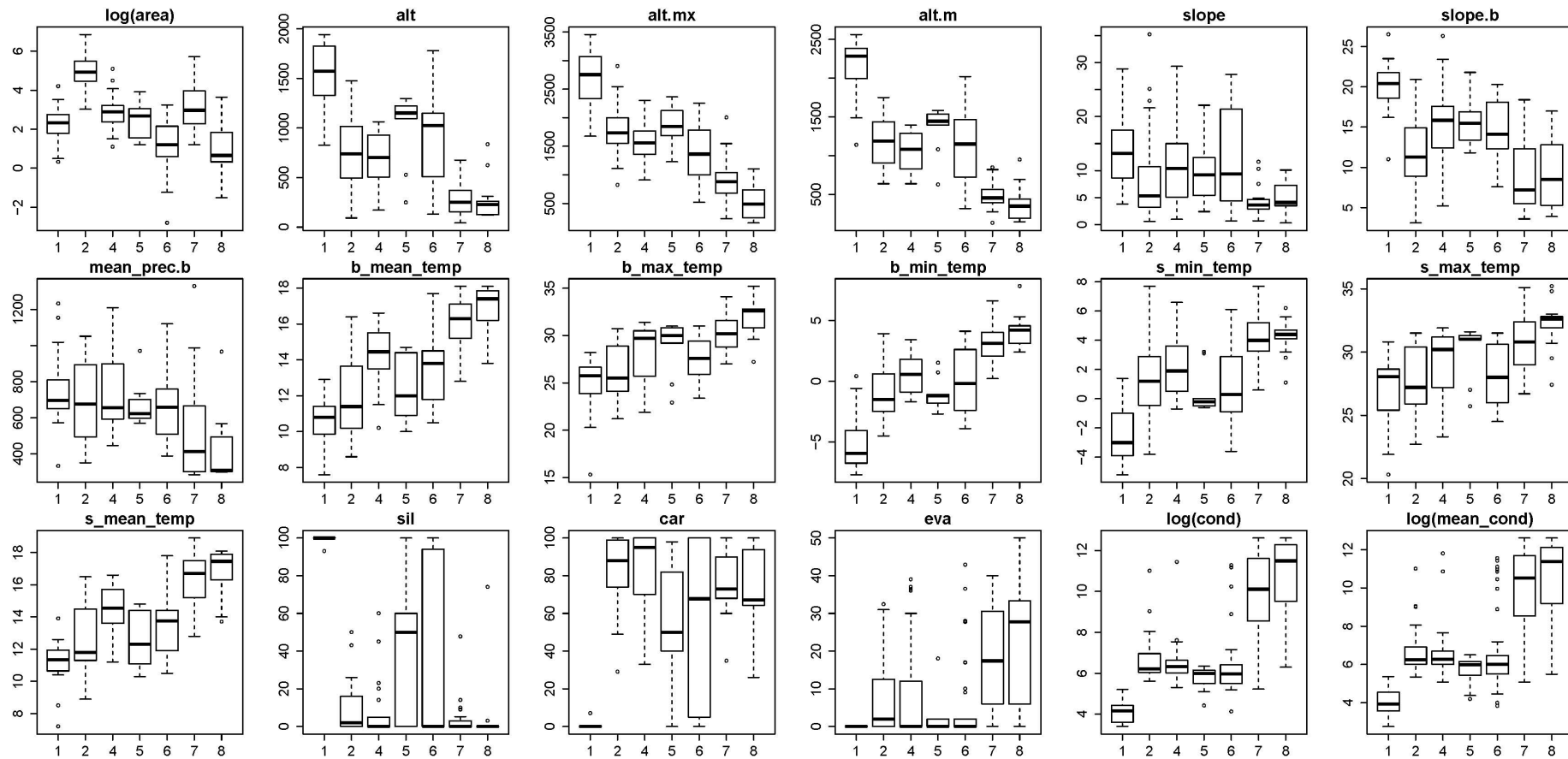


Fig. 2 Boxplot of the environmental features of the main stream types for the Spanish dataset using the environmental classification procedure. See Appendix A for further details and labels about environmental features. Hydrology was excluded because it was a categorical variable.

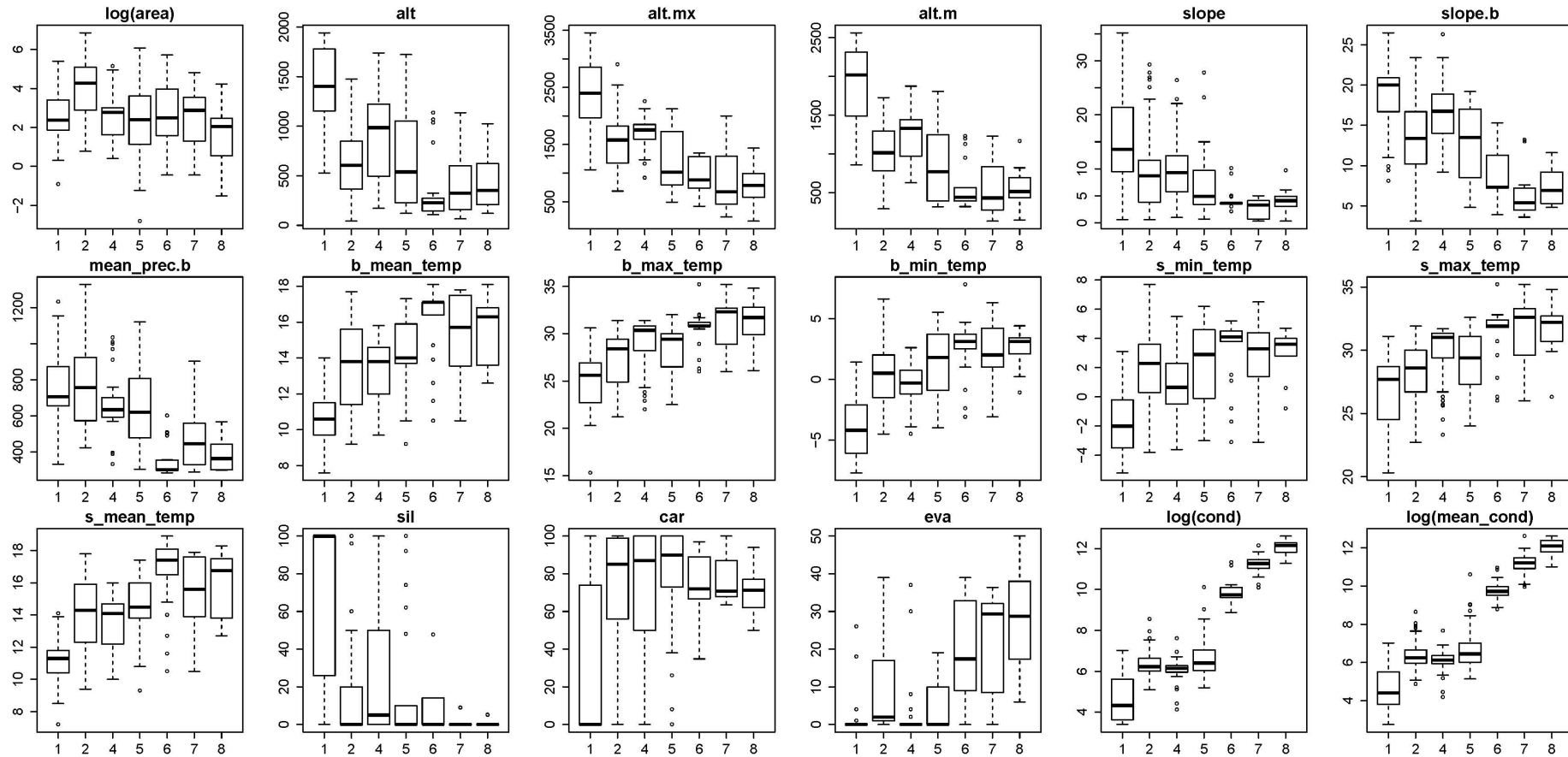


Fig. 3 Boxplot of the environmental features of the main stream types for the Spanish dataset using the biological classification procedure. See Appendix A for further details and labels about environmental features. Hydrology was excluded because it was a categorical variable.

The Random Forest model that predicted the environmental cluster showed higher predictive capacity (percentage of correct classification=0.91; accuracy=0.95, Kappa coefficient=0.94), in comparison with the model that predicted the biological cluster (percentage of correct classification=0.80; accuracy=0.85, Kappa coefficient=0.81). The percentage of correct classification varied among types, with types 1, 5 and 8 displaying the higher percentages of correct classification in both models (Table 2). The variable importance for Random Forest models revealed that the five more important predictors of environmental classes were area, hydrology, maximum altitude, basin mean maximum temperature and the percentage of siliceous lithology (see Appendix E), while mean conductivity, conductivity, basin mean maximum temperature, maximum altitude and basin area were the five most important variables to predict biological classes (Appendix E). In Italy, expert-based, environmental and biological models detected six (types 1, 4, 5, 6, 7 and 8), four (types 2, 4, 6 and 7) and five (types 2, 5, 6, 7 and 8) stream classes, respectively, whereas for Morocco, the same classifications found seven (types 1, 2, 3, 4, 5, 6 and 7), four (2, 4, 7 and 8) and six (types 2, 4, 5, 6, 7 and 8) stream classes, respectively.

Table 2 Performance of environmental and biological Random Forest models. Percentage of correct classification (PCC) is shown for each class and for the whole model. Accuracy and Kappa coefficient are shown for each model. See methods for further details about model construction.

Stream class	Environmental	Biological
T1	0.94	0.84
T2	0.98	0.76
T4	0.90	0.78
T5	0.90	0.89
T6	0.93	0.73
T7	0.82	0.74
T8	0.88	0.85
PCC	0.91	0.80
Accuracy	0.95	0.85
Kappa	0.94	0.81

Biological validation of stream classifications

For family and species data, the predicted biological cluster showed the highest CS values for Spanish dataset, while for Italian and Moroccan data, the expert based classifications displayed the highest CS values (Table 3). Environmental cluster showed consistently the lowest CS values for all datasets, with the exception of the CS using turnover for Moroccan dataset, where environmental and expert-based classifications yielded the highest values. Species data showed higher CS values for the three regions in comparison with family data, using both total beta-diversity and turnover similarities. In particular, the CS based on turnover similarities showed the highest values for each region and classification type.

Models relating total beta-diversity and turnover dissimilarities with the distance in stream class were all significant, explaining varying amounts of variance (Table 4). Turnover models showed higher slopes in comparison with total beta-diversity, reflecting the high contribution of species replacement to beta-diversity changes between classes. For total beta-diversity and turnover, biological classification explained the highest amounts of variance for Spanish ($P < 0.001$, $r^2 = 0.37$; $P < 0.001$; $r^2 = 0.39$) and Moroccan data ($P < 0.001$, $r^2 = 0.24$; $P < 0.001$; $r^2 = 0.25$), while expert based classification accounted for the highest amount of variance in Italian data ($P < 0.001$, $r^2 = 0.57$; $P < 0.001$; $r^2 = 0.59$).

Table 3 Classification strength (CS) for each classification type, region and beta-diversity component at family and species level. W: within-class similarity; B: Between-class similarity. Values in bold represent the highest value for each region. For Spanish data, we also compared observed classes (obtained from clusters) and predicted classes (obtained from Random Forest models).

Classification		Beta-diversity			Turnover		
		W	B	CS	W	B	CS
<i>Families</i>							
Spain	Expert	0.52	0.33	0.19	0.63	0.54	0.10
	Environmental predicted	0.49	0.35	0.14	0.64	0.54	0.11
	Environmental observed	0.48	0.35	0.13	0.64	0.54	0.10
	Biological predicted	0.53	0.33	0.20	0.64	0.53	0.11
	Biological observed	0.54	0.33	0.21	0.65	0.53	0.12
Italy	Expert	0.61	0.30	0.31	0.70	0.48	0.22
	Environmental	0.45	0.32	0.13	0.57	0.51	0.05
	Biological	0.57	0.29	0.28	0.66	0.48	0.18
Morocco	Expert	0.59	0.34	0.24	0.74	0.63	0.11
	Environmental	0.39	0.37	0.02	0.68	0.57	0.11
	Biological	0.53	0.34	0.19	0.65	0.65	0.00
<i>Species</i>							
Spain	Expert	0.28	0.07	0.21	0.39	0.12	0.27
	Environmental	0.25	0.09	0.16	0.38	0.13	0.24
	Biological	0.29	0.06	0.22	0.40	0.11	0.30
Italy	Expert	0.54	0.13	0.42	0.67	0.20	0.47
	Environmental	0.38	0.18	0.20	0.47	0.26	0.21
	Biological	0.53	0.16	0.37	0.64	0.24	0.40
Morocco	Expert	0.37	0.12	0.25	0.50	0.16	0.34
	Environmental	0.17	0.12	0.06	0.23	0.17	0.05
	Biological	0.30	0.12	0.19	0.41	0.16	0.25

Table 4. Results of the models examining changes in beta-diversity components in response to differences in stream type for each region and classification type. Values in bold represent the higher value for each region.

Region	Classification	Intercept	P	Slope	P	F	r ²
<i>Beta-diversity</i>							
Spain	Expert	0.76	0.154	0.07	0.001	3911.7	0.26
	Environmental	0.78	1.000	0.05	0.001	2507.6	0.18
	Biological predicted	0.74	0.001	0.05	0.001	6770.9	0.37
Italy	Expert	0.50	0.334	0.24	0.001	605.4	0.57
	Environmental	0.68	1.000	0.06	0.001	50.0	0.10
	Biological	0.54	0.780	0.08	0.001	405.7	0.47
Morocco	Expert	0.72	0.996	0.05	0.001	104.0	0.20
	Environmental	0.82	0.991	0.02	0.010	10.6	0.03
	Biological	0.71	0.976	0.05	0.001	128.8	0.24
<i>Turnover</i>							
Spain	Expert	0.65	1.000	0.11	0.001	3843.1	0.25
	Environmental	0.66	1.000	0.07	0.001	2896.1	0.20
	Biological	0.61	1.000	0.07	0.001	7150.3	0.39
Italy	Expert	0.36	1.000	0.29	0.001	673.8	0.59
	Environmental	0.58	1.000	0.07	0.001	47.7	0.09
	Biological	0.40	0.999	0.11	0.001	506.8	0.52
Morocco	Expert	0.62	1.000	0.06	0.001	116.1	0.22
	Environmental	0.76	0.981	0.02	0.021	7.1	0.02
	Biological	0.61	1.000	0.07	0.001	137.3	0.25

Indicators of ecological status for saline stream types

Table 5 shows the Pearson correlation coefficients calculated between conventional and novel biological metrics and reference criteria (MRC) and human pressures (land-use intensification) for saline stream types. Conventional metrics (i.e. IBMWP, fam.ric, EPT, icm11a) showed a pattern contrary to which is expected in response to the increase of anthropogenic stress, displaying negative correlations with MRC and positive correlations with land-use intensification. Although results varied among stream types, novel metrics generally showed a better agreement with the expected response, since metrics indicating degradation (fams.deg, per.fams.deg, per.deg.col, pa.per.deg.col, pa.per.deg.col, deg.hem) showed negative correlations with MRC score or positive relationships with land-use intensification, while metrics indicating pristine conditions (sel.col, per.col, sel.col.pa, per.col.pa) were positively correlated with MRC or negatively correlated with land-use

intensification. For type 6 (hyposaline streams), MRC correlated positively with sel.col, per.col and sel.col.pa and negatively with fams.deg and per.fams.deg, while the percentage of irrigated agriculture show significant negative correlations with deg.col and deg.col.pa, and the percentage of urbanized land show negative significant correlation coefficients with per.col and per.col.pa and positive coefficients with fams.deg, per.fams.deg, deg.col, deg.col.pa and deg.hem. For type 7 (mesosaline streams), MRC only correlated significantly with fams.deg and per.fams deg, showing negative coefficients. In this stream type, the percentage of urbanized land show significant positive correlations with EPT, icm11a, fams.deg, per.fams.deg, deg.col, deg.col.pa, per.deg.col, pa.per.deg.col. For type 8 (hypersaline streams), MRC showed negative significant correlation coefficients with ibmwp, fam.ric, fams.deg and per.fams.deg, the percentage of irrigated agriculture show positive correlations with ibmwp and fam.ric, and the percentage of urbanized land show positive correlations with ibmwp, fam.ric and negative correlation coefficients with IASPT.

Table 5. Pearson correlation coefficients calculated between community structure metrics and indicators of natural conditions (MRC, Mediterranean Reference Criteria) and anthropogenic pressures (percentages of irrigated agriculture and urbanizations in the basin). T6: hyposaline streams; T7: mesosaline streams and T8: hypersaline streams. Significant relationships are in bold. NA: Correlation not available (all the indicator scores were zero). See Appendix B for details about metrics.

Metric	Mediterranean Reference Criteria			Percentage of irrigated agriculture			Percentage of urbanized basin		
	T6	T7	T8	T6	T7	T8	T6	T7	T8
<i>Conventional metrics</i>									
ibmwp	0.14	-0.15	-0.55	0.06	0.19	0.40	0.14	0.10	0.41
fam.ric	0.11	-0.16	-0.51	0.08	0.23	0.37	0.22	0.10	0.43
ept	0.16	-0.26	-0.32	-0.18	0.15	0.24	0.10	0.33	0.23
iaspt	0.20	0.14	0.06	-0.07	-0.12	-0.02	-0.23	-0.18	-0.39
sel.eptcd	0.25	-0.02	NA	-0.26	0.04	NA	-0.28	0.18	NA
icm11a	0.16	-0.26	-0.35	-0.17	0.15	0.26	0.10	0.32	0.24
<i>Novel metrics</i>									
sel.col	0.55	-0.11	-0.19	-0.15	-0.02	0.03	0.06	0.29	0.34
per.col	0.44	0.14	-0.05	-0.03	0.00	-0.17	-0.46	-0.13	-0.23
sel.col.pa	0.51	-0.26	-0.17	-0.26	0.15	0.10	-0.04	0.24	0.27
per.col.pa	0.35	0.12	0.00	0.05	0.02	-0.16	-0.44	-0.17	-0.28
fams.deg	-0.37	-0.40	-0.47	0.18	0.23	0.34	0.55	0.35	0.34
per.fams.deg	-0.54	-0.37	-0.46	0.22	0.17	0.34	0.51	0.41	0.33
deg.col	0.36	-0.19	0.14	-0.41	-0.05	0.11	0.57	0.40	-0.04
deg.col.pa	0.35	-0.04	0.10	-0.43	-0.20	0.15	0.54	0.38	-0.04
per.deg.col	0.27	-0.23	0.00	-0.35	-0.02	0.21	0.19	0.42	-0.02
pa.per.deg.col	0.25	-0.15	0.00	-0.36	-0.11	0.21	0.23	0.47	-0.01
deg.hem	0.01	-0.12	0.28	-0.26	0.03	-0.13	0.60	0.14	-0.08
per.deg.hem	-0.32	-0.20	0.23	-0.22	0.09	-0.12	0.26	0.15	-0.09
deg.hem.pa	-0.03	-0.03	0.29	-0.29	-0.07	-0.12	0.58	0.08	-0.08
pa.per.deg.hem	-0.28	-0.14	0.27	-0.27	0.02	-0.13	0.33	0.11	-0.09

Discussion

Classification of western Mediterranean streams

This is the first work providing stream classifications that cover the large environmental and biological diversity of the streams of the western Mediterranean. In addition, these classifications were biologically validated and enable the automatic typification of large datasets. Other advantage is that these classifications can be applied automatically to large datasets, using environmental predictors, which can be obtained easily from online databases (including digital layers) or simple field surveys. These properties are useful to extend biomonitoring tools to the whole Mediterranean basin and to make WFD more cost-effective in order to preserve biodiversity.

Our different classification procedures resulted in broadly similar stream types, especially when comparing expert-based and biological classifications. Previous typifications of Mediterranean streams that used expert criteria (European Commission 2005), environmental (Sánchez-Montoya *et al.* 2007; Belmar *et al.* 2011; 2013b) or biological data (Poquet *et al.* 2009) yielded between four and five (including large watercourses) freshwater classes, which are similar to the four freshwater classes defined in our expert-based and biological classifications (types 1, 2, 4 and 5). The expert-based and biological classifications also yielded three types of saline watercourses as reported in earlier works (Gutiérrez-Cánovas *et al.* 2008; Arribas *et al.* 2009; Millán *et al.* 2011). However, our environmental classification showed some differences, which were particularly evident for freshwater temporary watercourses and saline streams. This classification detected just two types of saline streams with similar conductivity means and great dispersion, and a heterogeneous class including freshwater temporary and perennial streams and hyposaline streams. The most important variables to discriminate stream classes were the water electric conductivity, altitude, hydrology and lithology. In fact, conductivity has been recognised as important environmental filter for aquatic organisms (Díaz *et al.* 2008; Millán *et al.* 2011) and a driver of speciation over evolutionary time (Arribas *et al.* 2014). The capacity of organisms to osmoregulate determines their distribution along salinity gradients (Bradley 2008; Carbonell *et al.* 2012; Céspedes *et al.* 2013). Hydrological regime is another important filter that configure macroinvertebrate composition in Mediterranean streams (Belmar *et al.* 2013a). Organisms adapted to strong flow seasonality and droughts show a set of adaptations to cope with the changing physical conditions (Lytle & Poff 2004; Bonada *et al.* 2007b). Altitude reflects the combined natural stressors

associated with elevation gain, including reduced nutrient concentrations, oxygen concentrations and temperature (Jacobsen 2004).

In our study, turnover drove almost all the community changes (i.e. beta diversity) among stream classes in each classification. This result provides strong biological support to the delimitation of the seven stream types provided by these classifications for the western Mediterranean. However, these classifications showed varying biological concordance. Our analyses indicated that the expert-based classification showed the best overall performance both at family and species levels, whereas environmental classification displayed the lowest biological representativeness. The biological classification showed the highest CS values only for Spanish dataset. Other studies also detected that environmental classifications based on site clustering have a weak capacity to predict biological communities (Hawkins & Vinson 2000; Heino & Mykra 2006). Snelder *et al.* (2012) also found that the biological classifications predicted with Random Forest models show limited advantages over other classification methods. Therefore, we recommend the use of the expert-based classification to classify streams of the western Mediterranean, since their compromise between rapid application and accuracy. For the Spanish datasets, the use of the predictable biological classification can yield accurate predictions too.

Biological indicators of ecological status in saline streams

Saline streams of the Mediterranean basin occur in areas where agricultural and urban uses have increased enormously during the last 30 years, resulting in an anthropogenic reduction of salinity (i.e. dilution stress), organic enrichment and water withdrawal in most streams (Millán *et al.* 2011; Cooper *et al.* 2013). Furthermore, future climatic scenarios predict an increase of the aridity in the Mediterranean basin (Sala *et al.* 2000), which may affect to the distribution and abundance of these ecosystems, and especially to those exhibiting low, intermittent or ephemeral discharges.

In this context, the novel biological metrics proposed here were useful to detect anthropogenic impacts, becoming a promising tool to monitor the ecological status of saline stream types. Contrastingly, we detected that conventional freshwater biomonitoring metrics like the popular EPT, family richness, IBMWP, IASPT or the recent ICM-11a, failed in detecting ecological integrity or impairment in saline stream types, as detected in a previous work (Gutiérrez-Cánovas *et al.* 2008). These metrics increase as the studied community resembles to which is found under freshwater perennial flow pristine conditions, which differ strongly from the set of organisms occurring at saline

streams. Most of these biomonitoring indicators are related with taxonomic richness or other diversity measures (e.g. Munné & Prat 2009; Sánchez-Montoya *et al.* 2010; Bruno *et al.* 2014a), because they are based in the core idea that human alterations will result in depauperate communities (Odum 1985). However, other natural filters as historical events (i.e. glaciations) or hard environmental filtering (i.e. altitude, salinity) may also result in depauperated communities (Jacobsen & Marin 2008; Weiher *et al.* 2011; Baselga *et al.* 2012). Therefore, we strongly recommend using these conventional metrics only in the freshwater types (1, 2, 3, 4 and 5) and not using in saline streams (types 6, 7 and 8) of the western Mediterranean, also taking care when applying to other naturally stressed aquatic ecosystems around the world (Elliott & Quintino 2007).

Bioassessment protocols for naturally stressed ecosystems should take these special features into account by selecting metrics that work better than diversity measures in detecting anthropogenic impacts (Elliott & Quintino 2007). For example, metrics that reflected the composition of taxa with high affinity to natural saline conditions (e.g. per.col) or those appearing under degraded status (e.g. fams.deg) showed the best performance in this study. However, these metrics responded differently in relation with the human pressure type and the stream class, which is useful to detect the cause of the degradation. Many metrics worked quite well for hyposaline and mesosaline streams in detecting anthropogenic impacts, whereas there are few yielding significant correlations for hypersaline streams. The reason could be that there are not many examples of hypersaline streams showing high or intermediate impacts, constraining our capacity to detect degradation for this class (Millán *et al.* 2011). Besides these biological indicators based on composition and structure of macroinvertebrate communities, other functional indicators such as biofilm biomass, total biomass of primary producers and Production : Respiration ratio can be useful to distinguish between chronic and pulse dilution disturbances in saline streams (Gutiérrez-Cánovas *et al.* 2009; Gutiérrez-Cánovas *et al.* 2012). Therefore, integrating functional and structural indicators into an assessment tool can be a good strategy to detect the different type of impacts to which saline streams can be exposed. Further research should be focused in the development of a new tool based on the structural and functional indicators that worked effectively in saline streams.

Concluding remarks

From a broad perspective, the three classification procedures provided here give support to our objective of covering the natural environmental and

biological diversity in streams of the western Mediterranean, demonstrating also that the use of species turnover component was useful to validate ecological classifications. Moreover, these classification procedures enable an automatic cost-effective typification of large datasets of streams of the western Mediterranean. We deliver open source functions to implement these procedures, which require widely available environmental variables as predictors. Among these classifications, we suggest to use the expert-based or the predictable biological classifications as they showed the best classification strength, and a good compromise between high species turnover between classes and low within-class species heterogeneity. Finally, we suggest using IBMWP, EPT or ICM-11 metrics only in freshwater types (here, types 1, 2, 3, 4 and 5), because we demonstrated that these metrics did not work well in the saline types (6, 7 and 8). Many of the novel metrics proposed here worked much better to assess the ecological status of saline streams (e.g. fams.deg). The results of this study will help environmental managers to perform a better assessment of the ecological status of Mediterranean streams, especially for saline streams, which are frequently composed of unique species showing high habitat affinity, high degree of endemism and remarkable phylogenetic diversity.

Acknowledgements

We would like to thank P. Abellán, F. Picazo, I. Ribera and D. Sánchez-Fernández for their substantial contribution in sampling and identifying aquatic Coleoptera samples used in this study. We are grateful to N. Prat, M. Sánchez-Montoya and all the other members of Guadalmed project for providing data about the macroinvertebrate family composition and site electrical conductivity of most of the Spanish freshwater streams. We also thank to G. Castelli and F. Formica for helping in surveying Italian saline streams and M. El Alami, M. El Haissoufi, O. Lmohdi, for collecting and identifying most of the Moroccan samples. We are also grateful to O. Belmar and M. Sánchez-Montoya for their insightful comments on earlier versions of this manuscript. CG-C and PA were partially supported by two pre-doctoral grants from the Fundación Séneca (Agencia Regional de Ciencia y Tecnología, Región de Murcia) and from the Ministerio de Educación (Spain, FPU program), respectively. Saline stream surveys were financed by the Ministerio de Ciencia e Innovación (Spain) through I+D+i project no. CGL2006- 04159/BOS (AM).

References

- Abellán, P., Millán, A. & Ribera, I. (2009). Parallel habitat-driven differences in the phylogeographical structure of two independent lineages of Mediterranean saline water beetles. *Molecular Ecology*, 18, 3885-3902.
- Abellán, P., Sánchez-Fernández, D., Velasco, J. & Millán, A. (2007). Effectiveness of protected area networks in representing freshwater biodiversity: the case of a Mediterranean river basin (south-eastern Spain). *Aquatic Conservation: Marine and Freshwater ecosystems*, 361-374.
- Arribas, P., Andújar, C., Abellán, P., Velasco, J., Millán, A. & Ribera, I. (2014). Tempo and mode of the multiple origins of salinity tolerance in a water beetle lineage. *Molecular Ecology*, 23, 360-373.
- Arribas, P., Andujar, C., Sánchez-Fernández, D., Abellan, P. & Millan, A. (2013). Integrative taxonomy and conservation of cryptic beetles in the Mediterranean region (Hydrophilidae). *Zoologica Scripta*, 42, 182-200.
- Arribas, P., Gutiérrez-Cánovas, C., Abellán, P., Sánchez-Fernández, D., Picazo, F., Velasco, J. *et al.* (2009). Tipificación de los ríos salinos ibéricos. *Ecosistemas*, 18, 1-13.
- Bailey, R.C., Reynoldson, T.B., Yates, A.G., Bailey, J. & Linke, S. (2007). Integrating stream bioassessment and landscape ecology as a tool for land use planning. *Freshwater Biology*, 52, 908-917.
- Baselga, A. (2010). Partitioning the turnover and nestedness components of beta diversity. *Global Ecology and Biogeography*, 19, 134-143.
- Baselga, A., Lobo, J.M., Svenning, J.C., Aragón, P. & Araújo, M.S. (2012). Dispersal ability modulates the strength of the latitudinal richness gradient in European beetles. *Global Ecology and Biogeography*, 21, 1106-1113.
- Baselga, A. & Orme, D.L. (2012). betapart: an R package for the study of beta diversity. *Methods in Ecology and Evolution*, 3, 808-812.
- Belmar, O., Bruno, D., Martínez-Capel, F., Barquín, J. & Velasco, J. (2013a). Effects of flow regime alteration on fluvial habitats and riparian quality in a semiarid Mediterranean basin. *Ecological Indicators*, 30, 52-64.
- Belmar, O., Velasco, J., Gutiérrez-Cánovas, C., Mellado-Díaz, A., Millan, A. & Wood, P.J. (2013b). The influence of natural flow regimes on

- macroinvertebrate assemblages in a semiarid Mediterranean basin. *Ecohydrology*, 6, 363-379.
- Belmar, O., Velasco, J. & Martínez-Capel, F. (2011). Hydrological Classification of Natural Flow Regimes to Support Environmental Flow Assessments in Intensively Regulated Mediterranean Rivers, Segura River Basin (Spain). *Environmental Management*, 47, 992-1004.
- Bennas, N., Abellán, P., Sánchez-Fernández, D. & Millán, A. (2008). *Ochthebius* (*Ochthebius*) *Lanarotis* Ferro, 1985 (Coleoptera, hydraenidae), un coleoptere endemique marocain specifique des milieux aquatiques hypersalin. *Boletín de la Sociedad Entomológica Aragonesa*, 43, 361-366.
- Bonada, N., Doledec, S. & Statzner, B. (2007a). Taxonomic and biological trait differences of stream macroinvertebrate communities between mediterranean and temperate regions: implications for future climatic scenarios. *Global Change Biology*, 13, 1658-1671.
- Bonada, N., Prat, N., Resh, V.H. & Statzner, B. (2006). Developments in aquatic insect biomonitoring: A comparative analysis of recent approaches. *Annual Review of Entomology*, 51, 495-523.
- Bonada, N., Rieradevall, M. & Prat, N. (2007b). Macroinvertebrate community structure and biological traits related to flow permanence in a Mediterranean river network. *Hydrobiologia*, 589, 91-106.
- Bonada, N., Zamora-Muñoz, C., Rieradevall, M. & Prat, N. (2005). Ecological and historical filters constraining spatial caddisfly distribution in Mediterranean rivers. *Freshwater Biology*, 50, 781-797.
- Bradley, T.J. (2008). Saline-water Insects: Ecology, Physiology and Evolution. In: *Aquatic insects: challenges to populations* (eds. Lancaster, J & Briers, RA). CAB International Oxfordshire OX10 8DE, UK, pp. 20-35.
- Breiman, L. (2001). Random forests. *Machine Learning*, 45, 5-32.
- Bruno, D., Belmar, O., Sánchez-Fernández, D., Guareschi, S., Millán, A. & Velasco, J. (2014a). Responses of Mediterranean aquatic and riparian communities to human pressures at different spatial scales. *Ecological Indicators*, 45, 456-464.
- Bruno, D., Belmar, O., Sánchez-Fernández, D. & Velasco, J. (2014b). Environmental determinants of woody and herbaceous riparian vegetation patterns in a semi-arid mediterranean basin. *Hydrobiologia*, 730, 45-57.

- Buffagni, A., Erba, S., Birk, S., Cazzola, M., Feld, C., Ofenbock, T. *et al.* (2005). *Towards european inter-calibration for the water framework directive: procedures and examples for different river types from the E.C. project STAR, 11th STAR deliverable. STAR contract No: EVK1-CT 2001-00089. IRSA, Rome (Italy).*
- Carbonell, J.A., Millán, A. & Velasco, J. (2012). Concordance between realised and fundamental niches in three Iberian Sigara species (Hemiptera: Corixidae) along a gradient of salinity and anionic composition. *Freshwater Biology*, 57, 2580-2590.
- Céspedes, V., Pallarés, S., Arribas, P., Millán, A. & Velasco, J. (2013). Water beetle tolerance to salinity and anionic composition and its relationship to habitat occupancy. *Journal of Insect Physiology*, 59, 1076-1084.
- Cooper, S.D., Lake, P.S., Sabater, S., Melack, J.M. & Sabo, J.L. (2013). The effects of land use changes on streams and rivers in mediterranean climates. *Hydrobiologia*, 719, 383-425.
- Cutler, D.R., Edwards, T.C., Jr., Beard, K.H., Cutler, A. & Hess, K.T. (2007). Random forests for classification in ecology. *Ecology*, 88, 2783-2792.
- Díaz, A.M., Alonso, M.L.S. & Gutiérrez, M.R.V.A. (2008). Biological traits of stream macroinvertebrates from a semi-arid catchment: patterns along complex environmental gradients. *Freshwater Biology*, 53, 1-21.
- Elliott, M. & Quintino, V. (2007). The estuarine quality paradox, environmental homeostasis and the difficulty of detecting anthropogenic stress in naturally stressed areas. *Marine Pollution Bulletin*, 54, 640-645.
- European Commission (2005). *Common Implementation Strategy for the Water Framework Directive (2000/60/EC). Guidance Document No. 14. Guidance on the Intercalibration Process 2004–2006.*
- Finocchiaro, M., Torrisi, M. & Ferlito, A. (2009). Caratterizzazione delle comunità di diatomee bentoniche del bacino idrografico del fiume Simeto (Sicilia orientale) mediante applicazione dell'Indice Diatomico di Eutrofizzazione/Polluzione (EPI-D). *Biologia Ambientale*, 23, 53-66.
- Gasith, A. & Resh, V.H. (1999). Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. *Annual Review of ecology evolution and systematics*, 30, 51-81.
- Gerecke, R. (1991). Taxonomische, faunistische und ökologische Untersuchungen an Wassermilben aus Sizilien, unter Berücksichtigung anderer aquatischer Invertebraten. *Lauterbornia*, 7, 1-303.

- Gutiérrez-Cánovas, C., Hernández, J., Millán, A. & Velasco, J. (2012). Impact of chronic and pulse dilution disturbances on metabolism and trophic structure in a saline Mediterranean stream. *Hydrobiologia*, 686, 225-239.
- Gutiérrez-Cánovas, C., Millán, A., Velasco, J., Vaughan, I.P. & Ormerod, S.J. (2013). Contrasting effects of natural and anthropogenic stressors on beta diversity in river organisms. *Global Ecology and Biogeography*, 22, 796-805.
- Gutiérrez-Cánovas, C., Velasco, J. & Millán, A. (2008). SALINDEX: A macroinvertebrate index for assessing the ecological status of saline "ramblas" from SE of the Iberian Peninsula. *Limnetica*, 2, 299-316.
- Gutiérrez-Cánovas, C., Velasco, J. & Millán, A. (2009). Effects of dilution stress on the functioning of a saline Mediterranean stream. *Hydrobiologia*, 619, 119-132.
- Hawkins, C.P. & Vinson, M.R. (2000). Weak correspondence between landscape classifications and stream invertebrate assemblages: implications for bioassessment. *Journal of the North American Benthological Society*, 19, 501-517.
- Heino, J. & Mykra, H. (2006). Assessing physical surrogates for biodiversity: Do tributary and stream type classifications reflect macroinvertebrate assemblage diversity in running waters? *Biological Conservation*, 129, 418-426.
- Jacobsen, D. (2004). Contrasting patterns in local and zonal family richness of stream invertebrates along an Andean altitudinal gradient. *Freshwater Biology*, 49, 1293-1305.
- Jacobsen, D. & Marin, R. (2008). Bolivian Altiplano streams with low richness of macroinvertebrates and large diel fluctuations in temperature and dissolved oxygen. *Aquatic Ecology*, 42, 643-656.
- Jáimez-Cuéllar, P., Vivas, S., Bonada, N., Robles, S., Mellado, A., Álvarez, M. *et al.* (2002). Protocolo GUADALMED (PRECE). *Limnetica*, 21, 187-204.
- Larsen, S. & Ormerod, S.J. (2010). Combined effects of habitat modification on trait composition and species nestedness in river invertebrates. *Biological Conservation*, 143, 2638-2646.
- Lichstein, J.W. (2007). Multiple regression on distance matrices: a multivariate spatial analysis tool. *Plant Ecology*, 188, 117-131.
- Linke, S., Norris, R.H., Faith, D.P. & Stockwell, D. (2005). ANNA: A new prediction method for bioassessment programs. *Freshwater Biology*, 50, 147-158.

- Lytle, D.A. & Poff, N.L. (2004). Adaptation to natural flow regimes. *Trends in Ecology & Evolution*, 19, 94-100.
- Margat, J. (1961). *Les eaux salées au Maroc, Hydrogéologie et Hydrochimie. Note et mémoire du service géologique*. Edition du service géologique du Maroc.
- Millán, A., Velasco, J., Gutiérrez-Cánovas, C., Arribas, P., Picazo, F., Sánchez-Fernández, D. *et al.* (2011). Mediterranean saline streams in southeast Spain: What do we know? *Journal of Arid Environments*, 75, 1352-1359.
- Moreno, J.L., Aboal, M., Vidal-Abarca, M.R. & Suarez, M.L. (2001). Macroalgae and submerged macrophytes from fresh and saline waterbodies of ephemeral streams ('ramblas') in semiarid south-eastern Spain. *Marine and Freshwater Research*, 52, 891-905.
- Munné, A. & Prat, N. (2009). Use of macroinvertebrate-based multimetric indices for water quality evaluation in Spanish Mediterranean rivers: an intercalibration approach with the IBMWP index. *Hydrobiologia*, 628, 203-225.
- Odum, E.P. (1985). Trends expected in stressed ecosystems. *Bioscience*, 35, 419-422.
- Olden, J.D., Joy, M.K. & Death, R.G. (2006). Rediscovering the species in community-wide predictive modeling. *Ecological Applications*, 16, 1449-1460.
- Poquet, J., Alba-Tercedor, J., Punti, T., Sánchez-Montoya, M.d.M., Robles, S., Alvarez, M. *et al.* (2009). The MEDiterranean Prediction And Classification System (MEDPACS): an implementation of the RIVPACS/AUSRIVAS predictive approach for assessing Mediterranean aquatic macroinvertebrate communities. *Hydrobiologia*, 623, 153-171.
- Prat, N. (2002). El Proyecto GUADALMED. *Limnetica*, 21, 1-4.
- R-Development-Core-Team (2013). R: a language and environment for statistical computing. R Foundation for Statistical Computing Vienna, Austria.
- Ros, M.D., Marín-Murcia, J.P. & Aboal, M. (2009). Biodiversity of diatom assemblages in a Mediterranean semiarid stream: implications for conservation. *Marine and Freshwater Research*, 60, 14-24.
- Sala, O.E., Chapin, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R. *et al.* (2000). Biodiversity - Global biodiversity scenarios for the year 2100. *Science*, 287, 1770-1774.

- Sánchez-Fernández, D., Abellán, P., Mellado, A., Velasco, J. & Millán, A. (2006). Are water beetles good indicators of biodiversity in Mediterranean aquatic ecosystems? The case of the segura river basin (SE Spain). *Biodiversity and Conservation*, 15, 4507-4520.
- Sánchez-Fernández, D., Bilton, D.T., Abellán, P., Ribera, I., Velasco, J. & Millán, A. (2008). Are the endemic water beetles of the Iberian Peninsula and the Balearic Islands effectively protected? *Biological Conservation*, 141, 1612-1627.
- Sánchez-Fernández, D., Lobo, J.M., Abellán, P. & Millán, A. (2011). Environmental niche divergence between genetically distant lineages of an endangered water beetle. *Biological Journal of the Linnean Society*, 103, 891-903.
- Sánchez-Montoya, M.D.M., Puntí, T., Suárez, M.L., Vidal-Abarca, M.D.R., Rieradevall, M., Poquet, J.M. *et al.* (2007). Concordance between ecotypes and macroinvertebrate assemblages in Mediterranean streams. *Freshwater Biology*, 52, 2240-2255.
- Sánchez-Montoya, M.M., Vidal-Abarca, M.R., Puntí, T., Poquet, J.M., Prat, N., Rieradevall, M. *et al.* (2009). Defining criteria to select reference sites in Mediterranean streams. *Hydrobiologia*, 619, 39-54.
- Sánchez-Montoya, M.M., Vidal-Abarca, M.R. & Suárez, M.L. (2010). Comparing the sensitivity of diverse macroinvertebrate metrics to a multiple stressor gradient in Mediterranean streams and its influence on the assessment of ecological status. *Ecological Indicators*, 10, 896-904.
- Snelder, T., Barquin Ortiz, J., Booker, D., Lamouroux, N., Pella, H. & Shankar, U. (2012). Can bottom-up procedures improve the performance of stream classifications? *Aquatic Sciences*, 74, 45-59.
- Strobl, C., Hothorn, T. & Zeileis, A. (2009). Party on! *R Journal*, 1, 14-17.
- Velasco, J., Millán, A., Hernández, J., Gutiérrez-Cánovas, C., Abellán, P., Sánchez-Fernández, D. *et al.* (2006). Response of biotic communities to salinity changes in a Mediterranean hypersaline stream. *Saline Systems*, 2, 12.
- Weiher, E., Freund, D., Bunton, T., Stefanski, A., Lee, T. & Bentivenga, S. (2011). Advances, challenges and a developing synthesis of ecological community assembly theory. *Philosophical Transactions of the Royal Society B-Biological Sciences*, 366, 2403-2413.

Wright, J.F., Furse, M.T. & Moss, D. (1998). River classification using invertebrates: RIVPACS applications. *Aquatic Conservation-Marine and Freshwater Ecosystems*, 8, 617-631.

Supporting Information

Appendix 3.1 Environmental variables used in this study.

Appendix 3.2 Conventional and novel metrics used in this study to detect ecological integrity and anthropogenic impacts.

Appendix 3.3 R functions to classify Mediterranean streams using different methods (expert based, environmental cluster and biological cluster) and to calculate the bioassessment metrics used in this study (see Appendix B).

Available online at:

https://www.dropbox.com/s/16hevc78wnoi1vg/Appendix_3.3.R

Appendix 3.4 R script with the code necessary to run the analysis conducted in this study. Available online at:

https://www.dropbox.com/s/5yaabqxobe4flc0/Appendix_3.4.R

Appendix 5.5 Plots showing variable importance for the Random Forest models predicting the environmental and biological classifications.

Chapter 3: Performance of classifications and bioindicators for Mediterranean streams

Appendix 3.1 Environmental variables used in this study. Labels, units and data range for each region are shown.

Variable	Label	Unit	Full dataset	Spain	Morocco	Italy
<i>Reach level</i>			Range	Range	Range	Range
Site maximum air temperature	s_max_temp	°C	20.3-38.8	20.3-35.2	26.7-31.5	26.2-38.8
Site mean air temperature	s_mean_temp	°C	7.2-20.2	7.2-18.9	13.5-18.1	15-20.2
Site minimum air temperature	s_min_temp	°C	-5.2-11.9	-5.2-7.7	3.9-8	2-11.9
			ephemeral, intermittent, permanent	ephemeral, intermittent, permanent	ephemeral, intermittent, permanent	ephemeral, intermittent, permanent
Hydrology	Hydro	categorical	seasonal	seasonal	seasonal	seasonal
Site slope	s_slope	degrees	0.3-35.2	0.3-35.2	1.5-16.8	0.6-23.3
Water electrical conductivity	Cond	μS cm ⁻¹	15.8-300,000	15.8-300,000	201-225,000	97-120,000
Mean water electrical conductivity	mean_cond	μS cm ⁻¹	30-300,000	30-300,000	374-200,000	97-120,000
<i>Basin level</i>						
Basin maximum air temperature	b_max_temp	°C	15.3-37.9	15.3-35.2	23.8-28.6	26.4-37.9
Basin mean air temperature	b_mean_temp	°C	7.6-19.9	7.6-18.1	11.2-16.9	13.5-19.9
Basin mean precipitation	mean_prec	mm	53-1328	284-1328	467-1082	53-952
Basin minimum air temperature	b_min_temp	°C	-7.7-12.9	-7.7-7.8	-0.7-9.9	-0.5-12.9
Calcareous surface	Carb	percentage	0-100	0-100	34-100	42-100
Evaporitic surface	Evap	percentage	0-66	0-50	0-66	0-58

Chapter 3: Performance of classifications and bioindicators for Mediterranean streams

Siliceous surface	Sil	percentage	0-100	0-100	0-52	0-50
Basin area	Area	km ²	0.06-2570.55	0.06-955.22	0.78-528.92	0.36-2570.55
Basin slope	b_slope	degrees	0.8-26.5	3.1-26.5	3.5-12.1	0.8-25.6
Maximum basin altitude	max_alt	m	158-3,456	158-3,456	502-3,308	230-2,147
Mean basin altitude	mean_alt	m	89-2,564	131-2,564	284-1,489	89-1,279
Minimum basin altitude	min_alt	m	0-1,942	42-1,942	85-1,189	0-1,026

Chapter 3: Performance of classifications and bioindicators for Mediterranean streams

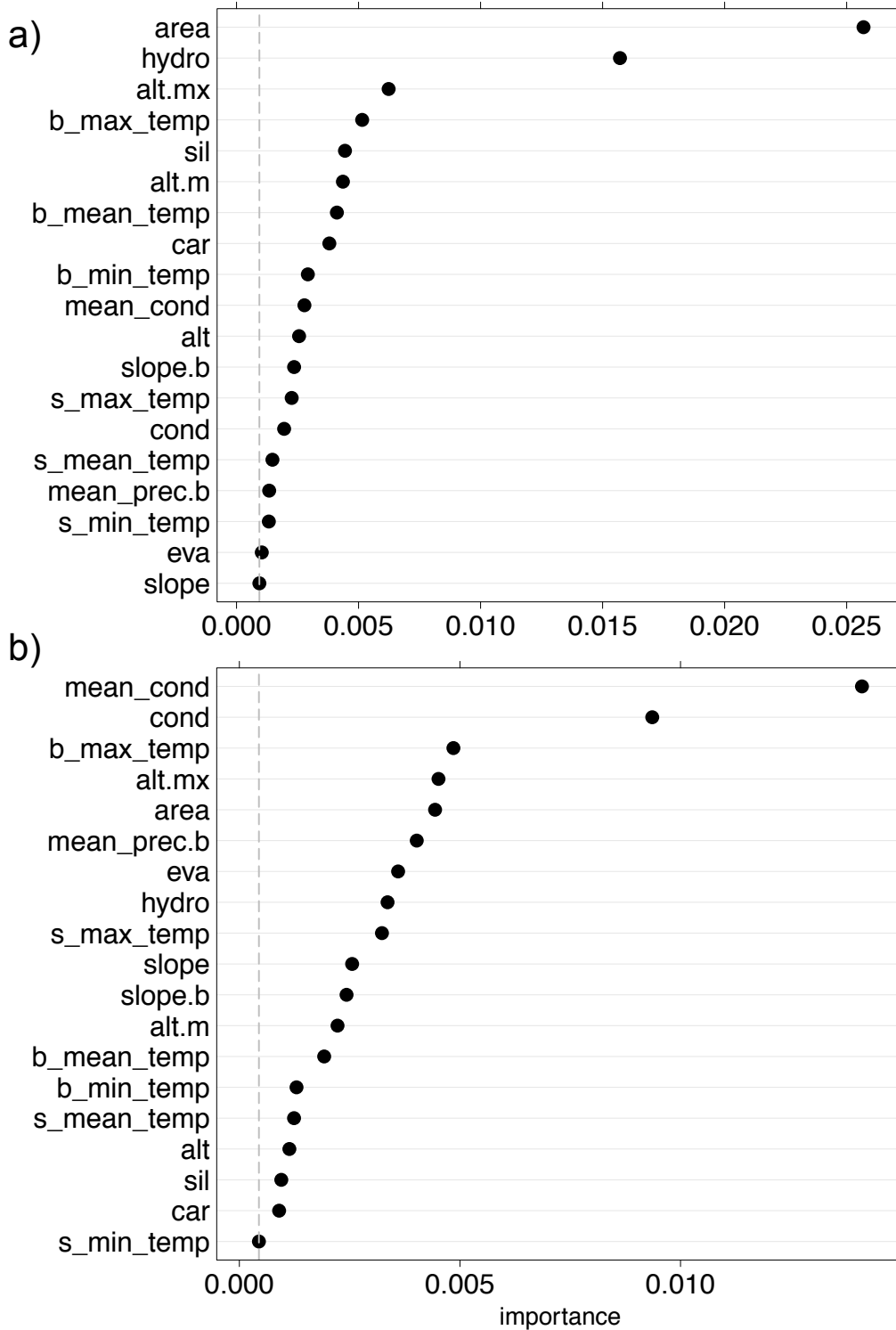
Appendix 3.2 Conventional and novel metrics used in this study to detect ecological integrity and anthropogenic impacts.

Metric	Description	Taxonomic level	Expected response to anthropogenic stress	References
ibmwp	Iberian Bio-Monitoring Working Party (sum of the scores of each family occurring in the sample, representing the differential tolerance to organic pollution)	Family	Decrease	Alba-Tercedor & Sánchez-Ortega (1988)
och	Richness of Odonata, Coleoptera and Hemiptera families	Family	Decrease	Sánchez-Montoya et al 2007
per.och	och / number of families	Family	Decrease	Sánchez-Montoya et al 2007
fam.ric	Family richness	Family	Decrease	Barbour et al 1999; Munné and Prat, 2009
ept	Richness of Ephemeroptera, Plecoptera and Trichoptera families	Family	Decrease	Barbour et al 1999; Munné and Prat, 2010
iaspt	IBMWP value / number of families	Family	Decrease	Alba-Tercedor & Sánchez-Ortega (1988)
sel.eptcd	Percentage of families of Leptophlebiidae, Ephemerellidae, Chloroperlidae, Nemouridae, Leuctridae, Philopotamidae, Limnephilidae, Psychomyiidae, Sericostomatidae, Elmidae, Dryopidae and Athericidae	Family	Decrease	Munné and Prat, 2009
icm11a	Multimetric index proposed to be used in Mediterranean streams ($0.15 \cdot \text{fam.ric} + 25 \cdot \text{ept} + 0.35 \cdot \text{iaspt} + 0.25 \cdot \text{sel.eptcd}$)	Family	Decrease	Munné and Prat, 2009
sel.col	Abundance of the Enochrus, Ochthebius and Nebrioporus genera, which have affinity for saline streams	Genus	Decrease	Proposed in this paper
per.col	sel.col / number of Coleoptera individuals	Genus	Decrease	Proposed in this paper
sel.col.pa	Richness of the Enochrus, Ochthebius and Nebrioporus genera, which have affinity for saline streams	Genus	Decrease	Proposed in this paper
per.col.pa	sel.col.pa / number of Coleoptera genera	Genus	Decrease	Proposed in this paper

Chapter 3: Performance of classifications and bioindicators for Mediterranean streams

	Richness of the Ancyliidae, Baetidae, Hydrobiidae, Hydropterygidae families, which are related with dilution stress and organic enrichment.	Family	Increase	Proposed in this paper
fams.deg				
per.fams.deg	fams.deg / number of families	Family	Increase	Proposed in this paper
	Abundance of the Dryops, Laccophilus, Haliplus, Yola, Hydroglyphus genera, which are related with dilution stress and organic enrichment	Genus	Increase	Proposed in this paper
deg.col				
deg.col.pa	deg.col / abundance of Coleoptera individuals	Genus	Increase	Proposed in this paper
	Richness of the Dryops, Laccophilus, Haliplus, Yola, Hydroglyphus genera, which are related with dilution stress and organic enrichment	Genus	Increase	Proposed in this paper
per.deg.col				
pa.per.deg.col	per.deg.col / number of Coleoptera genera	Genus	Increase	Proposed in this paper
	Abundance of Micronecta, Nepa and Notonecta genera, which are related with dilution stress and organic enrichment	Genus	Increase	Proposed in this paper
deg.hem				
per.deg.hem	deg.hem / abundance of Hemiptera individuals	Genus	Increase	Proposed in this paper
	Abundance of Micronecta, Nepa and Notonecta genera, which are related with dilution stress and organic enrichment	Genus	Increase	Proposed in this paper
deg.hem.pa				
pa.per.deg.hem	deg.hem.pa / richness of Hemiptera genera	Genus	Increase	Proposed in this paper

Appendix 3.5 Plots showing variable importance for the Random Forest models predicting the environmental (a) and biological classifications (b).



Chapter 4:

Contrasting effects of natural and anthropogenic stressors on beta-diversity in river organisms

Published in Global Ecology and Biogeography:

Gutiérrez-Cánovas, C.; Millán, A; Velasco, J.; Vaughan, I.P. & Ormerod, S.J. Contrasting effects of natural and anthropogenic stressors on beta-diversity in river organisms. *Global Ecology and Biogeography* 22(7): 796-805.

Contrasting effects of natural and anthropogenic stressors on beta-diversity in river organisms

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Abstract

We hypothesised that mechanisms underlying beta-diversity in rivers would differ between gradients where i) natural stressors result in progressive species turnover with high specificity and ii) anthropogenic stressors result in the loss of specialist taxa thus giving rise to nestedness. We analysed five data sets describing benthic macroinvertebrates sampled along natural (altitude in the Himalayan Mountains; salinity in the Iberian Peninsula) and anthropogenic (acidity in Great Britain, metals in Great Britain, land use in the Iberian Peninsula) stress gradients. Predictions were tested by fitting models relating species richness and beta-diversity components (total, turnover and nestedness dissimilarities) to putative stress intensity (i.e. the degree to which a particular environmental constraint filters species occurrence). Stress intensity accounted for most of the variability in species richness ($r^2 = 0.64\text{--}0.93$) - which declined with increasing stress. Dissimilarity in community composition between locations increased with the difference in stress intensity for all datasets. For natural stressors, beta-diversity patterns mainly reflected species turnover, whilst for anthropogenic stressors beta-diversity mainly reflected nesting of subsets of species as stress intensity increased. Our results support the hypothesis that natural and anthropogenic stressors generate contrasting patterns in beta-diversity that arise through different mechanisms. Therefore, different conservation strategies should be applied depending on the historical persistence of the stressor.

Introduction

The assessment of beta-diversity has now become a central topic in macroecology and is progressively identifying key patterns at broad spatial extents as well as the mechanisms that might be responsible (Qian *et al.* 2005). Approaches which decompose beta-diversity into turnover and nestedness components (Baselga 2010) are particularly powerful for testing hypotheses about biogeographical and ecological processes at regional to continental scales. Recent examples include assessments of the effects of species turnover along well known gradients in productivity and altitude (e.g. Melo *et al.* 2009; Andrew *et al.* 2012), or of processes related to re-colonisation in areas previously affected by glaciation (Baselga *et al.* 2012). So far, however, few studies provide satisfactory explanations for beta-diversity gradients at smaller scales, for example within and among river basins or within regions. In such cases, available evidence suggests that ecological and anthropogenic factors play a major role in configuring beta-diversity (Bonada *et al.* 2005), especially when they result from stressor effects on organism distribution – in other words, environmental constraints which filter species occurrence (Odum 1985). Such effects are consistent with niche theory, which predicts that species should be filtered more from abiotically stressful habitats (i.e. habitats whose environmental conditions constrain the number of occurring species, which must have specific biological attributes) in comparison to more benign environments within the same region (Weiher & Keddy 1995). Studies aiming to test this prediction have yielded unclear results and now require further investigation (Swenson 2011).

Although the relationship between species diversity and stress intensity probably differs in shape and magnitude depending on whether the stressor is natural or anthropogenic in origin (Pettrin *et al.* 2007), theoretical and empirical evidence suggests that taxon richness generally correlates negatively with stress intensity (Odum 1985; Smith *et al.* 2009; Millán *et al.* 2011). Such richness gradients are ultimately translated into dissimilarities in assemblage composition that create beta-diversity (i.e. changes in species composition among locations). However, knowledge is still limited about how different mechanisms create beta-diversity under different circumstances, and in particular the balance between the effects of species turnover and nestedness. For example, along richness gradients where sites differ in stress intensity and share a low proportion of species, assemblage dissimilarities will arise due to species turnover (Fig. 1a); species will exhibit a high degree of specificity along the gradient and occur as specialists that are adapted to local conditions. Alternatively, where richness gradients are a consequence of communities

being reduced to nested subsets of those at richer sites, beta-diversity gradients are more likely to arise from nestedness (Fig. 1b); in this case, sites share a higher percentage of species because taxa occurring at poorer sites are stress-tolerant generalists (i.e. species that cover almost the whole stress gradient). As a result, the occurrence of one or other of these mechanisms should depend on the existence of specialist species adapted to particular stress intensity.

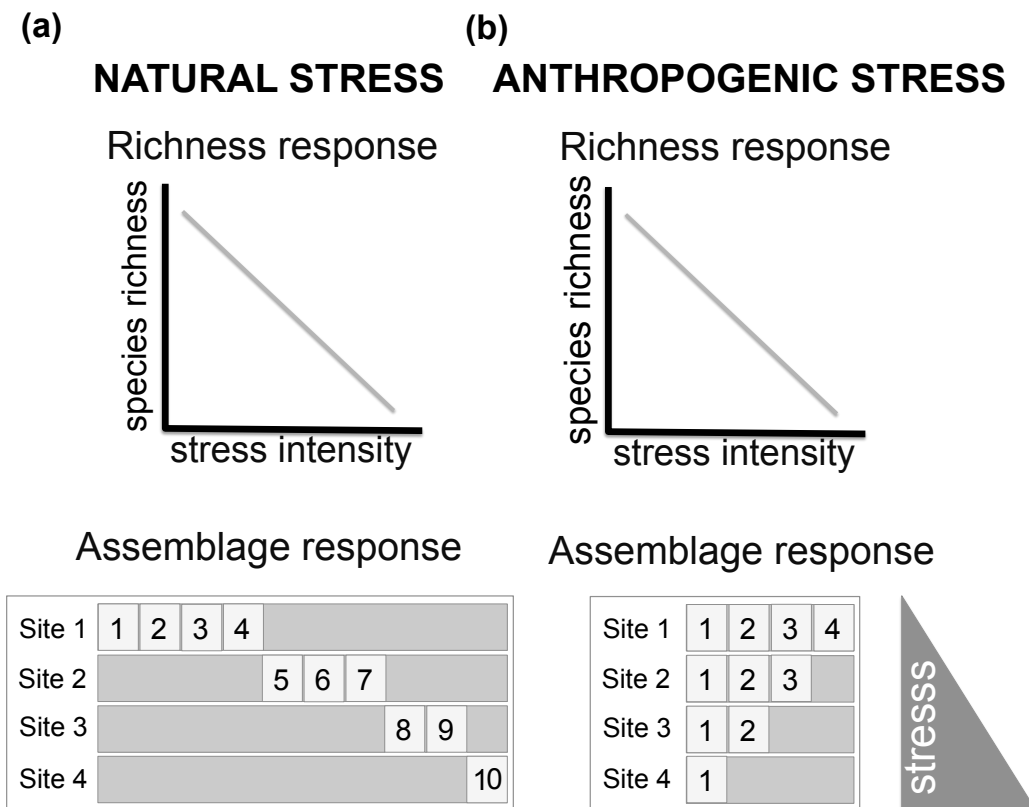


Fig. 1 Scheme describing the hypothesised mechanisms explaining beta-diversity gradients caused by natural (a) and anthropogenic (b) stressors.

Natural stress gradients that have persisted over evolutionary time are expected to promote speciation through the development of specialised biological traits, which allow some species to outperform other tolerant taxa and result in a richer pool of specialists. This is a core idea of the Evolutionary Species Pool hypothesis (Taylor *et al.* 1990), which predicts that historically more persistent and abundant habitats should contain a more native species in comparison with rare or less persistent habitats. In fact, stress is regarded as a powerful catalyst of speciation processes (Parsons 2005). As a consequence, there should be few specialists for any anthropogenic stressors that have limited occurrence in nature. Examples include synthetic toxic compounds or heavy metals, whose distribution and occurrence has been increased by mining

(e.g. Iwasaki & Ormerod 2012). In contrast, long-established natural stressors are expected to be tolerated by numerous specialist species. Contrastingly, different studies have demonstrated how certain stress types extirpated species having specific combinations of biologic traits (Larsen & Ormerod 2010), resulting in ordered extinctions along the stress gradient. Following this rationale, generalist species should occupy anthropogenically stressed habitats in the absence of specialists, causing nested assemblages. Empirical evidence shows already how marked nesting develops along human-induced stress gradients (Fernández-Juricic 2002; González-Oreja *et al.* 2012; Picazo *et al.* 2012a).

Rivers are particularly effective environments in which to examine beta-diversity patterns and mechanisms. First, they are well defined habitats that have persisted over geological timescales, allowing long-term speciation (e.g. Abellán *et al.* 2009). Secondly, they are characterised by marked gradients caused by contrasting effects that are either natural (e.g. altitude, downstream progression, natural water quality) (Moss *et al.* 1987) or anthropogenic (e.g. acidification, pollution) (Bonada *et al.* 2006). Thirdly, they are characterised by large biological diversity, for example among macroinvertebrates, that can also be readily sampled and identified (Bonada *et al.* 2006). Benthic macroinvertebrates also represent an important fraction of stream biodiversity and their assemblage composition and richness exhibit a strong correlation with environmental change at reach and basin levels (Strayer 2006). Fourthly, despite some contrasts in evolutionary history among regions, broadly similar groups of aquatic invertebrates are present – particularly across the Palearctic and northern hemispheric regions covered by this study. There is clear evidence that organisms such as Ephemeroptera, Trichoptera and Plecoptera respond in similar ways to major anthropogenic stressors throughout large areas of the world (Iwasaki & Ormerod 2012). Finally, many river data sets along environmental gradients are available to test hypotheses about processes that determine beta-diversity. Several have arisen from our own large-scale research (Buckton *et al.* 1998; Manel *et al.* 2000; Hirst *et al.* 2002; Millán *et al.* 2011). In the context of the Evolutionary Species Pool hypothesis, we postulated in this paper that mechanisms underlying beta-diversity in rivers would differ between gradients where i) natural stressors result in progressive species turnover with high specificity and ii) anthropogenic stressors result in the loss of specialist taxa thus creating nestedness. We tested this hypothesis using five sets of benthic macroinvertebrate data collected along natural (altitude, salinity) and anthropogenic (acidity, metals, land uses) stress gradients. Specifically, we tested three predictions that 1) increased stress should reduce species richness, irrespective of the particular stressor (*Prediction 1*); 2) beta-diversity patterns

caused by natural stressors result primarily from species turnover (*Prediction 2*); and 3) beta-diversity patterns caused by anthropogenic stress are driven primarily by species nestedness (*Prediction 3*).

Materials and methods

Data sources

We investigated five datasets describing gradients in altitude, salinity, acidity, metal concentration and land use along which aquatic macroinvertebrates were collected (See Appendixes 4.1 and 4.2 in Supporting Information). We assumed that stressors showing a high temporal variability would result in complex patterns of beta-diversity (e.g. flow permanence). Therefore, we only selected chronic abiotic stressors, which act as perennial habitat filters for the regional species pool. For each dataset, we selected biogeographically defined regions where ecological factors are assumed to have affected aquatic macroinvertebrates assemblages more than historical factors (e.g. Bonada *et al.* 2005). All rivers drained catchments with natural or semi-natural vegetation, or low intensity agriculture, and we reduced the risk of other potential confounding factors by selecting sampling locations as far as possible of similar altitude, stream order, gradient, climate, conductivity, lithology and substratum, except where these were the effects under test (e.g. altitude-climate, conductivity-lithology, crops/urbanisations-land uses). Anthropogenic stress datasets (i.e. acidity, metals, land use) comprised streams ranging from minimally disturbed sites to those highly affected by the putative stressor, but not showing other significant human or natural perturbations.

We selected two datasets with contrasting characteristics (natural *vs* anthropogenic stress) from the Iberian Peninsula to ensure that the observed beta-diversity patterns were associated to ecological features and not to differences in the species pool, resulting from different biogeography. In addition, we selected datasets from other areas to have spatial replication for the natural (Himalayan mountains) and anthropogenic stress gradients (Great Britain).

The altitudinal dataset was collected in the Indian and Nepali Himalaya (see Manel *et al.* 2000 for further details), encompassing a major part of the Himalaya and Middle Hills. Samples were taken during autumn and winter 1994–1996 over a wide range of altitude (350 – 4,695 m a.s.l.). We used altitude to indicate the combined natural stressors associated with altitudinal gain over

this large range, including reduced nutrient concentrations, oxygen concentrations and temperature.

The salinity dataset originated from the south (Guadalquivir, Guadiana and Sur basins) and southeast (Júcar and Segura basins) of the Iberian Peninsula. Sites differed in the degree of water mineralisation (24 to 315,200 $\mu\text{S cm}^{-1}$) as a result of differences in basin lithology (Millán *et al.* 2011). We used water electrical conductivity as indicator of the osmotic stress likely at elevated salinity.

The pH dataset came from catchments in upland mid- and north Wales (UK) (Buckton *et al.* 1998), underlain by base-poor rocks composed from Ordovician or Silurian shales and mudstones, or igneous formations. The post-industrial acidification of approximately half of Welsh streams has resulted from high rainfall (2-3 m p.a.) polluted moderately by anthropogenic acidity linked to sulphur and nitrogen compounds. Two surveys were undertaken in 1984 and 1995, respectively, at sites selected to give a large enough acid-base gradient (4.9 to 6.9 pH units) to permit the assessment of acid effects on aquatic biota. At each site, pH was measured by combination gas electrode. Mean annual pH (October to September) was used to characterise the acidification of each site in each year because this provides a synoptic variable representing the array of chronic and episodic hydrochemical effects involved in acidification (Weatherley & Ormerod 1991).

The metal dataset came from Cornwall and Wales (UK), at 20-415 m (Hirst *et al.* 2002). These regions have a history of localised metal mining around ore-bearing lithologies, which have resulted in locally high metal concentrations and biological impairment in streams receiving mine drainage. The streams were commonly affected by zinc, copper and/or lead at concentrations exceeding safe limits for macroinvertebrates for at least one of these metals (Iwasaki & Ormerod 2012). Chemical and biological surveys at these sites were conducted in spring (March and April) 1997. Water samples were taken for analyses of major ions and total organic carbon (DC-180 Automated TOC Analyser, Dohrmann, Mason, OH, USA). We used copper as a proxy of total metal toxicity at these sites because (1) other studies found this metal to be the most relevant to reduced richness and assemblage patterns in streams polluted by metal mixture (Cain *et al.* 2004; Stockdale *et al.* 2010) and (2) alternative metrics have weaker relationships with invertebrate richness, as Cumulative Criterion Unit (e.g. Hirst *et al.* 2002), or involve complex calculations that affect interpretation (Stockdale *et al.* 2010). We also used dissolved inorganic carbon as a variable in regressions investigating putative metal effects because it can reduce metal toxicity (Stockdale *et al.* 2010).

The land use dataset was collected from the south (Guadalquivir, Guadiana and Sur basins) and southeast (Segura basin) of the Iberian Peninsula. Sites differed in the intensity of the anthropogenic land uses. We measured land use intensity at each sampling point as the percentage sum of the area of crops under irrigation or urban land in the adjacent 5 km upstream catchment (i.e. the intersect between the entire catchment and a 5 km radius buffer centred on the sampling point). These two variables have been associated with nutrient enrichment and habitat impairment at the studied scale (Monteagudo *et al.* 2012). Land uses were obtained from the land use map that most closely matched the year that the invertebrate sample was taken (Corine Land Cover 1990, 2000 and 2006 layers). Initially, both this and the salinity datasets contained only records of Coleoptera and Hemiptera species, and were supplemented with data from grey literature to incorporate a wider range of species belonging to other taxonomic groups (See Appendix 4.2). These additional data came from surveys of streams occurring in the same region and under the same environmental circumstances. There is a small risk of effects from differences in sampling methods used, but we suggest that these are outweighed by the value of including more taxa. Any such effects would also be minimised by the pooling procedure described below.

All invertebrates were identified to species level, except for the Himalayan altitudinal dataset where identification was restricted to family level due to taxonomic difficulties in this region. Non-native species were removed from the datasets. Although taxonomic resolution can affect the resulting beta-diversity pattern, there was no evidence that this confounded the current analysis: the explicit focus of the analysis was turnover in composition, and any effects revealed at family level implies unequivocally that species belonging to these families will also turnover along the same environmental gradient.

Data analysis

For statistical analyses, our goal was to gather the species occurring at each stress class in streams of similar conditions within the same region but mainly differing in the focal stress gradient. Therefore, we split each stress gradient into 10 classes (hereafter called “stress classes”) using the deciles of the stressor frequency distribution as boundaries. For each dataset, macroinvertebrate samples were pooled within the corresponding stress classes to give species composition matrices with 10 rows (i.e. stress classes) and S columns, where S is the number of species (Table 2). This procedure was conducted to permit the use of the enlarged salinity and land use datasets, which included more comprehensive taxonomic information in comparison with the initial dataset

but with an important number of samples not embracing all the taxonomical groups. As explanatory variables, for each dataset, we used the mean stressor value of the samples included in each stress class. For the metal dataset we also calculated the maximum inorganic carbon value of the samples included within each metal stress class. Prior to statistical analyses, conductivity, copper and inorganic carbon were ln-transformed and pH was multiplied by -1 to make it comparable with the other stressors, given that acidity reduces pH values. All explanatory variables were z-standardised (mean=0, SD=1).

Relationships between species richness and the stress intensity were examined using General Linear Models (GLM), assuming a Poisson error distribution of the dependent variable. For the metals dataset, we also included inorganic carbon as explanatory variable because it may influence the final stress intensity of copper, as mentioned before. We tested the significance of linear and quadratic coefficients for each stressor using a forward stepwise procedure, retaining the final model that minimised the Akaike Information Criteria (AIC), showing all the explanatory variables significant.

We employed Baselga's (2010) approach to explore which mechanisms best explained variation in species composition along the stress gradients. This method requires the calculation for each dataset of three different dissimilarity matrices based on species composition matrix as follows: (1) the total pairwise beta-diversity is considered as the Sørensen dissimilarity between two communities due to differences in stress intensity (β_{sor}); (2) species turnover can be measured using the Simpson dissimilarity index (β_{sim}) and (3) species nestedness-resultant dissimilarity (β_{nes}) can be estimated by subtracting the turnover effect from the total beta-diversity ($\beta_{nes} = \beta_{sor} - \beta_{sim}$). Next, we standardised (mean=0, SD=1) stressors prior to calculate the matrix of Euclidean environmental distances (hereafter, environmental distance) for each one. Finally, the relationships between total beta-diversity, turnover and nestedness dissimilarity matrices and the matrices of environmental distance due to stress intensity differences were examined using multiple regression models for distance matrices (hereafter MRM, Lichstein 2007). This method is conceptually similar to traditional multiple regression but with all variables being distance matrices instead of raw data and *P*-values being calculated through permutation tests (1000 runs). For the metal dataset, we also built models including the distance matrix based on differences in inorganic carbon as predictor of beta-diversity, turnover and nestedness dissimilarity matrices. All statistical analyses were carried out using the statistical computing software R (R Development Core Team, 2012).

Results

Effect of natural and anthropogenic stressors on species richness

Stress intensity predicted much of the variation in the species richness for both the natural (Altitude $r^2=0.92$; Salinity $r^2=0.93$) and anthropogenic datasets (Acidity $r^2=0.91$; Metal $r^2=0.64\%$; Land uses $r^2=0.83$) (Table 1). Stress intensity reduced species richness within all datasets but the response differed among the stressors (Fig. 2). For natural stressors, there were significant non-linear relationships, with species richness peaking at moderate stress intensities. Contrastingly, for anthropogenic stress only linear coefficients were significant and species richness decreased with stress intensity. For the metal richness model, inorganic carbon concentration was also a significant predictor of taxon richness (slope=0.2104; $P<0.01$).

Effect of natural and anthropogenic stressors on beta-diversity

Stress difference accounted for much of the variation in the total beta-diversity in the natural (Altitude $r^2=0.82$, $P<0.001$; Salinity $r^2=0.83$, $P<0.001$) and anthropogenic datasets (Acidity $r^2=0.53$, $P<0.01$; Metal $r^2=0.44$, $F=33.1$, $P<0.01$; Land use $r^2=0.65$, $P<0.001$) (Table 2). Positive regression coefficients indicated that community dissimilarity increased with distance along the stress gradient, but there were clear contrasts between natural and anthropogenic stressors in the character of the relationships (Fig. 3). Natural stressors were correlated with community turnover (Altitude $r^2=0.57$, $P<0.001$; Salinity $r^2=0.77$, $P<0.001$), unlike anthropogenic stressors (Acidity $r^2=0.00$, $P=0.546$; Metal $r^2=0.04$, $P=0.247$; Land uses $r^2=0.00$, $P=0.721$). Conversely, distance along natural stress gradients was not significantly related to dissimilarities due to community nestedness (Altitude $r^2=0.13$, $P=0.057$; Salinity $r^2=0.03$, $P=0.310$). For anthropogenic stressors, pairwise differences in stress intensity were a good predictor of dissimilarities due to community nestedness (Acidity $r^2=0.51$, $P<0.001$; Metal $r^2=0.31$, $P<0.05$; Land use $r^2=0.45$, $P<0.001$). For the anthropogenic cases, regression coefficients were significantly positive indicating marked nesting as pairwise stress differences increased. Metal models did not include the environmental distance matrix due to inorganic carbon differences as significant predictor.

Table 1 Results of the GLM analyses relating ln-transformed standardised species richness to natural and anthropogenic stressors. Intercepts, slopes and r^2 values are displayed.

	Intercept	Linear coefficient	Quadratic coefficient	r^2
<i>Natural stressors</i>				
Altitude	4.00***	0.42*	-0.54*	0.92
Salinity	4.29***	1.87***	-2.63***	0.93
<i>Anthropogenic stressors</i>				
Acidity	3.82***	-0.24***		0.91
Metals ¹	3.44***	-0.30***		0.64
Land use	4.54***	-0.60***		0.83

¹The Metals model also includes ln-transformed inorganic carbon as a significant explanatory variable, although its coefficient was omitted in this table. Significance levels: *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

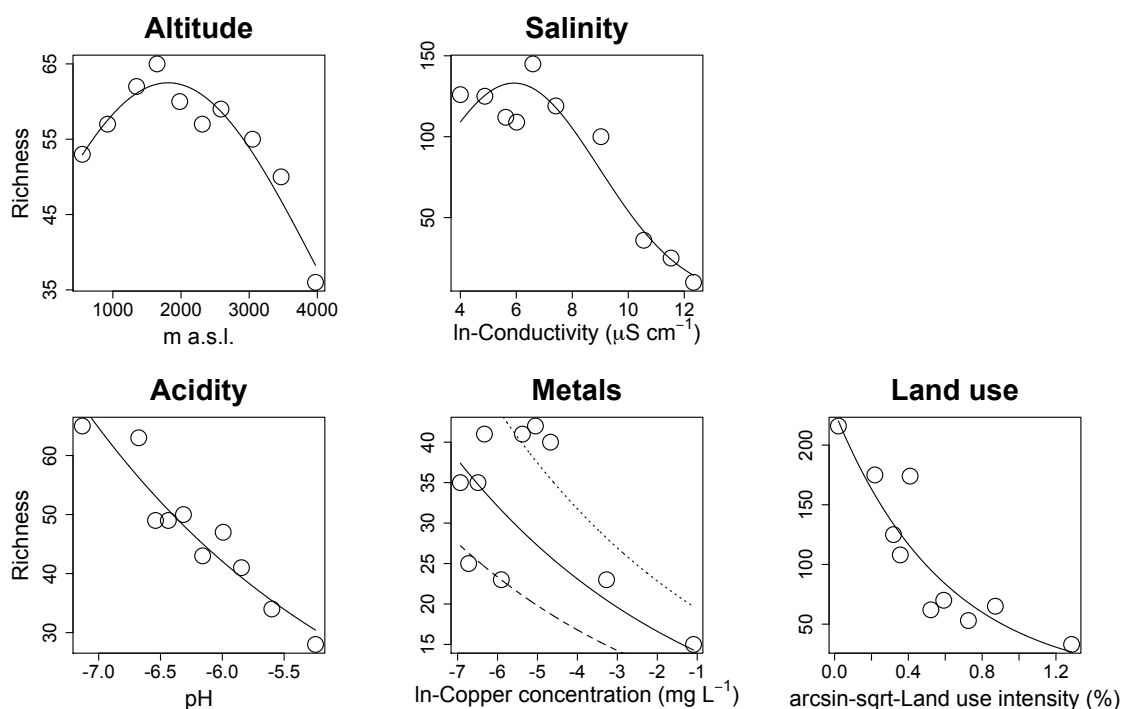


Fig. 2 Richness response to natural (altitude, salinity) and anthropogenic stressors (metals, acidity, land use). The Metals model also includes ln-transformed inorganic carbon (ln-ic) as a significant explanatory variable. Three ln-ic values were selected to display this effect on taxon richness (dashed line: ln-ic=-0.5, solid line: ln-ic=1 and dotted line: ln-ic=2.5). For the other models, solid lines represent the fitted values of the Table 1 models.

Table 2 Results of the multiple regression models for distance matrices relating beta-diversity components with stressors. Intercepts, slopes, significance tests and r^2 of the MRM relating total beta-diversity, turnover and nestedness-resultant dissimilarities with matrices of environmental distance due to natural and anthropogenic stress intensity differences are shown.

	Intercept	Linear coefficient	r^2
Beta-diversity			
<i>Natural stressors</i>			
Altitude	0.08	0.11***	0.82
Salinity	0.46***	0.24***	0.83
<i>Anthropogenic stressors</i>			
Acidity	0.46*	0.11**	0.53
Metals [¶]	0.28	0.06*	0.43
Land use	0.46***	0.11***	0.65
Turnover			
<i>Natural stressors</i>			
Altitude	0.05	0.08***	0.57
Salinity	0.34	0.26***	0.77
<i>Anthropogenic stressors</i>			
Acidity	0.39	-0.01	0.00
Metals [¶]	0.20	-0.02	0.04
Land use	0.37	0.01	0.00
Nestedness			
<i>Natural stressors</i>			
Altitude	0.03	0.03	0.13
Salinity	0.13	-0.03	0.03
<i>Anthropogenic stressors</i>			
Acidity	0.07	0.12***	0.51
Metals [¶]	0.08	0.08*	0.31
Land use	0.09	0.11***	0.45

[¶]We built the Metals models including also the distance matrix based on differences in inorganic carbon as predictor of beta-diversity, turnover and nestedness dissimilarity matrices, although its coefficient was omitted in this table because they were non-significant. Significance levels: *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

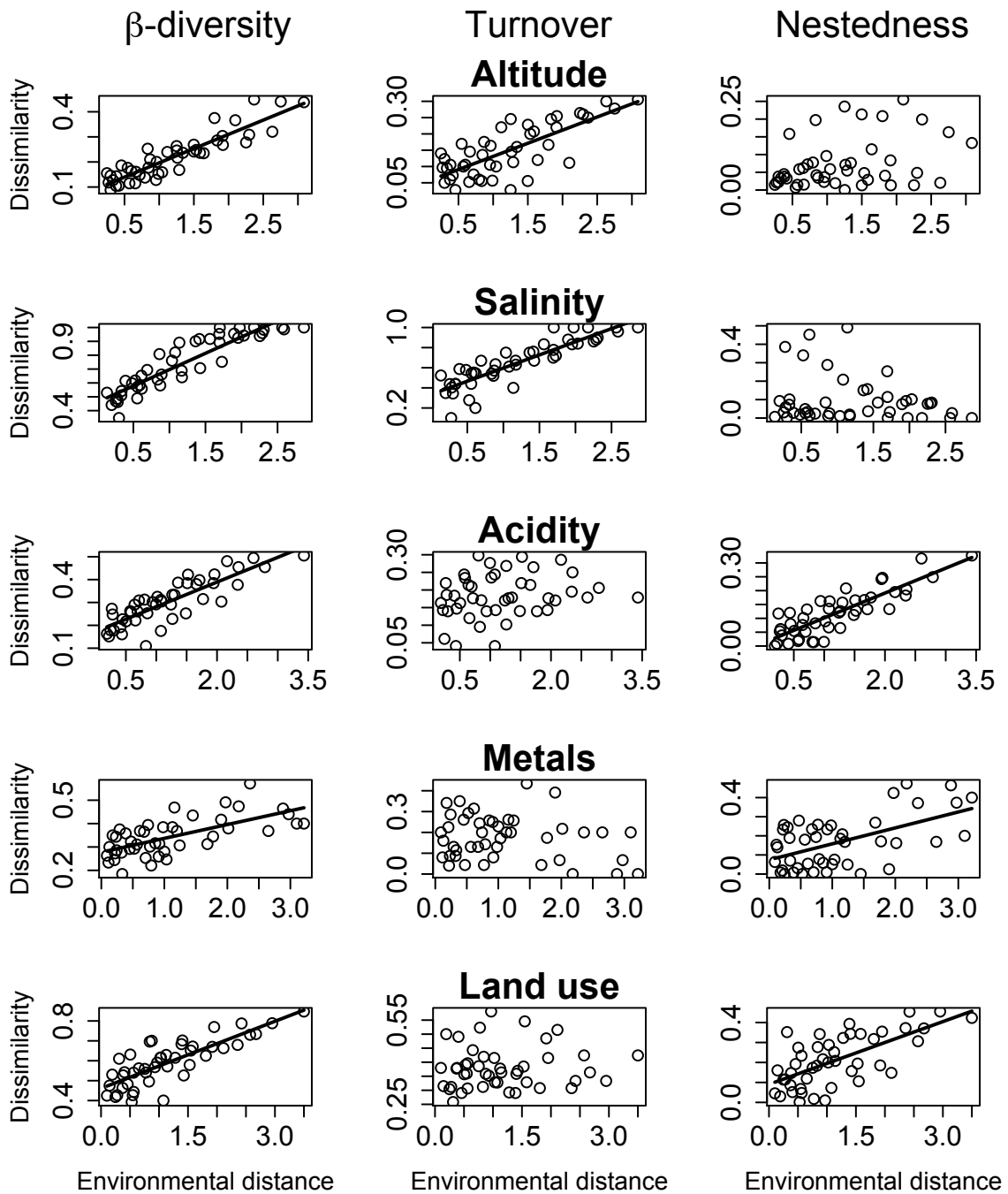


Fig. 3 Plots showing the response of beta-diversity, turnover and nestedness dissimilarities in community composition to the environmental distance matrices based on differences in natural (altitude, salinity) and anthropogenic stressors (acidity, metals, land use). Solid lines represent the fitted values of the significant models from Table 2.

Discussion

These results illustrate how both natural and anthropogenic stressors reduce species richness as expected (*Prediction 1*). However, they appear to generate different beta-diversity patterns most likely through differences in the way that species are filtered from regional species pools to form local assemblages (Swenson 2011). Despite the contrasting magnitudes and shape of the relationship between species richness responses and stress intensity (Gray 1989; Petrin *et al.* 2007), our results match the general situation in which a reduced number of species inhabit chronically stressed habitats (Rapport *et al.* 1985; Millán *et al.* 2011). At larger scales, the blend of historical and biological processes through which gradients in richness develop can depend on the regional context and the taxonomic group in question. For instance, Qian *et al.* (2005) found that spatial variation created by geological history produced larger changes in angiosperm beta-diversity than prevailing ecological conditions in temperate North America and eastern Asia. Similarly, differences in the recolonisation capacities of organisms following recent glaciations have led to different regional species pools and beta-diversity patterns in Europe (Svenning & Skov 2007; Arribas *et al.* 2012; Baselga *et al.* 2012). However, these among-regions differences in species pools should not affect to the small-scale beta-diversity patterns observed here, because these macroecological studies point to dispersion capacity rather than stress tolerance to explain the post-glacial recolonisation and the ranges of species distributions of Europe. In fact, we can currently find a mixture of stress sensitive, tolerant and specialist organisms in Northern Europe (e.g. Petrin *et al.* 2007), which would allow the ecological responses we required to test our predictions. Nonetheless, having two datasets with contrasting stress characteristics (natural and anthropogenic) within the same region controlled for the potential effect caused by differences in the regional species pool.

The main novelty of the present study was in revealing likely contrasting mechanisms between natural or anthropogenic stressors through which beta-diversity was structured. Supporting *Prediction 2*, natural stressors caused turnover along the beta-diversity gradient, which involved organisms showing a high specificity to given segments of the stress gradient (i.e. altitude or salinity). Such specificity has been observed in species occupying different naturally stressed terrestrial (Scarano 2002; Mandeel 2006) and aquatic habitats (e.g. Dunson & Travis 1991; Millán *et al.* 2011). Stressors are considered to enhance evolution, with specialisation then expected to arise as result of the adaptation process over geological time (Dunson & Travis 1991; Parsons 2005; Bickford *et al.* 2007). It is important to highlight that altitude dataset results

supported *Prediction 2*, despite using family level data, which is a more conservative approach (see Materials and Methods section for a full explanation). This finding matches with the results of other studies, which observed species of lichens, plants and animals showing narrow distributions and increasing endemic species occurrence along the altitudinal gradient of the Himalayan Mountains (Vetaas & Grytnes 2002; Baniya *et al.* 2010; Bhatt *et al.* 2012). In contrast, the anthropogenic stressors appeared to exclude sensitive species progressively whilst tolerant generalist species were present along almost the whole gradient. This resulted in beta-diversity gradients that were best explained by nesting (*Prediction 3*). Previous studies have also shown significantly nested assemblages along other human-induced disturbance gradients without finding stress specialists (Fernández-Juricic 2002; González-Oreja *et al.* 2012; Picazo *et al.* 2012a). More particularly, anthropogenically salinised sites in Germany, where there was no historical occurrence of saline streams, are inhabited by exotic or tolerant fauna rather than specialised species like those in the Iberian data (Arle & Wagner in press)

A natural question arises: why generalist taxa are infrequent at naturally stressed sites, when they apparently tolerate a wide range of anthropogenic stressors. On the one hand, stressed environments offer advantages for potential colonisers in terms of low competition for resources (Odum 1985). Thus, when a stressed habitat emerged during evolutionary history, probably it had to be occupied first by the best-adapted taxa within the available regional pool, and generalist species would be good candidates for two main reasons: first, there is evidence to suggest that many generalist species show a high degree of inter-individual variation allowing the species to occupy a wide spectrum of habitats (Bolnick *et al.* 2007); second, it has been hypothesised that a constitutive higher expression of coping proteins or other physiological mechanisms in response to stress can explain why species belonging to some particular phylogenetic groups tend to colonise stressful habitats (Bradley 2008; Buchwalter *et al.* 2008; Garbuz *et al.* 2008). These mechanisms probably arose in response to ancestral environmental constraints for the group being conserved likely because some baseline stress of such a type has historically remained within the focal region (Buchwalter *et al.* 2008). At the sites where stress persisted over geological timescales (i.e. natural stressors), stress specialists would have emerged, likely evolving from generalist ancestors that exhibit pre-adaptations or suitability for stressful habitats. For example, many Coleoptera and Diptera species have aquatic stages adapted to a wide array of stress types. The larvae of the Chironomidae species are extremely ubiquitous in being able to withstand both natural and anthropogenic stresses (Danks 2008; Gutiérrez-Cánovas *et al.* 2012). There are other good examples among species of the

families Stratiomyidae and Culicidae (Diptera) or Dytiscidae and Hydraenidae (Coleoptera), which can inhabit different aquatic habitats with extreme salinity or thermal conditions (Bradley 2008; Garbuz *et al.* 2008; Picazo *et al.* 2012b). Later, specialist probably replaced generalists, since under strong selection (i.e. stressful conditions), specifically stress-suited taxa would show competitively superior fitness (van Tienderen 1997). Although the new specialised coping traits would involve a higher energetic cost in comparison with tolerant strategies (Bradley 2008), this should be compensated given the high degree of unexploited food resources occurring at stressful habitats. Ultimately, stress specialists may be confined within specific environmental conditions due to deleterious mutations resulting in the loss of some functions that are unnecessary under such circumstances but would matter at more common environments (Kawecki *et al.* 1997).

Conservation implications

The contrasting effects of turnover and nestedness on beta-diversity patterns have three clear implications for species and habitat conservation – particularly for ecosystems such as rivers that are characterised by marked environmental gradients.

First, and most obviously, where anthropogenic stressors cause nested assemblage patterns at regional scales, the major priority will usually be to protect the richest, unpolluted and intact sites that are likely to be relict environments containing a large proportion of the remaining species pool. Where such nesting is apparent, there are likely to be attendant implications for meta-population dynamics, gene flow and variability which could be particularly important in the light of current global change (Brown *et al.* 2009). Additionally, under these circumstances habitat or ecosystem-scale restoration or stress mitigation becomes important in arresting further loss. In the case of acidification investigated here, there are ongoing challenges in returning impacted sites to former hydrochemical and ecological conditions (Ormerod & Durance 2009). Other sources of species nesting in rivers appear to have arisen from catchment-scale modification and replacement of semi-natural habitats with conservation implications that could become major, large-scale restoration challenges in future (Larsen & Ormerod 2010). In these cases, there are fundamental questions about how nested species losses could ever be recovered by restoration.

Second, when beta-diversity is caused by species turnover along natural gradients, conservation strategies would ideally aim to protect sufficient

locations to capture the entire natural stress gradient, for example from headwaters to estuaries throughout whole river basins (Strayer 2006; Abellán *et al.* 2007). While this approach is now applied through the conservation of rivers within basins, less attention is given to conserving naturally stressed ecosystems where gradients in conditions arise among basins, for instance saline inland waters in the Western Mediterranean (Sánchez-Fernández *et al.* 2008; Millán *et al.* 2011).

Third, our results illustrate how naturally stressed ecosystems contribute to regional species pools by supporting the unique species that these sites contain as a result of local evolutionary processes (Abellán *et al.* 2009). Such naturally stressed ecosystems are often considered as unproductive ecologically or economically (e.g. saline landscapes, high mountains, arid lands or deserts) and many have been neglected when designing conservation and biomonitoring programs (Millán *et al.* 2011).

In combination, these implications for conservation illustrate the importance of understanding how stressors configure beta-diversity patterns generally, and we advocate further investigations of the consequences for conservation strategies, ecological networks and large-scale ecosystem function.

Acknowledgements

We would like to thank O. Belmar, D. Bruno, J. Carbonell, S. Guareschi and D. Sánchez-Fernández for kindly providing macroinvertebrate data of Segura River Basin, Dr Heike Rothfritz for allowing access to the metals data, and to the very many people involved in collecting large-scale data on the effects of acidification in Wales and altitudinal variation in the Himalayan Mountains. We are grateful to N. Bonada for helping to identify some Trichoptera species of salinity dataset and P. Arribas for her helpful comments. C.G-C. was partially supported by a predoctoral grant from the Fundación Séneca (Agencia Regional de Ciencia y Tecnología, Región de Murcia). The salinity surveys were financed by *Ministerio de Ciencia e Innovación (Spain)*, through I+D+I project ref: CGL2006-04159/BOS. A. Baselga, D. Currie and two anonymous referees provided insightful, generous and helpful comments on the manuscript.

References

- Abellán, P., Millán, A. & Ribera, I. (2009). Parallel habitat-driven differences in the phylogeographical structure of two independent lineages of Mediterranean saline water beetles. *Molecular Ecology*, 18, 3885-3902.
- Abellán, P., Sánchez-Fernández, D., Velasco, J. & Millán, A. (2007). Effectiveness of protected area networks in representing freshwater biodiversity: the case of a Mediterranean river basin (south-eastern Spain). *Aquatic Conservation: Marine and Freshwater ecosystems*, 361-374.
- Andrew, M.E., Wulder, M.A., Coops, N.C. & Baillargeon, G. (2012). Beta-diversity gradients of butterflies along productivity axes. *Global Ecology and Biogeography*, 21, 352-364.
- Arle, J. & Wagner, F. (in press). Effects of anthropogenic salinisation on the ecological status of macroinvertebrate assemblages in the Werra River (Thuringia, Germany). *Hydrobiologia*.
- Arribas, P., Velasco, J., Abellán, P., Sánchez-Fernández, D., Andujar, C., Calosi, P. *et al.* (2012). Dispersal ability rather than ecological tolerance drives differences in range size between lentic and lotic water beetles (Coleoptera: Hydrophilidae). *Journal of Biogeography*, 39, 984-994.
- Baniya, C.B., Solhoy, T., Gauslaa, Y. & Palmer, M.W. (2010). The elevation gradient of lichen species richness in Nepal. *Lichenologist*, 42, 83-96.
- Baselga, A. (2010). Partitioning the turnover and nestedness components of beta diversity. *Global Ecology and Biogeography*, 19, 134-143.
- Baselga, A., Lobo, J.M., Svenning, J.C., Aragón, P. & Araújo, M.S. (2012). Dispersal ability modulates the strength of the latitudinal richness gradient in European beetles. *Global Ecology and Biogeography*, 21, 1106-1113.
- Bhatt, J.P., Manish, K. & Pandit, M.K. (2012). Elevational gradients in fish diversity in the himalaya: water discharge is the key driver of distribution patterns. *Plos One*, 7, e46237.
- Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K. *et al.* (2007). Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution*, 22, 148-155.
- Bolnick, D.I., Svanback, R., Araujo, M.S. & Persson, L. (2007). Comparative support for the niche variation hypothesis that more generalized

- populations also are more heterogeneous. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 10075-10079.
- Bonada, N., Prat, N., Resh, V.H. & Statzner, B. (2006). Developments in aquatic insect biomonitoring: A comparative analysis of recent approaches. In: *Annual Review of Entomology*, pp. 495-523.
- Bonada, N., Zamora-Muñoz, C., Rieradevall, M. & Prat, N. (2005). Ecological and historical filters constraining spatial caddisfly distribution in Mediterranean rivers. *Freshwater Biology*, 50, 781-797.
- Bradley, T.J. (2008). Saline-water Insects: Ecology, Physiology and Evolution. In: *Aquatic insects: challenges to populations* (eds. Lancaster, J & Briers, RA). CAB International Oxfordshire OX10 8DE, UK, pp. 20-35.
- Brown, L.E., Cereghino, R. & Compin, A. (2009). Endemic freshwater invertebrates from southern France: Diversity, distribution and conservation implications. *Biological Conservation*, 142, 2613-2619.
- Buchwalter, D.B., Cain, D.J., Martin, C.A., Xie, L., Luoma, S.N. & Garland, T. (2008). Aquatic insect ecophysiological traits reveal phylogenetically based differences in dissolved cadmium susceptibility. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 8321-8326.
- Buckton, S.T., Brewin, P.A., Lewis, A., Stevens, P. & Ormerod, S.J. (1998). The distribution of dippers, *Cinclus cinclus* (L.), in the acid-sensitive region of Wales, 1984-95. *Freshwater Biology*, 39, 387-396.
- Cain, D.J., Luoma, S.N. & Wallace, W.G. (2004). Linking metal bioaccumulation of aquatic insects to their distribution patterns in a mining-impacted river. *Environmental Toxicology and Chemistry*, 23, 1463-1473.
- Danks, H.V. (2008). Aquatic Insect Adaptations to Winter Cold and Ice. In: *Aquatic insects: challenges to populations* (eds. Lancaster, J & Briers, RA). CAB International Oxfordshire OX10 8DE, UK, pp. 1-19.
- Dunson, W.A. & Travis, J. (1991). The role of abiotic factors in community organization. *American Naturalist*, 138, 1067-1091.
- Fernández-Juricic, E. (2002). Can human disturbance promote nestedness? A case study with breeding birds in urban habitat fragments. *Oecologia*, 131, 269-278.
- Garbuz, D.G., Zatssepina, O.G., Przhiboro, A.A., Yushenova, I., Guzhova, I.V. & Evgenev, M.B. (2008). Larvae of related Diptera species from thermally contrasting habitats exhibit continuous up-regulation of heat shock proteins and high thermotolerance. *Molecular Ecology*, 17, 4763-4777.

- González-Oreja, J.A., De La Fuente-Díaz-Ordaz, A.A., Hernández-Santín, L., Bonache-Regidor, C. & Buzo-Franco, D. (2012). Can human disturbance promote nestedness? Songbirds and noise in urban parks as a case study. *Landscape and Urban Planning*, 104, 9-18.
- Gray, J.S. (1989). Effects of environmental stress on species rich assemblages. *Biological Journal of the Linnean Society*, 37, 19-32.
- Gutiérrez-Cánovas, C., Hernández, J., Millán, A. & Velasco, J. (2012). Impact of chronic and pulse dilution disturbances on metabolism and trophic structure in a saline Mediterranean stream. *Hydrobiologia*, 686, 225-239.
- Hirst, H., Juttner, I. & Ormerod, S.J. (2002). Comparing the responses of diatoms and macroinvertebrates to metals in upland streams of Wales and Cornwall. *Freshwater Biology*, 47, 1752-1765.
- Iwasaki, Y. & Ormerod, S.J. (2012). Estimating safe concentrations of heavy metals from inter-continental field data on river macroinvertebrates. *Environmental Pollution*, 166, 182-186.
- Kawecki, T.J., Barton, N.H. & Fry, J.D. (1997). Mutational collapse of fitness in marginal habitats and the evolution of ecological specialisation. *Journal of Evolutionary Biology*, 10, 407-429.
- Larsen, S. & Ormerod, S.J. (2010). Combined effects of habitat modification on trait composition and species nestedness in river invertebrates. *Biological Conservation*, 143, 2638-2646.
- Lichstein, J.W. (2007). Multiple regression on distance matrices: a multivariate spatial analysis tool. *Plant Ecology*, 188, 117-131.
- Mandeel, Q.A. (2006). Biodiversity of the genus *Fusarium* in saline soil habitats. *Journal of Basic Microbiology*, 46, 480-494.
- Manel, S., Buckton, S.T. & Ormerod, S.J. (2000). Testing large-scale hypotheses using surveys: the effects of land use on the habitats, invertebrates and birds of Himalayan rivers. *Journal of Applied Ecology*, 37, 756-770.
- Melo, A.S., Rangel, T.F.L.V.B. & Diniz-Filho, J.A.F. (2009). Environmental drivers of beta-diversity patterns in New-World birds and mammals. *Ecography*, 32, 226-236.
- Millán, A., Velasco, J., Gutiérrez-Cánovas, C., Arribas, P., Picazo, F., Sánchez-Fernández, D. *et al.* (2011). Mediterranean saline streams in southeast Spain: What do we know? *Journal of Arid Environments*, 75, 1352-1359.

- Monteagudo, L., Luis Moreno, J. & Picazo, F. (2012). River eutrophication: Irrigated vs. non-irrigated agriculture through different spatial scales. *Water Research*, 46, 2759-2771.
- Moss, D., Furse, M.T., Wright, J.F. & Armitage, P.D. (1987). The prediction of the macroinvertebrate fauna of unpolluted running-water sites in Great-Britain using environmental data. *Freshwater Biology*, 17, 41-52.
- Odum, E.P. (1985). Trends expected in stressed ecosystems. *Bioscience*, 35, 419-422.
- Ormerod, S.J. & Durance, I. (2009). Restoration and recovery from acidification in upland Welsh streams over 25 years. *Journal of Applied Ecology*, 46, 164-174.
- Parsons, P.A. (2005). Environments and evolution: interactions between stress, resource inadequacy and energetic efficiency. *Biological Reviews*, 80, 589-610.
- Petrin, Z., Laudon, H. & Malmqvist, B. (2007). Does freshwater macroinvertebrate diversity along a pH-gradient reflect adaptation to low pH? *Freshwater Biology*, 52, 2172-2183.
- Picazo, F., Bilton, D.T., Moreno, J.L., Sánchez-Fernández, D. & Millán, A. (2012a). Water beetle biodiversity in Mediterranean standing waters: assemblage composition, environmental drivers and nestedness patterns. *Insect Conservation and Diversity*, 5, 146-158.
- Picazo, F., Millán, A. & Dolédec, S. (2012b). Are patterns in the taxonomic, biological and ecological traits of water beetles congruent in Mediterranean ecosystems? *Freshwater Biology*, 57, 2192-2210.
- Qian, H., Ricklefs, R.E. & White, P.S. (2005). Beta diversity of angiosperms in temperate floras of eastern Asia and eastern North America. *Ecology Letters*, 8, 15-22.
- R-Development-Core-Team (2012). R: a language and environment for statistical computing. R Foundation for Statistical Computing Vienna, Austria.
- Rapport, D.J., Regier, H.A. & Hutchinson, T.C. (1985). Ecosystem behaviour under stress. *American Naturalist*, 125, 617-640.
- Sánchez-Fernández, D., Bilton, D.T., Abellán, P., Ribera, I., Velasco, J. & Millán, A. (2008). Are the endemic water beetles of the Iberian Peninsula and the Balearic Islands effectively protected? *Biological Conservation*, 141, 1612-1627.

- Scarano, F.R. (2002). Structure, function and floristic relationships of plant communities in stressful habitats marginal to the Brazilian Atlantic rainforest. *Annals of Botany*, 90, 517-524.
- Smith, M.D., Knapp, A.K. & Collins, S.L. (2009). A framework for assessing ecosystem dynamics in response to chronic resource alterations induced by global change. *Ecology*, 90, 3279-3289.
- Stockdale, A., Tipping, E., Lofts, S., Ormerod, S.J., Clements, W.H. & Blust, R. (2010). Toxicity of proton-metal mixtures in the field: Linking stream macroinvertebrate species diversity to chemical speciation and bioavailability. *Aquatic Toxicology*, 100, 112-119.
- Strayer, D.L. (2006). Challenges for freshwater invertebrate conservation. *Journal of the North American Benthological Society*, 2, 271-287.
- Svenning, J.-C. & Skov, F. (2007). Could the tree diversity pattern in Europe be generated by postglacial dispersal limitation? *Ecology Letters*, 10, 453-460.
- Swenson, N.G. (2011). The role of evolutionary processes in producing biodiversity patterns, and the interrelationships between taxonomic, functional and phylogenetic biodiversity. *American Journal of Botany*, 98, 472-480.
- Taylor, D.R., Aarssen, L.W. & Loehle, C. (1990). On the relationship between r/K selection and environmental carrying-capacity - a new habitat templet for plant life-history strategies. *Oikos*, 58, 239-250.
- van Tienderen, P.H. (1997). Generalists, specialists, and the evolution of phenotypic plasticity in sympatric populations of distinct species. *Evolution*, 51, 1372-1380.
- Vetaas, O.R. & Grytnes, J.A. (2002). Distribution of vascular plant species richness and endemic richness along the Himalayan elevation gradient in Nepal. *Global Ecology and Biogeography*, 11, 291-301.
- Weatherley, N.S. & Ormerod, S.J. (1991). The importance of acid episodes in determining faunal distributions in Welsh streams. *Freshwater Biology*, 25, 71-84.
- Weiher, E. & Keddy, P.A. (1995). Assembly rules, null models, and trait dispersion - new questions front old patterns. *Oikos*, 74, 159-164.

Supporting Information

Appendix 4.1. Number of sites, location and main environmental descriptors of the natural and anthropogenic stress datasets.

Appendix 4.2. Sampling and taxonomic features and sources of the datasets.

Supporting Information

Appendix 4.1. Number of sites, location and main environmental descriptors of the natural and anthropogenic stress datasets.

Dataset	Sites	Study area	Climate	Altitudinal range (m a.s.l.)	Conductivity range ($\mu\text{S cm}^{-1}$)
<i>Natural stress</i>					
<i>Altitude</i>	165	Indian and Nepali Himalaya and Middle Hills	Temperate, alpine	350–4,695	9–413
<i>Salinity</i>	77	South and southeast of the Iberian peninsula	Mediterranean	90–1,200	24–315,200
<i>Anthropogenic stress</i>					
<i>Acidity</i>	102	Wales (UK)	Temperate	50–500	28–202
<i>Metals</i>	51	Cornwall and Wales (UK)	Temperate	20–415	34–463
<i>Land uses</i>	63	South and southeast of the Iberian peninsula	Mediterranean	23–1150	24–2,500

Appendix 4.2. Sampling and taxonomic features and sources of the datasets.

Dataset	Sampling method	Sampled habitats	Sampling period	Sampled seasons	Taxonomic orders	Taxon number	Data source
<i>Natural stress</i>							
Altitude	kick-net	riffle, margins	1994-1996	autumn, winter	COL, EPH, DIP, HEM, LEP, MOL, PLE, TRI, TRC	83 ^a	Manel <i>et al.</i> , 2000
Salinity	kick-net, surber	multi-habitat	1982-2010	spring, summer, autumn, winter	COL, CRU, EPH, HEM, HIR, MOL, ODO, OLI, PLE, TRI, TRC	347 ^b	Own data; Alba_Tercedor & Jiménez-Millán, 1985; Zamora-Muñoz & Alba-Tercedor 1992; Gallardo-Mayenco, 1994; Picazo-Muñoz, 1995; Zamora-Muñoz <i>et al.</i> , 1996; Moreno, 2002; Vivas, 2003
<i>Anthropogenic stress</i>							
Acidity	kick-net	riffle	1984, 1995	spring	COL, CRU, EPH, DIP, HEM, HIR, ODO, MEG, MOL, PLE, TRI, TRC	90 ^b	Buckton <i>et al.</i> , 1998
Metals	kick-net	riffle	1997	spring	COL, CRU, EPH, DIP, HIR, PLE, TRI, TRC	69 ^b	Hirst <i>et al.</i> , 2002
Land uses	kick-net, surber	multi-habitat	1982-2010	spring, summer, autumn, winter	COL, CRU, EPH, HEM, HIR, MOL, ODO, OLI, PLE, TRI	356 ^b	Own data; Alba_Tercedor & Jiménez-Millán, 1985; Zamora-Muñoz & Alba-Tercedor, 1992; Picazo-Muñoz, 1995; Zamora-Muñoz <i>et al.</i> , 1996; Vivas, 2003; Sánchez-Fernández <i>et al.</i> , 2006.

^a Family level; ^b Species level. COL: Coleoptera, CRU: Crustacea, EPH: Ephemeroptera, HEM: Hemiptera, HIR: Hirudinea, LEP: Lepidoptera, MEG: Megaloptera, MOL: Mollusca, ODO: Odonata, PLE: Plecoptera, TRI: Trichoptera, TRC: Tricladida.

References from Appendix 4.2

- Alba-Tercedor, J. & Jiménez-Millán, F. (1987) *Evaluación de las variaciones de la calidad de las aguas del Río Guadalfeo basada en el estudio de las comunidades de macroinvertebrados acuáticos y de los factores físico-químicos*. LUCDEME III. Monografía 48 del ICONA.
- Buckton, S.T., Brewin, P.A., Lewis, A., Stevens, P. & Ormerod, S.J. (1998) The distribution of dippers, *Cinclus cinclus* (L.), in the acid-sensitive region of Wales, 1984-95. *Freshwater Biology*, **39**, 387-396.
- Gallardo-Mayenco, A. (1994) Freshwater macroinvertebrate distribution in two basins with different salinity gradients (Guadalete and Guadaira river basins, south-western Spain). *International Journal of Salt Lake Research* **3**: 75-91.
- Hirst, H., Juttner, I. & Ormerod, S.J. (2002) Comparing the responses of diatoms and macroinvertebrates to metals in upland streams of Wales and Cornwall. *Freshwater Biology*, **47**, 1752-1765.
- Manel, S., Buckton, S.T. & Ormerod, S.J. (2000) Testing large-scale hypotheses using surveys: the effects of land use on the habitats, invertebrates and birds of Himalayan rivers. *Journal of Applied Ecology*, **37**, 756-770.
- Moreno, J.L. (2002) *Hábitats, recursos tróficos y estructura de la comunidad de macroinvertebrados bentónicos en un arroyo salino del Sureste Ibérico (Rambla del Reventón)*. PhD Thesis, Universidad de Murcia.
- Picazo-Muñoz, J. (1995) *Caracterización y calidad de las aguas de los cauces de la cuenca del Río Guadiana Menor. Aspectos físico-químicos y macroinvertebrados acuáticos*. PhD Thesis, Universidad de Granada.
- Sánchez-Fernández, D., Abellán, P., Mellado, A., Velasco, J. & Millán, A. (2006) Are water beetles good indicators of biodiversity in Mediterranean aquatic ecosystems? The case of the Segura river basin (SE Spain). *Biodiversity and Conservation*, **15**: 4507-4520.
- Vivas, M.S. (2003) *Comunidades de macroinvertebrados de los Ríos Aguas y Almanzora: relaciones con la evaluación del estado ecológico*. PhD Thesis, Universidad de Almería.
- Zamora-Muñoz, C. & Alba-Tercedor, J. (1992) *Caracterización y calidad de las aguas del Río Monachil*. Junta de Andalucía, Consejería de Cultura y Medio Ambiente.

Chapter 4: Beta diversity along natural and anthropogenic stress gradients

Zamora-Muñoz, C., Madrid-Vinuesa, F. & Alba-Tercedor, J. (1996) *Estudio preliminar del efecto del embalse de canales en la comunidad de macroinvertebrados acuáticos del Río Genil*. (ed. by Chacón, J. and J.L. Rosua) pp. 187-203. Actas de la Conferencia internacional de Sierra Nevada: Conservación y desarrollo sostenible.

Chapter 5:

Similarity in the difference: non-random changes in community functional features along natural and anthropogenic stress gradients

Submitted to Ecology Letters:

Gutiérrez-Cánovas, C.; Sánchez-Fernández, D.; Velasco, J.; Millán, A. & N. Bonada. Similarity in the difference: non-random changes in community functional features along natural and anthropogenic stress gradients.

Similarity in the difference: non-random changes in community functional features along natural and anthropogenic stress gradients

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Abstract

The effect of stressors on biodiversity can vary in relation to the degree to which biological communities have adapted over evolutionary time. We compared the responses of functional features of stream insect communities along chronic stress gradients with contrasting historical persistence. Water salinity and land use intensification were used as examples of natural (historically persistent) and anthropogenic (modern environmental filter) stressors, respectively. A new trait-based approach was applied to quantify functional diversity components and functional redundancy within the same multidimensional space, using metrics at the taxon and community levels. We found similar functional responses along natural and anthropogenic stress gradients. In both cases, the mean taxon functional richness and functional similarity increased with stress, whereas community functional richness and functional redundancy decreased. Despite the differences in evolutionary persistence, both chronic stressors act as strong non-random environmental filters, producing convergent functional responses. These results can improve our ability to predict functional effects of novel stressors at ecological and evolutionary scales.

Introduction

Predicting ecosystem responses to environmental change is one of the most challenging tasks that ecologists face. The effects of stressors on biodiversity can vary in relation to the degree to which biological communities have adapted over evolutionary time (Buchwalter *et al.* 2008; Garbuz *et al.* 2008; Gutiérrez-Cánovas *et al.* 2013). Natural stressors, such as drought in arid regions or low pH in regions with an acidic geology, persist over evolutionary time, allowing species to acquire adaptations to survive in these environments (Badyaev 2005; Arribas *et al.* 2014). Conversely, relatively modern anthropogenic stressors, such as pollution or invasive species, introduce unfamiliar environmental conditions that do not persist sufficiently to trigger evolutionary responses (Buchwalter *et al.* 2008; Pool & Olden 2012).

Ecological theory predicts non-random species sorting in association with environmental filters, determined by species traits (Southwood 1977; Shipley *et al.* 2006; Weiher *et al.* 2011; Mouillot *et al.* 2013). As traits conferring stress tolerance arise via evolution in response to historical persistent environmental filters (Buchwalter *et al.* 2008), it is likely that the functional responses of communities subjected to natural, historically persistent stressors are different from those related to new anthropogenic stressors. Thus, stress may filter traits differently during colonisation and adaptation, resulting in dissimilar traits and different patterns of functional features along gradients with contrasting historical persistence. However, previous studies have documented that traits conferring natural stress tolerance can also grant a species the ability to tolerate novel stressors (Badyaev 2005; Buchwalter *et al.* 2008; Arribas *et al.* 2014). In such a case, a subset of biological features will be shared among the different organisms that inhabit naturally and anthropogenically stressed environments (Southwood 1977; Odum 1985; Stanton *et al.* 2000). These commonalities should result in similar trends of functional features along stress gradients, irrespective of the degree to which a species has adapted and specialised over evolutionary time (i.e. to a natural or anthropogenic stressor). In this case, stress would act as a strong non-random environmental filter that configures trait evolution and community functional properties.

Functional measures at the community level are related to ecosystem functioning and stability (Hooper *et al.* 2005) and are able to reflect non-random community assembly processes (Villéger *et al.* 2008). For example, whole-community functional diversity measures, such as functional richness and divergence, or functional redundancy, are predicted to decrease with increasing

environmental filtering (Weiher *et al.* 2011; Mouillot *et al.* 2013). However, these measures are generally estimated using mean trait values for each taxon, despite the fact that some studies have demonstrated the importance of within-taxon trait variability to better explain community assembly (Stubbs & Wilson 2004; Violle *et al.* 2012). Therefore, the advantages of using within-taxon functional measures are twofold: (1) improving whole-community measures and (2) allowing the estimation of new functional measures at the taxon level. As an example of the first case, within-taxon trait information provides a direct measure of community functional overlap among taxa (i.e. functional redundancy), eliminating the need to classify species into functional groups, which may result in some loss of information (Villéger *et al.* 2008). Regarding the second case, taxon functional richness can be estimated as the functional space filled by the variability exhibited by each taxon (Stubbs & Wilson 2004). Taxa showing higher functional richness are more likely to be present in a wider range of environmental conditions than more functionally homogeneous taxa, which are thought to be more sensitive to environmental change (Scoville & Pfrender 2010; Latta *et al.* 2012). Functional similarity is an additional within-taxon measure, which indicates the mean relative overlap between pairs of taxa in a community, thereby allowing detection of community assembly rules in relation to mean trait similarity. For example, limited similarity of coexisting species due to interspecific competition prevails under non-stressful conditions (MacArthur & Levins 1967; Stubbs & Wilson 2004), whereas intense habitat filtering increases functional similarity (Weiher *et al.* 2011; Maire *et al.* 2012).

We explored changes in community functional features along chronic stress gradients with contrasting historical persistence. In particular, we compared changes in stream insect communities along a water salinity gradient (i.e. as a natural, evolutionary persistent stress) and across different land uses (i.e. as an anthropogenic, modern stress) as stress gradients using within-taxon functional measures at the taxon (mean taxon functional richness and mean functional similarity) and whole-community (community functional richness, functional dispersion, and functional redundancy) levels. We selected streams as focal ecosystems because they are (1) well-defined and stable habitats over geological time scales, allowing long-term speciation (e.g. Arribas *et al.* 2014); and (2) characterised by marked environmental gradients in relation to which taxonomic and functional responses are detected (e.g. Díaz *et al.* 2008; Gutiérrez-Cánovas *et al.* 2013).

Materials and Methods

To study the functional responses of aquatic insects to natural and anthropogenic stresses, we chose one example of each type. As a natural stressor, we selected water salinity due to (1) its persistence over evolutionary time scales in the study region (Arribas *et al.* 2014) and because (2) the relationships between salinity and the taxonomic and functional attributes of aquatic fauna are well known (Díaz *et al.* 2008; Millán *et al.* 2011; Arribas *et al.* 2014). As an anthropogenic stressor, we chose land-use intensification because (1) it is one of the most important stressors that affects aquatic ecosystems worldwide, and (2) it is closely associated with increasing human populations (Scanlon *et al.* 2007). Because stressors showing high temporal variability may yield complex ecosystem responses (Gutiérrez-Cánovas *et al.* 2013), we only selected chronic environmental stressors, which act as perennial habitat filters for the regional species pool.

We focused our study on western Mediterranean streams (Iberian Peninsula and Italy), gathering data from published work, grey literature, and our own resources (see Table S1 in Supporting Information). We selected this study area because (1) the regional pool of aquatic taxa is well-known in terms of taxonomy, biological traits, and ecological responses (Díaz *et al.* 2008; Bonada & Dolédec 2011; Millán *et al.* 2011); and (2) it presents the natural (water salinity) and anthropogenic (land-use intensification) stressors examined in this study (Bonada & Resh 2013).

Environmental datasets

The Salinity dataset included unpolluted (i.e. drained catchments mainly covered with natural or semi-natural vegetation or non-irrigated crops), small (width <10 m), mid-mountain and lowland streams (<1,200 m a.s.l.) with a permanent flow (see Appendix 5.1 in Supporting Information); these criteria resulted in a total of 105 sites. The salinity gradient ranged from electrical conductivities of 100 $\mu\text{S cm}^{-1}$ to near 300,000 $\mu\text{S cm}^{-1}$. We used the electrical conductivity of water as an indicator of osmotic stress.

The Land-use dataset included perennial, freshwater (conductivity <2,500 $\mu\text{S cm}^{-1}$), small and intermediate (width <40 m), mid-mountain and lowland streams (<1,200 m a.s.l.) (see Table S1), resulting in a total of 102 sites. This dataset contained streams that drain from natural vegetation catchments to highly modified catchments. We measured anthropogenic land-use intensity as the sum of the percent areas of crops under irrigation and urban land in the

catchment 5 km upstream of each sampling point (i.e. the intersection between the entire catchment and a 5 km radius buffer centred on the sampling point). These two land uses have been associated with nutrient enrichment and habitat degradation at the scale we studied (Monteagudo *et al.* 2012). Land uses were obtained from the most up-to-date land-use map that predated the time when the invertebrate sample was obtained (Corine Land Cover 1990, 2000 and 2006 layers).

Biological datasets

We obtained community composition data from the sites where water salinity (105 sites) or land-use data were available (102 sites), as indicated above. At each site, the taxonomic composition was obtained by pooling up to four samples that corresponded to different seasons. This approach is adequate to represent the mean annual community, even when samples are collected from only a single season (Bonada *et al.* 2007). We focused our analysis on genera belonging to the main insect orders present in aquatic habitats (i.e. Coleoptera, Ephemeroptera, Hemiptera, Odonata, Plecoptera, and Trichoptera), as genus-level information is sufficiently accurate to study the functional diversity of rivers (Dolédec *et al.* 2000; Gayraud *et al.* 2003). We excluded dipterans due to the paucity of detailed trait information on these species and their complex taxonomy (Pfenninger *et al.* 2007). The groups of insects considered in this analysis show contrasting ecological responses to stress that are associated with different biological traits (Harrison & Dobson 2008), some of which are good surrogates for macroinvertebrate biodiversity in aquatic habitats (Sánchez-Fernández *et al.* 2006; Dijkstra *et al.* 2014).

To characterise the functional properties of the communities, we used a database containing information on 13 biological traits at the genus level (e.g. morphology, life history, aquatic stage, dispersal, resistance forms, respiration, locomotion, and feeding habits) (see Appendix 5.2). Eleven out of these 13 biological traits were obtained from Tachet *et al.* (2002). The other two traits (i.e. body form and the degree of body sclerotisation; see Appendix 5.3), were codified for this study based on taxonomic expertise and published taxonomic keys (Tachet *et al.* 2002). Each genus was coded according to its affinity with each trait category using a fuzzy coding approach that accounted for within-genus variation (Chevenet *et al.* 1994). More details on the environmental and biological datasets used here can be found in Statzner *et al.* (2007).

Data analysis

We employed the following three matrix types to characterise the functional space: a presence/absence taxonomic matrix (hereafter, *sites x taxa*); a matrix containing the fuzzy coded trait profile for each taxon (hereafter, *taxa x traits*); and the resulting community-level trait matrix (hereafter, *sites x traits*) that represents the mean trait profile for each site.

We defined the functional space as a multidimensional Euclidean space where the axes summarised the variability of a subset of relevant traits that responded to both stressors, assuming that some trait features may be necessary to establish and maintain viable populations in stressful habitats. To define this space for each type of stressor, we calculated the Pearson correlation coefficients between trait categories and the stress intensity for each dataset separately using the *sites x traits* matrices. We retained the minimum set of traits with an average absolute coefficient of $R \geq |0.30|$ for both datasets (see Appendix 5.2 and 5.4). Trait selection is necessary in all types of analyses to illustrate functional patterns clearly (Villéger *et al.* 2008). Our aim was to use the same suite of traits to explore the response of functional features to both environmental gradients. Although this suite of traits might be different if only one type of stressor is considered, it is useful to illustrate how similar functional patterns would emerge in response to chronic stress when considering common relevant traits.

The retained set included the number of generations per year, body form, and degree of sclerotisation, to characterise functional niches (see Appendix 5.4). The number of generations per year is positively related to resilience to disturbance (Southwood 1977). Body form and the degree of sclerotisation play a fundamental role in dispersion, as fully sclerotised and robust adults are able to avoid desiccation during flight (Harrison & Dobson 2008). Body form is also related to hydraulic stress (Lamouroux *et al.* 2004), and the degree of sclerotisation is related to resistance against physical and chemical impacts (Hopkins & Kramer 1992).

We developed different R functions (R-Development-Core-Team 2013) to estimate five functional measures based on within-taxon trait information to account for the changes in the functional space at the taxon and at the whole-community levels (see Appendixes 5.5 and 5.6 for details). Mean taxon functional richness and mean functional similarity were calculated at the taxon level, while functional richness, functional dispersion and functional redundancy were quantified at the whole-community level.

The relationship between these functional measures and stressor gradients was tested using generalised linear models (GLMs), assuming a Gaussian distribution of the dependent variables (see Appendix 5.7). We only evaluated linear relationships between dependent and independent variables to test our initial predictions. For the mean functional similarity, we selected sites with a taxonomic richness greater than 1 to avoid potential bias, as the mean functional similarity=1 when taxonomic richness=1. The mean functional similarity and functional redundancy were log-transformed to improve linearity with stressors. The predictors were z-standardised (mean=0 and standard deviation=1) after appropriate transformations (log-transformation for conductivity and arcsin-square-root-transformation for the percentage of land-use intensity).

Null models

A relationship between stress and functional variables can be found simply as a result of an underlying taxonomic richness gradient (Villéger *et al.* 2008; 2011). This uncertainty is due to the probability of finding more or fewer trait categories solely as a consequence of the number of taxa (i.e. sampling effect). To assess the non-randomness of the observed trends, empirical parameters should be distinct from those resulting from relating the stress intensity to functional variables obtained after randomising communities with the same richness gradient and taxon frequency of occurrence but a random composition.

To perform this assessment, we assembled 999 artificial communities in which taxa were drawn randomly from the taxonomic pool for each dataset with fixed row (taxon richness) and column totals (taxon frequencies) (Ulrich & Gotelli 2007). For each simulation, we calculated the mean taxon functional richness, mean functional similarity, community functional richness, functional dispersion, and functional redundancy and re-examined their relationship with the putative stressor to obtain the intercept and slope for each relationship (i.e. using the same procedure as for the empirical data). We examined the null model's statistical significance using an exact two-tailed test to estimate the probability that the observed value would be significantly ($\alpha=0.05$) greater or smaller than the null distribution.

All statistical analyses were performed in R (ade4, plyr and vegan libraries; R-Development-Core-Team 2013).

Results

The models relating functional features to natural and anthropogenic stressors were all significant and showed similar patterns, regardless of the type of stressor (Table 1 and Fig. 1). In general, more deviance was explained in the Salinity dataset models (16.1-71.6%) than in the Land-use dataset models (12.8-40.7%). The functional features that were best explained by stress were community functional richness (Salinity: 71.6%, Land-use: 36.4%) and functional dispersion (Salinity: 69.1%, Land-use: 40.7%), followed by the mean functional similarity (Salinity: 65.1%, Land-use: 19.0%). Stress explained less of the deviance in the mean taxon functional richness (Salinity: 35.9%, Land-use: 12.8%) and functional redundancy (Salinity: 16.1%, Land-use: 22.9%).

Functional features showed similar trends in response to natural and anthropogenic stressors (Fig. 1, Table 1). The mean taxon functional richness and mean functional similarity (both measured at the taxon level) showed positive slopes in relation to stress intensity for the Salinity and Land-use datasets. In contrast, the three features measured at the whole-community level (i.e., community functional richness, functional dispersion and functional redundancy) showed negative slopes in relation to stress intensity in both datasets (Fig. 1).

The empirical model parameters were generally distinct from those obtained from models relating simulated functional attributes to stressors, reflecting non-random changes along natural and anthropogenic stress gradients (Table 2); 9 out of 10 intercepts and slopes for the empirical models were significantly distinct from the null distributions.

For the Salinity dataset, the intercept of the mean taxon functional richness models was indistinguishable from the null distribution (z -score=1.32; P =0.105), whereas for the Land-use dataset, the slope of the functional dispersion (z -score=-1.41; P =0.087) model was not significantly different from the null distribution. For both datasets, the empirical slopes showed significantly higher values for the mean taxon functional richness (Salinity: z -score=4.04, P =0.001; Land-use: z -score=3.37, P =0.001) and mean functional similarity (Salinity: z -score=7.02, P =0.001; Land-use: z -score=2.48, P =0.006), while the slope of the empirical models relating functional richness (Salinity: z -score=-6.42, P =0.001; Land-use: z -score=-1.72, P =0.048) and functional redundancy (Salinity: z -score=8.54, P =0.001; Land-use: z -score=1.75, P =0.049) to stress showed significantly lower values.

Table 1 Results of generalised linear models relating the response of community functional features to natural (i.e. salinity) and anthropogenic (i.e. land use) stressors. The statistical significance of the linear coefficients (P) and the percentage of explained deviance are also shown for each model.

Dataset	Model	P	Explained deviance
<i>Mean taxon functional richness</i>			
Salinity	$y=3.13+0.47x$	<0.001	35.9
Land-use	$y=3.01+0.30x$	<0.001	12.8
<i>Mean functional similarity</i>			
Salinity	$y=-1.53+0.35x$	<0.001	65.1
Land-use	$y=-1.59+0.16x$	<0.001	19.0
<i>Community functional richness</i>			
Salinity	$y=15.06-3.14x$	<0.001	71.6
Land-use	$y=15.69-2.14x$	<0.001	36.4
<i>Functional dispersion</i>			
Salinity	$y=3.38-0.41x$	<0.001	69.1
Land-use	$y=3.50-0.27x$	<0.001	40.7
<i>Functional redundancy</i>			
Salinity	$y=5.47-0.52x$	<0.001	16.1
Land-use	$y=5.27-0.59x$	<0.001	22.9

Salinity dataset: $n=105$; Land-use dataset: $n=102$.

Table 2 Results of two-tailed significance tests examining the differences between the empirical parameters of models that relate functional diversity components to natural (i.e. salinity) and anthropogenic (i.e. land use) stresses and the null distribution of parameters from models relating the same stress gradients to functional features calculated from 999 artificial communities built with fixed row and column totals (see Methods). Z-values (z) and significance (P) are reported.

Model	Mean taxon functional richness		Mean functional similarity		Community functional richness		Functional dispersion		Functional redundancy	
	z	P	z	P	z	P	z	P	z	P
<i>Salinity</i>										
intercept	1.32	0.105	3.09	0.001	-8.20	0.001	-5.55	0.001	7.43	0.001
slope	4.04	0.001	7.02	0.001	-6.42	0.001	-4.54	0.001	8.54	0.001
<i>Land-use</i>										
intercept	3.49	0.001	2.50	0.005	-3.83	0.003	-3.11	0.003	2.60	0.011
slope	3.37	0.001	2.48	0.006	-1.72	0.048	-1.41	0.087	1.75	0.049

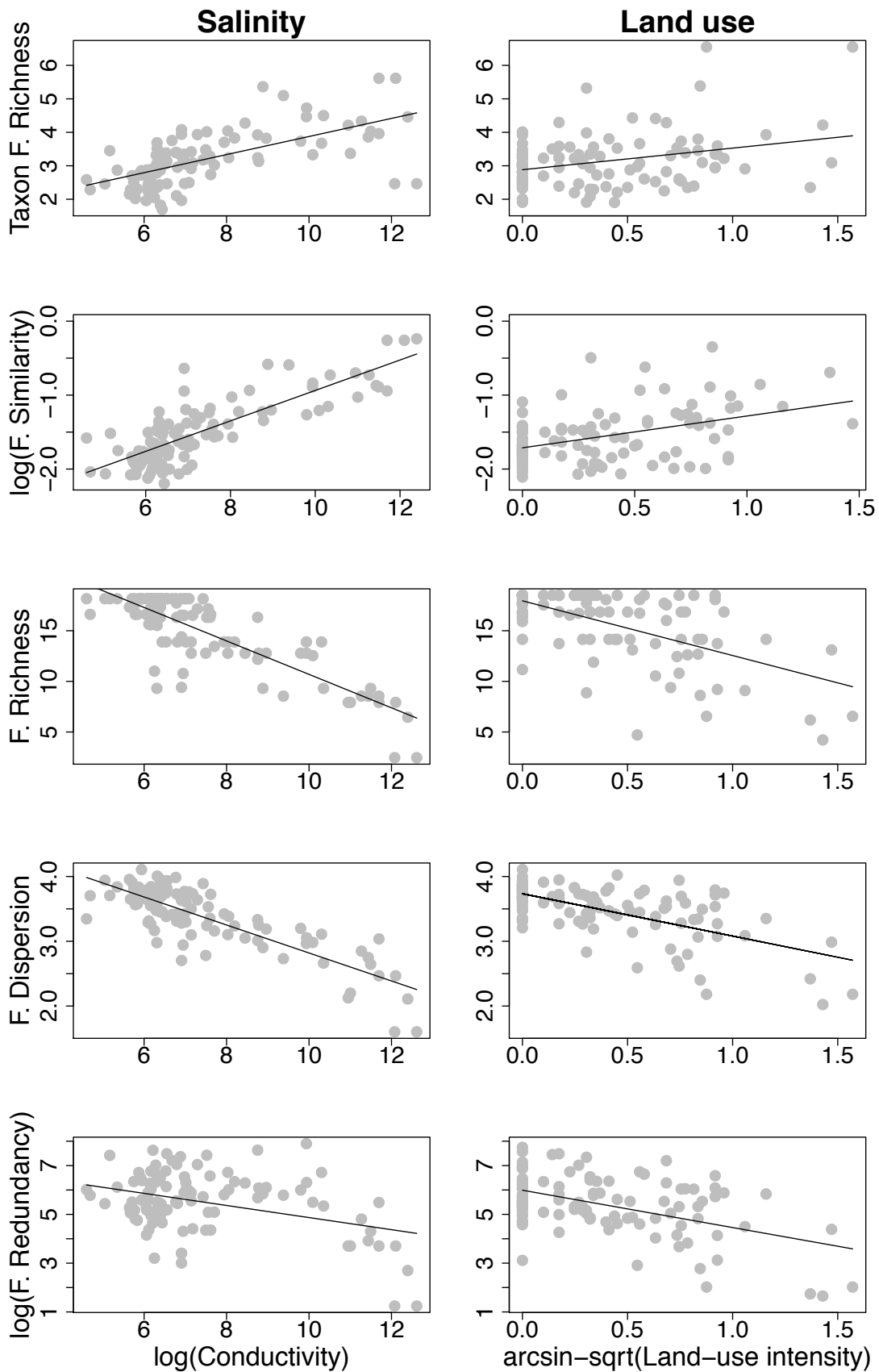


Fig. 1 Plots showing the responses of functional measures to natural (i.e. salinity) and anthropogenic (i.e. land use) stressors. Solid lines represent the fitted values of generalised linear models (see Table 1 for model details). Salinity dataset: $n=105$; Land-use dataset: $n=102$.

Discussion

The functional properties of communities responded similarly along natural and anthropogenic gradients of stress. These changes included a greater mean taxon functional richness and mean functional similarity, and a lower community functional richness and functional redundancy associated with increased stress. These results highlight the importance of deterministic processes of trait filtering during the different stages of colonisation and the response and adaptation to chronic stress over time. The similarity of the responses observed here indicates that the two types of stress constrain functional features of organisms and communities in an analogous manner, regardless of the evolutionary adaptations acquired over geological time, and stress would therefore act as a template that configures trait evolution and community functional properties. Furthermore, this is the first study reporting how the functional features of communities respond along a gradient of natural stress covering a large region.

At the taxon level, we found that both types of stress progressively selected taxa showing more trait variability (higher mean taxon functional richness) and more homogeneous functional traits (higher mean functional similarity). Previous studies have found that trait variability can facilitate adaptation to novel or stressful environments, enhancing ecological tolerance (Badyaev 2005; Scoville & Pfrender 2010; Latta *et al.* 2012). In fact, adaptation to stressful habitats could arise from ecological generalisation (Latta *et al.* 2012), where colonisers would benefit from low predation pressure and low interspecific competition. Similarly, we found higher mean functional similarity among taxa as stress increased. For each trait, habitat filtering reduces overdispersion, producing a convergence toward the optimum value (Shiple *et al.* 2006; Weiher *et al.* 2011). Accordingly, recent studies have found that stress causes functional homogenisation of plants (e.g. Maire *et al.* 2012), fish (e.g. Pool & Olden 2012), and birds (e.g. Luck & Smallbone 2011).

We also found similar trends for functional measures estimated at the whole-community level (community functional richness and functional redundancy) in response to natural and anthropogenic stressors. This result indicates that stress is a factor that constrains community assembly similarly during the colonisation process and over evolutionary time. In benign environments, assembly rules shift over evolutionary time as evolution enhances functional diversification, progressively filling the functional space (Villéger *et al.* 2011). In this study, the stress intensity reduced the range of the functional space filled by the community, due to non-random environmental filtering. This process

sorted the species located in certain parts of the functional space, particularly those occurring at extreme positions along the axes. Hence, for functional dispersion, we found that the slope of the Land-use dataset was indistinguishable from those resulting from null models. Previous studies have reported both random and non-random reductions in functional richness with increased anthropogenic stress. For example, Flynn *et al.* (2009) detected non-random changes in functional richness with land-use intensification for animals but not for plants.

Our results also showed that stress diminished functional redundancy to a greater extent than expected by chance, revealing non-random filtering of taxa with traits placed in the most sensitive regions of the functional space and producing a net reduction in niche overlap among taxa, while the remaining species were progressively more similar (i.e. mean functional similarity increased). Other studies have found a decrease in functional redundancy along anthropogenic stress gradients for plants (e.g. Laliberté *et al.* 2010), soil microbes and invertebrates (e.g. Salminen *et al.* 2001), and aquatic invertebrates (e.g. Schmera *et al.* 2012). Species are able to colonise and adapt to less stressful habitats through trait diversification and speciation, increasing thus functional redundancy over evolutionary time as a result of increasing both the number of species and functional identities (Villéger *et al.* 2011). Conversely, the similarity of the response to modern and historically persistent stressors supports the idea that habitat filtering constrains trait evolution in organisms residing in stressful habitats, resulting in high inter-taxon similarity, as observed here, but reducing the chances for species to colonise or adapt to these environments, resulting in low taxonomic richness (e.g. Gutiérrez-Cánovas *et al.* 2013).

Recent evidence suggests that some lineages are more likely to withstand stressful conditions than expected by chance due to the presence of certain traits that enhance stress tolerance (Garbuz *et al.* 2008; Arribas *et al.* 2014). Therefore, the initial selection of traits is often critical when estimating the functional properties of communities along environmental gradients (Villéger *et al.* 2008), as the choice of unimportant traits can increase the probability of finding random response patterns. First, there is a probability of finding a significant functional pattern in response to environmental change simply as a result of the underlying relationship between taxonomic and functional diversities (e.g. the probability of finding rare traits in depauperated communities can be lower just by chance and not due to environmental filtering). Second, unimportant traits may obscure the functional pattern arising in response to stress, leading to a loss of explanatory power. For these reasons, we selected a suite of traits that are relevant to passing environmental filters along the studied gradients, using

empirical data and mechanistic links. Additionally, we tested the non-randomness of the relationship between functional features and stress using null models where functional features were recalculated using simulated communities with a fixed taxon number and frequency. We found that multivoltinism, a sclerotised body, and flattened and cylindrical forms may allow organisms to colonise and survive in habitats subject to modern sources of stress (Southwood 1977; Buchwalter *et al.* 2008) or stimulate adaptation through exaptation (i.e. a shift in the function of one trait during evolution to cope with novel conditions) over evolutionary time (Arribas *et al.* 2014). The selected traits should be plesiomorphic (i.e. shared within members of the lineage) rather than apomorphic (i.e. an innovation within the lineage) because they are common in most organisms facing stresses of a different nature, including anthropogenic stressors (Southwood 1977; Herbst 2001). For these reasons, we suggest that the similarity in the functional trends found here may arise from analogous assembly rules operating during colonisation and over time.

Here, we demonstrated how chronic stress can be a major driver of non-random changes in the functional features of communities, irrespective of whether the stressor is natural (i.e. persistent over evolutionary time) or anthropogenic (i.e. new for the species pool). Our results suggest that ecosystems under intensified stress will exhibit functionally homogeneous communities composed of taxa showing wider functional niches. Functional homogenisation can have consequences for ecosystem functions, particularly decreases in or a loss of stability of those functions in which stress-sensitive species are involved (Hooper *et al.* 2005). In addition, functionally specialised species are more likely to be lost due to stress intensification (Clavel *et al.* 2011; Pool & Olden 2012). The interaction between the intensification of human activities and the effects of ongoing global change will increase the number and the intensity of novel stressors that ecosystems will face in the near future. The approach employed here can be useful to anticipate to the functional consequences of novel stressors, contributing to making ecology a more predictive science.

Acknowledgements

We would like to thank P. Abellán, P. Arribas, O. Belmar, D. Bruno, J.A. Carbonell, S. Guareschi, S. Pallarés and F. Picazo for their contributions in obtaining and identifying most of the macroinvertebrate samples used in this study. We are also grateful to P. Arribas, C.E. Gray, F.T. Maestre, G.

Woodward, and L. Zhao for their insightful comments on earlier versions of this manuscript. CG-C was partially supported by a pre-doctoral grant from the Fundación Séneca (Agencia Regional de Ciencia y Tecnología, Región de Murcia). DS-F was supported by the “Juan de la Cierva” program of the Spanish Ministry of Economy and Competitiveness. The salinity surveys were financed by the Ministerio de Ciencia e Innovación (Spain) through I+D+I project no. CGL2006- 04159/BOS (AM). NB acknowledges the BioFresh EU-funded project -Biodiversity of Fresh-water Ecosystems: Status, Trends, Pressures, and Conservation Priorities (7th FWP contract No 226874)-.

References

- Arribas, P., Andújar, C., Abellán, P., Velasco, J., Millán, A. & Ribera, I. (2014). Tempo and mode of the multiple origins of salinity tolerance in a water beetle lineage. *Molecular Ecology*, 23, 360–373.
- Badyaev, A.V. (2005). Stress-induced variation in evolution: from behavioural plasticity to genetic assimilation. *Proceedings of the Royal Society B-Biological Sciences*, 272, 877-886.
- Bonada, N. & Dolédec, S. (2011). Do mediterranean genera not included in Tachet et al. 2002 have mediterranean trait characteristics? *Limnetica*, 30, 129-141.
- Bonada, N., Doledec, S. & Statzner, B. (2007). Taxonomic and biological trait differences of stream macroinvertebrate communities between mediterranean and temperate regions: implications for future climatic scenarios. *Global Change Biology*, 13, 1658-1671.
- Bonada, N. & Resh, V. (2013). Mediterranean-climate streams and rivers: geographically separated but ecologically comparable freshwater systems. *Hydrobiologia*, 719, 1-29.
- Buchwalter, D.B., Cain, D.J., Martin, C.A., Xie, L., Luoma, S.N. & Garland, T. (2008). Aquatic insect ecophysiological traits reveal phylogenetically based differences in dissolved cadmium susceptibility. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 8321-8326.
- Chevenet, F., Dolédec, S. & Chessel, D. (1994). A fuzzy coding approach for the analysis of long-term ecological data. *Freshwater Biology*, 31, 295-309.

- Clavel, J., Julliard, R. & Devictor, V. (2011). Worldwide decline of specialist species: toward a global functional homogenization? *Frontiers in Ecology and the Environment*, 9, 222-228.
- Díaz, A.M., Alonso, M.L.S. & Gutiérrez, M.R.V.A. (2008). Biological traits of stream macroinvertebrates from a semi-arid catchment: patterns along complex environmental gradients. *Freshwater Biology*, 53, 1-21.
- Dijkstra, K.D.B., Monaghan, M.T. & Pauls, S.U. (2014). Freshwater Biodiversity and Aquatic Insect Diversification. *Annual Review of Entomology*, 59, 143-163.
- Dolédec, S., Olivier, J.M. & Statzner, B. (2000). Accurate description of the abundance of taxa and their biological traits in stream invertebrate communities: effects of taxonomic and spatial resolution. *Archiv Fur Hydrobiologie*, 148, 25-43.
- Flynn, D.F.B., Gogol-Prokurat, M., Nogeire, T., Molinari, N., Richers, B.T., Lin, B.B. *et al.* (2009). Loss of functional diversity under land use intensification across multiple taxa. *Ecology Letters*, 12, 22-33.
- Garbuz, D.G., Zatssepina, O.G., Przhiboro, A.A., Yushenova, I., Guzhova, I.V. & Evgen'ev, M.B. (2008). Larvae of related Diptera species from thermally contrasting habitats exhibit continuous up-regulation of heat shock proteins and high thermotolerance. *Molecular Ecology*, 17, 4763-4777.
- Gayraud, S., Statzner, B., Bady, P., Haybachp, A., Scholl, F., Usseglio-Polatera, P. *et al.* (2003). Invertebrate traits for the biomonitoring of large European rivers: an initial assessment of alternative metrics. *Freshwater Biology*, 48, 2045-2064.
- Gutiérrez-Cánovas, C., Millán, A., Velasco, J., Vaughan, I.P. & Ormerod, S.J. (2013). Contrasting effects of natural and anthropogenic stressors on beta diversity in river organisms. *Global Ecology and Biogeography*, 22, 796-805.
- Harrison, S.S.C. & Dobson, M. (2008). Evolutionary drivers and the ecological traits of adult aquatic insects. In: *Aquatic insects: challenges to populations* (eds. Lancaster, J & Briers, RA). CAB International Publishing Wallingford, pp. 250-267.
- Herbst, D.B. (2001). Gradients of salinity stress, environmental stability and water chemistry as a templet for defining habitat types and physiological strategies in inland salt waters. *Hydrobiologia*, 466, 209-219.
- Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S. *et al.* (2005). Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecological Monographs*, 75, 3-35.

- Hopkins, T.L. & Kramer, K.J. (1992). Insect cuticle sclerotization. *Annual Review of Entomology*, 37, 273-302.
- Laliberté, E., Wells, J.A., DeClerck, F., Metcalfe, D.J., Catterall, C.P., Queiroz, C. *et al.* (2010). Land-use intensification reduces functional redundancy and response diversity in plant communities. *Ecology Letters*, 13, 76-86.
- Lamouroux, N., Dolédec, S. & Gayraud, S. (2004). Biological traits of stream macroinvertebrate communities: effects of microhabitat, reach, and basin filters. *Journal of the North American Benthological Society*, 23, 449-466.
- Latta, L.C., Weider, L.J., Colbourne, J.K. & Pfrender, M.E. (2012). The evolution of salinity tolerance in *Daphnia*: a functional genomics approach. *Ecology Letters*, 15, 794-802.
- Luck, G.W. & Smallbone, L.T. (2011). The impact of urbanization on taxonomic and functional similarity among bird communities. *Journal of Biogeography*, 38, 894-906.
- MacArthur, R. & Levins, R. (1967). Limiting similarity convergence and divergence of coexisting species. *American Naturalist*, 101, 377-385.
- Maire, V., Gross, N., Boerger, L., Proulx, R., Wirth, C., Pontes, L.d.S. *et al.* (2012). Habitat filtering and niche differentiation jointly explain species relative abundance within grassland communities along fertility and disturbance gradients. *New Phytologist*, 196, 497-509.
- Millán, A., Velasco, J., Gutiérrez-Cánovas, C., Arribas, P., Picazo, F., Sánchez-Fernández, D. *et al.* (2011). Mediterranean saline streams in southeast Spain: What do we know? *Journal of Arid Environments*, 75, 1352-1359.
- Monteagudo, L., Moreno, J.L. & Picazo, F. (2012). River eutrophication: Irrigated vs. non-irrigated agriculture through different spatial scales. *Water Research*, 46, 2759-2771.
- Mouillot, D., Graham, N.A.J., Villegier, S., Mason, N.W.H. & Bellwood, D.R. (2013). A functional approach reveals community responses to disturbances. *Trends in Ecology & Evolution*, 28, 167-177.
- Odum, E.P. (1985). Trends expected in stressed ecosystems. *Bioscience*, 35, 419-422.
- Pfenninger, M., Nowak, C., Kley, C., Steinke, D. & Streit, B. (2007). Utility of DNA taxonomy and barcoding for the inference of larval community structure in morphologically cryptic *Chironomus* (Diptera) species. *Molecular Ecology*, 16, 1957-1968.

- Pool, T.K. & Olden, J.D. (2012). Taxonomic and functional homogenization of an endemic desert fish fauna. *Diversity and Distributions*, 18, 366-376.
- R-Development-Core-Team (2013). R: a language and environment for statistical computing. R Foundation for Statistical Computing Vienna, Austria.
- Salminen, J., van Gestel, C.A.M. & Oksanen, J. (2001). Pollution-induced community tolerance and functional redundancy in a decomposer food web in metal-stressed soil. *Environmental Toxicology and Chemistry*, 20, 2287-2295.
- Sánchez-Fernández, D., Abellán, P., Mellado, A., Velasco, J. & Millán, A. (2006). Are water beetles good indicators of biodiversity in Mediterranean aquatic ecosystems? The case of the segura river basin (SE Spain). *Biodiversity and Conservation*, 15, 4507-4520.
- Scanlon, B.R., Jolly, I., Sophocleous, M. & Zhang, L. (2007). Global impacts of conversions from natural to agricultural ecosystems on water resources: Quantity versus quality. *Water Resources Research*, 43.
- Schmera, D., Baur, B. & Eros, T. (2012). Does functional redundancy of communities provide insurance against human disturbances? An analysis using regional-scale stream invertebrate data. *Hydrobiologia*, 693, 183-194.
- Scoville, A.G. & Pfrender, M.E. (2010). Phenotypic plasticity facilitates recurrent rapid adaptation to introduced predators. *Proceedings of the National Academy of Sciences of the United States of America*, 107, 4260-4263.
- Shipley, B., Vile, D. & Garnier, E. (2006). From plant traits to plant communities: A statistical mechanistic approach to biodiversity. *Science*, 314, 812-814.
- Southwood, T.R.E. (1977). Habitat, templet for ecological strategies - Presidential-address to British-Ecological-Society, 5 January 1977. *Journal of Animal Ecology*, 46, 337-365.
- Stanton, M.L., Roy, B.A. & Thiede, D.A. (2000). Evolution in stressful environments. I. Phenotypic variability, phenotypic selection, and response to selection in five distinct environmental stresses. *Evolution*, 54, 93-111.
- Stubbs, W.J. & Wilson, J.B. (2004). Evidence for limiting similarity in a sand dune community. *Journal of Ecology*, 92, 557-567.

- Tachet, H., Richoux, P., Bournaud, M. & Usseglio-Polatera, P. (2002). *Invertébrés d'eau douce. Systematique, biologie, écologie (2nd corrected impression)*. CNRS éditions, Paris.
- Ulrich, W. & Gotelli, N.J. (2007). Null model analysis of species nestedness patterns. *Ecology*, 88, 1824-1831.
- Villéger, S., Mason, N.W.H. & Mouillot, D. (2008). New multidimensional functional diversity indices for a multifaceted framework in functional ecology. *Ecology*, 89, 2290-2301.
- Villéger, S., Novack-Gottshall, P.M. & Mouillot, D. (2011). The multidimensionality of the niche reveals functional diversity changes in benthic Marine biotas across geological time. *Ecology Letters*, 14, 561-568.
- Violle, C., Enquist, B.J., McGill, B.J., Jiang, L., Albert, C.H., Hulshof, C. *et al.* (2012). The return of the variance: intraspecific variability in community ecology. *Trends in Ecology & Evolution*, 27, 244-252.
- Weiher, E., Freund, D., Bunton, T., Stefanski, A., Lee, T. & Bentivenga, S. (2011). Advances, challenges and a developing synthesis of ecological community assembly theory. *Philosophical Transactions of the Royal Society B-Biological Sciences*, 366, 2403-2413.

Supporting Information

Appendix 5.1 Description of the datasets gathered for this study, including references.

Appendix 5.2 Pearson correlation coefficients between community-weighted biological trait categories expressed as percentages and the stress intensity.

Appendix 5.3 Fuzzy coded affinities of each taxon to biological trait categories. Available online at:

https://www.dropbox.com/s/os2tl412jfsbfdy/Appendix_5.3.xls

Appendix 5.4 Mean absolute Pearson correlation coefficients between trait categories and the stress intensity for each trait.

Appendix 5.5 Procedure used to calculate functional niche features.

Appendix 5.6 R functions for estimating functional measures using fuzzy coded trait data. Available online at:

https://www.dropbox.com/s/zisopktozg5q7c4/Appendix_5.6.R

Appendix 5.7 R script for studying the effect of stressors on functional measures. Available online at:

https://www.dropbox.com/s/xc6inm0g28vtwry/Appendix_5.7.R

Appendix 5.1 Description of the datasets gathered for this study, including references.

Dataset	Altitude (m.a.s.l.)	Channel width (m)	Conductivity ($\mu\text{S cm}^{-1}$)	Stream flow	Land-use
Salinity	< 1200	< 10	100-300,250	Permanent	Natural or semi-natural
Land-use intensification	< 1200	< 40	109-2,450	Permanent	From natural to intensified agricultural and urban catchments

References

- Alba-Tercedor, J., Capitán-Vallvey, L.F. & Espigares-García, M. (1990). *Estudio de las condiciones ecológicas, sanitarias, químicas y de calidad de las aguas de la cuenca media-alta del río Guadalquivir*. Report to the Dirección General de Obras Hidráulicas, Confederación Hidrográfica del Guadalquivir, Granada.
- Alba-Tercedor, J. & Jiménez-Millán, F. (1987). *Evaluación de las variaciones de la calidad de las aguas del Río Guadalfeo basada en el estudio de las comunidades de macroinvertebrados acuáticos y de los factores físico-químicos*. LUCDEME III. Monografía 48 del ICONA., Granada.
- Bonada, N., Rieradevall, M. & Prat, N. (1996). Biodiversitat y qualitat de l'aigua a la riera de Sant Cugat (Collserola, Barcelona). In: *I Jornades sobre la recerca en els sistems naturals de Collserola: aplicacions a la gestió del Parc* (eds. Limona, F, Espelta, JM, Guix, JC, Mateos, E & Rodríguez-Tejeiro, JD). Consorci Parc de Collserola Barcelona, pp. 45-49.
- Ferrito, V. (1994). Les macroinvertebrés benthiques de la rivièrè Simeto (Sicile) et de quelques-uns de ses afluent. *Ann. Limnol.*, 33-56.
- Gallardo, A. & Toja, J. (1989). Efecto de la contaminación orgánica en los macroinvertebrados acuáticos en la cuenca del río Guadaira (Sevilla, SW España). In: *Actas Colóquio Luso-Espanhol Ecología Bacias Hidrográficas Recursos Zoológicos* (ed. Machado-Cruz, J). Instituto de Zoologia e estação de zoologia marítima Dr. Augusto Nobre, Faculdade de Ciências, Universidade do Porto Porto, pp. 163-170.
- Gallardo-Mayenco, I. (1993). *Respuesta de macroinvertebrados Xuviales a la salinidad. Comparación de las cuencas de los río Guadaira y Guadalete*. PhD Thesis, Universidad de Sevilla, Sevilla.
- Gumiero, B. (1993). *Analisi degli effetti del disturbo di uno sbarramento artificiale sulla comunita' di macrozoobentos in due corsi idrici dell'Apennino Tosco-Emiliano*. PhD Thesis, Università di Bologna Bologna.

- Moreno, J.L. (2002). *Hábitats, recursos tróficos y estructura de la comunidad de macroinvertebrados bentónicos en un arroyo salino del Sureste Ibérico (Rambla del Reventón)*. PhD Thesis, Universidad de Murcia, Murcia.
- Moreno, J.L., Millán, A., Suárez, M.L., Vidal-Abarca, M.R. & Velasco, J. (1997). Aquatic Coleoptera and Heteroptera assemblages in waterbodies from ephemeral coastal streams ("ramblas") of south-eastern Spain. *Archiv fur Hydrobiologie*, 141, 93-107.
- Ortega, M., Suárez, M.L., Vidal-Abarca, M.R., Gómez, R. & Ramírez-Díaz, L. (1991). Aspect of postflood recolonization of macroinvertebrates in a "Rambla" of south-east Spain ("Rambla del Moro": Segura River Basin). *Verh. Int. Ver. Th*, 24, 1994-2001.
- Picazo-Muñoz, J. (1995). *Caracterización y calidad de las aguas de los cauces de la cuenca del Río Guadiana Menor. Aspectos físico-químicos y macroinvertebrados acuáticos*. PhD Thesis, Universidad de Granada, Granada.
- Rieradevall, M. & Prat, N. (1997). *La qualitat biològica de les aigües del Parc Natural de Sant Llorenç del Munt i la Serra de l'Obac*. Barcelona.
- Solá, C. (2004). *Impacte de l'accident miner d'Aznalcóllar sobre el riu Guadiamar. Recuperació de la comunitat de macroinvertebrats i bioacumulació de metalls pesants*. PhD Thesis, Universitat de Barcelona, Barcelona.

Appendix 5.2 Pearson correlation coefficients between community weighted biological trait categories expressed as percentages and the stress intensity.

Biological trait	Category	log-Conductivity	arcsin-sqrt-Land-use intensity
Maximal potential size (mm)	≤ 2.5	0.73	0.21
	> 2.5-5	0.17	0.28
	> 5-10	0.07	-0.05
	> 10-20	-0.65	-0.16
	> 20-40	-0.63	-0.42
	> 40-80	-0.07	-0.30
Life cycle duration (year)	≤ 1	-0.72	-0.02
	> 1	0.72	0.01
Reproductive cycles (year-1)	<1	-0.70	-0.42
	1	-0.75	-0.42
	> 1	0.82	0.49
Aquatic stages	egg	-0.84	0.05
	larva	-0.74	-0.06
	nymph	-0.70	-0.47
	adult	0.87	0.20
Reproduction	ovoviviparity	0.09	0.12
	isolated eggs, free	-0.42	0.31
	isolated eggs, cemented	-0.80	0.11
	clutches, cemented or fixed	-0.17	-0.17
	clutches, free	-0.4	-0.16
	clutches, in vegetation	0.28	-0.07
	clutches, terrestrial	0.62	0.01
Dispersal	aquatic passive	-0.34	0.11
	aquatic active	-0.63	-0.22
	aerial passive	-0.75	0.00
	aerial active	0.77	0.02
Resistance forms	eggs, statoblasts	-0.72	0.16
	cocoons	-0.22	-0.18
	diapause	0.12	0.14
	none	0.43	-0.16
Respiration	tegument	-0.84	-0.37
	gill	-0.87	-0.07
	plastron	0.84	-0.06
	spiracle	0.51	0.20
	hydrostatic vesicle	0.55	0.27

Locomotion substrate relation	and flier	0.85	0.28
	surface swimmer	-0.11	-0.04
	full water swimmer	0.47	0.34
	crawler	-0.75	-0.21
	burrower	0.55	-0.24
	interstitial	-0.71	0.14
	temporarily attached	-0.64	-0.30
	permanently attached	-0.25	-0.19
Food	microorganisms	0.63	0.28
	detritus < 1mm	-0.62	-0.06
	dead plant >= 1mm	0.22	0.06
	living microphytes	-0.21	-0.24
	living macrophytes	-0.13	-0.16
	dead animal >= 1mm	-0.17	0.03
	living microinvertebrates	0.45	0.29
	living macroinvertebrates	0.09	0.05
	vertebrates	-0.10	-0.07
Feeding habits	shredder	-0.66	0.29
	scraper	-0.20	-0.27
	filter-feeder	0.14	-0.23
	piercer	-0.57	-0.09
	predator	0.27	0.20
	parasite	0.24	0.03
Body form*	flattened	-0.20	0.53
	cylindrical	0.77	-0.25
	streamlined	-0.85	-0.37
Sclerotization degree*	slight	-0.60	-0.57
	partial	0.63	0.12
	full	0.15	0.45

Appendix 5.4 Mean absolute Pearson correlation coefficients between trait categories and the stress intensity for each trait. In bold, the average absolute coefficients $R \geq |0.30$.

Biological trait	log- Conductivity	arcsin-sqrt-Land-use intensity
Maximal potential size	0.39	0.24
Life cycle duration	0.72	0.01
Reproductive cycles	0.75	0.44
Aquatic stages	0.79	0.20
Reproduction	0.40	0.14
Dispersal	0.63	0.09
Resistance forms	0.37	0.16
Respiration	0.72	0.20
Locomotion and substrate relation	0.54	0.22
Food	0.29	0.14
Feeding habits	0.35	0.18
Body form	0.61	0.38
Sclerotization degree	0.46	0.38

Appendix 5.5 Procedure used to calculate functional niche features.

First, we performed a principal components analysis (PCA) using the *taxa x traits* matrix to obtain a set of uncorrelated axes that represent the variability of the trait categories contained in the raw matrix (Fig. S1a). To do this, we selected the significant axes resulting from applying the "Broken Stick rule" (Jackson 1993). We retained four axes in both datasets, representing 90.2% and 90.1% of the variance for the Salinity and Land-use trait datasets, respectively. These axes constituted the dimensions of the functional space in which we quantified functional measures. Second, we built several simulated binary matrices with one category per trait, following a random sampling based on the probability that each category be present in a randomly generated, simulated individual belonging to that genus (i.e. trait fuzzy code profile, expressed as a percentage of occurrence) (Fig. S1b). These matrices were projected onto the generated functional space to create clouds of points that simulate the suite of potential trait combinations that comprise inter- and intra-genus functional variability (Fig. S1c). This procedure was repeated several times to produce between 3 to 2000 points per genus for examining potential asymptotic behaviour along this gradient. We selected the minimum number of simulated points that achieved a Pearson correlation coefficient of $R \geq 0.95$ using the functional niche descriptors calculated with 2000 points per taxon (assumed as a sufficient number to represent the full species variability). In our case, 25 simulated points were enough to reach the proposed criteria in both datasets. Finally, once we obtained points representing the different trait combinations in each community and a defined functional space, we calculated taxon functional richness, functional similarity, functional richness, and functional redundancy as the areas between pairs of axes and functional dispersion as the average distance to the community centroid for each pair of axes (Laliberte & Legendre 2010). We estimated the taxon niche as the functional space filled by all the simulated points projected for all axis pairs, representing the taxon functional richness (Fig S1d). Taxon functional richness was estimated as the average of the taxon functional richness of each community. Functional similarity was obtained by averaging the percentage of inter-taxon niche overlap for all taxon pairs in each axis pair for each community (Fig. S1e). Functional richness was estimated as the functional space filled by the points of all of the taxa present at each community for all axis pairs (Fig. S1f). Functional dispersion (Laliberté and Legendre 2010) was estimated by averaging the Euclidean distance from each simulated point to the community centroid (Fig. S1g). Functional redundancy was calculated as the sum of the overlapping niche regions for all taxon pairs in each axis pair at each site (Fig. S1h). This is a new measure of functional redundancy that eliminates the necessity of making a previous

functional classification of data, which may involve information loss (Villéger *et al.* 2008).

References

Laliberte, E. & Legendre, P. (2010). A distance-based framework for measuring functional diversity from multiple traits. *Ecology*, 91, 299-305.

Jackson, D.A. (1993). Stopping rules in principal component analysis: a comparison of heuristical and statistical approaches. *Ecology*, 74, 2204-2214.

Villéger, S., Mason, N.W.H. & Mouillot, D. (2008). New multidimensional functional diversity indices for a multifaceted framework in functional ecology. *Ecology*, 89, 2290-2301.

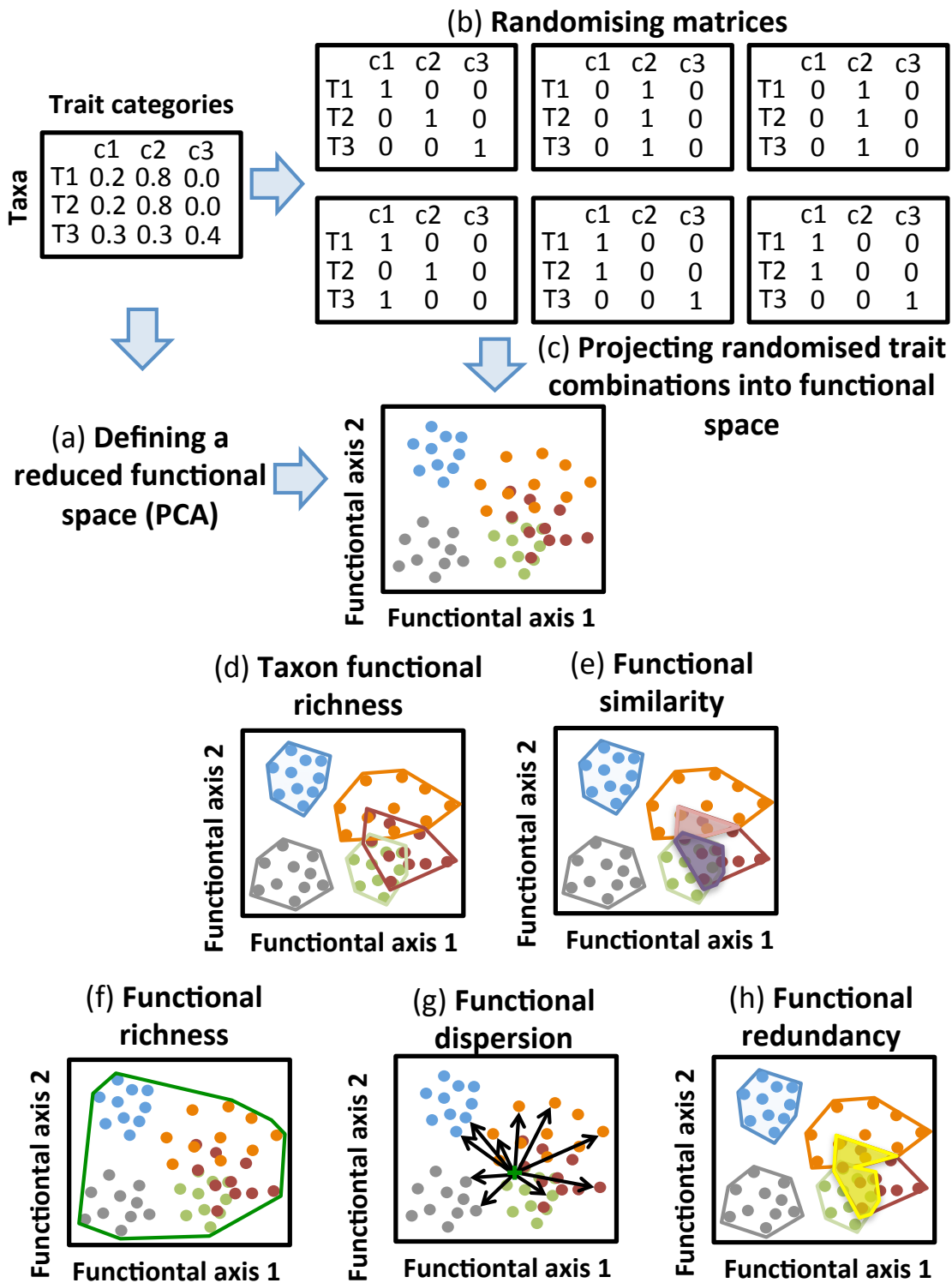


Fig. S1 Procedure used to calculate functional niche features.

General conclusions

Chapter 1

1. Metabolic rates were best explained by climatic variables, with higher rates presented during spring and summer.
2. Conductivity and discharge accounted for changes in the biomass of primary producers and consumers, which peaked during the diluted period.
3. Epipellic biofilm biomass was the only variable showing significant differences between diluted and recovered periods, and acted as a good indicator of pulse dilution stress for saline streams.

Chapter 2

4. Pulse dilution increased predator biomass but had no significant effect on production : respiration ratios (P/R).
5. Chronic dilution significantly decreased P/R values as well as consumer and producer biomasses.
6. The relative importance of the different functional feeding groups was unaffected by either pulse or chronic dilution stress.
7. Isotopic analyses revealed that macroinvertebrates relied upon aquatic primary producers in both reaches, despite the high common reed inputs at the *chronically disturbed reach*.
8. Pulse disturbances had minor effects on ecosystem function, while chronic dilution produced biomass depletion and a change from an autotrophic to a heterotrophic ecosystem.
9. P/R , and primary producer and consumer biomasses are good indicators of chronic dilution stress for saline streams.

Chapter 3

10. Expert-based and biological classification yielded similar stream classes, while environmental classification displayed important discrepancies.
11. Biological classification showed the highest classification strength for Spanish data, while expert-based evaluations displayed the highest values for Moroccan and Italian data. Environmental classification presented the poorest classification strength in almost all cases.
12. Novel indicators suggested in this study (fams.deg) worked much better at detecting ecological integrity and anthropogenic degradation than the conventional metrics (e.g. IBMWP, ICM-11a, EPT, family richness).
13. The results of this study will improve the capacity of environmental managers to assess the ecological status of Mediterranean watercourses, especially for saline streams.

Chapter 4

14. Stress intensity accounted for most of the variability in species richness, which declined with increasing stress.
15. Dissimilarity in community composition among locations increased with the difference in stress intensity for all datasets. For natural stressors, beta-diversity patterns mainly reflected species turnover, whilst for anthropogenic stressors beta diversity mainly reflected nesting of subsets of species as stress intensity increased.
16. Our results support the hypothesis that natural and anthropogenic stressors generate contrasting patterns in beta diversity that arise through different mechanisms.

Chapter 5

17. Similar functional responses to natural and anthropogenic stressors were found.
18. In both stress gradients studied, mean taxon functional richness and similarity increased with stress, whereas community functional richness and redundancy decreased.
19. Our results suggest that communities may display predictable functional changes in response to novel chronic stressors that ecosystems will face in the future, which can increase our ability to predict biological responses to environmental change.