Departamento de Ecología e Hidrología
Facultad de Biología
Universidad de Murcia

Use of Iberian water beetles in biodiversity conservation

Uso de los coleópteros acuáticos ibéricos en la conservación de la biodiversidad

Tesis doctoral

David Sánchez Fernández

2008

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Memoria presentada para optar al grado de doctor en Biología por el licenciado

David Sánchez Fernández

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E HIDROLOGÍA

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Que la Tesis Doctoral titulada "Uso de los coleópteros acuáticos ibéricos en la conservación de la biodiversidad", ha sido realizada por D. David Sánchez Fernández, bajo la inmediata dirección y supervisión de D. Andrés Millán Sánchez, D. Ignacio Ribera Galán y Dª Josefa Velasco García, y que el Departamento ha dado su conformidad para que sea presentada ante la Comisión de Doctorado.

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La presentación de la Tesis Doctoral titulada "Uso de los coleópteros acuáticos ibéricos en la conservación de la biodiversidad", realizada por D. David Sánchez Fernández, bajo mi inmediata dirección y supervisión, en el Departamento de Ecología e Hidrología, y que presenta para la obtención del grado de Doctor por la Universidad de Murcia.

En Murcia, a 9 de abril de 2008

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***"Si Dios es el autor de todas las criaturas,
hay que reconocer que siente una extraordinaria
simpatía por los escarabajos".***

John B. S. Haldane

A mis padres

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INTRODUCCIÓN

Actualmente, uno de los principales problemas ambientales es la aceleración en la tasa de extinción de especies asociada con actividades humanas, hecho que provoca una pérdida irreversible de información biológica y que puede tener consecuencias impredecibles (Kerr y Currie, 1995; Purvis y Hector, 2000). En este sentido, la conservación de la diversidad biológica ha llegado a convertirse en una preocupación global y un objetivo ineludible. Esta circunstancia se ha visto plasmada en la Convención sobre Diversidad Biológica (United Nations Environment Programme, 2005).

Existe un amplio consenso científico en el reconocimiento del alto grado de amenaza al que está sometida la biodiversidad acuática, por encima de otros ecosistemas (Allan y Flecker, 1993; Master *et al.*, 1998; Ricciardi y Rasmussen, 1999). Además, durante las próximas décadas, probablemente aumentaran las presiones humanas sobre los recursos acuáticos, poniendo todavía más especies en peligro (Strayer, 2006). La pérdida de biodiversidad acuática es especialmente preocupante en el caso de la Península Ibérica, un área de alto interés biogeográfico, reconocida como una de las regiones europeas más interesantes en términos de diversidad de especies (Médail y Quézel, 1999; Domínguez-Lozano *et al.*, 1996; Reyjol *et al.*, 2007). Esta región presenta una amplia diversidad de ecosistemas acuáticos, abarcando desde arroyos de cabecera, pasando por pozas, humedales y ramblas hipersalinas, hasta salinas interiores y costeras. Muchos de estos ecosistemas son únicos tanto por la presencia de especies raras y endémicas como por sus características ecológicas (Ribera, 2000; Ribera *et al.*, 2003; Gómez *et al.*, 2005).

Por otro lado, el paisaje en esta región ha estado sometido a una fuerte influencia humana durante cientos de años, dando lugar a una progresiva pérdida de especies y hábitats acuáticos. La transformación de los tradicionales paisajes agrícolas extensivos a agricultura intensiva se ha acelerado en las últimas décadas, y la actual expansión de tierras irrigadas en esta zona está incrementando las demandas de agua para uso agrícola, dando lugar a la reducción de caudales naturales en ríos y arroyos, sobreexplotación de acuíferos, pérdida de fuentes y la disminución de reservas de agua en embalses (Martínez-Fernández y Esteve, 2005). A pesar de estos evidentes cambios, tan rápidos como destructivos, la biodiversidad acuática goza de una prioridad muy baja en iniciativas de conservación llevadas a cabo por organizaciones gubernamentales, tanto a escala nacional como internacional (Balmford *et al.*, 2002; Saunders *et al.*, 2002).

Así, la conservación de los ecosistemas y la biota acuática en la Península Ibérica se ha convertido en una delicada y urgente tarea. En este sentido, se hace necesario identificar aquellas áreas con alta biodiversidad y/o que albergan especies amenazadas con el objetivo de asignar prioridades de conservación (Margules y Pressey 2000; Moore *et al.*, 2003). Para intentar aproximarnos a algo tan complejo como es la medida de la biodiversidad, una de las estrategias más utilizadas es el uso de indicadores o sustitutos de biodiversdidad. Entre estos, se suelen utilizar medidas de biodiversidad a escala amplia (datos climáticos y de tipos de vegetación), características del hábitat (naturalidad o tipismo), rangos taxonómicos altos (géneros o familias) o taxones indicadores (Noss, 1990; Williams, 1996). En este último caso, se utilizan grupos con taxonomía bien conocida que han sido suficientemente estudiados en un área determinada, asumiendo que sus patrones de riqueza, rareza y endemicidad son similares a los del resto de grupos menos conocidos (Reyers y Jaarsveld, 2000).

Con frecuencia, los invertebrados acuáticos han sido utilizados como indicadores del estado ecológico o la calidad de los hábitats, sobre todo con relación al enriquecimiento por nutrientes o la presencia de determinados contaminantes (Wright *et al.*, 2000). Sin embargo, se ha prestado poca atención a la identificación de posibles taxones indicadores de biodiversidad acuática (Paszkowski y Tonn 2000; Heino 2002; Briers y Biggs 2003). Para identificar áreas prioritarias de conservación, tradicionalmente se han utilizado plantas y/o vertebrados, especialmente aves, mientras que los artrópodos han sido ignorados sistemáticamente en los estudios (Posadas et al., 2001; Serrano, 2002), a pesar de que representan sobre el 95% de todas las especies de fauna conocidas (Hull *et al.*, 1998; Palmer, 1999; Sluys, 1999). Dentro de los invertebrados acuáticos, los coleópteros son uno de los grupos más ricos. Se estima que podría haber actualmente en la tierra sobre unas 18.000 especies de coleópteros acuáticos (Jäch y Balke, 2008), siendo uno de los grupos más útiles para clasificar ecosistemas acuáticos en función de su interés de conservación (Jeffries, 1988; Foster *et al.*, 1990). Los coleópteros acuáticos constituyen potencialmente un grupo ideal para ser usado como indicadores de biodiversidad ya que cumplen muchos de los criterios propuestos en la literatura para la selección de este tipo de taxones indicadores (Noss, 1990; Pearson y Cassola, 1992; Pearson, 1994). A pesar de esto, se hace necesario elaborar test formales para evaluar si los potenciales grupos indicadores reflejan el conjunto de la biodiversidad acuática.

En este contexto, es imprescindible el uso de taxones indicadores adecuados para seleccionar áreas para la conservación de la biodiversidad acuática, que puedan medir, de esta manera, la efectividad de las áreas protegidas existentes en la conservación de la biodiversidad acuática e identificar vacíos de protección, es decir, áreas que están fuera de los espacios protegidos y que son interesantes para la conservación de la biodiversidad acuática (Scott *et al.*, 1993). Este último objetivo es crucial, ya que la designación de áreas protegidas ha estado basada históricamente en criterios de oportunismo o *ad hoc*, y por otro lado, los esfuerzos para conservar la biodiversidad acuática han sido escasos, creándose muy pocas áreas para conservarla específicamente (Saunders *et al.*, 2002).

Para proporcionar información científica sobre patrones y procesos de biodiversidad basados en estos taxones indicadores que permitan desarrollar estrategias de conservación adecuadas, es necesario disponer de bases de datos de calidad (Prendergast *et al.*, 1993; Soberón y Peterson, 2004; Guralnick *et al.*, 2007; Hortal *et al.*, 2007). Sin embargo, solo determinados países con suficientes recursos y con una larga tradición naturalista son capaces de producir buenos mapas de distribución para varios grupos taxonómicos basados en el desarrollo de muestreos suficientes (Lawton *et al.*, 1994; Griffiths *et al.*, 1999). Este no es el caso de los países mediterráneos, como España, en los que los inventarios para muchos de los grupos faunísticos, especialmente insectos, son incompletos o inexistentes (Ramos *et al.*, 2001), apareciendo vacíos importantes cuando se representa en un mapa toda la información disponible de especies de insectos. Esto es especialmente evidente cuando se representan amplias escalas espaciales. Este inconveniente puede ser solventado empleando métodos de modelado estadístico, que se basan en la información de áreas con inventarios fiables y, a partir de estas, son capaces de predecir determinados atributos de biodiversidad en el resto del territorio, (Hortal *et al.*, 2001; Ferrier, 2002; Lobo y Martín-Piera, 2002; Hortal *et al.*, 2004; Ferrier y Guisan, 2006; Lobo, 2008). Sin embargo, la incompleta cobertura tanto geográfica como ambiental de estas áreas adecuadamente muestreadas puede comprometer la utilidad de los modelos predictivos basados en ellas (Hortal y Lobo, 2006). Por lo tanto, se hace necesario incorporar en los estudios de biodiversidad tanto medidas del esfuerzo de muestreo como estimas de los posibles sesgos en el muestreo, con el objetivo de poder discriminar las áreas poco muestreadas de las que tienen inventarios fiables (Romo *et al.*, 2006).

Otras estrategias usadas con frecuencia para establecer prioridades de conservación, son aquellas basadas en la protección de especies amenazadas. En este sentido, los artrópodos (acuáticos o no) han recibido tradicionalmente escasa protección legal, presumiblemente debido a su reducido tamaño y a la relación evolutiva lejana con los humanos (Metrick y Weitzman, 1996), pero indudablemente también debido a la dificultad para categorizar sus especies con sistemas tradicionales de análisis de vulnerabilidad o riesgo de extinción (Samways, 1994; New, 1999). La falta generalizada de atención en protección se hace evidente a partir del número desproporcionadamente bajo de insectos listados como amenazados. Por ejemplo, sólo 623 especies de insectos aparecen como amenazados en la Lista Roja de la UICN (Unión Internacional para la Conservación de la Naturaleza; IUCN, 2006). Así, mientras que la efectividad de la protección legal directa para pequeños invertebrados puede ser debatida (Hutchings y Ponder, 1999; New y Sands, 2003), en la situación actual, la única forma de protección posible para estas especies es que éstas aparezcan en áreas protegidas, que normalmente han sido diseñadas en función de la presencia de determinados hábitats o taxones (principalmente vertebrados). Por lo tanto, es necesario evaluar la efectividad de las redes de reservas existentes (como la Red Natura 2000) en la protección de especies amenazadas de grupos no carismáticos como son los coleópteros acuáticos.

Objetivos

Considerando todo lo expuesto hasta ahora, el principal objetivo de esta tesis ha sido determinar el estado de conservación de la biodiversidad acuática en la Península Ibérica e Islas Baleares utilizando inventarios de escarabajos acuáticos. De esta manera, la presente tesis doctoral aborda distintas estrategias y metodologías desde una doble perspectiva, estableciendo prioridades de conservación tanto para especies como para espacios. Así, los objetivos específicos de la tesis fueron:

- Evaluar si los coleópteros acuáticos pueden ser utilizados como buenos indicadores de biodiversidad en ecosistemas acuáticos mediterráneos.

- Seleccionar áreas para la conservación de la biodiversidad acuática usando coleópteros acuáticos como indicadores de biodiversidad a escala regional.

- Compilar una base de datos de coleópteros acuáticos ibéricos y evaluar tanto el esfuerzo de muestreo desarrollado, como el grado de cobertura geográfica de los datos y, por último, estimar la cantidad y naturaleza de los posibles sesgos en la compilación de esta base de datos.

- Obtener una función basada en variables ambientales y espaciales capaz de predecir la distribución de la riqueza de especies en la Península Ibérica e Islas Baleares.

- Identificar las especies endémicas de coleópteros acuáticos más amenazadas de la Península Ibérica e Islas Baleares, y evaluar la efectividad de redes de reservas existentes (Natura 2000) en la protección de estas especies.

Estructura de la tesis

Esta tesis se basa en cinco artículos, cada uno de los cuales constituye un capítulo. Los capítulos 1 y 2 ya han sido publicados, los capítulos 3 y 5 están actualmente en prensa para ser publicados en revistas internacionales incluidas en el SCI, mientras que el capítulo 4 ha sido recientemente enviado a una revista de las mismas características. Así, la tesis está basada en los siguientes capítulos:

Capítulo 1	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.
Capítulo 2	Sánchez-Fernández D, Abellán P, Velasco J, Millán A. 2004. Selecting areas to protect the biodiversity of aquatic ecosystems in a semiarid Mediterranean region using water beetles. *Aquatic Conservation: Marine and Freshwater Ecosystems*. 14, 465-479.
Capítulo 3	Sánchez-Fernández D, Lobo JM, Abellán P, Ribera I, Millán A. 2008. Bias in freshwater biodiversity sampling: the case of Iberian water beetles. *Diversity and Distributions*. En prensa. DOI: 10.1111/j.1472-4642.2008.00474.x.
Capítulo 4	Sánchez-Fernández D, Lobo JM, Abellán P, Millán A. Assessing models for forecasting species richness of Iberian water beetles. (Enviado).
Capítulo 5	Sánchez-Fernández D, Bilton DT, Abellán P, Ribera I, Velasco J, Millán A. Are the endemic water beetles of the Iberian Peninsula and the Balearic Islands effectively protected? *Biological Conservation*. En prensa. DOI: 10.1016/jbiocon.2008.04.05

En el capítulo 1, se evaluaron los escarabajos acuáticos como potenciales indicadores de biodiversidad en ecosistemas acuáticos en una región mediterránea semiárida, la Cuenca del Río Segura (SE España). El valor indicador de los coleópteros se investigó examinando el grado en el que la riqueza de especies de este grupo se correlaciona con la riqueza de otros grupos de macroinvertebrados (plecópteros, tricópteros, moluscos, heterópteros y efemerópteros), así como a través de la evaluación de la eficiencia de las redes de reservas basadas en coleópteros acuáticos (seleccionadas por complementariedad) en la representación de la riqueza del resto de grupos. También se examinó si la riqueza de taxones altos de coleópteros acuáticos era capaz de predecir el conjunto de la riqueza de especies en ecosistemas acuáticos.

Una vez comprobado que los coleópteros acuáticos pueden ser utilizados como indicadores de biodiversidad acuática, en el capítulo 2 se seleccionaron las áreas prioritarias para la conservación de la biodiversidad acuática usando los coleópteros como indicadores en una región mediterránea semiárida, en este caso la Región de Murcia, de la que se dispone de información fiable para este taxón indicador. Esta selección de áreas, permitió detectar vacíos en la red de Espacios Naturales Protegidos (ENPs), a través de la superposición cartográfica de las áreas seleccionadas como prioritarias y los ENPs actualmente reconocidos o propuestos en la provincia de Murcia.

En el capítulo 3, se construyó una base de datos exhaustiva de coleópteros acuáticos ibéricos, con lo que se aumentó considerablemente la escala de trabajo. Analizando esta base de datos, se intentó determinar si estos datos pueden representar una imagen no sesgada de la diversidad y distribución de este grupo de especies. En primer lugar, se examinó la distribución del esfuerzo de muestreo, y se identificaron las áreas que se pueden considerar como adecuadamente muestreadas. También se estimó si estas áreas cubren las diferentes subregiones biogeográficas ambientales actualmente reconocidas en la Península Ibérica e Islas Baleares. Luego, se evaluó si grupos de variables ambientales, espaciales o relacionadas con el efecto de atracción ejercido por las áreas son capaces explicar los posibles sesgos en el muestreo. Finalmente, se identificaron las áreas clave donde se deberían concentrar futuros programas de muestreo.

Usando la misma base de datos del capítulo 3, y teniendo en cuenta algunos inconvenientes en los datos identificados en ese capítulo (datos relativamente escasos y sesgados), el objetivo general del capítulo 4 fue obtener una función basada en variables espaciales y ambientales capaz de predecir la distribución de la riqueza de especies en la Península Ibérica e Islas Baleares. En primer lugar se identificaron aquellas cuadrículas con inventarios relativamente bien muestreados atendiendo a diferentes valores de “completeness” (como de completos) de los mismos. Por otro lado, se utilizó como variable dependiente de las funciones predictivas los valores de riqueza de especies tanto estimados, a partir de curvas de acumulación, como los observados. Después de comparar la actuación de los modelos predictivos en estos diferentes escenarios, se generó un mapa predictivo y se describió la distribución de la riqueza de especies de coleópteros acuáticos para toda la Península Ibérica. Por último, se localizaron áreas insuficientemente muestreadas pero con elevada riqueza predicha, ya que estas áreas deben ser aquellas en las que los futuros muestreos serán más rentables.

En el Capítulo 5 se evaluó si las especies endémicas de coleópteros acuáticos ibéricas amenazadas están protegidas por la Red Natura 2000. En primer lugar, se identificaron los coleópteros acuáticos endémicos más amenazados en el área de estudio y se clasificaron de acuerdo con su vulnerabilidad o grado de amenaza. Posteriormente, se localizaron puntos calientes para estas especies amenazadas. Por último, se evaluó y discutió si la Red Natura 2000 es efectiva a la hora de proteger tanto a estas especies como a las áreas.

METODOLOGÍA

En el Capítulo 1 se seleccionaron cuarenta estaciones de muestreo que representaban la variedad de hábitats tipo de la Cuenca del Río Segura de las cuales existía información fiable para seis grupos de macroinvertebrados: coleópteros, heterópteros, moluscos, tricópteros, efemerópteros y plecópteros. Se utilizaron correlaciones de Spearman para evaluar las relaciones entre los patrones de riqueza de estos seis grupos de macroinvertebrados, así como para evaluar las relaciones entre la riqueza de cada grupo y un parámetro conocido como RR (Resto de Riqueza). Este parámetro se calcula como la riqueza total de macroinvertebrados (número de especies de los seis grupos) a la que se le resta el número de especies que aporta el grupo que estamos examinando. Por otro lado, se seleccionó una red de reservas en función de cada grupo indicador aplicando un algoritmo iterativo basado en el principio de la complementariedad. Finalmente, se calculó el porcentaje del RR y el número total de especies de cada grupo que se incluyen en cada una de las selecciones propuestas para cada taxon indicador, como una medida de su efectividad a la hora de proteger la biodiversidad acuática. Las correlaciones de Spearman también se usaron para evaluar si la riqueza de niveles taxonómicos superiores al de especie de coleópteros acuáticos estaban significativamente correlacionada con la riqueza de especies de los otros grupos y con los valores de RR. La eficiencia de las redes complementarias para estos niveles taxonómicos superiores fue evaluada siguiendo la misma metodología.

En el capítulo 2, se seleccionaron las áreas prioritarias para la conservación de la biodiversidad acuática en la Región de Murcia utilizando los coleópteros como indicadores de biodiversidad. Las unidades geográficas empleadas fueron cuadrículas UTM de 10x10 Km. Para evaluar si la riqueza de especies encontrada era adecuada, se estimo el número total de especies en el área de estudio mediante el ajuste de una función matemática a la curva de rarefacción, obteniendo así un valor asintótico que correspondería con el número total de especies estimadas en el área de estudio. Se seleccionaron las diez cuadrículas que en conjunto recogieron la mayor diversidad de coleópteros acuáticos aplicando un algoritmo iterativo basado en el principio de la complementariedad (Vane-Wright *et al.*, 1991). Utilizando un sistema de información geográfica se superpusieron estas cuadrículas por un lado con la actual red de Espacios Naturales Protegidos y por otro con la Red Natura 2000 con el objetivo de intentar detectar vacíos en la protección de la biodiversidad acuática en esta Región.

Los capítulos 3, 4 y 5 se basaron en la elaboración de una base de datos exhaustiva de coleópteros acuáticos ibéricos (ESACIB “EScarabajos ACuáticos IBéricos”) que compila toda la información taxonómica y faunística disponible, recogiendo más de 50000 citas fiables. En los Capítulos 3 y 4 se utilizaron cuadrículas UTM de 50x50 Km (n=257), mientras que en el Capítulo 5 se utilizaron cuadrículas UTM de 10x10 Km (n=6283). Además, en los capítulos 3 y 4 se usó la relación entre el número de especies observado y el estimado (obtenido a través del valor asintótico de las curva de colecta usando el número de registros como una medida del esfuerzo de muestreo) para analizar el grado de “completeness” de los inventarios en cada cuadrícula.

En el capítulo 3, una cuadrícula fue considerada como adecuadamente muestreada cuando sus valores de “completeness” fueron ≥70% (siguiendo a Jiménez-Valverde y Hortal, 2003). Una vez seleccionadas aquellas cuadrículas bien muestreadas, se calculó la proporción de estas cuadrículas en las diferentes regiones tanto fisioclimáticas (Lobo y Martín-Piera, 2002) como biogeográficas (Ribera, 2000). Por otro lado, para conocer las variables que podrían explicar la distribución del esfuerzo de muestreo se realizaron regresiones entre el número de registros y los valores de “completeness” de cada inventario frente a 26 variables divididas en 4 categorías: 17 variables ambientales, 2 espaciales, 4 de usos del suelo y 3 relacionadas con la accesibilidad y los posibles factores de atracción a los investigadores, que se denominaron “variables de atracción”. La importancia de cada grupo de variables se evaluó por medio de modelos lineales generalizados (GLM: McCullagh y Nelder, 1989; Crawley, 1993) y además se empleó un análisis de partición jerárquica (MacNally, 2000) para evaluar la importancia relativa de cada uno de los tipos de variables explicativas. Por ultimo, se utilizó un test de Mann-Whitney para identificar las variables que difieren significativamente entre las cuadrículas que pueden ser consideradas como bien muestreadas y el resto.

En el capítulo 4 se evaluó la actuación de diferentes modelos basados en grupos de cuadrículas seleccionadas en función de distintos puntos de corte según los valores de “completeness” (50%, 55%, 60%, 65%, 70%, 75%, y 80% del total de los valores predichos por las curvas de acumulación). En la función predictiva, se utilizaron como variables dependientes tanto la riqueza observada como la estimada por el valor asintótico de las curvas de acumulación. Se emplearon GLMs para modelar la variación de la riqueza de especies en función de las variables ambientales y espaciales más explicativas (McCullagh y Nelder 1989). Se utilizaron las 18 variables ambientales que podrían estar más relacionadas con la riqueza de especies a nuestra escala de trabajo. También se incorporó la localización espacial de cada cuadrícula (latitud y longitud) para incluir los efectos producidos por eventos históricos o por variables no consideradas con una estructural espacial, teniendo en cuenta así, los posibles efectos provocados por otras variables distintas a las puramente ambientales. Se seleccionó el modelo que fuera capaz de explicar el mayor porcentaje de “desviaza” con menor error predictivo. Con los valores obtenidos del modelo seleccionado, se puede calcular las diferencias entre la riqueza observada y la predicha, lo que nos permite diferenciar las áreas realmente pobres de las que están mal muestreadas, así como localizar áreas en las que se deberían de concentrar los futuros programas de muestreo.

En el capítulo 5 se aplicó el método desarrollado por Abellán *et al.* (2005) para asignar prioridades de conservación a 120 especies endémicas de coleópteros acuáticos de la Península Ibérica e Islas Baleares, modificando los valores para algunas variables. Este método se basa en la evaluación de seis criterios que hacen referencia a las características de las especies y los hábitats que ocupan: distribución general, distribución ibérica, rareza, persistencia, rareza del hábitat y amenaza o pérdida del hábitat. En total se incluyeron en el análisis más de 6500 citas (especie/cuadrícula/referencia) con información asociada de la abundancia, persistencia y hábitat tipo. Así, las especies se clasificaron en cuatro categorías en función de sus valores de vulnerabilidad: baja, moderada, alta y muy alta. Las especies de las clases de vulnerabilidad alta y muy alta se consideraron como especies prioritarias en términos de conservación. A través de la superposición cartográfica de los mapas de distribución de este grupo de especies, se pudieron localizar puntos calientes de especies prioritarias, que serían aquellas cuadrículas que contienen al menos un registro de tres de estas especies. Por último, se utilizó Arcview 3.2 (ESRI inc.) para realizar un análisis de huecos o vacíos ("gap analysis") y poder así determinar el grado de protección proporcionado por la Red Natura 2000 tanto para las especies como para las áreas. Este análisis se realizó mediante la superposición cartográfica de los mapas para cada especie y los de las áreas identificadas como puntos calientes de especies amenazadas, con el mapa de la Red Natura 2000 en la Península Ibérica e Islas Baleares.

RESULTADOS

Capítulo 1: En las 40 estaciones de muestreo seleccionadas de la Cuenca del Río Segura se registraron 295 especies pertenecientes a seis grupos de macroinvertebrados acuáticos. El grupo con mayor número de especies fue el de coleópteros, con 147, siendo los coleópteros y heterópteros los que presentaron una distribución más amplia en el área de estudio (apareciendo en 40 y 34 estaciones respectivamente), apareciendo en todos los habitats tipo definidos. Los resultados muestran que los patrones de riqueza de tricópteros, plecópteros, efemerópteros, moluscos y coleópteros estuvieron significativamente correlacionados ($p<0.01$) con sus respectivos valores de RR. La red de áreas complementarias seleccionadas por los coleópteros representó el mayor porcentaje de RR (84.46%), recogiendo, como mínimo, el 78% de las especies de cada grupo, seguida de las redes complementarias para plecópteros, tricópteros, moluscos y efemerópteros que recogieron, respectivamente, el 80.88, 78.69, 77.82, 71.93 y 71.92 % de sus valores de RR.

Capítulo 2: En la Región de Murcia se inventariaron 146 especies de coleópteros acuáticos, de las cuales 12 son endemismos ibéricos y 32 son especies raras (encontradas tan solo en una cuadrícula en el área de estudio). Estas 146 especies constituyen ya el 74% del número total estimado de especies, de acuerdo con el valor asintótico de la curva de rarefacción. Las 10 cuadrículas seleccionadas como prioritarias se localizaron, principalmente, en el noroeste (seis cuadrículas), así como en puntos aislados del este, sur y sureste de la Región de Murcia. Estas cuadrículas recogen el 95% (138 de 146) del total de especies de coleópteros acuáticos en el área de estudio, el 68% de las raras y el 100% de las especies endémicas y vulnerables.

Cuando se superpusieron las cuadrículas seleccionadas como prioritarias para la conservación de la diversidad acuática con la actual red de Espacios Naturales Protegidos (ENPs) se detectó un grado de solapamiento bajo, quedando sin ninguna protección las cuadrículas del noroeste. Sin embargo, cuando este solapamiento se hizo con la Red Natura 2000 para Murcia, se observó un grado de solapamiento mucho mayor, coincidiendo, al menos en parte, todas las cuadrículas con algún tipo de área protegida.

Capítulo 3: El análisis de la base de datos “ESACIB” mostró que el valor medio del número de registros y especies por cuadrícula UTM 50x50 km es de 197 y 48, respectivamente. Las cuadrículas en las que se llevó a cabo un mayor esfuerzo de muestreo y que presentaron valores de “completeness” más altos se encuentran ampliamente distribuidas por la Península Ibérica, mientras que las áreas menos muestreadas parecen concentrarse en el centro de la Península (con excepción de algunas cadenas montañosas como las Sierras de Gredos y Guadarrama) y la parte centro-sur de Portugal. El valor medio de “completeness” por cuadrícula fue del 46%. De un total de 257 cuadrículas, 56 presentaron valores de “completeness” por encima del 70%. Aunque aparecieron cuadrículas bien muestreadas por todo el territorio, éstas no presentaron una distribución equilibrada entre las regiones fisioclimáticas y biogeográficas. Por otro lado, las variables que explicaron un mayor porcentaje de la variabilidad en el número bruto de registros fueron el número de localidades tipo, la distancia a los principales centros de investigación, el rango de altitud y la altitud máxima. Las variables relacionadas con la atracción ejercida sobre los investigadores fueron las variables más relevantes, como demuestra que un modelo construido sólo con esas 3 variables fue capaz de explicar casi el 50% de la variabilidad total. Los resultados del análisis de partición jerárquica demostraron que este mismo grupo de variables tienen el mayor efecto medio (23.5%) después de su inclusión en todas las combinaciones de modelos construidos con los otros grupos de variables. Las cuadrículas bien muestreadas difieren significativamente del resto de cuadrículas en las siguientes variables: presentan mayor número de localidades tipo, rangos de altitud más amplios, mayor superficie protegida, altitud máxima, y precipitación anual y estival. Además están más cerca de los principales centros de investigación, tienen menor cantidad de superficie de secano, y menor temperatura media máxima e índice de aridez más bajo.

Capítulo 4: Los resultados de los modelos dependieron de los puntos de corte de “completeness” utilizados. La desvianza explicada osciló entre el 11.1 y el 60.1%, mostrando los valores más altos cuando se utilizan sólo las cuadrículas que presentan los porcentajes más altos de “completeness” (80%) y cuando se utiliza la riqueza estima como variable dependiente. Los porcentajes de error medio no difirieron significativamente entre los distintos modelos aplicados, aunque la menor variabilidad en las diferencias entre los valores observados y los predichos por el método de Jackniffe también sugieren que las mejores predicciones se alcanzan cuando la variable dependiente es elegida por el punto de corte de “completeness” más estricto. Por lo tanto, el modelo seleccionado es aquel que es capaz de explicar mayor porcentaje de “deviance”, en este caso, es el modelo construido usando como variable dependiente la riqueza estima por las curvas de acumulación en las que se ha registrado más de un 80% del total de especies estimado para la misma. De esta manera, el modelo final fue: S = EXP (5.04 + 0.24Amin – 0.22Amin2 + 0.04Latitude), siendo Amin el valor de la variable altitud mínima. Este modelo simple fue capaz de explicar el 60.1% del total de la variabilidad de la riqueza de especies con un porcentaje de error medio del 26.6%. Por lo tanto, los resultados deben ser interpretados con precaución. Este modelo se aplicó a la totalidad del área de estudio, obteniendo unos valores de riqueza de entre 67 y 179 especies por cuadrícula, pudiéndose diferenciar cinco zonas principales en el área de estudio. Posteriormente se examinaron las diferencias entre los valores observados y los predichos por el modelo. Estas diferencias permiten distinguir las áreas genuinamente pobres de las mal muestreadas y localizar áreas interesantes donde ubicar futuros programas de muestreo. Las áreas con las mayores diferencias fueron las de la zona centro de España y Portugal, algunos puntos aislados en los pre-Pirineos y un grupo de cuadrículas en el sureste ibérico.

Capítulo 5: De un total de 120 especies endémicas de la península Ibérica y Baleares, sólo dos se identificaron como especies con un grado de vulnerabilidad muy alto (*Ochthebius ferroi* y *Ochthebius javieri*), 71 con grado de vulnerabilidad alto, 46 con grado de vulnerabilidad moderado, y sólo una especie presentó un grado de vulnerabilidad bajo. Por lo tanto, entre los endemismos de la Península Ibérica e Islas Baleares, se pudieron identificar 73 especies prioritarias. Una vez superpuestos los mapas de distribución de estas especies, se localizaron 57 cuadrículas como puntos calientes de especies prioritarias. Treinta de estas cuadrículas contienen medios salinos y están localizados principalmente en la mitad sur de la Península Ibérica. El resto de puntos calientes están ampliamente distribuidos por todo el área de estudio, encontrándose asociados a arroyos de cabecera de los principales sistemas montañosos. Cuando se superpusieron los mapas de distribución de las especies prioritarias con la Red Natura 2000, se observó un alto grado de solapamiento. Las distribuciones de 22 especies, principalmente asociadas a sistemas montañosos, solapan completamente con el mapa de la Red Natura 2000. Por el contrario, el rango de distribución de cuatro especies se encuentra fuera de los límites actuales de la Red Natura 2000. Estas especies son: *Iberoporus cermenius*, *Hydraena quetiae*, *Limnebius monfortei* y *Ochthebius irenae*. En este capítulo, se ha podido constatar que 26 de los 57 hotspots están fuera de la Red Natura, o solapan en proporciones muy pequeñas. La mayoría de estos puntos calientes sin protección están localizados en áreas bajas, con ecosistemas salinos como principal hábitat acuático.

CONCLUSIONES

1. Las especies de coleópteros acuáticos han sido seleccionados como el mejor taxón indicador de biodiversidad acuática en áreas mediterráneas, de acuerdo con los resultados de las correlaciones de los patrones de riqueza y el alto número de especies de otros grupos recogido en sus redes de áreas complementarias.

2. Para conservar el mayor grado de biodiversidad acuática en la Región de Murcia, las siguientes áreas necesitan ser específicamente protegidas: a) los arroyos de cabecera del noroeste de la provincia; b) los tramos altos del Río Segura; c) las ramblas hipersalinas y costeras; las pozas rocosas y las charcas litorales en Calblanque y Cabo de Palos.

3. La actual red de Espacios Naturales Protegidos (ENPs) en la provincia de Murcia no incluye la mayor parte de los ecosistemas acuáticos que han demostrado tener la mayor diversidad de coleópteros. Sin embargo, la Red Natura 2000 protegerá, al menos en parte, las 10 cuadrículas con mayor biodiversidad acuática.

4. Se ha detectado una evidente falta de inventarios extensivos o completos para coleópteros acuáticos, ya que a pesar de ser uno de los grupos de invertebrados acuáticos mejor estudiados en la Península Ibérica e Islas Baleares, sólo una cuarta parte de las cuadrículas pueden ser consideradas como adecuadamente muestreadas.

5. Las áreas bien muestreadas en la Península Ibérica e Islas Baleares no presentaron una distribución equilibrada entre las regiones fisioclimáticas y biogeográficas, reflejando un cierto sesgo en la distribución del esfuerzo de muestreo. Estos sesgos pueden ser explicados por factores relativamente simples que afectan a la distribución del esfuerzo de muestreo, como puede ser el efecto de atracción producido por paisajes montañosos, con áreas protegidas y con especies nuevas para

la ciencia descritas recientemente, junto con la accesibilidad de las áreas, entendida como distancia a los principales centros de investigación.

6. En los modelos predictivos de la riqueza de especies se recomienda utilizar como variable dependiente los valores estimados de riqueza de especies y ser exigente en los criterios para seleccionar una cuadrícula como bien muestreada, a pesar de la posible pérdida de observaciones para el análisis.

7. El modelo seleccionado para predecir la riqueza de especies en la Península Ibérica e Islas Baleares fue capaz de explicar el 60.1% del total de la variabilidad de la riqueza con un porcentaje de error medio del 26.6%. Por lo tanto, los resultados deben ser interpretados con precaución, y se debe asumir que la estadística no puede siempre solventar los problemas derivados de la escasez de datos.

8. El esfuerzo de muestreo para validar y mejorar este modelo, actualmente, debe focalizarse en las áreas con alta riqueza predicha que no están bien inventariadas. Estas áreas están localizadas en el centro de España (desde los Montes de Toledo a Sierra Morena) y algunas zonas del noreste de Portugal (Serra de Megadouro), el sureste de la Península Ibérica (Sierra de los Filabres, cerca de Sierra Nevada) y las faldas de la parte sur de los Sistemas Ibérico y Central.

9. De las 120 especies de coleópteros acuáticos endémicos de la península Ibérica e Islas Baleares, sólo *Ochthebius ferroi* y *Ochthebius javieri* presentaron un grado de vulnerabilidad muy alto, 76 especies grado alto, 46 grado moderado y solo 1 especie presentó un grado bajo de vulnerabilidad.

10. Treinta de los 57 puntos calientes de especies endémicas prioritarias contienen medios salinos y se encuentran distribuidos principalmente en el sur de la Península Ibérica. El resto están ampliamente distribuidos en los principales sistemas montañosos con arroyos de cabecera como hábitat principal.

11. A pesar del alto grado de solapamiento entre los puntos calientes de especies endémicas amenazadas y la Red Natura 2000, el rango de distribución de cuatro especies está totalmente fuera de esta red. El análisis llevado a cabo, también revela que la Red Natura 2000 falla a la hora de proteger los cuerpos de agua salinos, a pesar de su distribución global restingada y alto interés de conservación.

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General Abstract

General Abstract

INTRODUCTION

Nowadays, among the numerous environmental problems, the most serious is undoubtedly the acceleration in the rate of species extinction associated with human activities, as it involves an irreversible loss of biological information with unpredictable consequences (Kerr and Currie, 1995; Purvis and Hector, 2000). In this sense, the conservation of biological diversity has become a global preoccupation and a commonly acknowledged goal, as illustrated by the Convention on Biological Diversity (United Nations Environment Programme, 2005).

There is widespread agreement that the biodiversity of inland waters is highly threatened, to a greater extent, many believe, than in any other ecosystem (Allan and Flecker, 1993; Master *et al.,* 1998; Ricciardi and Rasmussen, 1999). Furthermore, human pressures on freshwater resources are likely to increase in the coming decades, putting yet more species at risk (Strayer, 2006). The loss of freshwater biodiversity is particularly worrying in the Iberian Peninsula, an area of great biogeographic interest, being regarded as one of the richest European regions in terms of species diversity (Medail and Quezel, 1999; Domínguez-Lozano *et al.,* 1996; Reyjol *et al.,* 2007). This region comprises a wide range of aquatic ecosystems, from freshwater streams, ponds and wetlands to hypersaline *ramblas*, or continental and coastal salt-pans. Many of these ecosystems are unique because of their ecological characteristics and because of the presence of rare and endemic species (Ribera, 2000; Ribera *et al.,* 2003; Gómez et al., 2005). On the other hand, the landscape in this region has been subject to strong human influence for centuries, leading to the progressive loss of freshwater species and habitats. The transformation of agricultural landscapes, moving from extensive to intensive farming, has accelerated during the last decades, and the current expansion of irrigated lands in this area is increasing agricultural water demands far beyond available resources, leading to the exhaustion of natural flows in rivers and streams, aquifer overexploitation, loss of springs and wetlands and the depletion of water reserves in dams (Martínez-Fernández and Esteve, 2005). Despite the signs of rapid and destructive changes in inland water, freshwater biodiversity remains of low priority in global conservation initiatives carried out by governmental and intergovernmental organizations (Balmford *et al.,* 2002; Saunders *et al.,* 2002).

Thus, the conservation of aquatic ecosystems and freshwater biota in this region has become an urgent and critical task. In this sense, it is necessary to identify areas of high biodiversity and the most threatened species in order to assign conservation priorities (Margules and Pressey 2000; Moore *et al.,* 2003). In approaching something so complex as the measurement of biological diversity, surrogates of biodiversity are commonly used. Among them, broad-scale biodiversity measures (such as climatic or vegetation data), habitat features (naturalness or typicality), higher taxonomic groups (genera or families) or indicator taxa are frequently used (Noss, 1990; Williams, 1996). In the last case, taxonomically well known groups that have been sufficiently studied in the area are used, so their taxonomic richness, rarity and endemicity patterns are assumed to be indicative of similar patterns in less known groups (Reyers and Jaarsveld, 2000).

Freshwater invertebrates have been used extensively as indicators to monitor the status of habitats regarding nutrient enrichment or the presence of potential pollutants (Wright *et al*., 2000). However, little attention has been paid to the identification of possible indicator taxa for assessing freshwater biodiversity (Paszkowski and Tonn 2000; Heino 2002; Briers and Biggs 2003). To identify high-priority conservation areas using indicator taxa, researchers have traditionally used plants and/or vertebrates, especially birds, whereas arthropods have been systematically ignored in conservation studies (Posadas *et al*, 2001; Serrano, 2002), despite the fact that they represent around 95% of all known animal species (Hull *et al*., 1998; Palmer, 1999; Sluys, 1999). Among aquatic invertebrates, beetles are one of the richest groups. It is estimated that about 18,000 species of aquatic Coleoptera are roaming the earth at present (Jäch and Balke, 2008), being one of the most useful groups for ranking sites in relation to their conservation value in inland waters (Jeffries, 1988; Foster *et al*., 1990). They are a potentially ideal indicator of freshwater ecosystems biodiversity and meet most of the criteria proposed in the literature for the selection of indicator taxa (Noss, 1990; Pearson and Cassola, 1992; Pearson, 1994). In spite of that, formal tests are required to assess how well potential indicators taxa reflect the overall freshwater biodiversity.

In this context, the use of suitable indicator taxa to select areas for freshwater biodiversity conservation is a valuable tool to measure the extent to what existing protected areas represent freshwater biodiversity and to identify elements that need further protection (Scott *et al*., 1993). This is a crucial issue, since the designation of protected areas has historically been opportunistic or *ad hoc*, while efforts to conserve freshwater biodiversity have often been scarce and few protected areas have been created to protect aquatic biota (Saunders *et al*., 2002).

To provide reliable conservation strategies and scientific information on biodiversity patterns and processes based in these indicator taxa, good-quality databases are required (Prendergast *et al.,* 1993; Soberón and Peterson, 2004; Guralnick *et al.,* 2007; Hortal *et al*., 2007). Nevertheless, only countries with a long-standing tradition of natural history and sufficient resources are able to produce good distribution maps based on adequate sampling of a number of taxonomic groups (Lawton *et al*., 1994; Griffiths *et al*., 1999). This is not the case of Mediterranean countries as Spain, in which inventories of many animal groups, particularly insects, are incomplete or nonexistent (Ramos *et al*., 2001), appearing large gaps once all available information of insects is mapped, especially when wide spatial scales are considered. This drawback could be overcame using statistical modelling methods based in the information from areas considered as enough surveyed to forecast the distribution of biodiversity attributes in the remaining not well-surveyed territory (Hortal *et al.,* 2001; Ferrier, 2002; Lobo and Martín-Piera, 2002; Hortal *et al.,* 2004; Ferrier and Guisan, 2006; Lobo, 2008). However, the incomplete coverage of the geographic and environmental diversity of these adequately surveyed areas can compromise the utility of any predictive models based on them (Hortal and Lobo, 2006). Therefore, it is necessary to incorporate estimates of sampling bias and measures of sampling effort in biodiversity studies to discriminate poorly from well-surveyed areas and minimize their potential confounding effect (Romo *et al*., 2006).

Other commonly used approaches for setting conservation priorities are those based in protecting threatened species. In this sense, arthropods (freshwater or not) have traditionally received only minimal legislative protection, presumably because of their small size and distant evolutionary relationship to humans (Metrick and Weitzman, 1996), but also because of the difficulty involved in categorizing them using the widespread systems of vulnerability ranking (Samways, 1994; New, 1999). The overall lack of conservation attention is evident in the disproportionately few insects listed as threatened. For example, 623 species of insects are listed as threatened in the IUCN red list while the number of threatened vertebrate species is 5624 (IUCN, 2006). Thus, while the effectiveness of legal protection for small invertebrates may be debated (Hutchings and Ponder, 1999; New and Sands, 2003), in the current situation, the only protection available to these species is the extent to which they occur in protected areas designated on the basis of other taxa (principally vertebrates) or habitat features. Consequently, it is necessary to evaluate the effectiveness of existing reserve networks (such as Natura 2000) in protecting threatened, diverse and ‘non charismatic’ groups, such as water beetles.

Objectives

Considering all the exposed above, the main objective of this thesis was to determine the conservation status of freshwater biodiversity in the Iberian Peninsula and Balearic Islands using inventories of water beetles. This thesis involves different approaches and methods from the double perspective of setting conservation priorities for organisms and areas. Thus, the specific objectives are:

- To asses if water beetles can be used as reliable biodiversity indicators in Maditerranean aquatic ecosystems.

- To select areas for freshwater biodiversity conservation using water beetles as biodiversity surrogates at regional scale.

- To compile a database of Iberian water beetles, and to assess the survey effort, the degree of geographical coverage and the amount and nature of bias in this database.

- To obtain a function based on environmental and spatial variables able to predict species richness distribution in the Iberian Peninsula and Balearic Islands.

- To identify the most threatened endemic water beetles in the Iberian Peninsula and Balearic Islands and evaluate the effectiveness of existing reserve network (Natura 2000) in protecting them.

Thesis structure

This thesis is based on five articles, with each one of them constituting a chapter. Chapters 1 and 2 have already been published, chapters 3 and 5 are currently in press, to be published in international peer-reviewed journals indexed in SCI, and chapter 4 has been recently submitted to a similar journal. Thus, the thesis is based on the following papers:

Chapter 1	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.
Chapter 2	Sánchez-Fernández D, Abellán P, Velasco J, Millán A. 2004. Selecting areas to protect the biodiversity of aquatic ecosystems in a semiarid Mediterranean region using water beetles. *Aquatic Conservation: Marine and Freshwater Ecosystems*. 14, 465-479.
Chapter 3	Sánchez-Fernández D, Lobo JM, Abellán P, Ribera I, Millán A. 2008. Bias in freshwater biodiversity sampling: the case of Iberian water beetles. *Diversity and Distributions*. In press. DOI: 10.1111/j.1472-4642.2008.00474.x
Chapter 4	Sánchez-Fernández D, Lobo JM, Abellán P, Millán A. Assessing models for forecasting species richness of Iberian water beetles. (Submitted).
Chapter 5	Sánchez-Fernández D, Bilton DT, Abellán P, Ribera I, Velasco J, Millán A. Are the endemic water beetles of the Iberian Peninsula and the Balearic Islands effectively protected?. *Biological Conservation*. In press. DOI: 10.1016/jbiocon.2008.04.05

In Chapter 1, water beetles were examined for being used as potential biodiversity indicators in continental aquatic ecosystems in a semiarid Mediterranean region, the Segura river basin (SE Spain). The indicator value of water beetles was investigated by examining the degree to which their species richness patterns was correlated with other aquatic groups of macroinvertebrates (Plecoptera, Trichoptera, Mollusca, Heteroptera and Ephemeroptera), as well as by assessing the efficiency of water beetle area networks (selected by complementarity) in conserving overall groups richness. We also examined if the higher-taxon richness of water beetles are suitable for predicting overall species richness in aquatic ecosystems.

Once checked that water beetles can be used as surrogates for freshwater biodiversity, in Chapter 2 areas for freshwater biodiversity conservation were selected using water beetles as aquatic biodiversity indicators in a semiarid Mediterranean region (Region of Murcia), an area with reliable information about this surrogate taxa. The identification of such areas allowed the detection of gaps in the network of Protected Natural Spaces (PNSs) in the study area, through the cartographic superposition of these areas and the (PNSs) currently recognised or proposed in the province of Murcia.

In Chapter 3, an exhaustive database on Iberian water beetles was constructed, enlarging considerably the scale of the work. By analyzing this database, we aim to determine whether these data are able to provide an unbiased picture of the species diversity and distribution. Firstly, the distribution of sampling effort was examined, and the areas most likely to be well-surveyed were identified. We also assess whether these areas cover effectively the different biogeographical and environmental subregions previously recognized in the Iberian Peninsula with independent data. Then, the extent to which sampling bias can be explained by a suite of environmental, spatial or “attractiveness” variables was evaluated. Finally, we identified the key areas where the effort should be concentrated in future sampling programs.

Using the same database presented in Chapter 3, and taking into account some previous drawbacks in our data identified in this chapter (relatively scarce and biased), the general aim of Chapter 4 was to obtain a function based on environmental and spatial variables able to predict species richness distribution in the Iberian Peninsula and the Balearic Islands. Firstly, we discriminate those cells with relatively well-surveyed inventories according to different completeness criteria, and select both observed and asymptotic predicted species richness values in these squares as the dependent variable in predictive functions. After comparing and evaluating the performance of these different species richness scenarios, we generate a forecast map, describing the obtained species richness distribution of these insects for the entire Iberian Peninsula. Lastly, the location of those not enough surveyed cells with important predicted species richness values was examined in order to propose future areas of sampling.

In Chapter 5 the extent to which endemic Iberian and Balearic water beetles are protected by Natura 2000 network was determined. In this chapter we identify the most threatened endemic water beetles in the study area, by ranking species according to their conservation priority or degree of vulnerability. We also locate distributional hotspots for the most threatened species. Lastly, the extent to which the Natura 2000 network provides effective protection for these species and areas to evaluated and discussed.

METHODOLOGY

For Chapter 1, forty sites were selected to include the whole variety of water body types known within the Segura River Basin and taking into consideration the available information on the six well studied taxonomic groups of aquatic macroinvertebrates: Coleoptera, Heteroptera, Mollusca, Trichoptera, Ephemeroptera and Plecoptera. Spearman correlations were used to evaluate the relationship among species richness patterns of these six groups of macroinvertebrates and between the richness of each group and the total number of species found at a site (of all six groups examined) minus the number of species belonging to the considered indicator group (RR or Remaining Richness). Complementary networks for each indicator taxa were also selected by applying an iterative algorithm based on the complementarity principle. Finally, the RR percentage and the total number of species represented in each network were calculated as a measure of their effectiveness for preserving biodiversity. Spearman correlation was also used to assess whether the higher taxon richness of water beetles is significantly correlated with the species richness of the other groups and with the RR value. The efficiency of the complementary network selected according to the higher taxa of water beetles was also evaluated using the same methodology as above.

In Chapter 2, the high-priority areas in the Region of Murcia for freshwater biodiversity conservation were selected using water beetles as aquatic biodiversity indicators. We used UTM 10x10 Km cells as geographical units. In order to evaluate the degree of completeness of the observed species richness, the expected total number of species in the study area was estimated by fitting the rarefaction curve to a mathematical function to find the asymptotic value that would correspond to the expected total number of species in the study area. Ten cells showing the greatest conservation interest for water beetles were selected by applying an iterative algorithm based on the complementarity principle (Vane-Wright *et al*., 1991). To detect gaps in freshwater biodiversity conservation, these selected areas were superimposed on the current network of Protected Natural Spaces (PNSs) and future Natura 2000 network for the study area using a geographical information system software.

Chapters 3, 4 and 5 are based on an exhaustive database of Iberian water beetle records (ESACIB "EScarabajos ACuáticos IBéricos") compiling of all available taxonomic and distributional data, and containing around 50,000 reliable records. In Chapter 3 and 4, 50 x 50 Km UTM squares were used as geographic units (n = 257), while in Chapter 5, 10x10 UTM squares were used (n= 6283). In Chapter 3 and 4, the ratio of recorded to estimated species richness (the asymptotic score Collector's curves using number of records as a mesure of sampling effort) was used as a measure of completeness of each cell inventory. In Chapter 3, a UTM cell was considered to be adequately sampled when the completeness values were ≥70% (following Jiménez-Valverde and Hortal, 2003). Once adequately prospected cells were selected, we examined the proportion of well surveyed squares for physicoclimatic (Lobo and Martín-Piera, 2002) and biogeographical (Ribera, 2000) subregions. Raw number of database records and cell completeness values of well surveyed cells were regressed against 26 explanatory variables that could potentially explain the distribution of the sampling effort, divided into 4 categories: seventeen environmental, two spatial, four land-use and three variables related to the accessibility and appeal for researchers. The importance of each subgroup of variables was assessed by using Generalised Linear Models (GLM: McCullagh and Nelder, 1989; Crawley, 1993), and the relative importance of each type of explanatory variable was also measured using a hierarchical partitioning procedure (MacNally, 2000). A Mann-Whitney U-test was used to identify the variables that differ significantly between cells considered well-surveyed and not well-surveyed.

In Chapter 4, the performance of distribution models according different completeness criteria to discriminate those cells with relatively well-surveyed inventories (50%, 55%, 60%, 65%, 70%, 75%, and 80% of total Clench predicted values) was assessed, selecting both observed and asymptotic predicted species richness values in these cells as the dependent variable in predictive functions. A GLM procedure was used to model variation in species richness as a function of the most significant environmental and spatial explanatory variables (McCullagh and Nelder 1989). To account for environmental factors affecting species richness, we use 18 environmental variables that could potentially be related to species richness at our working scale. We also included the spatial location of each cell (latitude and longitude), to include effects due either to historic events or unconsidered variables with a spatial structure, as they may aid to include the effects of different variables from purely environmental ones. The model explaining the highest percentage of deviance and with the highest predictive power was selected. Thus, the difference between the richness predicted by the selected model and the observed richness was used to distinguish genuinely poor from badly sampled areas, and to identify the areas where the effort should be concentrated in future sampling programs.

In chapter 5, the method developed by Abellán *et al.* (2005) was applied for assessing conservation priorities for the 120 species of water beetle endemics of the Iberian Peninsula and Balearic Islands, modifying the scoring of some variables. This evaluation is based on a set of six species and habitat attributes: general distribution, Iberian distribution, rarity, persistence, habitat rarity and habitat loss. In total, more than 6,500 records (species/site/reference, with associated information on persistence, abundance and habitat type) were included in the analyses. Thus, species were grouped into four vulnerability categories according to their overall vulnerability scores: low, moderate, high and very high. Species assigned to high and very high categories were considered high-priority taxa in conservation terms. Distribution maps of all these high-priority conservation species were overlapped to detect ‘hotspots’ of threatened endemic water beetles, these being defined as squares containing a record of at least three of those species. A gap analysis was conducted to evaluate the degree of protection of the high-priority species and hotspots achieved by the Natura 2000 network in the study area by overlapping the distribution maps of individual species and hotspots with the Natura 2000 network map using Arcview 3.2 (ESRI inc.).

RESULTS

In Chapter 1, a total of 295 species belonging to six groups of aquatic macroinvertebrates were recorded in the 40 sampling sites of the Segura river basin. Coleoptera was the richest group with 147 species, and Coleoptera and Heteroptera were the most widespread groups in the study area (appearing in 40 and 34 sites respectively), present in all four types of habitat described. Results shows that the

Trichoptera, Plecoptera, Ephemeroptera, Mollusca and Coleoptera species richness patterns were significantly correlated ($p<0.01$) with their respective *RR* values. Area networks for Coleoptera selected by complementarity represented the highest RR percentage (84,46 %) and contained more than 78% species of each group, followed by the complementary networks of Plecoptera, Trichoptera, Heteroptera, Mollusca and Ephemeroptera, with 80.88, 78.69, 77.82, 71.93 and 71.92 % of their *RR* values respectively.

Results of chapter 2 show that 146 water beetle species were recorded in the province of Murcia, of which 12 are Iberian endemics and 32 rare species (found only in one grid cell in the study area). The 74% of the expected total species was already recorded in the study area, according with the asymptotic value of the rarefaction curve. The 10 grid cells selected as high-priority conservation areas were located fundamentally in the northwest (six squares) and isolated points of the east, south, and southeast of the study area. These grid cells included 138 of the 145 (95%) species of aquatic beetles of the area, the 68% of the rare species, and 100% of the endemic and vulnerable species. When superimposing the grid cells selected as high-priority conservation areas with the current network of PNSs, a very low overlapping was detected, remaining without any protection the Northwest grid cells. However, when this overlapping process was repeated with the Nature 2000 Network of Murcia, it was observed the coincidence of the 10 grid cells, at least in part, with some type of protected area.

The analysis of the database “ESACIB” carried out in chapter 3 shows that the mean value of records and number of species per 50x50 km cell were 197 and 48 respectively. The cells with the higher sampling effort and completeness seem to be widespread in the Iberian Peninsula, while less surveyed cells occur mainly in central Spain (with the exception of the Sierra de Guadarrama and Sierra de Gredos) and south-central Portugal. The mean value of completeness by cells was around 46%. From a total of 257 cells, 56 had completeness values higher than 70%. There are well-surveyed cells across the whole Iberian territory, although they are not evenly distributed amongst both biogeographical or physioclimatic subregions. The number of type localities, distance from main research centres, altitudinal range and maximum altitude were the variables that accounted for the highest variability in the number of database records. “Attractiveness” variables seemed to be the most influential; a complete model including these variables explained almost 50% of the total variability. The results of the hierarchical partitioning demonstrated that the attractiveness variables had the highest average effect after inclusion in all models (around 23.5%). Well-surveyed cells significantly differed from the rest in a number of variables: they had a higher number of type localities, wider altitude range, larger protected surface, higher maximum altitude, and higher annual and summer precipitation. They were also closer to the main research centres, had less surface of non-irrigated crops, and a lower maximum mean temperature and aridity index.

Chapter 4 shows that modelling results seem to depend of the completeness threshold used. Explained deviance oscillates from 11.1% to 60.1%, showing the highest values when the data of the cells with higher completeness percentages (80%) and when species richness derived from accumulation curves are used as dependent variable. Mean error percentages do not significantly differ between competing models, although the lower variability in the differences between observed and predicted Jackknife values also suggest that better model predictions are obtained when the dependent variable is chosen by the most restrictive completeness threshold. Therefore, we selected the model that is able to explain the highest percentage of deviance; the model build using the cells with more than 80% of completeness and the species richness values estimated by the accumulation curves as dependent variable. Thus, the final model was: S = EXP (5.04 + 0.24Amin – 0.22Amin^2 + 0.04Latitude), being Amin the value of the variable minimum altitude. This simple model was able to explain 60.1% of total deviance, with a high mean Jackniffe predictive error (26.6%). However, these results should be interpreted carefully due to the low percentage of variance explained. When this model was applied to the entire study area, predicted species richness ranged from 67 to 179, being able to distinguish five main areas across the whole Iberian territory. In order to locate suitable areas in where to carry out future sampling programs, we examined the differences between predicted and observed richness (i.e. number of species left to be recorded in each cell). Areas with the highest differences where concentrated in central Spain and Portugal, and some isolated cells in pre-Pyrenees and a set of cells in southeast Spain.

In chapter 5, from a total of 120 endemic species only two species were identified as being of very high vulnerability (*Ochthebius ferroi and Ochthebius javieri*), 71 were identified as high vulnerability, 46 as moderate, and a single remaining species was assigned low vulnerability status. Therefore, amongst Iberian Peninsula and Balearic Island endemics, 73 high-priority species were identified. Once the individual distributional maps of these species were superimposed, 57 cells were identified as hotspots of high-priority species. Thirty of these hotspots contain saline systems and are mainly located in the southern half of the Iberian Peninsula. The rest of hotspots are widespread in streams of mountainous areas. When the distribution maps of individual species were superimposed on the Natura 2000 network map, a high degree of overlap was detected. Distributions of 22 species overlapping completely with Natura 2000 network, which occur mainly in mountainous areas. On the other hand, the distribution of four species is totally outside the existing Natura 2000 network. These species are *Iberoporus cermenius, Hydraena quetiae, Limnebius monfortei* and *Ochthebius irenae.* In this chapter, we were able to identify 26 of the 57 hotspots that are currently outside of the Natura 2000 network. Most of these 'missing' hotspots were in lowland areas, with saline streams or saltpans as their main aquatic ecosystem type.

CONCLUSIONS

1. Water beetles have been selected as the best surrogate taxa for freshwater biodiversity in Mediterranean areas according with the correlation of richness patterns and the high number of species containing in their complementary networks.

2. To preserve the highest degree of biodiversity in the aquatic ecosystems of the province of Murcia, the following areas need to be protected: a) the head water streams in the north-west of the province; b) the uppermost reaches of the Segura River; c) the hypersaline and coastal *ramblas* and; d) the rock-pools and coastal ponds in Calblanque and Cabo de Palos.

3. The present network of Protected Natural Spaces (PNS) in the province of Murcia does not include many of the aquatic ecosystems shown to have the highest biodiversity of beetles. However, the "Natura 2000" network will protect the ten grid cells of highest aquatic ecosystems biodiverstity, or at least, part of them.

4. A lack of complete and extensive inventory data for aquatic taxa was detected since just a quarter of the Iberian and Balearic 50x50 km UTM grid cells can be considered well prospected for water beetles, despite the fact that they could be probably considered one of the best studied groups of freshwater invertebrates in the region.

5. The well surveyed areas in the Iberian Peninsula are not evenly distributed across biogeographical and physicoclimatic subregions, reflecting some geographical

bias in the distribution of sampling effort. These biases can be explained by relatively simple variables affecting collector activity, such as the perceived “attractiveness” of mountainous landscapes and protected areas with recently described species, and accessibility of sampling sites (distance from main research centres).

6. It is recommended the use of estimated richness scores as dependent variable and to be stringent in the thresholds to consider cells as well-surveyed, despite the possible loss of observations for the analyses.

7. The model selected to forecast water beetles species richness in the Iberian Peninsula was able to explain 60.1% of the total deviance, with a high mean Jackniffe predictive error (26.6%) Hence, these results should be interpreted carefully, since show that statistics cannot always efficiently overcome the scantiness of the data.

8. The sampling effort to validate and improve this model must be actually focused on the areas of high predicted species richness that were not well inventoried, such as Central Spain (from Montes de Toledo to Sierra Morena) and some areas in the Northeast Portugal (Serra de Megadouro), Southwest of the Iberian peninsula (Sierra de los Filabres, close to Sierra Nevada), and southern foothills of the Iberian Central Systems.

9. Of the 120 species of water beetles endemic of the Iberian Peninsula and Balearic Islands, only *Ochthebius ferroi* and *O. javieri* were identified as being extremely vulnerable, 71 highly vulnerable and 46 moderately vulnerable, with only a single species identified as having low vulnerability status.

10. Thirty of the fifty-seven hotspots identified as hotspots of high-priority species contain saline systems mainly located in the southern half of the Iberian Peninsula. The rest of hotspots are widespread in mountainous areas with streams as main habitat.

11. Despite a high degree of concordance between hotspots of threatened endemic species and Natura 2000 sites, the distribution of four species falls completely outside the network. The analysis also reveals that Natura 2000 fails to protect saline water bodies, despite their high conservation interest and narrow global distribution.

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Use of Iberian water beetles in biodiversity conservation

Uso de los coleópteros acuáticos ibéricos en la conservación de la biodiversidad

Chapter 1

Are water beetles good indicators of biodiversity in Mediterranean aquatic ecosystems? The case of the Segura river basin (SE Spain)

Abstract

Water beetles were examined for use as potential biodiversity indicators in continental aquatic ecosystems in a semiarid Mediterranean region, the Segura river basin (SE Spain). The indicator value of water beetles was investigated by examining the degree to which their species richness patterns was correlated with other groups (Plecoptera, Trichoptera, Mollusca, Heteroptera and Ephemeroptera), and the efficiency of water beetle area networks (selected by complementarity) in conserving overall groups richness. The species richness patterns of Coleoptera, Ephemeroptera, Plecoptera and Trichoptera were significantly correlated with the *Remaining Richness value* (RR), defined as the total number of species found at a site (of all six groups examined) minus the number of species belonging to the considered indicator group. Area networks for Coleoptera selected by complementarity represented the highest RR percentage (84.46 %) and contained more than 78 % species of each group. Furthermore, water beetles meet most of the criteria proposed in the literature for choosing biodiversity indicator taxa. In our study, the correlation values and the percentage of species represented by family, genus and species complementary networks were similar and we suggest that the higher taxa of water beetles (genera or families) can be used as biodiversity surrogates for cost-effective practical surveys.

INTRODUCTION

The maintenance of biodiversity has become one of the principal goals of conservation, so that it is necessary to identify particularly valuable areas for conservation on which to focus more detailed effort (Margules and Pressey, 2000; Myers *et al.,* 2000; Moore *et al.,* 2003). Practical resources for measuring the overall biodiversity within a given area are limited (Williams and Gaston, 1994; Kerr *et al.,* 2000), and areas of high biological diversity are increasingly identified by means of biodiversity surrogates (Humphries *et al.,* 1995; Caro and O´Doherty, 1999). Among such surrogates, a wide range of biodiversity measures (such as climatic or vegetation data), higher taxonomic groups (genera or families) or indicator taxa are frequently used (Noss, 1990; Williams, 1996; Reyers and Jaasverld, 2000; Heino *et al.,* 2005).

Little attention has been paid to identifying possible indicator taxa for assessing freshwater biodiversity (Paszkowski and Tonn, 2000; Heino, 2002; Briers and Biggs, 2003). Nevertheless, several aquatic macroinvertebrate groups, such as Odonata and Coleoptera, have been suggested as indicator taxa for monitoring population trends in other species, and for identifying areas of high regional biodiversity (Davis *et al.,* 1987; Foster *et al.,* 1990; Foster and Eyre, 1992; Sánchez-Fernández *et al.,* 2004a; Heino *et al.,* 2005). However, the extent to which these or other taxa represent the biodiversity content of freshwater ecosystems has not been assessed (this is also the problem with the vast majority of studies that rely on bioindicators, which have generally used charismatic taxa). In this sense, several *a priori* suitability criteria have been proposed for the selection of indicators (Noss, 1990; Pearson, 1994; McGeoch, 1998).

Biodiversity indicator taxa, in general, are groups of organisms with a sound taxonomy that have been well surveyed in a region. It is assumed that patterns of species richness, endemism, rarity or vulnerability in these taxa are indicative of similar patterns of unsurveyed taxa in the region (Pearson, 1994). Nevertheless, the existence of a significant correlation between species or taxa richness does not necessarily indicate the extent to which sites selected on the basis of the indicator taxa represent wholesale species richness across all sites (Briers and Biggs, 2003).

Such indicator taxa, so called biodiversity surrogates are useful for identifying areas for conservation management. Several methods for selecting areas of high biodiversity conservation value have been advocated, including hotspots of richness, hotspots of rarity and complementary areas (Williams, 1996). As many authors have pointed out (Faith and Walker, 1996; Williams *et al.,* 1996, Howard *et al.,* 1998; Abellán *et al.,* 2005b), complementarity approaches are more effective than others methods involving scoring or richness and rarity hotspots to represent conservation targets, and should be integrated into the methodology for evaluating potential biodiversity indicators (Kati *et al.,* 2004). Complementarity can provide an effective answer concerning where conservation efforts should be concentrated (Broocks *et al.,* 2001; Sauberer *et al.,* 2004).

In some cases, the number of higher taxonomic groups in a region is used as a surrogate for the number of local species within the same clade, given that a relationship between these different taxonomic levels can be established. The advantage of this approach is that the number of families or genera can be documented more rapidly than the number of species (Williams and Gaston 1994; Caro and O´Doherty, 1999; Baldi, 2003; Villaseñor *et al.,* 2004). Moreover, aquatic organisms are usually larval stage forms, whose identification at species level is often problematic and sometimes impossible.

These kinds of criteria may help in the selection of indicators for species and higher taxa, but formal tests are required to assess how well the chosen indicators reflect the overall biodiversity. We attempt to evaluate the use of water beetles, the most studied group of insects in the study area, as biodiversity indicators in freshwater ecosystems of the Segura river basin (southeast Spain). The study area is a region of special interest, because, despite being one of the most arid zones of Europe, it has a high diversity of aquatic ecosystems and a rich and endemic biota (Médail and Quézel, 1997; Myers *et al.,* 2000; Abellán *et al.,* 2005a). Furthermore, most of these habitats are of special significance on a European scale and some of them are very unusual, such as hypersaline streams (Moreno *et al.,*1997; Sánchez-Fernández *et al.,* 2004a).

The aim of this study is to answer the following questions:

(i) Is species richness in water beetles correlated with overall species richness and, particularly, with the species richness of five macroinvertebrate groups (Heteroptera, Mollusca, Trichoptera, Ephemeroptera and Plecoptera)?

(ii) Are complementary sets of sites based on water beetles more efficient in capturing the greatest number of species than those based on the other macroinvertebrate groups?

(iii) Is the higher-taxon richness of water beetles suitable for predicting overall species richness in aquatic ecosystems?

METHODS

Study area and data set

The study was performed in the Segura river basin, a Mediterranean region located in the southeast of the Iberian Peninsula and encompassing an area of 18815 km² (Figure 1). Climatic patterns range from humid in the northwest mountains to semiarid in the rest of the study area. The geology ranges from limestone at the uplands headwaters to salt-rich tertiary marl in mid and lowlands, which also define the environmental conditions of the waterbodies in the area, allowing a high heterogeneity in the aquatic ecosystems present (Millán *et al.,* 1996; Moreno *et al.,* 1997).

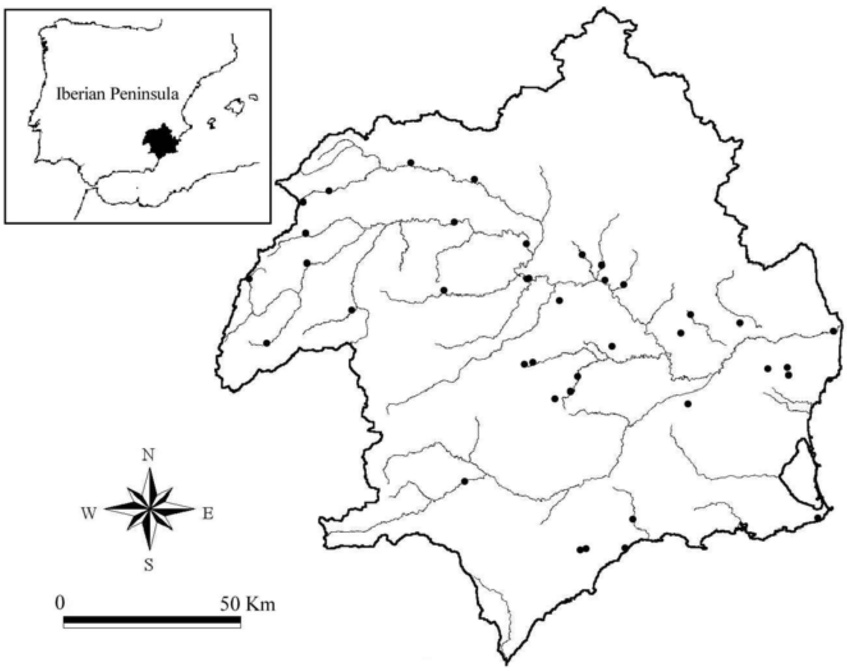

Figure 1. Map of the study area (Segura river basin) showing the main water courses. Locations of sampling localities are indicated with a black solid dot.

Forty sites were selected to include the whole variety of water body types known within the study area and taking into consideration the available information on the six well studied taxonomic groups of macroinvertebrates in the study area: Coleoptera, Heteroptera, Mollusca, Trichoptera, Ephemeroptera and Plecoptera. The sites selected were grouped into four types of habitat: lotic freshwater (19 sites), lentic freshwater (10 sites), lotic saline (10 sites) and lentic saline waters (1 site).

Data on the species were obtained from our own fieldwork and literature records: Mollusca (Gómez 1988); Ephemeroptera and Plecoptera (Ubero-Pascal 1996); Coleoptera (Sánchez-Fernádez *et al.,* 2004b); Heteroptera (Millán *et al.,* 1988); Trichoptera (Bonada *et al.,* 2004). Macroinvertebrates were sampled with a D-frame net (500 µm mesh) and sampling collection generally took around 30 minutes per site. Samples were preserved in 75 % ethanol and taken to the laboratory for identification. Although not visited with a regular frequency, each site was surveyed at least twice and most were sampled on five occasions. Thus, all published and unpublished data currently known to us were included.

Data analysis

Spearman correlations were used to evaluate the relationship between species richness patterns of the different groups of macroinvertebrates, and between the richness of each group and the total number of species found at a site (of all six groups examined) minus the number of species belonging to the considered indicator group a parameter (*RR* or *Remaining Richness*). This procedure avoids giving higher weight in the correlation of the groups with a greater number of species. Spearman correlations were used because data are not normally distributed. Statistical analyses were performed using Statistica for Windows (Release 4.5). We also examined how the habitat factor affects these relationships by analyzing data subsets corresponding to three of the four different habitat types (lotic freshwater, lentic freshwater and lotic saline waters). Saline lentic systems were not analyzed due to the insufficient number of sites.

Complementary networks for each indicator taxa were selected by applying an iterative algorithm based on the complementarity principle. This principle refers to the degree to which an area contributes otherwise unrepresented features (e.g. species) to a set of areas (Vane-Wright *et al.,* 1991). We therefore used complementarity to maximise the number of species represented within a given number of areas (10 sampling sites) for each group of macroinvertebrates. The algorithm is a modification of that proposed by Kirkpatrick (1983) and it is applied as described below:

In a first step, the site with the greatest number of species was selected. The next site selected was that with the highest number of species not included in the first site (thus providing the greatest number of species by complementarity). In case of equality, the site selected was the one with the greater richness of species (included or not in the first site selected). This procedure was repeated until 10 sites (an arbitrary number) had been selected. Thus, a complementary network for each group was obtained. Finally, the RR percentage and the total number of species represented in each network were calculated as a measure of their effectiveness for preserving biodiversity.

Spearman correlation was also used to assess whether the higher taxon richness of water beetles is significantly correlated with the species richness of the other groups and with the RR value. The efficiency of the complementary network selected according to the higher taxa of water beetles was also evaluated using the same methodology as above.

RESULTS

We recorded 57 families, 138 genera and 295 species in 40 sampling sites of the Segura river basin (Table 1). Coleoptera and Heteroptera species were widespread in the study area (40 and 34 sites respectively), and were present in all four types of habitat described. Ephemeroptera and Mollusca species were absent from one type of habitat (lentic saline waters) but appeared in 35 and 26 sites, respectively. Trichoptera species were found in 27 sites, including two types of habitat (freshwater and saline lotic waters). Plecoptera species were less widespread, being confined to freshwater lotic ecosystems and only appearing in 13 sites (Table 2).

Table 1. Number of families, genera and species of the six groups recorded in the study area.

	Families	Genera	Species
Coleoptera	10	52	147
Heteroptera	11	17	29
Ephemeroptera	7	8	10
Plecoptera	10	20	35
Mollusca	5	9	23
Trichoptera	14	32	51
Total	57	138	295

Table 2. Number of sites with the presence of each group in the different types of habitat.

	Lotic-freshwater (n =19)	Lentic-freshwater (n =10)	Lotic-saline (n =10)	Lentic-saline (n =1)	All sites
Coleoptera	19	10	10	1	40
Heteroptera	16	7	9	1	33
Ephemeroptera	19	7	9	0	35
Plecoptera	13	0	0	0	13
Mollusca	17	5	4	0	26
Trichoptera	18	3	0	0	21

Table 3 shows that the Trichoptera, Plecoptera, Ephemeroptera, Mollusca and Coleoptera species richness patterns were significantly correlated ($p<0.01$) with their respective *RR* values. The strongest correlation across groups was found between Trichoptera and Ephemeroptera, followed by Trichoptera and Plecoptera, and Ephemeroptera and Plecoptera. Coleoptera was significantly correlated with all groups, with the exception of Mollusca and Ephemeroptera. Mollusca species richness was significantly correlated with Ephemeroptera, Plecoptera and Trichoptera. Heteroptera species richness was significantly correlated only with Coleoptera.

Table 3. Results of pairwise Spearman correlation coefficients for the species richness of the six groups of taxa studied (40 sampling sites). * p < 0.05, ** p < 0.01, *** p < 0.001.

	Coleoptera	Heteroptera	Ephemeroptera	Plecoptera	Mollusca	Trichoptera	RR
Coleoptera	-	0.50**	0.28	0.50***	0.14	0.51***	0.52***
Heteroptera		-	0.06	0.22	-0.10	0.13	0.31
Ephemeroptera			-	0.78***	0.67***	0.88***	0.54***
Plecoptera				-	0.52***	0.86***	0.73***
Mollusca					-	0.68***	0.42**
Trichoptera						-	0.75***

Pairwise Spearman correlation coefficients of the species richness in each type of habitat are shown in Table 4. In lotic freshwater ecosystems, the results were similar to those obtained for combined habitats, with the same groups significantly correlated with RR values and the highest correlation was shown by Plecoptera. The strongest correlation across taxa was between Plecoptera and Trichoptera species richness patterns. In saline lotic systems and lentic freshwater systems, none of the correlations with RR values was significant, although the highest values were shown by Coleoptera.

Table 4. Pairwise Spearman correlation coefficient of the species richness in each type of habitat.* p < 0.05, ** p < 0.01, *** p < 0.001.

Lotic-freshwater (n=19)	Coleoptera	Heteroptera	Ephemeroptera	Plecoptera	Mollusca	Trichoptera	Total	RR
Coleoptera	-	0.55*	0.41	0.70***	0.06	0.68**	0.87***	0.76***
Heteroptera		-	0.08	0.22	-0.07	0.22	0.45	0.33
Ephemeroptera			-	0.78***	0.12	0.72***	0.70***	0.68**
Plecoptera				-	0.09	0.88***	0.89***	0.87***
Mollusca					-	0.19	0.20	0.16
Trichoptera						-	0.90***	0.78***
Lenitic-freshwater (n =10)								
Coleoptera	-	0.60	-0.11	-	-0.27	0.27	0.99***	0.56
Heteroptera		-	-0.24	-	-0.53	-0.25	0.65	0.55
Ephemeroptera			-	-	0.30	0.62	-0.06	-0.17
Plecoptera				-	-	-	-	-
Mollusca					-	0.29	-0.32	-0.41
Trichoptera						.	0.27	0.23
Lotic-saline (n =10)								
Coleoptera	-	0.39	-0.30	-	0.07	-	0.91***	0.33
Heteroptera		-	-0.35	-	-0.48	-	0.63	0.18
Ephemeroptera			-	-	0.15	-	-0.19	-0.35
Plecoptera				-		-		
Mollusca					-	-	-0.04	-0.18
Trichoptera						-		

Concerning the complementarity method, our results showed that the complementary network using overall species, maximised the representation of the total species (92 %), as was expected. Within groups, the Coleoptera complementary network captured the highest *RR* percentage (84.46 %), representing more than 78 % species of each group, followed by Plecoptera, Trichoptera, Heteroptera, Mollusca and Ephemeroptera complementary networks with 80.88, 78.69, 77.82, 71.93 and 71.92 % of their *RR* values respectively. Trichoptera and Heteroptera complementary networks also retained a high percentage of species of each other group (upper 73 %). In general, the percentage of species of one group represented by any other group was not less than 48% (Table 5).

Table 5. Percentage of species that would be represented in complementary networks for each group. (H: Heteroptera; E: Ephemeroptera; C: Coleoptera; M: Mollusca; P: Plecoptera; T: Tricoptera)

	Complementary network of						
	H	E	C	M	P	T	Overall species
% Heteroptera	86.21	48.28	93.10	58.62	55.17	79.31	93.10
% Ephemeroptera	82.86	100	80	82.86	80	82.86	82.86
% Coleoptera	76.19	65.31	96.60	70.07	80.27	74.83	92.52
% Mollusca	90	90	90	100	80	90	90
% Plecoptera	73.91	86.96	91.30	78.26	100	91.30	100
% Trichoptera	78.43	94.12	78.43	74.51	98.04	100	92.16
% Total	78.64	75.25	90.17	72.88	82.37	82.37	91.86
% RR	77.82	71.92	84.46	71.93	80.88	78.69	-

Table 6 shows that family, genus and species richness was significantly correlated with RR, and family richness showed the highest Spearman correlation coefficient. The percentage of species represented in the complementary networks of the beetle species, genera and families is shown in Table 7. In general, the correlation values and percentage of species represented by the family, genus and species complementary networks were similar.

Table 6. Spearman correlation coefficient between beetles richness at different taxonomic levels (families, genera and species), and the species richness of the other groups and RR value in the study area (40 sampling sites). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

		Complementary network of	
	Coleoptera Species	Coleoptera Genera	Coleoptera Familes
% Heteroptera	93.1	89.66	86.21
% Ephemeroptera	77.14	88.57	82.86
% Coleoptera	95.24	93.20	88.44
% Mollusca	90	70.0	80
% Plecoptera	91.3	86.96	86.96
% Trichoptera	78.43	80.39	84.31
% Total	90.17	88.73	86.44
% RR	84.46	83.09	83.28

Table 7. Percentage of species represented in complementary networks using different taxonomic levels of water beetles (families, genera and species).

		Complementary network of	
	Coleoptera Species	Coleoptera Genera	Coleoptera Familes
% Heteroptera	93.1	89.66	86.21
% Ephemeroptera	77.14	88.57	82.86
% Coleoptera	95.24	93.2	88.44
% Mollusca	90	70	80
% Plecoptera	91.3	86.96	86.96
% Trichoptera	78.43	80.39	84.31
% Total	90.17	88.73	86.44
% RR	84.46	83.09	83.28

DISCUSSION

It is assumed that if species counts for a potential indicator taxa are strongly correlated with counts for other taxa, then, on average, regions where many species of the indicator taxa occur will also be characterised by high species counts of other taxa. In general terms, when Plecoptera, Trichoptera, Ephemeroptera or Coleoptera show high species richness, there is also a high degree of overall species richness. However, the status of particular taxa as indicators may vary with habitat type, and hence taxa that are good indicators in ponds may not necessarily be good indicators for lakes or other water bodies (Davis *et al.,* 1987; Sahlen and Ekestubbe, 2001; Briers and Biggs, 2003). This study was carried out to select indicators of biodiversity in a variety of water bodies, rather than in one particular habitat type. If we only examine the degree of congruence of species richness patterns, Plecoptera or Trichoptera could be proposed as the best biodiversity indicators, despite the fact that they only occur in one or two types of habitat. In other words, only lotic freshwater systems would be selected if we used Plecoptera as indicator group, and other sites of special interest, such as saline streams or ponds, from which Plecoptera and Trichoptera are normally absent, would be excluded even though they contain specialised communities of rare or endemic species, perhaps with a low overall species richness (Moreno *et al.,* 1997; Abellán *et al.,* 2005a). Coleoptera, on the other hand, present higher correlations than Heteroptera, the only other group found across all the habitat types analysed.

Nevertheless, examining the correlation between species richness patterns is only one of the possible ways to evaluate biodiversity indicators (Kati *et al.,* 2004). In this sense and taking into consideration that environmental heterogeneity is one of the main factors generating biological diversity (Cellot *et al.,* 1994; Huston, 1994; Kati *et al.,* 2004), the complementary network of water beetles encompasses the gradient of environmental heterogeneity and thus constitutes a reliable local aquatic biodiversity surrogate in semiarid Mediterranean regions.

Thus, water beetles can be selected as the best indicator group for selecting areas of high biodiversity in aquatic ecosystems from the Segura river basin, because their species richness patterns are significantly correlated with RR and, furthermore, their complementary network contains the greatest proportion of RR (84.46 %) and more than 78 % of the species of each group. Moreover, although Plecoptera, Ephemeroptera and Trichoptera showed higher correlation values with RR, the Coleoptera richness relationship was more consistent between habitats.

Biodiversity indicators will be useful if they cover a reasonably wide geographic range (Wilcox, 1984; Caro and O´Doherty, 1999) and occur in a broad range of habitat types. Within a geographic area, they should have high habitat fidelity because their absence (in the face of habitat disturbance) may be a sensitive indicator of the absence of other species (Panzer *et al.,* 1995). In our case, water beetles comprise a great number of species, they show high functional diversity and they are capable of colonising a wide variety of habitats (Ribera and Foster, 1993). In fact, together with Heteroptera, they are the only groups that were present in all the habitat types. Furthermore, beetles appeared in all 40 sites sampled. Thus, they met most of the criteria proposed in the literature for choosing indicator taxa (Noss, 1990; Pearson and Cassola, 1992; Pearson, 1994).

Importantly too, beetles are taxonomically and faunistically well-know in the Iberian peninsula (Ribera *et al.,* 1998; Ribera, 2000), and their importance as indicators of the spatial and temporal changes that take place in aquatic systems has been demonstrated (Bournaud *et al.,* 1992; Richoux, 1994). They have also been used for ranking sites in relation to their conservation value (Jeffries 1988; Foster *et al.,* 1990).

Two types of biodiversity indicator can be differentiated depending, on the spatial scale: indicators at local scale (alpha-diversity) and indicators for biodiversity conservation at a regional or national scale (Duelli and Obrist, 2003). In the first case, we suggest using Plecoptera or Trichoptera as biodiversity indicators in lotic freshwater systems, and Coleoptera in lotic saline and lentic systems, whether saline or freshwater. In the second case, the value of the measurable units of biodiversity depends on their rarity or uniqueness with regard to a higher level area, and water beetles can be selected as the best indicator group for selecting areas for conservation. In this context, areas with the highest aquatic biodiversity could be identified by using water beetles as a surrogate and then applying an iterative algorithm of complementarity. This algorithm makes an integrated selection of a network of sites which, in a complementary way, sometimes omits sites with a higher richness than others which would merely provide redundant information. Furthermore, this method presents the additional advantage of flexibility in the selection process, which means that new criteria can be included in the model, such as the proximity to already protected areas, the presence of at least two populations of each species, the irreplaceable character of some of these areas, etc. (Margules *et al.,* 2002). We emphasise the importance of applying the principle of complementarity, instead of only testing the correlation of species richness patterns, when assessing the value of potential indicators for biodiversity conservation.

One limitation to the generalization of the results from this study is that it only aquatic invertebrate groups were considered, and the extent to which invertebrate taxa represent other groups, such as fish or plants, was not assessed. Nevertheless, in running waters, the congruence of species richness among bryophytes, macroinvertebrates and fish is generally low (Paavola *et al.,* 2003; Heino *et al.,* 2005).

Since it is difficult to express the components of a system representing a landscape or community using one group of species only, great care should be taken in the selection of areas based only on one group as a surrogate of total biodiversity and before extrapoling to other biological groups. The trend is to choose different, poorly related and representative of the different components of the system to study (Halffter *et al.,* 2001) or to use overall available data of species in a zone. Thus, water beetles could be taken as a suitable complementary surrogate in biodiversity research.

The large number of species of water beetles would make survey work more difficult, and this might be a problem for their use as surrogates of aquatic biodiversity, this problem can be overcome using higher taxon richness values (genus or family). In our study, the correlation values and the percentage of species represented by family, genus and species complementary networks were similar. Several studies support the idea of a relationship between the number of higher taxa and the numbers of species in a given area (Williams and Gaston, 1994; Williams *et al.,* 1997; Baldi, 2003) suggesting that the former would act as a good surrogate in more cost-effective practical surveys.

Although a group could be a good indicator of biodiversity in one geographical area, it may not be representative of species richness patterns elsewhere, due to biogeographical or climatic constraints (Su *et al.,* 2004), but it could be valid for regions with similar environmental and ecological features. Our results suggest that water beetles are a good biodiversity indicator, both at local and regional scales, and can be used for the rapid and inexpensive monitoring of biodiversity in aquatic ecosystems of Mediterranean areas. In this sense, these results provide conservationists and managers with an efficient tool for identifying priority areas for freshwater biodiversity conservation in the Mediterranean Basin.

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Chapter 2

Selecting areas to protect the biodiversity of aquatic ecosystems in a semiarid Mediterranean region using water beetles

Abstract

In this work, carried out in the province of Murcia, a representative semiarid area of the Iberian Peninsula, water beetles were used as indicators to identify the aquatic ecosystems with the highest interest for conservation. For that purpose, an iterative algorithm of complementarity based on the richness of these aquatic Coleoptera was applied. Complementarity refers to the degree to which an area, or set of areas, contributes otherwise unrepresented attributes to a set of areas. This principle was used to maximise the number of represented species within a given number of areas. Only the species subsets whose taxonomic status, presence and distribution in the study area are well known were used. A total of 146 species were employed, of which 12 are Iberian endemics and 32 rare species (found only in one grid cell in the study area). The highest correlation was generally shown by species richness with endemic, rare and vulnerable species richness. Thus, basing conservation strategies on species richness appears to be an effective criterion. To preserve the highest degree of biodiversity in the aquatic ecosystems of the study area, the following need to be protected: a) the head water streams in the north-west of the province; b) the uppermost reaches of the Segura River; c) the hypersaline and coastal *ramblas*; d) the rock-pools and coastal ponds. The present network of Protected Natural Spaces (PNS) in the study area does not include many of the aquatic ecosystems shown to have the highest biodiversity of beetles. However, the future “Natura 2000” network will protect the ten grid cells of highest aquatic ecosystems biodiverstity, or at least, part of them.

INTRODUCTION

The Mediterranean basin is considered one of the most important hotspots of biodiversity in the world (Myers *et al*., 2000). The southeast of the Iberian Peninsula is a Mediterranean region of special interest because, although it is located in the most arid zone of Europe, it comprises a wide range of aquatic ecosystems, from freshwater streams, ponds and wetlands to hypersaline *ramblas* (ephemeral streams, for detail see Moreno *et al*., 1997) or continental and coastal salt-pans. Many of these ecosystems are unique because of their ecological characteristics and because of the presence of rare and endemic species (*Moreno et* al, 1997; Millán *et al*., 2002; Sánchez-Fernández *et al*., in press). Nevertheless, the region has been subjected for centuries to the severe influence of human activities, leading to marked alterations and the spatial reduction, even disappearance, of some habitats, especially aquatic ones (Médail and Quézel, 1999). Any remaining ecosystems, therefore, should be high on the list of conservationist priorities.

During the last decade and following the “Río Conference”, there has been a growing awareness of the need to conserve species and habitats in many parts of the world. In this context, the identification of areas of high biodiversity on all scales (world-wide, national or regional) has become indispensable in order to assign conservation priorities (Gärdenfors, 2001). In approaching something so complex as the measurement of biological diversity, surrogates of biodiversity are commonly used. Among them, broad-scale biodiversity measures (such as climatic or vegetation data), habitat features (naturalness or typicality), higher taxonomic groups (genera or families) or indicator taxa are frequently used (Noss, 1990; Williams, 1996). In the last case, taxonomically well known groups that have been sufficiently studied in the area are used, so their taxonomic richness, rarity and endemicity patterns are assumed to be indicative of similar patterns in less known groups (Reyers and Jaarsveld, 2000).

Freshwater invertebrates have been used extensively as indicators to monitor the status of habitats as regards nutrient enrichment or the presence of potential pollutants (Wright *et al*., 2000). To identify high-priority conservation areas, researchers have used plants and/or vertebrates, but only on few occasions have invertebrates been taken into account for this propose (Posadas *et al*, 2001; Serrano, 2002), despite the fact that they represent around 95% of all known animal species (Hull *et al*., 1998; Palmer, 1999; Sluys, 1999). Among invertebrates, beetles are the richest group, representing one third of all described species (Ribera *et al*., 2002). Among invertebrates, water beetles are one of the most useful groups for ranking sites in relation to their conservation value (Jeffries, 1988; Foster *et al*., 1990). They are a potentially ideal indicator of freshwater ecosystems biodiversity and meet most of the criteria proposed in literature for the selection of indicator taxa: Taxonomically well-known and stable; biology and general life history well understood; populations are easily surveyed and manipulated; groups and related species should occupy a breadth of habitats and a broad geographical range; specialisation of each population within a narrow habitat; patterns observed in the indicator taxon are reflected in other related and unrelated taxa (Noss, 1990; Pearson and Cassola, 1992; Pearson, 1994).

Water beetles include a great number of species and show high functional diversity, they are capable of colonising a wide variety of habitats (Ribera and Foster, 1993). Furthermore, the taxonomic and faunistic knowledge of beetles is ample (Ribera *et al*., 1998; Ribera, 2000), and their importance as indicators of the spatial and temporal changes that take place in aquatic systems has been demonstrated (Bournaud *et al*., 1992; Richoux, 1994). However, a single taxonomic group cannot reflect the different components of an ecosystem, and the current trend is to choose little-related complementary taxa that may be considered representative of different components of the system (Halffter *et al*., 2001). In this sense, the water beetles used in this work are presented as a complement for the establishment, management and conservation of the scarce aquatic ecosystems included in the Protected Natural Spaces (PNSs) of the study area (Sánchez-Fernández *et al*., 2003).

The objectives of this study were to assess priority areas for biodiversity conservation in freshwater ecosystems in a semiarid Mediterranean region using water beetles as indicators, and to detect gaps in the network of Protected Natural Spaces (PNSs) in the study area, through the cartographic superposition of the high-priority conservation areas and the PNSs currently recognised or proposed in the province of Murcia.

STUDY AREA

The study is confined to a semi-arid region of SE Spain (Region of Murcia) covering 11,137 km² and with an annual average rainfall around 300 mm and an annual average temperature above 16 ºC. The analyses used cells defined by Universal Transverse Mercatore (UTM) coordinates as analytical units. Sixty-six of the 144 UTM 10 x 10 km grid cells of the Region of Murcia were sampled, covering 45.8% of the total regional surface (Figure 1). They included most of the aquatic ecosystems of the study area. In these 66 grid cells, 227 sampling sites were taken. Sites selected were grouped into types of habitat based on environmental and ecological parameters, according to Millán *et al*. (1996, 2002). Sixteen types of habitat were distinguished: *head water streams*; *middle reach streams*; *middle course of rivers*; *channelized river reaches; river reaches influenced by dams*; *eutrophic streams*; *ramblas (ephemeral streams)*; *springs*; *irrigation channels*; *reservoirs*; *irrigation pools*; *pools*, *ponds and other wetlands*; *rice-fields*; *continental salt-pans*; *coastal salt-pans* and *rocks pools*.

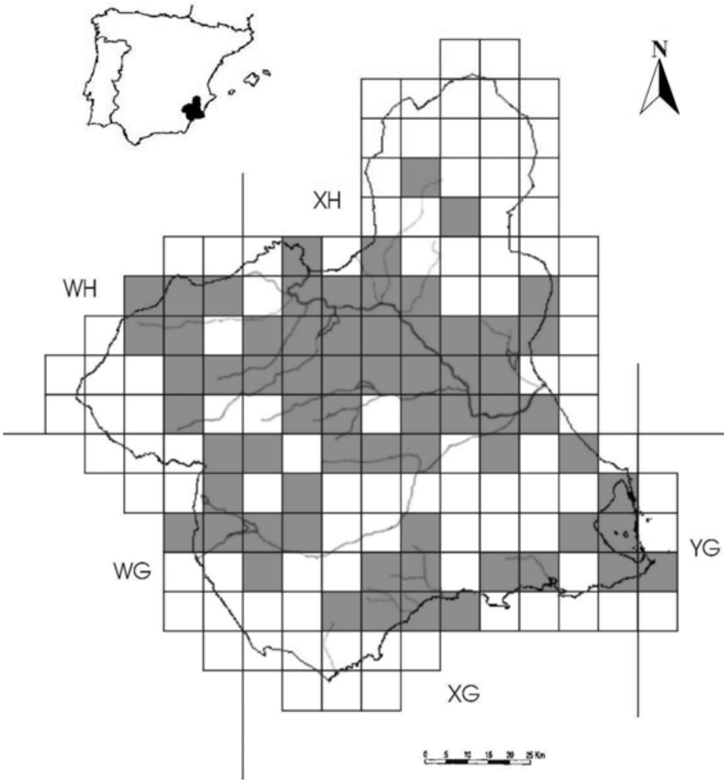

Figure 1. Location of the study area (province of Murcia) and sampled surface in UTM 10 x 10 km grid cells.

METHODS

The information on species occurrence (cumulative species richness) came from two sources: the literature and fieldwork samples collected between 1981 and 2002. Beetles were sampled with a D-frame net (500 µm mesh) and sampling collection generally took 30 minutes per site. Samples were preserved in 70 % ethanol and taken to the laboratory for processing, identification and enumeration. Although not visited with regular frequency, each site was surveyed at least twice and most were sampled five times. With this information a data base that included species identity, number of collected specimens, location, date, and collector was built up. Over 3000 records of aquatic beetles in the study area were included.

High-priority areas for conservation were identified using only the species subset (146) whose taxonomic status, presence and distribution in the study area were sufficiently known. Thus, species of semiaquatic coleoptera (phytophilous and shore water beetles: see Jäch, 1998) were not included in the analysis. In order to evaluate whether the species richness found was right, the expected total number of species of water beetles in the study area was estimated. The first step was to calculate the sample-based rarefaction curve, which shows the cumulative number of species collected with increasing sampling effort (number of samples). For this purpose the software PRIMER v.5 DIVERSE subroutine (Clarke and Gorley, 2001), based on a matrix of species abundance for each sample was used. The second step was to fit the rarefaction curve to a mathematical function to find the asymptotic value that would correspond to the expected total number of species in the province of Murcia. For this, the Curve Expert V. 1.34 software (Copyright 1995-1997 by Daniels Hyams) was used. Pearson correlations were used to determine the degree of correlation of the species richness, endemic species richness, rare species richness and vulnerable species richness. Statistical analyses were performed using Statistica for Windows (Release 4.5).

Categories and criteria of the International Union for the Conservation of Nature to define conservation priorities (IUCN, 2001) require species presence to be expressed in surface units. Thus, the sampling stations were included, according to their location, in UTM 10 x 10 km grid cells. Ten grid cells showing the greatest conservation interest for water beetles were selected by applying an iterative algorithm based on the complementarity principle (Vane-Wright *et al*., 1991). The number of grid cells selected represented between 5-15% of the total surface. This percentage was chosen because it represents the target proportion of land preserved for nature conservation in the European Union (Rey-Benayas and de la Montaña, 2003). According to Abellán *et al*. (2003), the algorithm based on the complementarity principle is an effective method for selecting high-priority areas for conservation using this group of insects. The algorithm is a modification of that proposed by Kirkpatrick (1983) and it is applied according to the following rules: In a first step, the grid cell with the greatest number of species is selected. In the case of equality, the cell with the higher number of rare species is selected (considered as species occurring only in one grid cell). The next cell selected is that with the highest number of species not included in the first grid cell (thus providing the greatest number of species by complementarity). In case of equality, the grid cell with a greater number of rare species, is once again selected, and if the ambiguity persists, that containing the greatest richness of species. This procedure is repeated until 10 grid cells have been selected.

To detect gaps in current and planned conservation priorities, ten grid cells selected on the current and future network of Protected Natural Spaces (PNSs) of the study area were superimposed using a geographical information system software (GRASS, 5.0.0 pre3 version, Mandrake Soft, 2002). Protected Natural Spaces is a network of spaces declared in the province of Murcia by a regional law (Law 4/92) based on a national law (Law 4/89). Places were selected in function of their landscapes, richness, their fauna and flora, or their unspoiled state. They can support nature conservation programmes because land use is restricted in them. The future network of PNS of the Region of Murcia (Baraza *et al*., 1999) will include (SPA) Special Protection Areas under the Bird directive (EU Council Directive 92/43/EEC 1992) and (SCI) Sites of Community Importance (EU Council Directive 79/409/EEC 1999).

RESULTS

Selection of high-priority conservation areas

Fifteen families, 63 genera and 159 species of aquatic beetles were found (Table 1 and Annex), mostly belonging to the families Dytiscidae, Hydrophilidae and Hydraenidae. This number of species represents 25% of the total species in the Iberian peninsula. However, there was no doubt about the taxonomic status, presence and distribution of only 146 of the species recorded in the study area and these were the species taken into account for the selection of high-priority areas.

Table 1. Number and percentage of genera and endemic species of aquatic and semiaquatic beetle families from the study area. (*) species whose presence in Murcia needs confirmation.

Suborder	**Family**	**Genus**	**Species**	**Number of Endemics**	**% Endemics**
Adephaga	Gyrinidae	3	6		
	Haliplidae	2	3		
	Noteridae	1	1		
	Dytiscidae	23	50	4	30,77
Polyphaga	Helophoridae	1	7(1)	1	7,69
	Georissidae	1	1		
	Hydrochidae	1	4	2	15,38
	Hydrophilidae	11	32 (3)		
	Hydraenidae	4	32(2)	5	38,46
	Scirtidae	2	2		
	Elmidae	8	14	1	7,69
	Dryopidae	2	3		
	Limnichidae	1	1		
	Heteroceridae	2	2		
	Curculionidae	1	1		
TOTAL	**15**	**63**	**159 (6)**	**13**	**100,00**

Figure 2 shows the rarefaction curve of species and samples. This curve was adjusted to a sublogistic model (MMF or Morgan-Mercer-Flod Model; Morgan *et al*., 1975) with a high correlation coefficient (r = 0.999) and a standard deviation (S) of 0.1747, according to the following formula:

$$y = ab + cxd / b + xd$$

where a = - 6.94, b = 20.82, c = 196.95 (expected richness) and d = 0.63. The asymptotic curve value and, therefore, the expected total number of species in the study area, was around 197. Thus, we estimated that 74% of the expected total species number had been recorded. Of these 146 species (see annex), 12 were Iberian endemics, representing 10% of the Peninsula's total endemic species, and 32 were rare species. Furthermore, 13 vulnerable species at regional level, 1 at national level and 2 at international level were detected. Vulnerable species were defined by taking into consideration a set of six variables: general distribution, endemicity, rarity, persistence, habitat rarity and habitat loss (for detail see Abellán *et al*., 2003; Sánchez-Fernández *et al*., 2003).

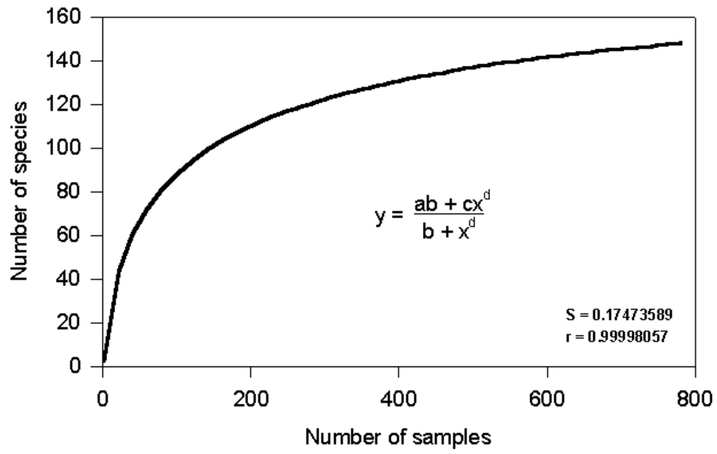

Figure 2. Rarefaction curve of aquatic beetle species in the province of Murcia.

Maps of species richness, endemicity, rarity and vulnerability at regional level are represented in Figures 3, 4, 5 and 6, respectively. As can be seen, they all show the highest values in the northwest of the study area.

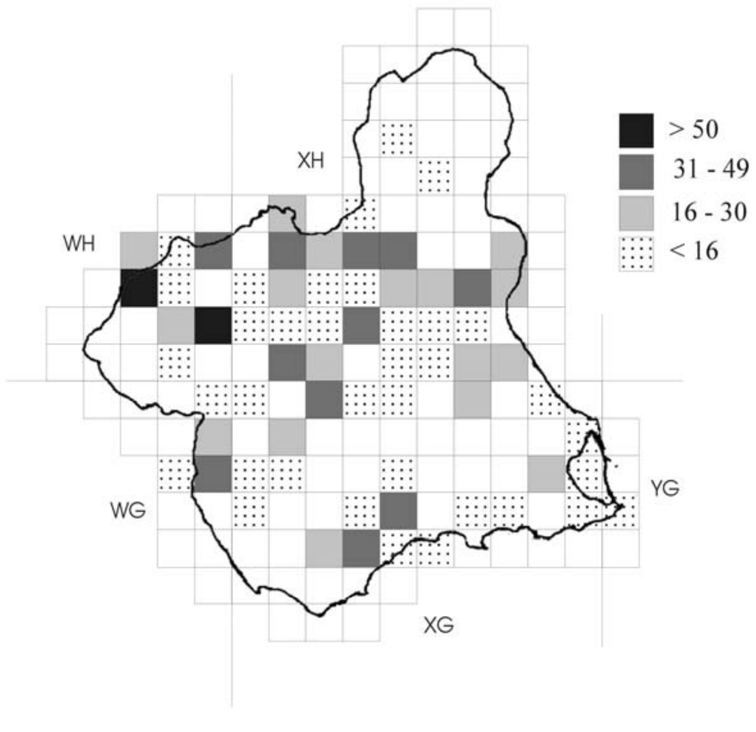

Figure 3. Species richness in UTM 10 x 10 km grid cells.

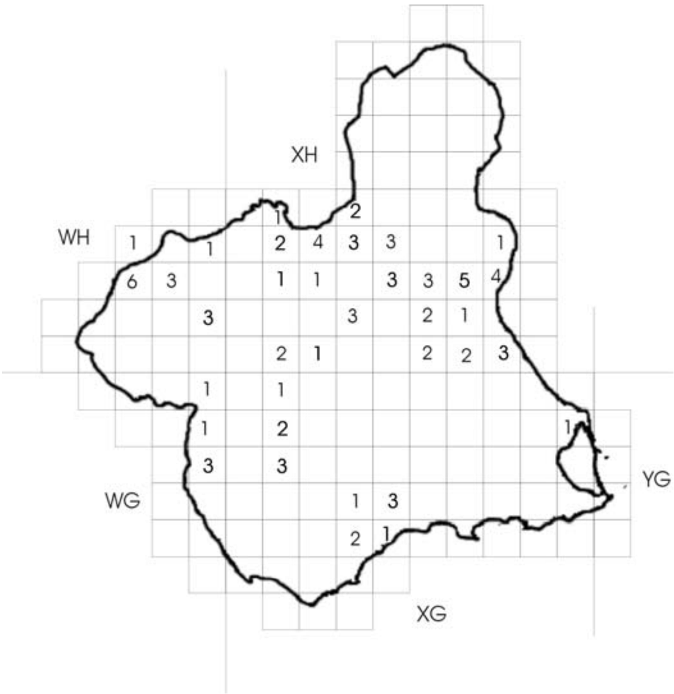

Figure 4. Endemic species richness in UTM 10 x 10 km grid cells.

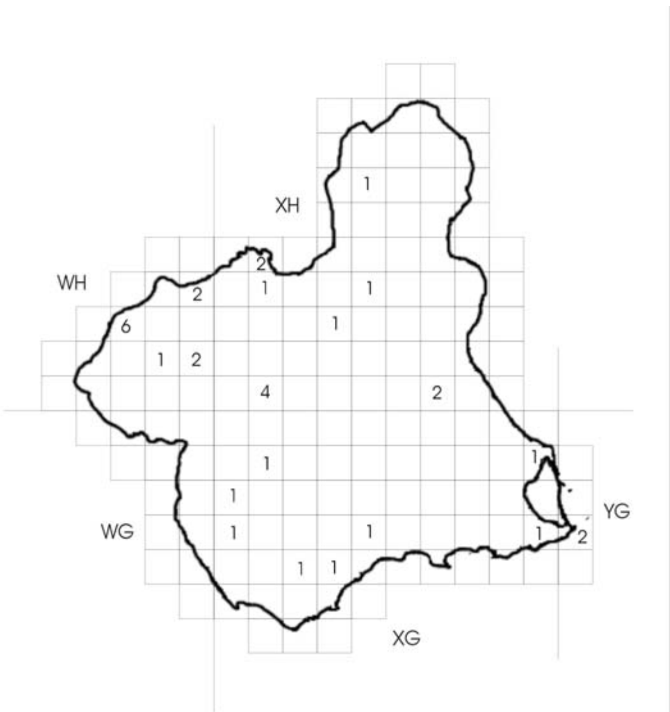

Figure 5. Rare species richness in UTM 10 x 10 km grid cells.

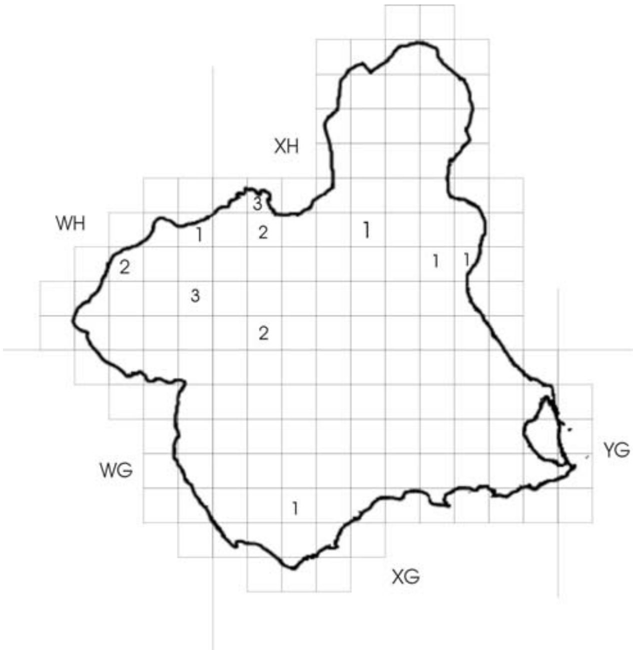

Figure 6. Vulnerable species richness, at regional scale, in UTM 10 x 10 km grid cells.

Table 2 shows the extent to which species richness, endemicity, rarity and vulnerability are correlated in the study area. All correlations were significant (*P* < 0.05), but species richness produced the highest degree of correlation with the other variables. This indicates that areas showing a high species richness are also areas with a high probability of finding endemic, rare and vulnerable species.

Table 2. Correlation matrix for species richness (SR), endemic species richness (ESR), rare species richness (RSR) and vulnerable species richness (VSR) in UTM 10 x 10 km grid cells sampled in the study area. All correlations were significant (*P* < 0.05).

	SR	**ESR**	**RSR**
ESR	0,75		
RSR	0,55	0,34	
VSR	0,54	0,38	0,65

The 10 grid cells selected as high-priority conservation areas were basically located in the northwest (WH72, WH91, WH93, XH10, XH13, XH14), and isolated points of the east (XH62), south (XG35) and southeast (WH96, YG06) of the study area (Figures 7 and 8). These selected 10 grid cells, included 12 of the 16 types of habitat defined. The four habitats that were not represented (channelled river sections, rivers influenced by dams, irrigation pools and irrigation channels) are human-made systems cannot therefore be considered to be in regression or threatened. Furthermore, these habitats contained species of low conservation interest. The ten grid cells also included 138 of the 146 (95%) species of water beetle of the study area, 68% of rare species and, more importantly, 100% of the endemic and vulnerable species at regional, national and international level (Sánchez-Fernández *et al*., 2003).

Gap detection in the Protected Natural Spaces Network

When the grid cells selected as high-priority conservation areas were superimposed on the current network of PNS (Figure 7), a very low degree of overlap was detected. The northwest grid cells, including WH72, which showed the highest richness of species, rare species and endemic species values, were totally unprotected. The only aquatic ecosystems that are currently protected are Ajauque and Rambla Salada (hypersaline *rambla*) it the northeast, Calblanque, Monte de las Cenizas and Peña del Aguila (a matrix of rock pools, coastal salt-pans and ponds) in the southeast, and the Segura River at Cañaverosa (a river reach with a well developed riparian forest). However, when we repeated this overlapping process with the future Nature 2000 Network of Murcia (Figure 8), at least part of all 10 grid cells was contained in some type of protected area.

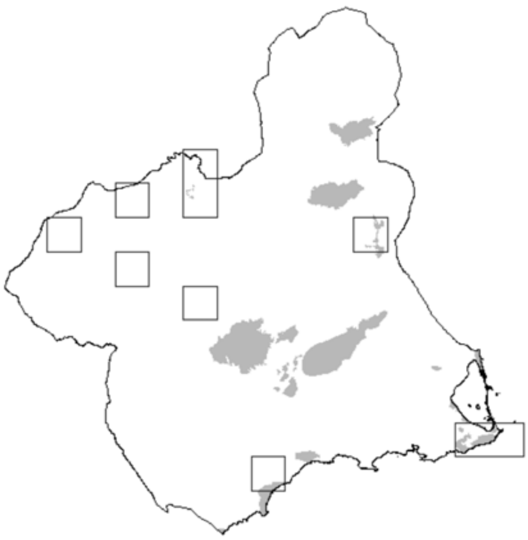

Figure 7. Overlapping of the high-priority areas for conservation (ten grid cells selected) and the current Protected Natural Spaces Network (shaded surface) in the Region of Murcia.

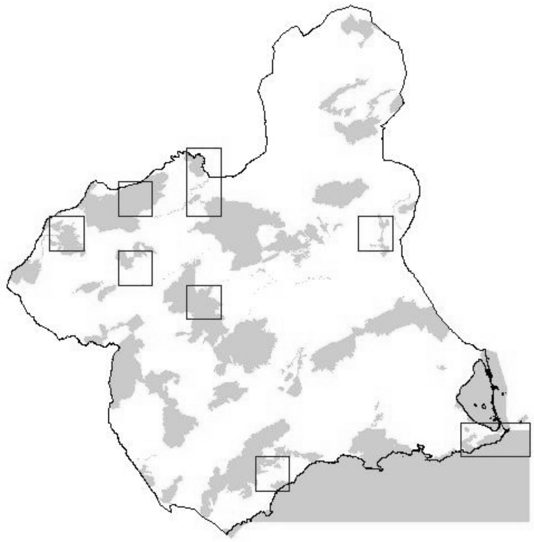

Figure 8. Overlapping of the high-priority areas for conservation (ten grid cells selected) and the future Natura 2000 Network in the Region of Murcia (shaded surface).

DISCUSSION

Despite all the efforts so far to study water beetles in the study area, it is still possible to find new species in the province of Murcia, since the estimated total species number was around 197, meaning that about 25% of species remain to be found. However, to incorporate three new species, we estimate that around 100 more samples would have to be collected, which gives some idea of the difficulty involved. Thus, the sampling effort and the current inventory seem adequate for the purpose of this work.

This research was carried out with accumulated species richness data, some of them may be old and not totally representative of current conditions, but such data still provide information concerning the potential capacity of the sites sampled to support a variety of species. Selection of areas is generated essentially from local data, without considering species distribution outside the study area. Therefore, this condition might underweight the importance of locally common habitats and species that are rare elsewhere, while over-weighting the importance of generally common species or habitats that are scarce in the region. However, this work is focused on a local scale and must be understood in this context, serving as a complement for a wider scale studies.

The iterative method employed in selecting the high-priority conservation areas does not value each grid cell separately and so does not establish categories based on the conservation interest of each grid cell in particular. Instead, it makes an integrated selection of a network of grid cells which, in a complementary way, harbour the greatest number of species, sometimes omitting grid cells with a higher richness than other grids in the selection, but which merely provide redundant information. The use of an iterative algorithm of complementarity also presents the additional advantage of flexibility in the selection process, which means that new criteria can be included in the model, such as the proximity to already protected areas, the presence of at least two populations of each species, the irreplaceable character of some of these areas, etc. (Margules *et al*., 2002). In this sense, the criteria used in the iterative algorithm of complementarity applied seem to be right. Furthermore, the presence in the grid cells selected of 95% of the water beetles recorded in the study area, 68% of rare species and 100% of endemics and vulnerable species can be explained by the high environmental heterogeneity detected in these grids.

The high correlation coefficient shown by species richness with endemic, rare and vulnerable species richness strongly suggest that this criterion is the most important for prioritising areas destined for conservation in semiarid regions. The highest richness, rarity and endemicity values were found in the northwest, where 6 of the 10 grid cells selected were situated. This area mainly includes headwater streams but also stream reaches in middle lowland areas (WH72, WH91, WH93, XH10), as well as upper reaches and rice-fields of the Segura River (XH13, XH14). The northwest of Murcia is the highest part of the study area. It presents a supramediterranean climate, with more frequent rainfall and lower temperatures than the rest of the region with its meso and thermomediterranean climate. These grid cells, furthermore, contain freshwater and well preserved ecosystems, which are infrequent in Mediterranean zones of the Iberian peninsula (Gasith and Resh, 1999).

The eastern zone includes the XH62 grid cell, which contains hypersaline *ramblas* and wetlands. The richness of this grid cell was not high, but the endemic value of the species and their singularity were the highest, with five Iberian endemics and nine Iberonorthafrican species appearing, most of them of a great importance as hypersaline water indicators. This is the case of *Rambla Salada* whose salinity values reach 100 g/l in natural conditions. However, at present does not surpass 50 g/l, with sections that usually present values close to and even lower than 12 g/l because of the freshwater which leaks from the Tajo-Segura transfer channel and from the surrounding irrigated crops. Such saline *ramblas*, still relatively common in the study area, are singular ecosystems in a European context. Furthermore, they are probably the most threatened aquatic ecosystems in the Mediterranean region due to non-point source pollution processes, water sweetening as a consequence of the expansion of intensive agriculture, and speculative property company activities in the zone (Martínez-Férnandez *et al*., 2000).

The richness and endemicity values in the southern zone were modest. However, rarity values were not, since these grid cells (WH96, YG06) contained some types of habitat that are not frequent in Murcia, such as the littoral rocks pools or abandoned coastal salt-pans. These are ephemeral environments, with high variations in salinity, and few organisms are capable of colonising them. Therefore, these habitats, as well as the species that inhabit them, are of high conservation interest since they show adaptive strategies of high ecological value (Greenwood and Wood, 2003).

The southern zone (XG35) is represented by the presence of coastal *ramblas* with saline streams and some freshwater streams, such as the *Rambla de Miñarros*. This freshwater habitat is an uncommon environment in Mediterranean coastal zones with a semi-arid climate, and seems to act as a regional biogeographical island and as a refuge for northern species (Moreno *et al*., 1997).

Of all the grid cells studied, WH72 stands out because of the high number of species (73), endemics (6) and rare species found (6). Compared with the other grid cells, it presents great environmental heterogeneity since it contains very different habitat types, ranging from head freshwater streams to continental salt-pans or saline streams. As a result, freshwater and saline water ecosystems appear relatively close, which considerably increases the number of species (Townsend *et al*., 1997; Millán *et al*., 2001 a and b). Furthermore, the habitats in this grid cell were the least affected by human activity. Of note in this grid cell are some headwater stream sites, particularly the Alharabe stream, which are places of exceptional conservation value. In this last habitat, over 50 species may be found in a single sampling, emphasising the importance of this locality, which is currently threatened by a dam, 200 metres upstream of the sampling station.

The poor coincidence of the Protected Natural Spaces in the study area with the high priority conservation areas for water beetles suggests that the current network of PNS is insufficient to protect the biodiversity of the aquatic ecosystems in Murcia. However, it seems that these gaps will be remedied by the future Natura 2000 network because of the high coincidence between the areas proposed in this paper and those defined by birds (SPA) and habitats as a function of plant associations and the presence of given taxa (SCI). Although part of the grid cell WH91 will be under protection (SCI), the upper reaches of the Argos stream, which are of high conservation interest, will remain unprotected. Therefore, the amplification of the limits of SCI is proposed to include the upper reaches of the Argos stream.

The coincidence between the high priority conservation areas for water beetles and the Future Natura 2000 Network seems to confirm that the water beetles are adequate surrogates for aquatic biodiversity (Jeffries, 1988; Ribera and Foster, 1993; Pearson, 1994; Fairchild *et al*. 2000). Thus, the high-priority conservation areas defined here using aquatic beetles as biodiversity surrogates could well be taken into account when delimiting the PNSs of the study area. In the same way, the proposed methodology can be applied to other groups of insects that fulfil the requirements of biodiversity indicators, and in other Mediterranean zones where an adequate knowledge of such taxa is available.

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ANNEX

List of aquatic and semiaquatic beetles from the study area.

Species	
Gyrinus caspius Ménétries, 1832	R
Gyrinus dejeani Brullé, 1832	
Gyrinus distinctus Aubé, 1836	R
Gyrinus urinator Illiger, 1807	
Aulonogyrus striatus (Fabricius, 1792)	
Orectochilus villosus (Müller, 1776)	
Peltodytes rotundatus (Aubé, 1836)	
Haliplus lineatocollis (Marsham, 1802)	
Haliplus mucronatus Stephens, 1832	
Noterus laevis Sturm, 1834	
Laccophilus hyalinus (De Geer, 1774)	
Laccophilus minutus (Linnaeus, 1758)	
Laccophilus poecilus Klug, 1882	
Hyphydrus aubei Ganglbauer, 1892	
Hydrovatus cuspidatus (Kunze, 1818)	
Yola bicarinata (Latreille, 1804)	
Bidessus minutissimus (Germar, 1824)	
Hydroglyphus geminus (Fabricius, 1792)	
Hydroglyphus signatellus (Klug, 1834)	
Hygrotus confluens (Fabricius, 1787)	
Herophydrus musicus (Klug, 1833)	
Hydroporus discretus Fairmaire, 1859	
Hydroporus limbatus Aubé, 1836	
Hydroporus lucasi Reiche, 1866	
Hydroporus marginatus (Duftschmid, 1805)	
Hydroporus normandi Régimbart, 1903	R
Hydroporus pubescens (Gyllenhal, 1808)	
Hydroporus tessellatus Drapiez, 1819	
Graptodytes fractus (Sharp, 1880-82)	
Graptodytes ignotus (Mulsant, 1861)	R
Graptodytes varius (Aubé, 1836)	R
Stictonectes epipleuricus (Seidlitz, 1887)	E
Stictonectes optatus (Seidlitz, 1887)	
Deronectes fairmairei (Leprieur, 1876)	
Deronectes hispanicus (Rosenhauer, 1856)	
Deronectes moestus (Fairmaire, 1858)	
Stictotarsus duodecimpustulatus (Fabricius, 1792)	
Stictotarsus griseostriatus (De Geer, 1774)	R
Nebrioporus bucheti cazorlensis (Lagar, Fresneda & Hernando, 1987)	E, Vr, R
Nebrioporus clarki (Wollaston, 1862)	
Nebrioporus baeticus (Schaum, 1864)	E
Nebrioporus ceresyi (Aubé, 1836)	
Agabus biguttatus (Olivier, 1795)	
Agabus bipustulatus (Linnaeus, 1767)	
Agabus brunneus (Fabricius, 1798)	
Agabus didymus (Olivier, 1795)	

Annex *(continuation)*

Species	
Agabus nebulosus (Forster, 1771)	
Agabus nitidus (Fabricius, 1801)	R
Agabus paludosus (Fabricius, 1801)	
Agabus ramblae Millán & Ribera, 2001	
Ilybius meridionalis Aubé, 1836	R
Rhantus suturalis (McLeay, 1825)	
Colymbetes fuscus (Linnaeus, 1758)	
Meladema coriacea Castelnau, 1834	
Eretes sticticus (Linnaeus, 1767)	
Hydaticus leander (Rossi, 1790)	
Dytiscus circumflexus Fabricius, 1801	
Dytiscus pisanus Castelnau, 1834	
Cybister tripunctatus africanus Castelnau, 1834	R
Cybister lateralimarginalis (De Geer, 1774)	
Helophorus alternans Gené, 1836	Vr, R
Helophorus gr. *Maritimus* Rey, 1885	R
Helophorus brevipalpis Bedel, 1881	Vr, R
Helophorus flavipes Fabricius, 1792	*, R
Helophorus fulgidicollis Motschuslky, 1860	
Helophorus longitarsis Wollaston, 1864	
Helophorus seidlitzii Kuwert, 1885	E
Georissus gr. *crenulatus* (Rossi, 1794)	
Hydrochus flavipennis Küster, 1852	R
Hydrochus grandicollis Kiesenwetter, 1870	
Hydrochus ibericus Valladares, Díaz-Pazos & Delgado, 1999	E, Vr
Hydrochus nooreinus Henegouven & Sáinz-Cantero, 1992	E
Berosus affinis Brullé, 1835	
Berosus hispanicus Küster, 1847	
Berosus fulvus Kuwert, 1888	
Berosus guttalis Rey, 1883	R
Hemisphaera guignoti Shalberg, 1900	R
Chaetarthria seminulum seminulum (Herbst, 1797)	
Paracymus aeneus (Germar, 1824)	
Paracymus relaxus Rey, 1884	*
Anacaena bipustulata (Marsham, 1802)	
Anacaena globulus (Paykull, 1798)	
Anacaena lutescens (Stephens, 1829)	
Anacaena limbata (Fabricius, 1792)	
Laccobius gracillis intermittens Kiesenwetter in Heyden, 1870	
Laccobius atrocephalus Reitter, 1872	R
Laccobius bipunctatus (Fabricius, 1775)	
Laccobius hispanicus Gentili, 1974	
Laccobius moraguesi Régimbart, 1898	
Laccobius neapolitanus Rottenberg, 1874	
Laccobius obscuratus Rottenberg, 1874	
Laccobius sinuatus Motschulsky, 1849	
Helochares lividus (Forster, 1771)	
Enochrus melanocephalus (Olivier, 1792)	*

Annex *(continuation)*

Species	
Enochrus ater (Kuwert, 1888)	
Enochrus bicolor (Fabricius, 1792)	
Enochrus falcarius Hebauer, 1991	
Enochrus politus Küster, 1849	
Enochrus salomonis (Sahlberg, 1900)	
Enochrus segmentinotatus (Kuwert, 1888)	R
Enochrus testaceus (Fabricius, 1801)	*
Hydrochara flavipes (Steven, 1808)	Vr, R
Hydrophilus pistaceus (Castelnau, 1840)	
Coelostoma hispanicum (Küster, 1848)	
Hydraena exasperata Orchymont, 1935	E, Vr, R
Hydraena capta d´Orchymont, 1936	
Hydraena rufipennis, Boscá-Berga = claryi Jäch, 1994	
Hydraena hernandoi Fresneda & Lagar, 1990	R
Hydraena testacea Curtis, 1830	*, R
Limnebius furcatus Baudi, 1872	R
Limnebius maurus Balfour-Browne, 1978	
Limnebius oblongus Rey, 1883	
Calobius quadricollis (Mulsant, 1844)	R
Ochthebius dilatatus Stephens, 1829	
Ochthebius maculatus Reiche, 1872	
Ochthebius subinteger Mulsant & Rey, 1861	R
Ochthebius auropallens Fairmaire, 1879	
Ochthebius bifoveolatus Waltl, 1835	
Ochthebius corrugatus Rosenhauer, 1856	
Ochthebius cuprescens Guillenbau, 1893	
Ochthebius delgadoi Jäch, 1994	E
Ochthebius dentifer Rey, 1885	R
Ochthebius difficilis Mulsant, 1844	
Ochthebius glaber Montes & Soler, 1988	E, Vn, Vi
Ochthebius grandipennis Fairmaire, 1879	
Ochthebius marinus (Paykull, 1798)	
Ochthebius mediterraneus Ieniestea, 1988	
Ochthebius metallescens Rosenhauer, 1847	
Ochthebius montesi Ferro, 1984	E, Vr, Vi
Ochthebius nanus Stephens, 1829	R
Ochthebius notabilis Rosenhauer, 1856	
Ochthebius quadrifoveolatus Wollaston, 1854	
Ochthebius semotus d´Orychmont, 1942	*, R
Ochthebius tacapasensis baeticus Ferro, 1984	
Ochthebius tudmirensis Jäch, 1997	E
Ochthebius viridis 2 sensu Jäch, 1992	
Cyphon sp.	R, **
Hydrocyphon sp.	**
Potamophilus acuminatus (Fabricius, 1792)	
Stenelmis canaliculata (Gyllenhal, 1808)	R
Elmis aenea (Müller, 1806)	Vr
Elmis maugetii maugetii Latreille, 1798	

Annex *(continuation)*

Species	
Elmis rioloides (Kuwert, 1890)	R
Esolus pygmaeus (Müller, 1806)	R
Oulimnius troglodytes (Gyllenhal, 1827)	
Oulimnius tuberculatus perezi Sharp, 1872	E, Vr, R
Limnius intermedius Fairmaire, 1881	
Limnius opacus Müller, 1806	Vr
Limnius volckmari (Panzer, 1793)	
Normandia nitens (Müller, 1817)	
Normandia sodalis (Erichson, 1847)	
Riolus illiesi Steffan, 1958	R
Pomatinus substriatus (Müller, 1806)	
Dryops gracilis (Karsch, 1881)	
Dryops sulcipennis (Costa, 1883)	
Limnichus sp.	R, **
Heterocerus flexuosus (Stephens, 1828)	R, **
Augyles maritimus (Guérin-Méneville, 1844)	R, **
Bagous sp.	R, **

(*) Species whose presence in the Region of Murcia needs confirmation, (**) Semiaquatic beetles.
E: Iberian endemics, Vr: Species vulnerable at regional scale, Vn: Species vulnerable at national scale, Vi: Species vulnerable at international scale, R: Species rare in the study area.

Chapter 3

Bias in freshwater biodiversity sampling: the case of Iberian water beetles

Abstract

Extensive distributional databases are key tools in ecological research, and good-quality data are required to provide reliable conservation strategies and an understanding of biodiversity patterns and processes. Although the evaluation of databases requires the incorporation of estimates of sampling effort and bias, no studies have focused on these aspects for freshwater biodiversity data. We used here a comprehensive database of water beetles from the Iberian Peninsula and the Balearic Islands, and examine whether these data provide an unbiased, reliable picture of their diversity and distribution in the study area. Based on theoretical estimates using the Clench function on the accumulated number of records as a surrogate of sampling effort, about a quarter of the Iberian and Balearic 50x50 km UTM grid cells can be considered well prospected, with more than 70% of the theoretical species richness actually recorded. These well-surveyed cells are not evenly distributed across biogeographical and physicoclimatic subregions, reflecting some geographical bias in the distribution of sampling effort. Our results suggest that recording was skewed by relatively simple variables affecting collector activity, such as the perceived “attractiveness” of mountainous landscapes and protected areas with recently described species, and accessibility of sampling sites (distance from main research centres). We emphasise the importance of these evaluation exercises, which are useful to locate areas needed of further sampling as well as to identify potential biases in the distribution of current biodiversity patterns.

INTRODUCTION

Conservation assessment and biodiversity research require high quality data on species' distributions, these usually being in the form of extensive databases (Hortal *et al*., 2007). Only countries with a long-standing tradition of natural history and sufficient resources are able to produce good distribution maps based on adequate sampling of a number of taxonomic groups (Lawton *et al*., 1994; Griffiths *et al*., 1999). This is not the case with many Mediterranean countries, in which inventories of many animal groups, particularly insects, are incomplete or nonexistent (Ramos *et al*., 2001).

Hortal *et al*. (2007) noted two general drawbacks associated with of the use of biodiversity databases: 1) lack of survey-effort assessments (and lack of exhaustiveness in compiling data on survey effort), and 2) incomplete coverage of the geographic and environmental diversity that affects the distribution of the organisms. These problems render existing databases and/or atlases of limited use for accurately describing patterns of biodiversity (Prendergast *et al*., 1993; Dennis and Shreeve, 2003; Soberón *et al*., 2007), and compromise the utility of any predictive models based on them (Hortal and Lobo, 2006; Lobo *et al*., 2007). Therefore, it is necessary to incorporate estimates of sampling bias and measures of sampling effort in biodiversity studies to minimize their potential confounding effect (Romo *et al*., 2006).

A number of attempts have been made to explore these issues, using databases from a variety of regions and covering a diversity of taxonomic groups (e.g. Dennis *et al*., 1999, 2006; Lobo and Martín-Piera, 2002; Reddy and Dávalos, 2003, Romo *et al*., 2006). However, to date no study has focused on freshwater biodiversity, probably due to the paucity of inventory data for freshwater systems (Lévêque *et al*., 2005), especially in Mediterranean countries. Freshwater biodiversity may be particularly at risk in many regions of the world (e.g. Allan and Flecker, 1993; Master *et al*., 1998; Ricciardi and Rasmussen, 1999), and inland aquatic systems typically harbour a diverse biota, rich in endemic taxa. This is particularly the case in the Mediterranean Basin, which is considered as one of Earth's biodiversity hotspots (Quézel, 1995; Mittermeier *et al*., 1998; Myers *et al*., 2000). The factors affecting the quality of databases of freshwater organisms are likely to differ from those of terrestrial ones, as the sampling and collecting of data rarely overlap. With this work, we aim to provide a case study for one of the most diverse and well studied groups of freshwater macroinvertebrates in a highly diverse region, the aquatic Coleoptera of the Iberian Peninsula and the Balearics.

Water beetles have high species richness in the Mediterranean region, inhabiting virtually every kind of fresh and brackish water habitat, from the smallest ponds to lagoons and wetlands, and from streams to irrigation ditches, large rivers and reservoirs (e.g. Ribera *et al*., 1998; Ribera, 2000; Millán *et al*., 2002). Water beetles have been proposed as good surrogates of biodiversity in Mediterranean aquatic ecosystems (Sánchez-Fernández *et al*., 2006) and have been used to select priority areas for conservation in this region (Sánchez-Fernández *et al*., 2004; Abellán *et al*., 2005). In comparison to other groups of freshwater invertebrates in the Iberian Peninsula and the Balearic Islands, water beetles are well known in their systematic and biogeography (Ribera *et al*., 1998; Ribera, 2000; Millán *et al*., 2006).

By analyzing an exhaustive database on Iberian water beetles, we aim to determine whether these data are able to provide an unbiased picture of the species diversity and distribution. Firstly, we identify the most probable well-surveyed areas examining whether they effectively cover the different biogeographical and environmental subregions recognized in the Iberian Peninsula. We then examine the distribution of sampling effort, and the extent to which sampling bias can be explained by a suite of environmental variables. Finally, we identify the key areas where the effort should be concentrated in future sampling programs.

METHODS

Study area

The Iberian Peninsula and the Balearic Islands include a wide variety of biomes, relief, climates and soil types (Fig. 1). Although the Iberian Peninsula lies in the temperate zone, its rugged topography gives rise to a great diversity of climates, from semiarid Mediterranean, to oceanic in the northern fringe, and alpine in the high mountains. Mean annual temperatures oscillate between 2.2 and 19 ºC, and total annual precipitation between 203 and 2990 mm. Due to this great variety of relief and climate, the Iberian Peninsula includes an enormous diversity of vegetation types, from deciduous and coniferous forests to sclerophyllous woodlands or annual steppe grasslands (Rey-Benayas and Scheiner, 2002).

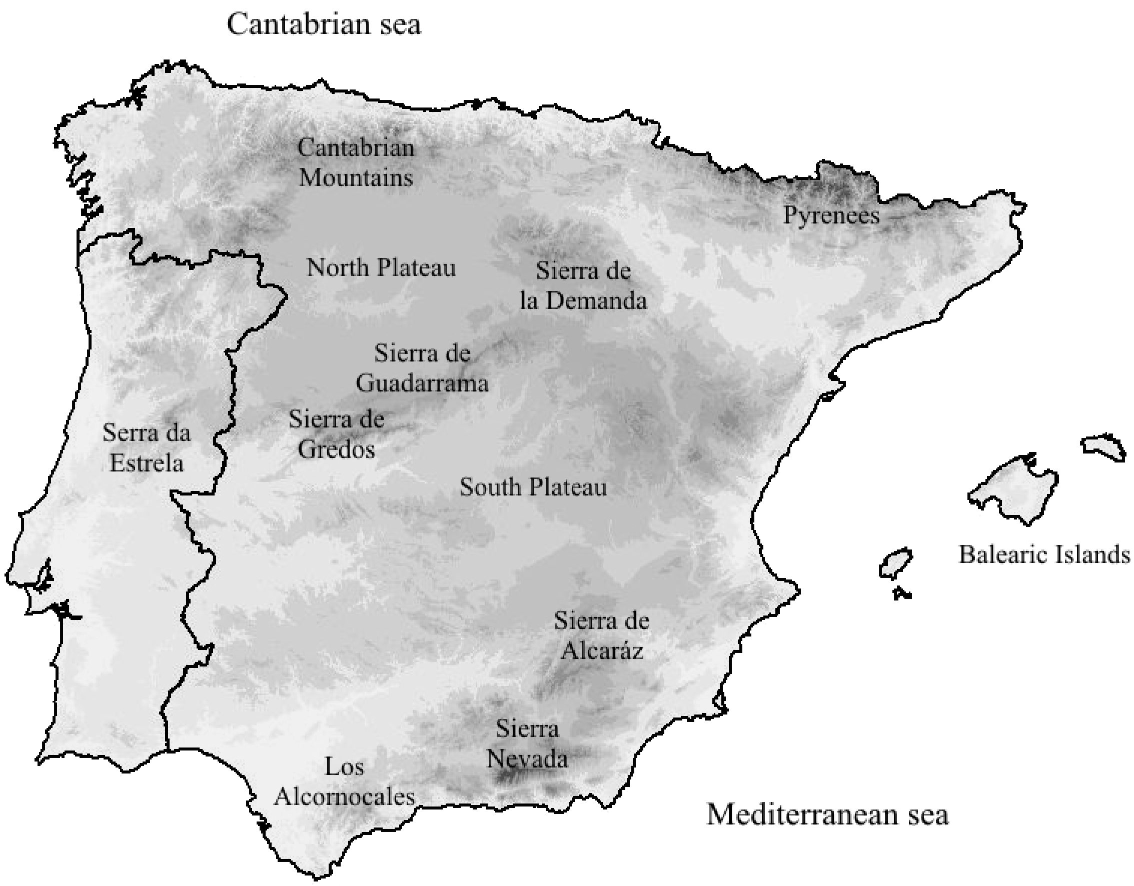

Figure 1. Study area with some locations referred to in text highlighted.

Source of biological data

This work is based on an exhaustive database of Iberian water beetle records (ESACIB "EScarabajos ACuáticos IBéricos") compiling of all available taxonomic and distributional data from the literature (485 bibliographic references and 34,504 database records) as well as museum and private collections, PhD theses and other unpublished sources (16,260 records). After deletion of records with taxonomic uncertainties or doubtful or imprecise localities, ESACIB contains around 50,000 workable records belonging to 511 species or subspecies of eleven families of water beetles (Table 1).

The database was originally referenced at a resolution of 100 km^2 (10x10 km cells), although for simplicity we used 50x50 Km UTM squares as geographic units (n = 257) in this study. This loss of resolution was necessary since only 35% of the 10x10 km cells contain data, and only 3.7% of these have twice the number of database records than species. Grid cells containing <15% of land were not considered, and database records from these cells added to the most environmentally similar neighbouring cell.

Assessing sampling effort

The number of database records in each cell was chosen as a surrogate of sampling effort, following Lobo and Martín-Piera (2002) and Hortal *et al*. (2004). Such an approach has been demonstrated to yield similar results to the use of other measures of sampling effort, such as data on number of individuals recorded, or number of traps employed (Hortal *et al*., 2006). We assumed the number of records in a grid cell is directly related to survey effort, and that the probability of species' occurrence correlates positively with the number of database records.

Collector's curves were used to identify grid squares with inventories complete enough to produce reliable richness scores. Collector's curves are generally considered a good approach to evaluate the quality of inventories (Soberón and Llorente, 1993; Jiménez-Valverde and Hortal, 2003; Hortal *et al*., 2004). These curves represent the expected accumulated number of species encountered within a certain geographical area as a function of a measure of the effort (number of records in this case) invested to collect them (Soberón and Llorente, 1993; Colwell and Coddington, 1994; Goteli and Colwell, 2001). The slope of the collector's curve determines the rate of species accumulation at a given level of sampling effort. This slope diminishes with sampling effort and as new species are found, reaching a hypothetical value of 0 when all species are detected. As the shape of this relationship depends on the order in which individuals were recorded, this order was randomized 100 times to obtain a smoothed accumulation curve (using the EstimateS 6.0 software package; Colwell, 2000). The Clench function was fitted to the smoothed data, and the asymptotic value (i.e. the species richness predicted for an ideally unlimited sample size) was computed. The ratio of recorded to predicted species richness (the asymptotic score) was used as a measure of completeness of each cell inventory. A UTM cell was considered to be adequately sampled when the completeness values were ≥70% (following Jiménez-Valverde and Hortal, 2003). Completeness values measured by different estimators can provide different richness estimations that in turn depend on the sampling effort accomplished (see Chiarucci *et al*., 2001; Hortal *et al*., 2006). Thus, our selection of well surveyed cells is not free of error being a compromise among the probability to choose them correctly and the number of cells able to be analyzed.

Physioclimatic and biogeographic subregions

We assessed the proportion of well surveyed 50 x 50 km cells (≥70% completeness) for each one of six previously delimited physicoclimatic subregions of the Ibero-Balearic area (Lobo and Martín-Piera, 2002). We also examined the degree of completeness for biogeographical subregions defined by Ribera (2000) using compositional information on Iberian water beetles, adding the Balearic Islands as a new subregion. The first regionalization allows us to consider the classic territories with different macro-environmental characteristics, while the second one has the capacity to reflect the differences due to causal variables difficult to quantify as those due to dispersal limitation or historical factors. For each subregion (both physicoclimatic and biogeographical) we computed the species richness and the associated number of database records.

Variables and sampling bias

We considered a total of 26 variables that could potentially explain the distribution of the sampling effort, divided into 4 categories: environmental, land-use, spatial and variables related to the "attractiveness" of the sites. The environmental variables included nine climatic (minimum and maximum monthly mean temperature, mean annual temperature, total annual rainfall, summer precipitation, number of days of sun per year, aridity, annual range of temperature variation, annual precipitation variation); four topographic (minimum, maximum and mean elevation, elevation range) and four lithological (percentage of area with clay, calcareous, and siliceous substrates, lithological diversity). The four land-use variables, selected to represent the degree of human disturbance, measure the coverage of the four human-induced landscapes, which are most common in the study area: urban and industrial areas, non-irrigated croplands, irrigated croplands, and anthropic pasturelands. We also included three variables (hereafter called "attractiveness" variables) related to the accessibility and appeal for researchers: distance from research centres (minimum distance from the central point of each square to the nearest main centre of research on water beetles in the study area; i.e. Barcelona, León and Murcia), number of type localities (where species new to science have been found), and coverage of protected land surface. Lastly, the central latitude and longitude of each cell were also included as spatial predictor variables.

Climate data (original resolution 1 km) are courtesy of the Spanish Instituto Nacional de Meteorología and the Portuguese Instituto de Meteorologia. Topographic variables were obtained from a Digital Elevation Model (Anon. 2000a), and the land-use data (original resolution 280 m) were provided by the European Environment Agency (Corine Programme 1985-1990, Anon. 2000b). Data on underlying geology were extracted from 1:200 000 scale geological maps (Anon. 1995); these were first digitized, and then superimposed on cells through the geographic information system IDRISI (Anon. 2003). Lithological diversity was estimated for each grid cell by applying the Shannon diversity index to the primary lithological variables.

Raw number of database records and cell completeness values of well surveyed cells were regressed against these 26 explanatory variables, and the importance of each subgroup of variables assessed by using Generalised Linear Models (GLM: McCullagh and Nelder, 1989; Crawley, 1993). All variables were standardised to mean = 0 and standard deviation = 1 to eliminate the effects of differences in measurement scale. We assumed a Poisson error distribution for the dependent variables, related to the set of predictor variables via a logarithmic link function. To evaluate potentially curvilinear relationships, the dependent variable was first related separately to either a linear, quadratic, or cubic function of each variable (Austin, 1980). Subsequently, a stepwise procedure was used to enter the variables into the model (Nicholls, 1989, Lobo and Martín-Piera, 2002). First, the linear, quadratic or cubic function of the variable that accounted for the most important change in deviance was entered. The remaining variables were added to the model sequentially according to their estimated explanatory capacity. The procedure was repeated iteratively until no more statistically significant explanatory variables remained ($p \leq 0.05$). At each step, the significance of the terms already selected was tested by submitting the new model to a backward selection procedure. The terms that became non-significant in this step were then excluded. After examining the individual contribution of each explanatory variable, four main complete models were constructed separately for each of the four types of explanatory variables: environmental (climatic and topographic variables), land use, “attractiveness” and spatial. Spatial variables were included as the third-degree polynomial equation of the central latitude and longitude (Trend Surface Analysis - see Legendre, 1993) in order to incorporate the influence of spatial structures arising from the effects of other historical, biotic or environmental variables not otherwise taken into account (Legendre and Legendre, 1998). A backward stepwise regression with the nine terms of the equation as predictor variables was performed to remove non-significant spatial terms. The Statistica package 6.1. (Anon. 2004) was used for all computations.

We measured the relative importance of each type of explanatory variable using a hierarchical partitioning procedure (MacNally, 2000). First, we calculated the percentage of explained deviance for each type of variable, as well as the variability explained by all possible variable combinations in which this type of variable participates. Subsequently, we calculated the average effect of inclusion of each type of variable in all models for which this type of variable was relevant. We took such averages as estimations of the independent contribution of each type of explanatory variable. A Mann-Whitney U-test was used to identify the variables that differ significantly between considered well-surveyed and not well-surveyed cells.

RESULTS

Analysis of the database

The mean value of records per 50x50 km cell was 197 and that of species 48 (Table 1). Most database records (86%) published after 1987 (Fig. 2). The family Dytiscidae shows the highest number of species and records, followed by Hydraenidae and Hydrophilidae (see Table 1). Species richness scores and number of database records were highly correlated (Spearman rank coefficient $rs = 0.95$; $n = 257$; $p \leq 0.001$), showing that the observed species richness in each square depends on the sampling effort.

Table 1. Total species richness (S), number of database records (DR), mean number of species (S_M) and mean number of database records (DR_M) per 50x50 km UTM cell (± SD) for each family of water beetles.

Family	S	DR	S_M	DR_M
Dryopidae	16	1143	4.5 ± 14.1	0.9 ± 1.3
Dytiscidae	171	22056	85.8 ± 146.7	20.4 ± 16.3
Elmidae	32	5561	21.6 ± 52.1	4.4 ± 4.8
Gyrinidae	10	1207	4.7 ± 9	1.4 ± 1.6
Haliplidae	17	2448	9.5 ± 18.2	2.1 ± 2
Helophoridae	34	1826	7.1 ± 16.3	2.1 ± 3.1
Hydraenidae	148	8204	31.9 ± 61.3	8.5 ± 8.3
Hydrochidae	11	626	2.4 ± 10.1	0.8 ± 1.2
Hydrophilidae	68	6790	26.4 ± 66.1	6.8 ± 6.9
Hygrobiidae	1	259	1 ± 3.1	0.3 ± 0.4
Noteridae	3	644	2.5 ± 11.3	0.5 ± 0.7
Total	511	50764	197.4 ± 408.3	48.2 ± 46.6

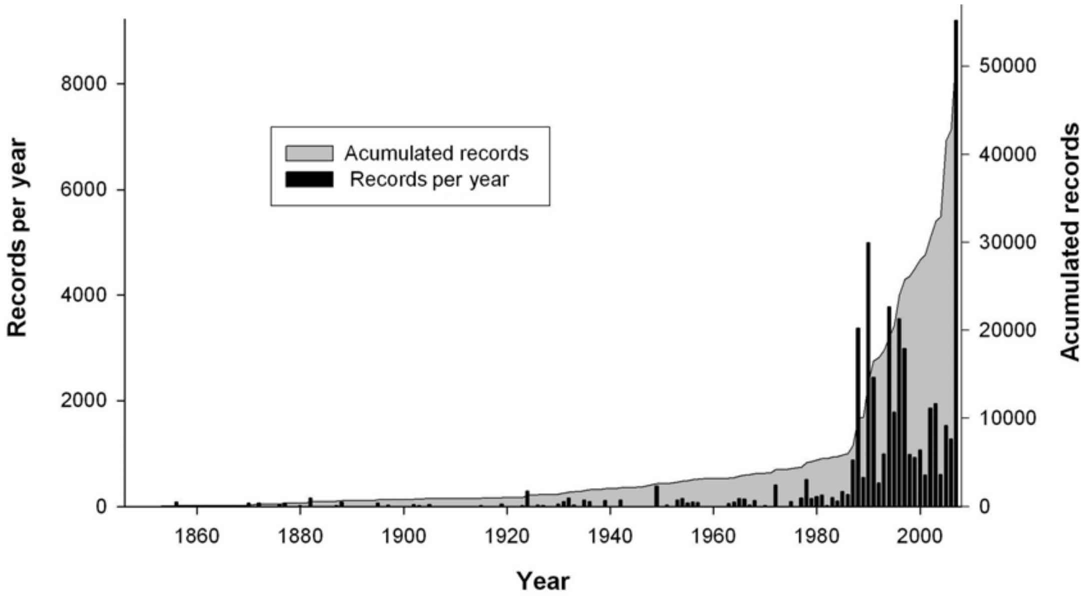

Figure 2. Historical variation in the number of database records and accumulated number of records of Iberian water beetles.

Geographic variation of completeness

The geographical patterns of the number of database records and completeness are quite similar (Fig. 3); cells with higher sampling effort and completeness seem to be widespread in the Iberian Peninsula, while less surveyed cells occur mainly in central Spain (with the exception of the Sierra de Guadarrama and Sierra de Gredos) and south-central Portugal (see Figs. 1 and 3). The mean value of completeness by cells was around 46% (mean ± SD; 45.4 ± 27.5). From a total of 257 cells, 56 had completeness values higher than 70% (considered as well-surveyed; Fig. 3), 26 of them reached scores of 80% or more and only 3 reached scores above 90%.

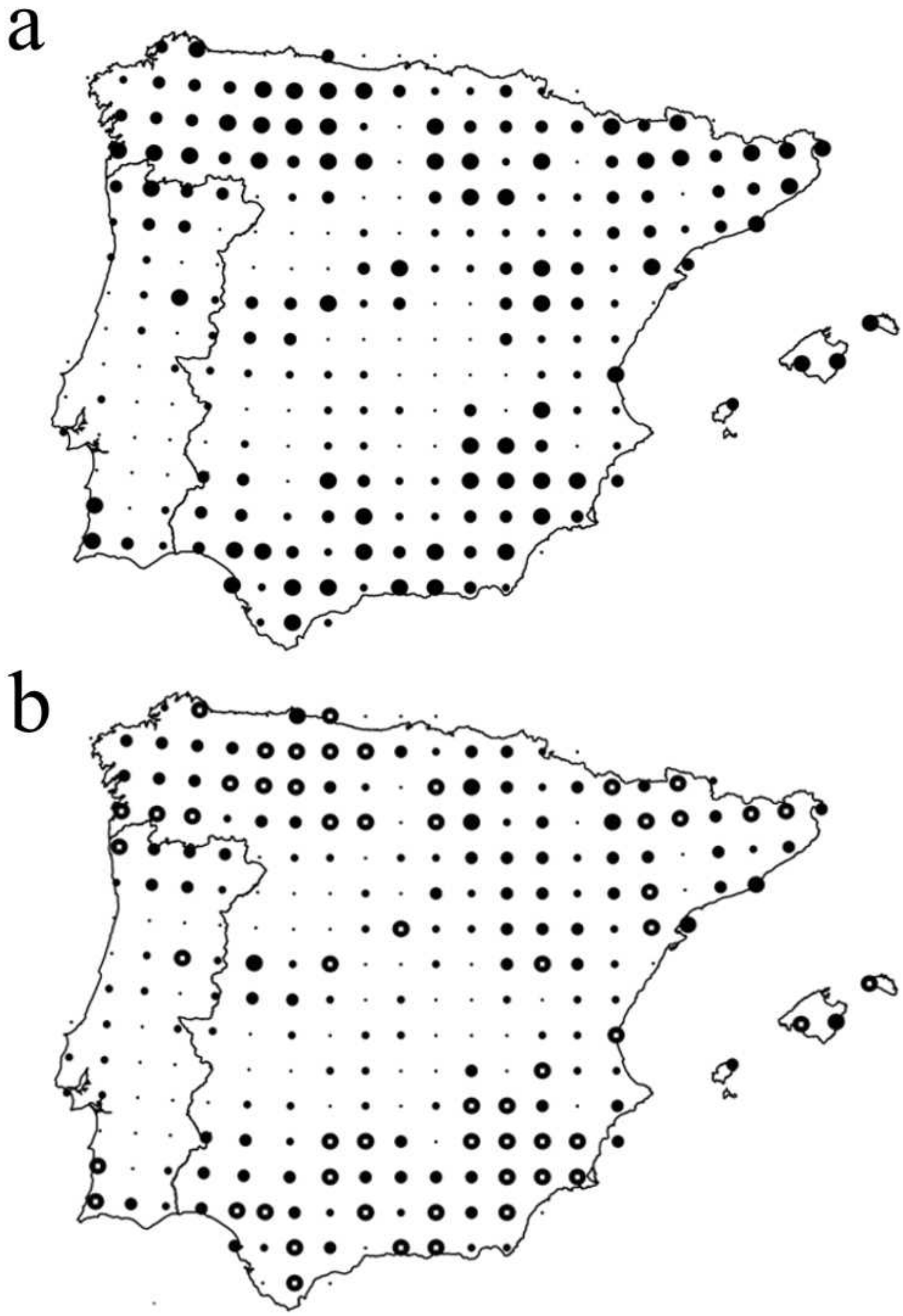

Figure 3. (a) Raw number of database records per cells, and (b) sampling completeness. Sampling completeness was estimated as the proportion of species actually recorded, to the number of species predicted by the accumulation curve adjusted by the Clench function (the asymptotic score of the relationships between the accumulated number of species and the increase in the number of database records). The varying diameter of symbols is proportional to sampling intensity on a scale of 4 categories (quartiles) in each range of values. White dots indicate those well surveyed cells where the proportion (number of species recorded/number of species predicted) equals or exceeds 70%.

There are well-surveyed cells across the whole Iberian territory, although they are not evenly distributed amongst both biogeographical (Chi-square test: χ^2 = 16.88; p=0.005; d.f = 5) or physioclimatic subregions (χ^2 = 12.37; p=0.05; d.f = 6) (Fig. 4). The percentages of considered well-surveyed cells in subregions oscillate from 10% to 50%, the best surveyed subregions being those located in the Balearic Islands, some mountain areas, northern areas close to the Cantabrian sea and the south-eastern subregion (see Table 2). The remaining subregions contained a roughly similar low proportion of well-surveyed cells.

Table 2. Number (N) of 50 x 50 km UTM cells, species (S), considered well-surveyed cells (WSC), database records (DR) and mean number (MN) of species and database records for each of the biogeographic and physicoclimatic Iberian subregions (see Ribera, 2000 and Lobo and Martín-Piera, 2002).

	N of cells	**N of species**	**N-WSC**	**N-DR**	**N-DR in WSC**
Biogeographical subregions					
Hercynian Iberia	132	403	19	17229	8917
Cantabrian	14	221	6	4256	3783
Pyrenean	44	352	9	10915	6776
SE Iberia	43	341	18	15435	12489
SW Iberia	20	150	2	988	625
Balearic Islands	4	145	2	1941	1491
Physioclimatic subregions					
Eurosiberian	35	256	8	5709	3655
West Mediterranean	28	228	5	3412	2141
East Mediterranean	31	309	7	8791	5810
Islands	4	145	2	1941	1491
North Plateau	46	305	6	6316	3257
South Plateau	76	345	12	10991	7462
Montane	37	377	16	13604	10265
Total	257	511	56	50764	34081

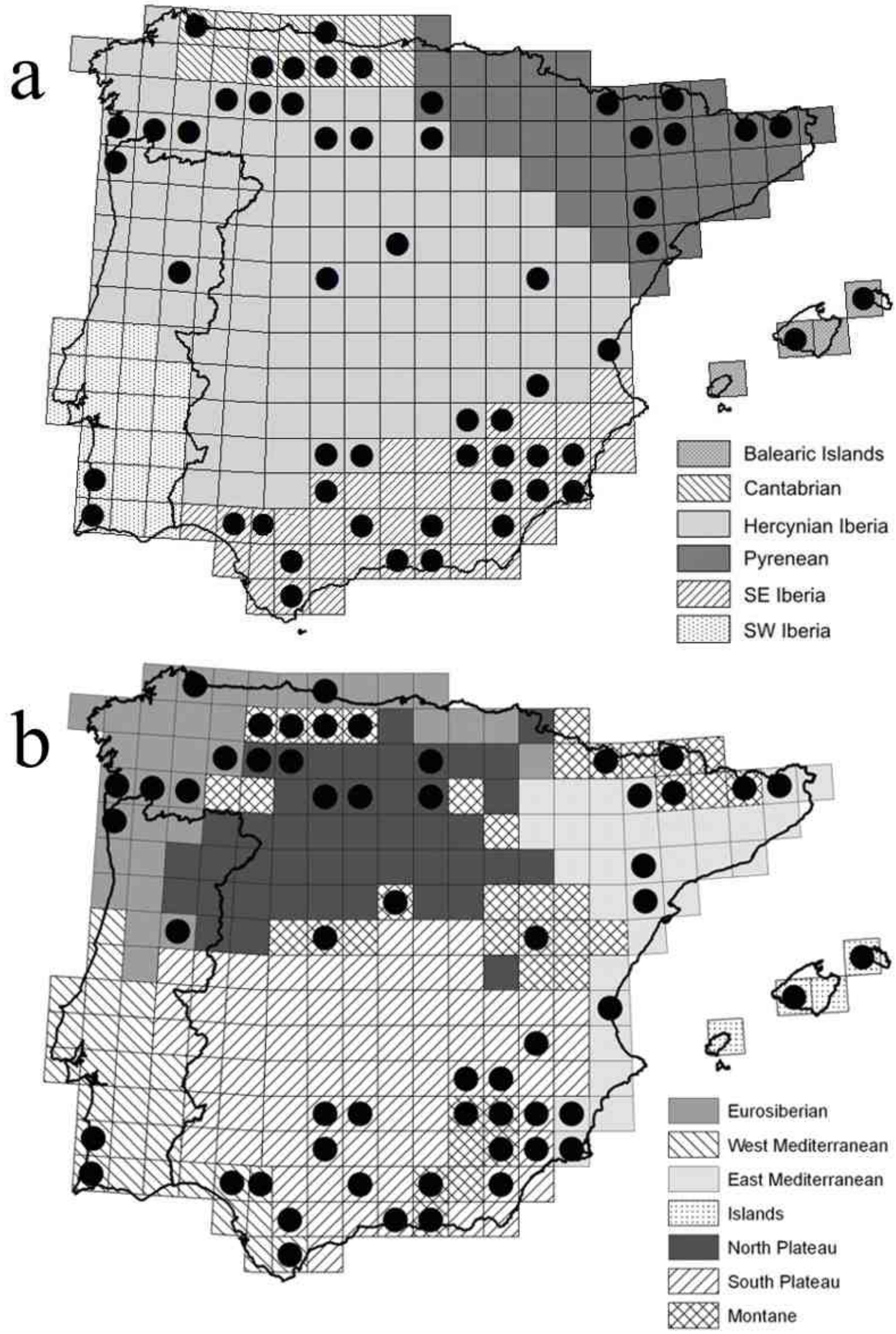

Figure 4. Distribution of well-surveyed 50x50 km UTM cells within (a) the bio-geographical subregions defined by Ribera (2000) and (b) the physicoclimatic subregions defined by Lobo and Martín-Piera (2002).

Variables and sampling bias

The number of type localities, distance from main research centres, altitudinal range and maximum altitude were the variables that accounted for the highest variability in the number of database records (Table 3). Attractiveness variables seemed to be the most influential; a complete model including these three significant variables explained almost 50% of the total variability. The variation in the number of database records was also highly linked to environmental variables (around 39% of total variability), while spatial and land use variables were less relevant. A stepwise regression model with all the significant variables accounted for almost 56% of the total variability in the number of database records (Table 3).

Table 3. Individual explanatory capacity of each one of the considered variables on the sampling effort variation in the 257 50 x 50 km UTM cells (measured as the raw number of database records), and explanatory capacity of the obtained models with all the considered variables of the same type (environmental, land use, spatial and attractiveness variables). Only those variables with a statistically significant effect (p<0.05) and a percentage of deviance >1.0 are represented. *Dev*: Deviance of each variable; *% Dev*: percentage of explained deviance on total variability in the number of database records.

Explanatory variables	***Dev***	***% Dev***	**function**
Environmental variables			
Altitude range	68866.8	18.67	cubic
Maximum altitude	70741	16.46	cubic
Mean altitude	78136.2	7.72	cubic
Summer precipitation	78803.4	6.94	cubic
Annual mean hours of sun	78954.2	6.76	cubic
Lithologic diversity	80584.5	4.83	cubic
Annual mean temperature	81017.1	4.32	cubic
Clay soils	81151.6	4.16	cubic
Aridity index	81552	3.69	cubic
Minimum mean temperature	81617.2	3.61	cubic
Siliceous soils	81850.2	3.34	cubic
Annual mean precipitation	82368.3	2.73	cubic
Maximum mean temperature	82589.2	2.46	cubic
Calcareous soils	82672.7	2.37	cubic
Temperature range	82993.1	1.99	cubic
Land use variables			
Anthropic pasturelands	77494.4	8.48	cubic
Non-irrigated crops	81628.5	3.6	cubic
Irrigated crops	83481.3	1.41	cubic
Attractiveness variables			
Number of type localities	57157.1	32.5	cubic
Distance from research centres	69556.6	17.86	cubic
Protected surface	77756.5	8.17	cubic
Full environmental model	51463.4	39.22	
Full land use model	70408.4	16.85	
Full spatial model	68354.3	18.22	
Full attractiveness model	42719	49.55	

The results of the hierarchical partitioning demonstrated that the attractiveness variables had the highest average effect after inclusion in all models (around 23.5%), followed by the averaged effect of environmental variables (14.6 %), spatial variables (8.3%) and land cover variables (5.3%). However, none of these variables are statistically significant when regressed against the completeness values in the previously considered well-surveyed cells (n = 56).

Well-surveyed cells significantly differed from the rest in a number of variables: they had a higher number of type localities, wider altitude range, larger protected surface, higher maximum altitude, and higher annual and summer precipitation. They were also closer to the main research centres, had less surface of non-irrigated crops, and a lower maximum mean temperature and aridity index (Table 4).

Table 4. Used explanatory variables with significant differences among well-surveyed cells (WSC) and remaining squares (RS) by using a Mann-Whitney U-test (n_1= 56; n_2= 201). The last two columns represent if the median score of each one of these variables is higher (+) or lower (-) for each one of the two groups of cells.

	U	p	WSC	RS
Number of type localities	3002	<0.00001	+	-
Altitude range	3350	<0.00001	+	-
Distance from research centres	3670	0.00007	-	+
Protected surface	3806	0.0002	+	-
Maximum altitude	3740	0.0001	+	-
Annual mean precipitation	4385	0.01	+	-
Summer precipitation	4400	0.01	+	-
Non-irrigated crops	4422	0.01	-	+
Maximum mean temperature	4501	0.02	-	+
Aridity index	4532	0.03	-	+

DISCUSSION

How complete is the water beetle database for the Iberian Peninsula and Balearic Islands?

To date, approximately half of the territory remains characterised by a remarkable scarcity of water beetles records (with < 50% of the predicted species recorded). The database ESACIB is less complete than other comparable databases of Iberian insects, probably due to the larger number of species, which are in general small and inconspicuous, with a limited appeal for amateur entomologists. Thus, in a database for butterflies (226 species), more than 68% of squares had completeness values >75%, and a third of the territory reached scores of 90% or more (Romo *et al*., 2006). For dung beetles (56 species), although the survey effort was low (15,740 records), 33% of the Iberian Peninsula could be considered well-surveyed (Lobo and Martín-Piera, 2002). Our results highlight the lack of complete and extensive inventory data for aquatic taxa (Lévêque *et al*., 2005), as water beetles could be probably be considered one of the best studied groups of freshwater invertebrates in the region. Despite the general incompleteness of the data, a quarter of the study area can already be considered well prospected (completeness values >70%).

Bias in the sampling of freshwater biodiversity

Even when a number of well-surveyed areas have been identified, unevenness in sampling effort may result in a partial (and biased) description of biodiversity variation (Dennis, 2001). In our case, well-surveyed cells are not evenly distributed across biogeographical or physicoclimatic subregions. Furthermore, the proportion of considered well-surveyed cells was also higher on islands and in montane areas (principally in Cantabria and SE Iberia), and lower in central Spain (principally both plateaus) and SW Iberia (Fig. 4).

As in others studies (Dennis and Thomas, 2000; Romo *et al*., 2006), our results demonstrate that the geographic variation in sampling effort is mainly related with attractiveness and environmental variables, and that these same variables significantly differ among well and not enough surveyed cells. Thus, although the sampling effort carried out within those cells considered as well surveyed does not seem to be biased, simple factors affecting the activity of collectors, such as perceived attractiveness of landscapes and accessibility of sampling sites would have deeply influenced the apparent observed species richness pattern. Two co-existing trends appear to occur: researchers tend to sample more intensely in accessible sites near their research centres, and select the study sites based on the presence of interesting species, and/or mountainous and protected areas.

Some of the sites located in mountainous areas with the highest survey effort were also the areas identified by Ribera (2000) as those with the highest conservation value for Iberian aquatic Coleoptera. These include medium altitude streams and freshwater lagoon of the pre-Pyrenees; Sierra de Alacaraz in SE Spain; streams in the Parque Natural de los Alcornocales (South Spain); Sierra de la Demanda (NE Spain, in Hercynian Iberia); eastern part of the provinces of Lugo and Orense (NW Spain); Serra da Estrela (N Portugal); Sierra de Guadarrama (central Spain); some National Parks such as Picos de Europa in the Cantabrian mountains, or Sierra Nevada in the South East; and some saline streams in SE Spain. The future use of distributional predictive models (see Hortal *et al*., 2001; Lobo and Martín-Piera, 2002) can help us to assess if the higher comparative species richness of more surveyed territories is real or some not enough surveyed cells become species-rich.

Where to sample next?

Results show that additional surveys of islands or mountainous areas would only further increase the unbalanced distribution of well-sampled areas across the region. Future surveys should be concentrated in currently recognized under-sampled subregions. As discussed above, well sampled and under-sampled cells differ significantly in a number of variables, and these differences should be borne in mind to diminish environmental bias in future surveys (Hortal and Lobo, 2005; Funk *et al*., 2005). To reduce further bias in collecting effort and improve both spatial and environmental coverage, further work should be concentrated in grid cells with completeness values close to 70%, located in central Spain, specially those on the north and south plateaus (Castilla León and Castilla La Mancha) and in south Portugal (Beja, Setubal, Portalegre and Santarém). This will increase the number of well-sampled squares in areas currently under-sampled, allowing the possibility of less biased analyses. Efforts should focus on water bodies on arid, low-elevation areas with a temperate Mediterranean climate and a high coverage of non-irrigated crops, which are in general far from research centres. The scarcity of water bodies in these areas, together with their low perceived attractiveness, could explain the low sampling effort invested on them to date.

Concluding remarks

We emphasize the importance of the evaluation of data quality as a preliminary step in biodiversity studies (see Reddy and Dávalos, 2003), assessing the degree of geographical coverage of existing faunistic data, and the amount and nature of any bias in its collection. Our results demonstrate that, as happens with other taxa and territories (Dennis *et al*., 1999; Dennis and Thomas, 2000; Zaniewski *et al*., 2002; Reutter *et al*., 2003; Graham *et al*., 2004; Soberón and Peterson, 2004; Martínez-Meyer, 2005; Romo *et al* ., 2006; Hortal *et al*., 2007; Lobo *et al*., 2007; Soberón *et al*., 2007), the available distributional information on Iberian water beetles is the result of a biased collection process influenced by sociological and environmental factors. More survey effort needs to be carried out to obtain a detailed and reliable representation of the diversity of Iberian water beetles. Despite this, our results reveal that all regions contain well-surveyed cells at the coarse resolution considered here, and both the number, and the lack of bias in the completeness percentages of these well-surveyed cells facilitate their use in biogeographical and conservation studies. In this sense, our results provide a basis for the design of efficient future field campaigns, since they allow the identification of genuinely under-sampled regions. The identification of well-surveyed cells is invaluable in modelling species' distributions, since taxa not recorded from these are likely to be genuinely absent (see Lobo, 2008). The discrimination of those localities with good quality distributional information, together with the use of these modelling techniques, will allow us to obtain a better picture of the distribution of water beetle diversity in the Iberian Peninsula in the future.

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Chapter 4

Assessing models for forecasting species richness of Iberian water beetles

Abstract

Using the information compiled for an exhaustive database of Iberian water beetles, and taking into account some previous weaknesses in our data (relatively scarce and biased), we set out to assess the possibility of obtaining a reliable prediction of species richness distribution in the Iberian Peninsula and Balearic Islands. We firstly discriminate those cells with relatively well-surveyed inventories according to different completeness criteria, selecting both observed and asymptotic predicted species richness values as the dependent variable in predictive functions. After comparing and evaluating the performance of the different species richness scenarios obtained, we generate a forecasting map describing the thus obtained species richness distribution of these insects for the study area. Lastly, we examine the location of insufficiently surveyed cells with important predicted species richness values in order to propose future areas of sampling. We recommend using estimated richness scores as dependent variable and greater stringency in setting the thresholds for considering cells as well-surveyed. The model selected explained 60.1% of total deviance with a high mean Jackniffe predictive error (26.6%). These results should be interpreted carefully, since they show that statistics cannot always efficiently overcome the scarceness of the data. On some occasions, these procedures simply provide an approach, and a greater survey effort is necessary to improve the accuracy of forecasting. Taking into account the limitations exposed, the use of these modelling techniques, combined with an accurate discrimination of the areas of high quality distributional information, will provide a better picture of the distribution of water beetle diversity in the Iberian Peninsula, being useful to locate those areas where more sampling effort is necessary. The sampling effort to validate and improve this model must be focused on the areas of high predicted species richness that are not well inventoried, such as Central Spain and some areas in the northeast Portugal, southwest of the Iberian peninsula, and the southern foothills of the Iberian Central Systems.

INTRODUCTION

Extensive biological databases are a primary tool in ecological and biogeographical research since good-quality data are required for biodiversity research to develop reliable conservation strategies and provide scientific information on biodiversity patterns and processes (Prendergast *et al.,* 1993; Soberón and Peterson, 2004; Guralnick *et al.,* 2007; Hortal *et al.,* 2007). Only countries with a long-standing tradition of natural history and sufficient resources are able to produce good distribution maps based on adequate sampling of a number of taxonomic groups (Lawton *et al.,* 1994; Griffiths *et al.,* 1999). This is not the case of Mediterranean countries such as Spain, in which inventories of many animal groups, particularly insects, are incomplete or nonexistent (Ramos *et al.,* 2001), being evident when all the available information on insects is mapped.

Therefore, a key question is to discriminate poorly from well-surveyed areas in order to orientate new sampling programmes and, when the localities considered as well surveyed represent the pool of environmental conditions in the considered territory (Kadmon *et al.,* 2004; Hortal and Lobo, 2005), this information can be used to forecast the distribution of biodiversity attributes in the remaining not well-surveyed territory (Hortal *et al.,* 2001; Ferrier, 2002; Lobo and Martín-Piera, 2002; Hortal *et al.,* 2004; Ferrier and Guisan, 2006; Lobo, 2008a).

The identification of well-surveyed areas is invaluable for modelling species distributions since predictive models are obtained from data gathered from these previously well-inventoried areas. In this context, the first step is to estimate the degree to which the data represent complete inventories on a given scale (Petersen and Meier 2003), which can be carried out using a variety of statistical techniques (Soberón and Llorente, 1993; Colwell and Coddington, 1994; Gotelli and Colwell, 2001, Rosenzweig *et al.,* 2003; Koellner *et al.,* 2004). Usually, an area is considered adequately sampled when its completeness score (number of species estimated/recorded) is above a specific arbitrary value. Therefore, the selection of well surveyed areas is always a delicate task not free from error (Chiarucci *et al.,* 2001; Hortal *et al.,* 2006), being a compromise between the probability of choosing the areas correctly and the number of areas that can be analyzed, while this selection might affect the performance of the distributional predictive model. However, the number of species recorded in each well-surveyed locality does not necessarily represent the “true” total number of species that may constitute its inventory. Thus, the asymptotic value generated by the use of species accumulation curves has also been used as an estimation of the species richness in well-surveyed localities in order to building interpolations for the whole territory (Hortal *et al.,* 2004).

Freshwater ecosystems are submitted to high rates of human alteration and therefore, of biodiversity loss (Allan and Flecker, 1993; Master *et al.,* 1998; Ricciardi and Rasmussen, 1999; Saunders *et al.,* 2002; Darwall and Vié, 2005). Furthermore, human pressures on freshwater resources are likely to increase in coming decades, putting yet more species at risk (Strayer, 2006). This is particularly true in the Mediterranean Basin, which is considered as one of Earth's biodiversity hotspots (Quézel, 1995; Mittermeier *et al.,* 1998; Myers *et al.,* 2000). In this study, we use Iberian water beetles as a focal group. From a taxonomic and biogeographical perspective, water beetles are perhaps one of the best known groups of invertebrates in the Iberian Peninsula and Balearic Islands (Ribera *et al.,* 1998; Ribera, 2000). These species groups are specially diversified in the Mediterranean region where they inhabit almost every kind of fresh and brackish water habitat, from the smallest ponds to lagoons and wetlands and from streams to irrigation ditches and reservoirs (e.g. Ribera *et al.,* 1998; Ribera, 2000; Millán *et al.,* 2002). Furthermore, they have been shown to be good indicator of the whole biological diversity existent in aquatic ecosystems (Bilton *et al.,* 2006; Sánchez-Fernández *et al.,* 2006) and have been successfully used in the selection of priority areas for conservation (Sánchez-Fernández *et al.,* 2004; Abellán *et al.,* 2005).

Using the information compiled for an exhaustive database on Iberian water beetles and taking into account some weaknesses in our data (relatively scare and biased) (see Sánchez-Fernández *et al.,* 2008), our general aim is to know if it is possible to obtain a reliable prediction on the species richness distribution of this animal group in the Iberian Peninsula at a resolution of 50 x 50 km UTM cells. We firstly discriminate those cells with relatively well-surveyed inventories according to different completeness criteria, selecting both observed and asymptotic predicted species richness values in these cells as the dependent variable in predictive functions. After comparing and evaluating the performance of the different species richness scenarios obtained, we generate a forecasting map describing the so obtained species richness distribution of these insects for the entire Iberian Peninsula. Lastly, we examine the location of insufficiently surveyed cells with important predicted species richness values in order to propose future areas of sampling.

METHODS

Study area

The study focuses on the Iberian Peninsula and Balearic Islands, two close, biogeographically related areas (López-Martínez, 1989), extending over 585 644 km^2 (Fig. 1). The territory includes a variety of biomes, relief, climates, and soil types, where altitude ranges from sea level to 3 483 m in the Sierra Nevada. Is one of the richest European regions in terms of animal species diversity (Williams, 2000), and particularly in endemic water beetles (Ribera, 2000; Ribera *et al.,* 2003), and is characterised by a wide range of ecosystem types, some of which are rare on a European scale.

Figure 1. Study area with some locations referred to in text highlighted.

Source of biological data

We used an exhaustive database of records of Iberian water beetles (ESACIB “EScarabajos ACuáticos IBéricos”). This database almost certainly represents the most complete information available for a major group of freshwater invertebrates in the study area. ESACIB contains over 50 000 records with associated location data for 510 species of water beetles, including all available geographical and biological data from the literature up to 2006, as well as from museum and private collections, PhD theses, and other unpublished sources. The database was originally referenced at a resolution of 100 km^2 (10x10 km cells), although for simplicity we use here 50x50 km UTM squares as geographical units (n = 257) (see details in Sánchez-Fernández *et al.,* 2008).

Adequately surveyed cells

In order to assess the performance of distribution models according to different completeness thresholds, the completeness values were calculated using the asymptotic score of the Clench function on the accumulated number of records. Many studies suggest the appropriateness of using the number of database records as a surrogate of sampling effort (see Lobo, 2008b). The ratio of recorded to asymptotic predicted species richness was used as a measure of the completeness of each cell inventory. Here, we have used seven completeness thresholds (50%, 55%, 60%, 65%, 70%, 75%, and 80% of total Clench predicted values) in order to select those cells that can be considered adequately surveyed (see Fig. 2). With the thresholds mentioned above, the number of well prospected cells were 121, 108, 92, 80 56, 42 and 26, respectively. It is important to remark that these well-surveyed cells are distributed across both physicoclimatic (Lobo and Martín-Piera, 2002) and biogeographical subregions (Ribera, 2000) (for details see Sánchez-Fernández *et al.,* 2008).

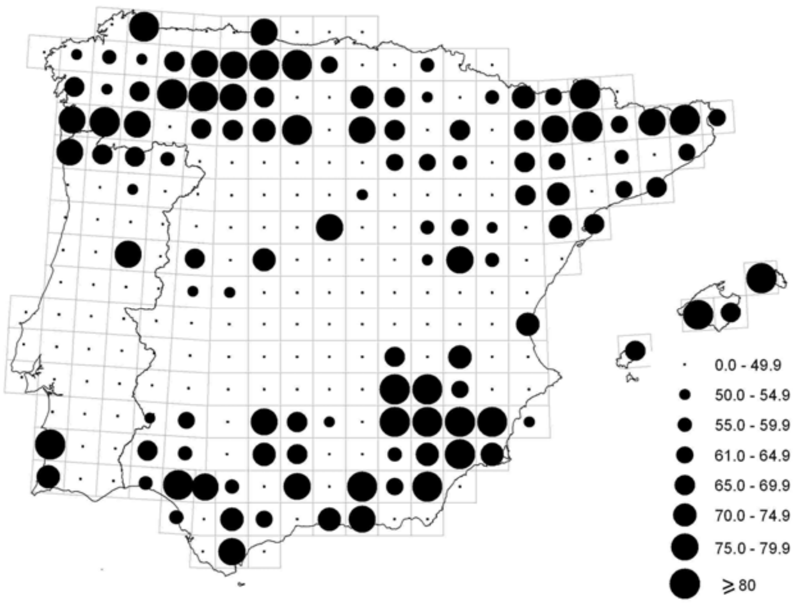

Figure 2. Sampling completeness (number of species recorded/number of species estimated by Clench function). Dot sizes indicate the different thresholds used to consider a cell as well surveyed cells.

Forecasting species richness

Selected explanatory variables

To account for the environmental factors affecting species richness, 18 environmental variables potentially related to species richness at our working scale (2500 km^2) were used. For each cell, nine climatic (minimum and maximum monthly mean temperature, mean annual temperature, total annual rainfall, summer precipitation, mean percentage of sunny hours per year, aridity, annual range of temperature variation, annual precipitation variation); four topographic (minimum, maximum and mean altitude, elevation range), four lithological variables (percentage of area with clay, calcareous, and siliceous substrates, lithological diversity) and one variable related with the surface of water bodies in each square were used. We also included the spatial location of each cell (latitude and longitude) as predictors in the model fitting procedure, to include effects due either to historic events or unconsidered variables with a spatial structure, since it was thought they may aid to include the effects of variables other than purely environmental. The sources of this environmental information are described in Lobo and Martín-Piera (2002).

Model building

A GLM procedure was used to model variation in species richness as a function of the most significant environmental and spatial explanatory variables (McCullagh and Nelder, 1989). Firstly, all explanatory variables were standardised to mean = 0 and standard deviation = 1 to eliminate the effects of differences in measurement scale. We assumed a Poisson error distribution for the dependent variables (the number of species), related to the set of predictor variables via a logarithmic link function. To evaluate potentially curvilinear relationships, the dependent variable was first related separately to either a linear, quadratic, or cubic function of each variable (Austin, 1980). Subsequently, a stepwise procedure was used to enter the variables into the model (Nicholls, 1989; Lobo and Martín-Piera, 2002). First, the linear, quadratic or cubic function of the environmental variable that accounted for the most important change in deviance was entered. The remaining variables were added to the model sequentially according to their estimated explanatory capacity. The procedure was repeated iteratively until no more statistically significant explanatory variables remained ($p \leq 0.05$). At each step, the statistical significance of the terms already selected was tested by submitting the new model to a backward selection procedure. The terms that became non-significant in this step were then excluded. Spatial variables were included after environmental variables as the third-degree polynomial equation of the central latitude and longitude (Trend Surface Analysis - see Legendre, 1993) in order to incorporate the influence of spatial structures arising from the effects of other historical, biotic or environmental variables not otherwise taken into account (Legendre and Legendre, 1998). A backward stepwise regression with the nine terms of the equation as predictor variables was performed to remove non-significant spatial terms. All the obtained models were checked for overdispersion (when the variability of the dependent variable exceeds the variability provided by the assumed distribution) in order to obtain better parameter estimates and related statistics. We also used Akaike's Information Criterion (AIC) to guarantee that the selected model by this stepwise procedure is the one that possesses lowest AIC values. The STATISTICA 6.1 package (Anon, 2004) was used for all statistical computations.

Model assessment

The selected final model was evaluated detecting outliers (cells with residual absolute values higher than mean ± standard deviation) and calculating the potential leverage (a measure of the distance of each observation from the centroid of the multi-dimensional space defined by the variables included in the model; Nicholls, 1989). Thus, residuals were examined to determine whether they were due to erroneous data, or to the environmental uniqueness of the cells. Whilst the latter were included with the rest of the observations in the final parameter estimation process, the former were eliminated and the modelling procedure carried out again.

The predictive power of the model was estimated with a Jackknife procedure; model parameters were estimated as many times as the number of considered adequately surveyed cells (n), deleting each cell singly once, and comparing the so obtained predicted values against the values of the dependent variable (observed richness or asymptotic species richness).The percentage of error for each cell value was subsequently calculated and the mean error percentage (MEP) for all the cells used to estimate the predictive error of competing model results.

Final model residuals were also checked for autocorrelation using Moran's I test (Legendre and Legendre, 1998). Whenever, as a result of this analysis, any spatial structure could be seen to remain in the residuals, such autocorrelation was taken to indicate the existence of at least one further variable not included in the analysis, with a spatially structured effect on species richness. The inclusion of the third degree polynomial of latitude and longitude after environmental variables is presumed to compensate for this influence.

Distinguishing genuinely poor from badly sampled areas

Once the best model was chosen, this was used to obtain a simulated map of water beetles species richness distribution in the Iberian Peninsula and Balearic Islands. The difference between the predicted and the observed richness was used to distinguish areas genuinely poor in species richness from those that have been badly sampled, and to identify the areas where the effort should be concentrated in future sampling programmes.

RESULTS

Selection of the best predictive model

Modelling results seemed to depend on the completeness threshold used. Explained deviance oscillates from 11.1% to 60.1%, the highest values being seen when the data of the cells with higher completeness percentages (80%) and species richness derived from accumulation curves were used (Fig. 3a). Mean error percentages did not significantly differ between competing models (Fig 3b), although the lower variability in the differences between the observed and predicted Jackknife also suggest that better model predictions are obtained when the dependent variable is chosen by the most restrictive completeness threshold.

Therefore, we selected the model that was able to explain the highest percentage of deviance: the model built using the cells with more than 80% completeness and the species richness values estimated by the accumulation curves as dependent variable. In this model, we found two outliers that were deleted. As the quadratic function of minimum altitude (Amin) accounted for the greatest change in deviance, this variable was the first to be included in the model. Once included, latitude alone added supplementary explanatory capacity. Thus, the final model was: $S = EXP\ (5.04 + 0.24Amin - 0.22Amin^2 + 0.04Latitude)$.This simple model is able to explain 60.1% of total species richness variability with a mean error percentage of 26.6% (95% confidence interval from 19.27% to 34.04%). Model residuals are not significantly autocorrelated at any one of the seven distance classes (lag = 80 km).

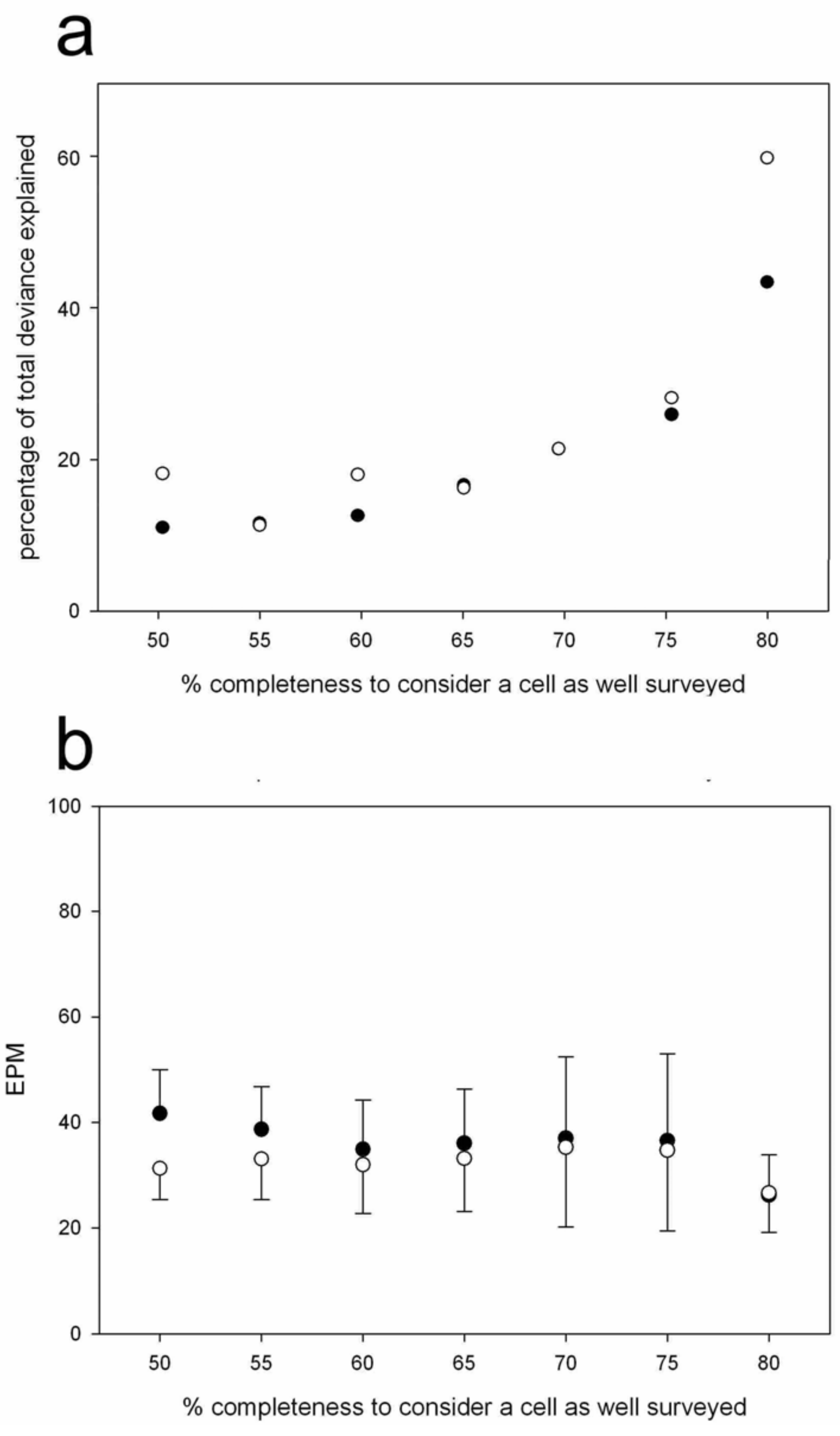

Figure 3. (A) Differences in the deviance explained by different models depending on the completeness threshold selected to consider a cell as adequately surveyed and using observed richness (black circles) and estimated richness by accumulation curves (Clench function) as dependent variable (white circles). (B) Mean error percentage (MEP) and confidence interval (95%) depending on the threshold used to consider a cell as adequately surveyed and using observed richness (white circles) and estimated richness by accumulation curves (Clench function) as dependent variable (black circles).

Species richness distribution pattern

When the final model was applied to the entire study area, the simulated geographic distribution pattern shows that high species richness areas can be found across the whole Iberian territory (Fig. 4). Predicted species richness ranged from 67 to 179 with hotspots of water beetles mainly concentrated in areas of medium-high altitude far from the coast, with the exclusion of high mountain areas (Fig. 4): We can distinguish five main areas: A) Northwest, from the western part of the Cantabrian Mountains to Serra de Megadouro in Portugal, including the Montes de León; B) Northeast, including the Pyrenees and the Iberian Central System; C) Central Spain, from the Iberian Central System (mainly Sierra de Gredos) to Sierra Morena, including Campo de Calatrava and Montes de Toledo); D) Southwest, including Serra de São Mamede in Portugal and Sierra de Aracena and Picos de Aroche in Spain, and E) Baetic Systems, including Sierra de Jabalones and Sierra de Filabres, but excluding the highest zones of Sierra Nevada.

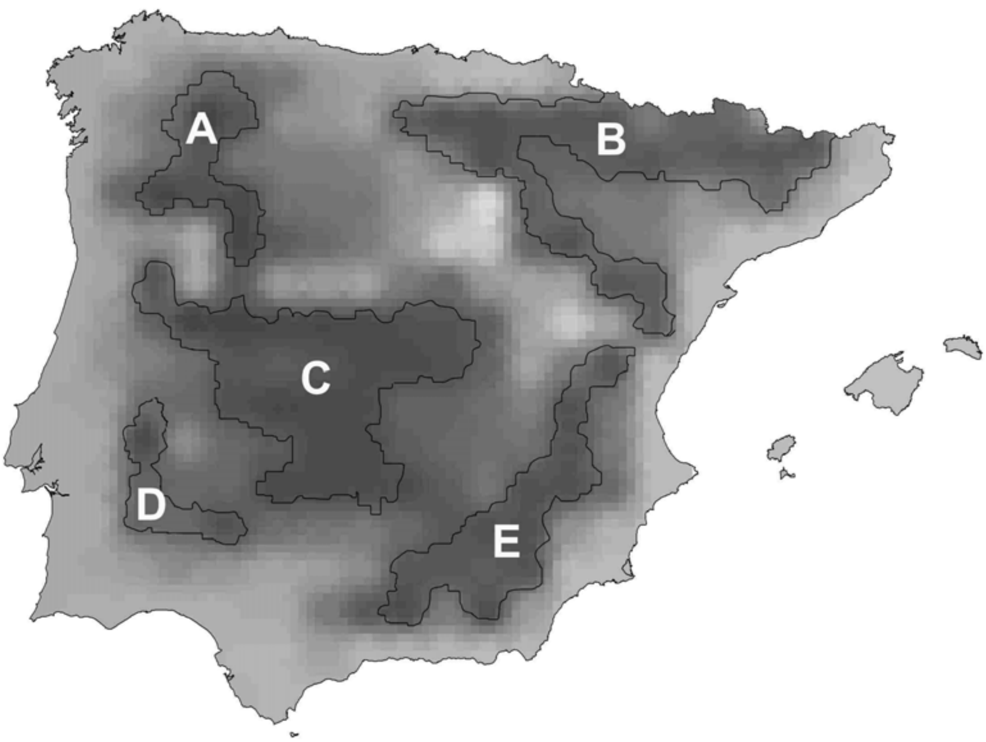

Figure 4. Predicted species richness of water beetles in the Iberian Peninsula and Balearic Islands. The results were interpolated at 10 km resolution.

Furthermore, the observed species richness pattern showed that the richest areas are widespread in the Iberian Peninsula: south-central Portugal, Balearic Islands, Cantabrian Mountains, Pyrenees and Baetic Systems (south-eastern region), while poor cells occur mainly in central Spain (with the exception of the Sierra de Guadarrama and Sierra de Gredos). The predicted species richness values differed greatly from the observed values (Spearman rank correlation coefficient; rs = -0.05; p = 0.44), and were also not correlated with the chosen sampling effort surrogate (rs = -0.09; p = 0.15). In order to locate suitable areas for future sampling programmes, figure 5 shows the differences between predicted and observed richness (i.e. number of species left to be recorded in each cell). The highest values were concentrated in central Spain and Portugal, and some isolated cells in pre-Pyrenees and a set of cells in southeast Spain.

DISCUSSION

Our results show that the use of local estimates of richness before applying the modelling procedure produces a better picture of species richness patterns (Hortal *et al.,* 2004). One general drawback associated with the use of biodiversity databases is the incomplete coverage of the geographical and environmental diversity which can affect the distribution of the organisms. These problems compromise the usefulness of any predictive models based on them (Hortal and Lobo, 2006; Lobo *et al.,* 2007). Selecting higher completeness values guarantees the appropriate choice of well-surveyed cells, but diminishes the number of cells able to be analyzed. According to our results, we recommend high stringency in setting the thresholds for considering cells as well-surveyed despite the possible loss of observations for the analyses, although all different biogeographical and environmental regions recognized in the study area must be represented at least once among the well surveyed areas.

Nevertheless, the simulated species richness pattern generated by an exigent selection of well surveyed cells should be interpreted with caution. Our model is able to explain a moderate percentage of total species richness variability (around 60%) similar to the obtained in the case of dung beetles (62%; see Lobo and Martín-Piera, 2002), the other Iberian insect group for which an analogous modelling procedure has been carried out. However, the model for water beetles only included two predictors, and was accomplished using only 26 observations from 257, and more importantly the mean prediction error for these observations was relatively high (27%) compared with the obtained for Iberian dung beetles (16%). All these results suggest that this distributional proposal should be considered a preliminary step towards a more definitive one, which will be possible when we possess a higher number of data on those insufficiently surveyed cells.

Our database does not seem to be so exhaustive; 50 000 database records on 510 species is not enough to have an adequate number of training localities well distributed across the Iberian environmental conditions (see Sánchez-Fernández *et al.,* 2008). Even at this wide resolution, approximately half of the Iberian territory remains characterised by a remarkable scarcity of water beetle records, with < 50% of the predicted species recorded. Unevenness in sampling effort may result in partial (and biased) descriptions of biodiversity variation (Dennis, 2001), a common drawback that limits the usefulness of existing databases and/or atlases for accurately describing biodiversity patterns (Prendergast *et al.,* 1993; Dennis and Shreeve, 2003; Hortal *et al.,* 2007; Soberón *et al.,* 2007). To produce an accurate map of the probable geographic distribution of Iberian water beetles it would be necessary to increase the number of adequately surveyed cells (>80% of completeness).

This study should thus be understood as a preliminary step, and more survey effort needs to be carried out to obtain a detailed and reliable representation of the diversity of Iberian water beetles. It should be noted that statistics cannot always efficiently overcome the scantiness of the data. Hence, on some occasions, these procedures provide just a first approach, and more survey effort is necessary in order to improve the accuracy of the forecasting. Nevertheless, the obtained results allow us to obtain a better picture of the distribution of water beetle diversity in the Iberian Peninsula and, especially, to locate the areas where more sampling effort is necessary.

The biogeographical diversity patterns for the majority of insects groups reflect the distribution of the areas investigated by entomologists (Dennis and Hardy, 1999), and, consequently, the most intensely surveyed areas should not necessarily be the richest ones. Our results show that the predicted patterns of Iberian water beetles richness differed greatly from the observed values, suggesting that entomologists' intuition could have failed in deciding where to focus sampling effort. As Sánchez-Fernández et al. (2008) point out, researchers have tended to sample water beetles more intensely in accessible sites near their research centres, and have selected the study sites based on the presence of interesting species, and/or mountainous and protected areas, ignoring other less attractive regions, but with high predicted richness. In this sense, our results provide a basis for the design of future sampling efforts, since they allow the identification of genuinely under-sampled but, potentially species rich regions. (Fig. 5). It is necessary to significantly increase the sampling effort in these areas to be able to accurately describe the spatial distribution of insects, even considering that Coleoptera are one of the best surveyed groups of freshwater biodiversity in the study area. Predictive model techniques such as those employed here, could be an effective and useful tool for designing sampling protocols. Furthermore, new field work data from less inventoried regions will allow us to validate the model and continually improve the predicted figures (Lobo *et al.,* 2004). The sampling effort to validate and improve this model must be focused on the areas of high predicted species richness that are not well inventoried, such as, Central Spain (from Montes of Toledo to Sierra Morena) and some areas in the northeast Portugal (Serra de Megadouro), southwest of the Iberian peninsula (Sierra de los Filabres, close to Sierra Nevada), and southern foothills of the Iberian Central Systems.

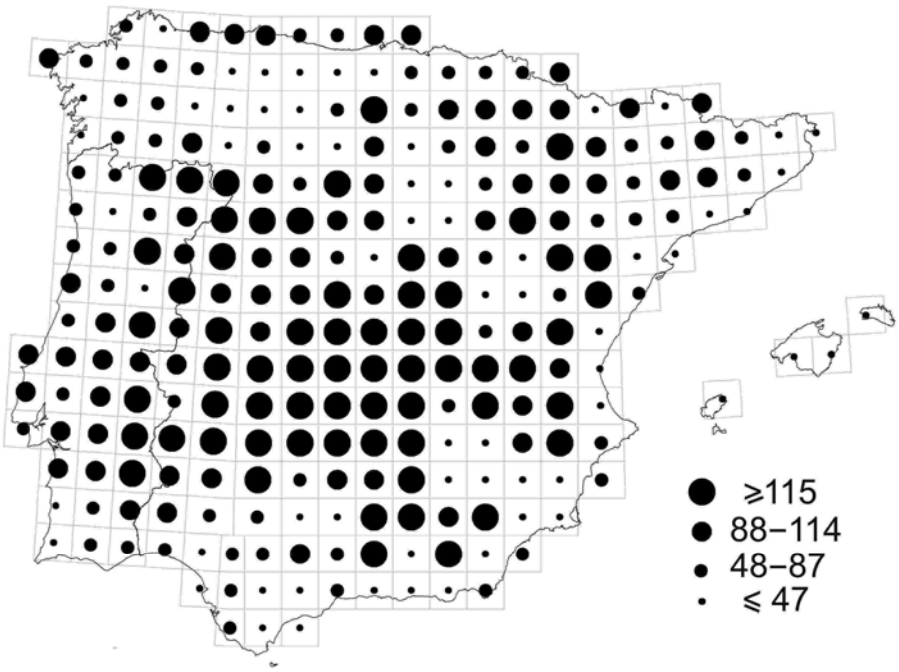

Figure 5. Differences between observed and predicted species richness values. The varying diameter of symbols is proportional to the number of species remaining to be recorded in each cell on a scale of four categories (quartiles).

Implications for conservation

Some of the hotspots of predicted richness identified by our models based solely on environmental and spatial variables are particularly threatened, especially those at low and medium altitude. These are the areas under most intense pressure and subjected to frequent changes in land use (Martínez-Fernández *et al.,* 2000), via dredging and stream canalization, drainage, urbanization and other human developments, pollution and loss of salinity (Gómez *et al.,* 2005; Velasco *et al.,* 2006). The extent to which the current species richness could have been affected by these processes is unknown, as there is no suitable reference data. Thus, any intensification of survey effort in these areas can provide lower species richness values than expected because of the impoverishment of natural assemblages. The predicted hotspots in mountainous areas are in general better preserved, since they are under less intensive management, and seem to be more appropriate for testing the validity of our conclusions.

Given the spatial scale studied, it is not possible to identify the sites most important for water beetle conservation in the study area. On the other hand, the use of predicted maps of species richness is not a sound strategy for identifying areas for conservation, even when models are accurate (Hortal *et al.,* 2004). To decide where and how to locate protected areas, other biodiversity components (e.g. faunal composition, vulnerability, rarity, endemism) must also be estimated, and used in combination with biogeographical and ecological information (Margules and Pressey, 2000).

Our results provide a preliminary picture of the distribution of water beetle species richness in the Iberian Peninsula and the Balearic Islands, a region of high diversity and intense human pressure. Water beetles have been identified as excellent surrogates of wider inland water biodiversity (Bilton *et al.,* 2006; Sánchez-Fernández *et al.,* 2006), and consequently, the results from this study are likely to be paralleled by other less well-known groups of freshwater macroinvertebrates, and are potentially useful for identifying areas of interest for the preservation freshwater biodiversity.

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Chapter 5

Are the endemic water beetles of the Iberian Peninsula and the Balearic Islands effectively protected?

Abstract

One of the most serious environmental problems is the current acceleration in the rate of species extinction associated with human activities, which is occurring particularly rapidly in freshwaters. Here we examine whether endemic water beetles are effectively protected by existing conservation measures in the Iberian Peninsula and the Balearic Islands, a region of high diversity and intense human pressure. We used an exhaustive database for aquatic beetles in the region to address such issues. Firstly, we identify the most threatened endemic taxa using a categorization system to rank species according to their conservation priority or vulnerability. Of the 120 endemic species of water beetles used in the analysis, only two (*Ochthebius ferroi* and *O. javieri)* were identified as being extremely vulnerable, 71 were highly vulnerable and 46 moderately vulnerable, with only a single species identified as having low vulnerability status. Since no Iberian species of aquatic Coleoptera has legal protection, the only conservation measure available for these species is the extent to which they occur in protected areas. Here we identify distributional hotspots for threatened endemic species, and evaluate the extent to which these are already included in the Natura 2000 network in Spain and Portugal. Despite a high degree of concordance between hotspots and Natura 2000 sites, the distribution of four species falls completely outside the network. The analysis also reveals that Natura 2000 fails to protect saline water bodies, despite their high conservation interest and narrow global distribution. The picture revealed here with water beetles is likely to be similar for others groups of freshwater macroinvertebrates, since Coleoptera are known to be good surrogates of aquatic biodiversity in the region. Finally, the degree of protection provided via Natura 2000, and the utility of red lists are discussed.

INTRODUCTION

Nowadays, among the numerous environmental problems, one of the most serious is undoubtedly the acceleration in the rate of species extinction associated with human activities, as it involves an irreversible loss of biological information with unpredictable consequences (Wilson, 1988; May *et al.*, 1995; Fontaine *et al.*, 2007). Biodiversity is unevenly distributed, and some areas and groups contain most of this biological information. In this context, conservation efforts should be focussed on areas of high biodiversity, with the highest number of threatened species (Kerr *et al.*, 2000; Margules and Pressey, 2000).

There is a widespread agreement that rates of biodiversity loss are greater in freshwater systems than in other ecosystems (Allan and Flecker, 1993; Master *et al.*, 1998; Ricciardi and Rasmussen, 1999; Saunders *et al.*, 2002; Darwall and Vié, 2005). Furthermore, human pressures on freshwater resources is likely to increase in the coming decades, putting yet more species at risk (Strayer, 2006). The most severe threat to freshwater species is habitat loss, followed by pollution and invasive species (IUCN, 2004). This can be particularly important in the Mediterranean Basin, which is considered as one of Earth’s hotspot areas for biodiversity (Mittermeier *et al.*, 1998; Myers *et al.*, 2000) and where landscapes have been subject to strong human influence for millennia (Naveh and Lieberman, 1984). The transformation of agricultural landscapes, moving from extensive to intensive farming, has accelerated during this century, leading to the progressive loss of inland water habitats (Hollis, 1995; Stoate *et al.*, 2001).

Here we use water beetles as an example of the wider freshwater biota since they are perhaps one of the best known groups of invertebrates, from a taxonomic and biogeographical perspective in the Iberian Peninsula and Balearic Islands (Ribera *et al.*, 1998; Ribera, 2000). Water beetles also have high species richness in the Mediterranean region and inhabit virtually every kind of fresh- and brackish water habitat, from the smallest ponds to lagoons and wetlands and from streams to irrigation ditches and reservoirs (e.g. Ribera *et al.*, 1998; Ribera, 2000; Millán *et al.*, 2002). Furthermore, they have been shown to be good indicators of the wider biodiversity in aquatic ecosystems (Bilton *et al.*, 2006; Sánchez-Fernández *et al.*, 2006) and have been used successfully to select priority areas for conservation (Sánchez-Fernández *et al.*, 2004a; Abellán *et al.*, 2005).

The Iberian Peninsula and the Balearic Islands are areas of great biogeographic interest, being regarded as one of the richest European regions in terms of species diversity (Médail and Quézel, 1997; Domínguez-Lozano *et al.*, 1996; Reyjol *et al.*, 2007). Insects in general and beetles in particular, make up the highest percentage of the biodiversity of this area. Close to 98% of the total Iberian fauna are invertebrates, and roughly 81% are insects (Ramos *et al.*, 2001). Iberian water beetles comprise 510 species and sub-species, 120 of which are endemic to the region. Few invertebrate species in the area have legal protection or are included in red lists. Only 10 species of water beetles are included in red lists at national or international level: *Acilius duvergeri* Gobert, 1874; *Cybister vulneratus* Klug, 1834; *Hydroporus lluci* (Fery, 1999); *Ochthebius glaber* Montes and Soler, 1988 and *O. montesi* Ferro, 1983 are included in the Red Book of Spanish invertebrates (Verdú and Galante, 2006) and *Acilius duvergeri* Gobert, 1874; *Ilybius hozgargantae* Burmeister, 1983; *Deronectes algibensis* Fery and Fresneda, 1988; *D. depressicollis* (Rosenhauer, 1856); *D. ferrugineus* Fery and Brancucci, 1997 and *Rhithrodytes agnus agnus* Foster, 1993 are included in the IUCN Red List (IUCN 2004). Since these were proposed, some changes have been suggested for the IUCN Red List. Abellán *et al.* (2005) proposed including *Ochthebius irenae* Ribera and Millán, 1998; *Ochthebius glaber* Montes and Soler, 1988 and *O. montesi* Ferro, 1983; and Ribera (2000) proposed the inclusion of *Rhithrodytes agnus argaensis* Bilton and Fery, 1996; *Stenelmis consobrina* Dufour, 1835 and *Potamophilus acuminatus* (Fabricius, 1792) and the exclusion of *Ilybius hozgargantae* Burmeister, 1983; *D. depressicollis* (Rosenhauer, 1856); and *D. ferrugineus* Fery and Brancucci, 1997, which have recently been shown to be more widespread than formerly thought.

None of the Ibero-balearic species of aquatic Coleoptera has legal protection, none being included in the National Catalogue of Threatened Species of Spain or Portugal, Annex II of the Bern convention or Annex II and IV of the Habitats Directive (92/43/EEC). Whilst the effectiveness of legal protection for small invertebrates may be debated (Hutchings and Ponder, 1999; New and Sands, 2003), in the current situation, the only protection available to these species is the extent to which they occur in protected areas designated on the basis of other taxa or habitat features. Most protected areas networks have been designated based on selected target species, or group of target organisms (typically plants and vertebrates). Such a strategy for establishing nature reserves may frequently lead to an under-representation of many important components of biodiversity (Linnell *et al.*, 2000), suggesting than that reserves are not always suited to protect ‘non charismatic’ organisms (Kati *et al.*, 2003;

Martínez *et al.*, 2006). Consequently, it is necessary to evaluate the effectiveness of existing reserve networks (such as Natura 2000) in protecting threatened, diverse and ‘non charismatic’ groups, such as water beetles. Despite these requirements, very few assessments of the effectiveness of protected area networks in maintaining any aspect of freshwater biodiversity have been carried out (e.g. Keith, 2000; Abellán *et al.*, 2006), despite the fact that species losses in these habitats are alarmingly high.

The work presented here aims to determine the extent to which endemic Iberian and Balearic water beetles are protected by existing conservation networks. As discussed above, aquatic Coleoptera are good surrogates of inland water biodiversity, so patterns which hold for this group are likely to apply to other aquatic taxa. The study has three specific objectives: 1) to identify the most threatened endemic water beetles in the study area, by ranking species according to their conservation priority or degree of vulnerability; 2) to locate distributional hotspots for the most threatened species; and 3) to evaluate the extent to which the Natura 2000 network provides effective protection for these species and areas.

METHODS

Study area

The study focuses on the Iberian Peninsula and Balearic Islands, two close, biogeographically related areas (López-Martínez, 1989) which extend over 585,644 km^2 (Fig. 1). The territory includes a variety of biomes, relief, climates, and soil types, where altitude ranges from sea level to 3,483 m in the Sierra Nevada. Although being entirely within the temperate zone, the rugged topography of the Iberian region gives rise to a great diversity of climates, from semiarid Mediterranean, to oceanic in the northern fringes, and alpine in the high mountains. The study area is one of the richest European regions in terms of animal species diversity (Williams, 2000), and particularly in endemic water beetles (Ribera, 2000; Ribera *et al.*, 2003) and is characterised by wide range of ecosystem types, some of which are rare on a European scale.

Figure 1. Study area, showing key locations referred to in the text.

All major inland aquatic habitat types are present within the Ibero-Balearic area, and here we divide inland waters into the following: headwater streams, rivers and middle reach streams, saline streams, springs, irrigation channels, rice-fields, artificial pools, reservoirs, lagoons, pools and ponds, and salt-pans (Millán *et al.*, 2002; Sánchez-Fernández *et al.*, 2004a).

Data set

We used an exhaustive database of records of Iberian water beetles (ESACIB) to assess the status and degree of protection afforded to endemic species in the area. This database almost certainly represents the most complete information available for a major group of freshwater invertebrates in the study area. ESACIB includes all available geographical and biological data from the literature up to 2006, as well as from museum and private collections, doctoral theses, and other unpublished sources. The database contains over 50 000 records with associated location data (10 x 10 UTM squares) for 510 species of water beetles. ESACIB also contains information on abundance, habitat and date of last record for Iberian endemic species.

We concentrated on the 120 species and well established subspecies of water beetle endemics of the Iberian Peninsula and Balearic Islands. We selected this subset to be able to assess degree of vulnerability in absolute terms, due to our understanding of their distribution throughout their ranges. In total more than 6,500 records (species/site/reference, with associated information on persistence, abundance and habitat type) were included in analyses.

Assessing conservation status of taxa

IUCN categories of threat (Endangered, Vulnerable, Rare, Indeterminate, etc.) are widely used in Red lists of endangered species, and have become an important tool in conservation action at international, national and regional levels. Existing definitions are largely subjective, however, and as a result evaluations made by different authors frequently differ, and may not accurately reflect actual extinction risk (Mace and Lande, 1991; Abellán *et al.*, 2005; Fitzpatrick et al, 2007). Furthermore, for many groups most species would have to be classified as data-deficient and, in the case of most invertebrates, where good quality historical or demographic data are lacking, it is inconceivable that there will ever be sufficient data for a sensible classification based on current IUCN evaluation techniques (Sutherland, 2000). As a consequence there remains a need for alternative objective methods with which to assess species' vulnerability, particularly ones that are applicable to invertebrates. Here we applied a method for prioritizing species and populations for conservation developed by Abellán *et al.* (2005), modifying some the scoring of some variables. This evaluation is based on a set of six species and habitat attributes: general distribution, Iberian distribution, rarity, persistence, habitat rarity and habitat loss. Each variable was scored 0-3 for each species, in order of increasing perceived risk (see Table 1). Variables were categorized and evaluated as follows:

1. General distribution. Five types of general distributional range (GD) were distinguished, from trans-Iberian to endemic (See Table 1 for more details). The highest scores going to species with the most restricted ranges. In our case, all species score 3 because all are endemic species, but this variable was maintained in order to keep the structure of the original methodology and to be able to compare absolute scores with future assessment of non endemic species.

2. Iberian distribution (ID). Here we overlapped the actual distribution of species with the biogeographical regions defined by Ribera (2000), including the Balearic

Islands as an additional region. Species' scores were based on the number of regions occupied, the highest scores being given to species restricted to a single region.

3. We evaluated Rarity (R) as a combination of three different aspects of rarity: rarity of occupancy (number of sites occupied), rarity of individuals within areas (density rarity), and habitat specificity (Rabinowitz *et al.* 1986; Gaston, 1994; see Table 1).

4. We evaluated the persistence (P) of a species as its temporal continuity in the study area (Abellán *et al.*, 2005). This was determined from the date of the last record (see Table 1).

5. Habitat rarity (HR) was considered since species restricted to locally scarce habitats are likely to be more vulnerable to local extinction. This was evaluated using an expert panel (see below).

6. Habitat loss (HL). This variable is also important as species that were once widespread can become rare or vulnerable through habitat loss (HL) or fragmentation. This was also evaluated using an expert panel.

Table 1. Variables used in species vulnerability analysis, and their rank values.

Variables	Score			
	0	1	2	3
General Distribution (GD)	Trans-Iberian species	Northern and Southern	Disjunct species	Endemic species
Iberian Distribution (ID)	Presence in 4 or more bio-geographical regions	Presence in 3 bio-geographical regions	Presence in 2 bio-geographical regions	Presence in 1 bio-geographical region
Rarity (R)	None of the 3 criteria exposed below	One of the criteria exposed below	Any two of the criteria exposed below	All the criteria
	gr (geographic rarity)	Small range size (less than 20 squares)		
	dr (demographic rarity)	Low abundance (less than 10 exemplars)		
	hs (habitat Specificity)	High habitat specificity (more than 75 % of total records in one habitat type)		
Persistence (P)	Last capture after 2001	Last capture between 1997 and 2001 (last 10 years)	Last capture between 1996 and 1987 (last 20 years)	Last capture before 1987 (more than 20 years)
Habitat Rarity (HR)	Rarity values of habitat type < 0.75	Rarity values of habitat type between 0.75 and 1.5	Rarity values of habitat type between 1.6 and 2.25	Rarity values of habitat type >2.25
Habitat Loss (HL)	Habitat loss values < 0.75	Habitat loss values between 0.75 and 1.5	Habitat loss values between 1.6 and 2.25	Habitat loss values >2.25

In the absence of an obvious quantitative way to evaluate the last two variables, we instead relied upon an “expert panel”. Surveys were sent to researchers working on freshwater ecosystems in the Iberian Peninsula including a wide range of workers to minimize local subjectivity. Individual researchers were asked to score the major inland aquatic habitat types (see above) according to their perception of their rarity, and the degree to which they are under threat within the Ibero-Balearic area. Scores (rarity/threat) ranged from 0 to 3, where 0 was very common/not threatened, 1 moderately common/minimally threatened, 2 moderately rare/threatened and 3 extremely rare/very threatened. We calculated the mean value of rarity and threat for each habitat type on the basis of the twenty-four returned sets of scores. Results from this expert panel are show in Table 2. We multiplied the rarity or threat scores of each habitat by the percentage occurrence of each species in each habitat to produce a habitat rarity (HR) score for each species. Values were then ranked into four categories, scored from 0 to 3.

Table 2. Rarity and threat scores for habitat types in the study area, according to the expert panel.

Habitat type	**Rarity**	**Threat**
Irrigation channels	0	0
Headwater streams	0	1
Rice-fields	2	1
Artificial pool	0	0
Reservoir	0	0
Spring	1	2
Lagoons	2	2
Pools, ponds	1	2
Saline streams	3	2
Rivers and middle reach streams	0	3
Salt-pans	2	2

We grouped species into four vulnerability categories according to their overall vulnerability scores: low (0–4); moderate (5–8); high (9–13); very high (14–18), following Abellán et al. (2005). Species assigned to high and very high categories were considered high-priority taxa in conservation terms.

Distribution maps of all these high-priority conservation species were overlapped to detect 'hotspots' of threatened endemic water beetles: these being defined as cells containing a record of at least three of those species.

Gap Analysis and effectiveness assessment

The Natura 2000 network forms the core of measures to protect biodiversity in Europe. Under the EC Habitats Directive (EU Council Directive 92/43/EEC), Member States are required to prepare, and propose to the European Commission, a national list of sites of community importance (pSCIs). These will eventually be designated by the Member States as special areas of conservation (SACs) (Article 4.4). These SACs, together with Special Protection Areas (SPAs) designated under the Birds Directive (79/409/EEC), will collectively form the future Natura 2000 network (Article 3.1 of the Habitats Directive). Four GIS data layers (SACs and SPAs for Spain and Portugal) supplied by national conservation agencies, were edited and combined to produce a single layer of current Natura 2000 networks areas in the Iberian Peninsula and the Balearics.

We conducted a gap analysis to evaluate the degree of protection of the high-priority species and hotspots achieved by the Natura 2000 network in the study area by overlapping the distribution maps of individual species and hotspots with the Natura 2000 network map using Arcview 3.2 (ESRI inc.). Here a square is considered protected when at least 25% of its area is within a Natura 2000 site. This threshold was considered appropriate since most aquatic habitats are highly influenced by processes occurring in their catchments.

RESULTS

Identification of threatened species

Of the 120 endemic species of water beetles used in the analysis, only two (1.7%) were identified as being of very high vulnerability, 71 (59.2%) were identified as high vulnerability, 46 taxa (38.3%) as moderate, and a single remaining species (0.8%) was assigned low vulnerability status (Table 3). As a result of these rankings, we were able to identify 73 high-priority species amongst Iberian Peninsula and Balearic Island endemics (with a vulnerability score of 9 or above).

Table 3. Vulnerability scores of variables used in vulnerability assessment. (GD, general distribution; ID, Iberian distribution; gr (geographic rarity); dr (demographic rarity); hs (habitat specificity), rarity; P, persistence; HR, habitat rarity; HL, habitat loss; VS: vulnerability score; CAT: Category). See appendix for species codes.

Code	**GD**	**ID**	**dr**	**gr**	**hs**	**R**	**P**	**HR**	**HL**	**SV**	**CAT**
Och.ferr	3	3	1	1	1	3	3	1	2	15	very high
Och.javi	3	3	1	1	1	3	2	1	2	14	very high
Och.anda	3	3	0	1	1	2	0	3	2	13	high
Och.caes	3	3	0	1	1	2	0	3	2	13	high
Och.mont	3	3	0	1	1	2	0	3	2	13	high
Aga.neva	3	3	0	1	1	2	0	2	2	12	high
Hdn.luca	3	3	1	1	1	3	0	0	3	12	high
Hdn.quet	3	3	1	1	1	3	0	0	3	12	high
Hep.joco	3	2	1	1	0	2	2	0	3	12	high
Hep.koro	3	3	0	1	0	1	2	1	2	12	high
Hyd.sier	3	3	0	1	1	2	0	2	2	12	high
Lib.hila	3	3	0	1	1	2	1	0	3	12	high
Och.cant	3	3	0	1	1	2	3	0	1	12	high
Hdn.alca	3	3	1	1	0	2	1	0	2	11	high
Hdn.alta	3	3	0	1	1	2	0	0	3	11	high
Hdn.mari	3	3	1	1	1	3	0	0	2	11	high
Hep.leon	3	2	1	1	1	3	0	1	2	11	high
Lib.mino	3	3	0	1	1	2	2	0	1	11	high
Lib.ordu	3	3	0	1	1	2	0	0	3	11	high
Neb.croc	3	3	0	1	0	1	1	0	3	11	high
Och.alba	3	3	0	1	1	2	2	0	1	11	high
Och.diaz	3	3	1	1	1	3	1	0	1	11	high
Och.glab	3	2	0	0	1	1	0	3	2	11	high
Och.pedr	3	3	1	1	0	2	2	0	1	11	high
Och.tudm	3	2	0	0	1	1	0	3	2	11	high
Ibe.cerm	3	3	0	1	1	2	0	1	2	11	high
Aga.pico	3	3	0	1	1	2	1	0	1	10	high
Der.cosg	3	3	0	1	0	1	1	0	2	10	high
Der.fost	3	3	0	1	1	2	1	0	1	10	high
Dry.cham	3	3	1	1	0	2	0	0	2	10	high

Code	GD	ID	dr	gr	hs	R	P	HR	HL	SV	CAT
Hch.inte	3	3	1	1	0	2	0	0	2	10	high
Hdn.alba	3	3	1	1	0	2	0	0	2	10	high
Hdn.isab	3	3	1	1	1	3	0	0	1	10	high
Hdn.lusi	3	3	1	1	0	2	0	0	2	10	high
Hdn.meca	3	3	1	1	1	3	0	0	1	10	high
Hdn.serv	3	3	1	1	0	2	0	0	2	10	high
Hdn.zeze	3	3	1	1	1	3	0	0	1	10	high
Hep.hisp	3	3	1	1	0	2	0	0	2	10	high
Hyt.fres	3	3	0	1	0	1	0	1	2	10	high
Lib.igna	3	3	0	1	0	1	0	0	3	10	high
Lib.mill	3	3	1	1	1	3	0	0	1	10	high
Lib.monf	3	3	1	1	0	2	0	0	2	10	high
Neb.baet	3	1	0	0	1	1	0	3	2	10	high
Och.gayo	3	3	0	1	0	1	1	0	2	10	high
Och.iren	3	2	0	1	0	1	0	2	2	10	high
Och.semo	3	3	1	1	0	2	0	0	2	10	high
Der.algi	3	3	0	1	1	2	0	0	1	9	high
Der.aube	3	2	0	1	0	1	1	0	2	9	high
Der.bran	3	3	0	1	1	2	0	0	1	9	high
Der.wewa	3	3	0	1	1	2	0	0	1	9	high
Hch.angi	3	2	1	1	0	2	0	0	2	9	high
Hdn.cata	3	3	0	1	0	1	0	0	2	9	high
Hdn.deli	3	3	0	1	1	2	0	0	1	9	high
Hdn.gadi	3	3	0	1	0	1	0	0	2	9	high
Hdn.madr	3	3	0	1	1	2	0	0	1	9	high
Hdn.marc	3	3	0	1	0	1	0	0	2	9	high
Hdn.mons	3	3	0	1	0	1	0	0	2	9	high
Hdn.tati	3	3	0	1	0	1	0	0	2	9	high
Hep.neva	3	2	0	1	0	1	1	0	2	9	high
Hyd.alha	3	3	0	1	1	2	0	0	1	9	high
Hyd.cant	3	3	0	1	0	1	0	0	2	9	high
Hyd.cons	3	3	0	1	0	1	0	0	2	9	high
Hyd.lluc	3	3	1	1	0	2	0	0	1	9	high
Ily.dett	3	3	0	1	0	1	0	0	2	9	high
Lab.glor	3	2	1	1	0	2	0	0	2	9	high
Lib.mont	3	3	0	1	0	1	0	0	2	9	high

Code	GD	ID	dr	gr	hs	R	P	HR	HL	SV	CAT
Lib.nanu	3	3	0	1	0	1	1	0	1	9	high
Och.delg	3	1	0	0	0	0	0	3	2	9	high
Oul.bert	3	2	0	0	1	1	0	0	3	9	high
Oul.echi	3	3	0	1	1	2	0	0	1	9	high
Rhi.agnu	3	3	0	1	1	2	0	0	1	9	high
Rhi.arga	3	3	0	1	1	2	0	0	1	9	high
Rhi.bima	3	2	1	1	1	3	0	0	1	9	high
Der.angu	3	2	0	1	0	1	0	0	2	8	moderate
Der.cost	3	2	0	1	1	2	0	0	1	8	moderate
Der.dela	3	3	0	0	0	0	0	0	2	8	moderate
Der.depr	3	3	0	0	1	1	0	0	1	8	moderate
Hdn.bale	3	3	0	1	0	1	0	0	1	8	moderate
Hdn.boli	3	2	0	1	0	1	0	0	2	8	moderate
Hdn.gava	3	3	0	1	0	1	0	0	1	8	moderate
Hdn.iber	3	2	0	0	0	0	0	0	3	8	moderate
Hdn.manf	3	2	0	1	1	2	0	0	1	8	moderate
Hep.bame	3	1	0	1	0	1	0	1	2	8	moderate
Hyd.brac	3	2	0	1	0	1	0	0	2	8	moderate
Hyd.gred	3	3	0	1	0	1	0	0	1	8	moderate
Hyd.paga	3	2	0	1	0	1	0	0	2	8	moderate
Och.bell	3	2	0	1	1	2	0	0	1	8	moderate
Oul.cyne	3	2	0	1	0	1	0	0	2	8	moderate
Stn.occi	3	2	0	0	0	0	0	0	3	8	moderate
Der.bico	3	2	0	0	0	0	0	0	2	7	moderate
Der.ferr	3	2	0	0	0	0	0	0	2	7	moderate
Grt.cast	3	1	0	0	0	0	0	1	2	7	moderate
Hch.iber	3	2	0	1	0	1	0	0	1	7	moderate
Hch.noor	3	2	0	0	1	1	0	0	1	7	moderate
Hdn.hisp	3	2	0	0	0	0	0	0	2	7	moderate
Hyd.bran	3	2	0	0	0	0	0	0	2	7	moderate
Hyd.neco	3	1	0	0	0	0	0	1	2	7	moderate
Hyd.vesp	3	1	0	0	0	0	0	1	2	7	moderate
Lib.hisp	3	2	0	0	0	0	0	0	2	7	moderate
Lib.iber	3	1	0	1	1	2	0	0	1	7	moderate
Lib.lusi	3	2	0	0	0	0	0	0	2	7	moderate
Neb.fabr	3	2	0	0	0	0	0	0	2	7	moderate

Code	GD	ID	dr	gr	hs	R	P	HR	HL	SV	CAT
Och.heyd	3	2	0	0	0	0	0	0	2	7	moderate
Stt.bert	3	2	0	0	0	0	0	0	2	7	moderate
Hdn.cori	3	1	0	0	0	0	0	0	2	6	moderate
Hdn.shar	3	1	0	0	0	0	0	0	2	6	moderate
Hyd.norm	3	0	0	0	0	0	0	1	2	6	moderate
Hyd.vage	3	0	0	0	0	0	0	1	2	6	moderate
Lib.cord	3	1	0	0	0	0	0	0	2	6	moderate
Lin.perc	3	1	0	0	0	0	0	0	2	6	moderate
Neb.buch	3	1	0	0	1	1	0	0	1	6	moderate
Neb.cari	3	1	0	0	0	0	0	0	2	6	moderate
Stt.iber	3	0	0	0	0	0	0	1	2	6	moderate
Hdn.afus	3	0	0	0	0	0	0	0	2	5	moderate
Hdn.unca	3	0	0	0	0	0	0	0	2	5	moderate
Hep.seid	3	0	0	0	0	0	0	0	2	5	moderate
Hyd.neva	3	0	0	0	0	0	0	0	2	5	moderate
Lib.gerh	3	0	0	0	0	0	0	0	2	5	moderate
Oul.tubp	3	0	0	0	0	0	0	0	2	5	moderate
Hyd.deci	3	0	0	0	0	0	0	0	1	4	low

The two most endangered species in the region (identified as of very high vulnerability) are both known only from their type series. They are *Ochthebius ferroi* and *Ochthebius javieri* (Hydraenidae). The former has not been recorded since its discovery in 1985 in a small spring located in the pre-Pyrenees (Betesa, Aragón), and the latter is a species found only once in a slightly brackish pond, a threatened habitat, at Cabo de Favàritx in Menorca (Balearic Islands).

Other than the obvious cases of extremely rare species, as those noted above, most of the high-priority species fall into two main groups: one includes those taxa that occur in habitats which are under immediate threat, and have high vulnerability scores as a consequence, despite being relatively widespread in the Iberian Peninsula (usually being found in more than 20 squares). This is the case with species inhabiting saline streams (e.g. *Nebrioporus baeticus*, *Ochthebius glaber*, *O. delgadoi, O. tudmirensis*) or rivers and middle reach streams (e.g. *Oulimnius bertrandi*). A second large group, is composed of species known from few localities which occur in habitats not under obvious immediate threat, usually located in headwater streams (e.g.

Hydraena isabelae, *H. mecai*, *H. zezerensis*, *Ochthebius albacetinus*, *O.cantabricus*, *Agabus picotae*, *Deronectes brannani*) or more rarely in lagoons or ponds in mountainous areas (e.g. *Agabus nevadensis*, *Helophorus leontis*).

Habitat rarity and threats

According to the results of the expert query the rarest habitats in the study area were saline streams followed by rice-fields, lagoons and salt-pans. The most threatened habitats were rivers and middle reach streams, followed by a group composed of springs, lagoons, pools and ponds, saline streams and salt-pans. On other hand, with the exception of rice fields and salt pans, both of have a long history in the region, no artificial habitats are rare or threatened (Table 2).

Spearman correlations were used to evaluate the relationship between final vulnerability score and the variables used in the assessment of vulnerability. Vulnerability score were determined principally by rarity (R) ($r=0.76$, $p<0.01$) and Iberian distribution (ID) ($r=0.72$, $p<0.01$). The vulnerability scores were not correlated with habitat loss (HL), probably due to the high number of restricted endemic species locates in headwater streams in mountainous areas, a habitat not considered under immediate threat. Furthermore, several of the species with low and moderate vulnerability appear in threatened habitats.

Hotspots of high-priority species

We identified 57 squares as hotspots of high-priority species (see Fig. 2). Thirty of these represent saline systems mainly located in the southern half of the Iberian Peninsula. Hotspots with the highest number of high-priority species (5 species and above) contain taxa with narrow distributional ranges, typically in headwater streams or lagoons in mountain areas. Key areas (see Fig.1) include: 1) Sierra de Guadarrama (Central Spain), with *Helophorus hispanicus*, *H. leontis*, *H. nevadensis*, *Hydrochus interruptus* and *Limnebius montanus*; 2) Sierra de Alcaráz (SE Spain), with *Hydraena mecai*, *H. servilia*, *Limnebius millani*, *Ochthebius albacetinus* and *O. semotus*; 3) Sierra Nevada (S Spain) with a total of eight high-priority species in two adjacent squares, four of them restricted to the Sierra Nevada itself (*Agabus nevadensis, Hydroporus sabaudus sierranevadensis*, *H. normandi alhambrae*, *Limnebius monfortei*); 4) Rambla Salada in Murcia (SE Spain), with five high-priority species found in a single saline stream system: *Ochthebus montesi*, *O. glaber*, *O. tudmirensis*, *O. delgadoi* and *Nebrioporus baeticus.* Remaining hotspots have fewer species, but again include species with narrow distributional ranges, and are mainly located in headwater streams from a range of Iberian regions such as Serra de Arga, Los Alcornocales, Serra da Estrela, Pre-Pyrenees, Sierra Morena, Sierra de Cazorla and Cordillera Cantabrica (see Fig.1).

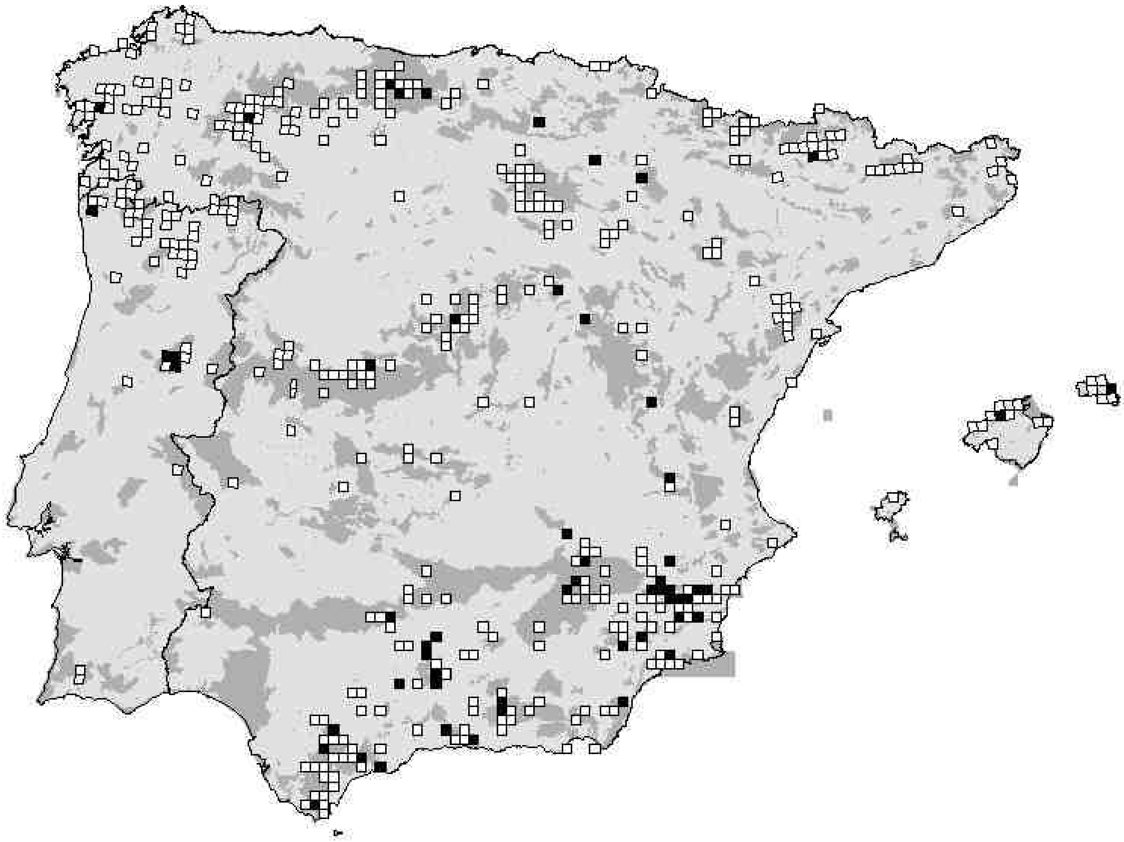

Figure 2. Location of the 57 squares recognized as hotspots of high-priority species (black) and remaining squares with high-priority species (white). Shaded surface represents the existing Natura 2000 network in the Iberian Peninsula and the Balearic Islands.

Gap Analysis

When the distribution maps of individual species were superimposed on the Natura 2000 network map, a high degree of overlap was detected (Figure 2 and Table 4), with the distributions of 22 species overlapping completely. These species occur mainly in mountainous areas, including the two species designed as having highest vulnerability. On the other hand, the distribution of four species is totally outside the existing Natura 2000 network. These species are *Iberoporus cermenius, Hydraena quetiae, Limnebius monfortei* and *Ochthebius irenae.* Another 9 species, including a number of predominantly lowland taxa and some associated with saline systems (*Hydrochus angusi*, *Hydraena alcantarana*, *H. lucasi*, *Nebrioporus baeticus, Ochthebius anadalusicus*, *O. delgadoi*, *O. glaber*, *O.tudmirensis* and *Oulimnius bertrandi*), have less than 40% of their distribution in protected areas.

Table 4. Percentage of overlap between distribution maps of high-priority species and the Natura 2000 network. Different thresholds (1%, 25%, 50%, 75% and 100%) are used to consider a square as protected. See appendix for species codes. (N: Number of squares with records for given species).

Code	**N**	**1%**	**10%**	**25%**	**50%**	**75%**	**100%**
Hyt.fres	10	90.00	80.00	70.00	70.00	40.00	10.00
Hyd.cant	2	100.00	100.00	100.00	100.00	50.00	50.00
Hyd.cons	6	100.00	100.00	100.00	100.00	83.30	66.70
Hyd.lluc	4	100.00	100.00	75.00	75.00	0.00	0.00
Hyd.alha	5	100.00	80.00	80.00	60.00	60.00	40.00
Hyd.sier	6	100.00	100.00	83.30	83.30	66.70	33.30
Ibe.cerm	1	100.00	100.00	0.00	0.00	0.00	0.00
Rhi.agnu	3	100.00	100.00	66.70	33.30	33.30	0.00
Rhi.arga	2	100.00	100.00	50.00	0.00	0.00	0.00
Rhi.bima	11	100.00	81.80	81.80	81.80	72.70	72.70
Der.algi	11	100.00	90.90	90.90	63.60	45.50	45.50
Der.aube	16	100.00	100.00	87.50	87.50	62.50	62.50
Der.bran	7	100.00	85.70	71.40	28.60	0.00	0.00
Der.cosg	18	100.00	100.00	94.40	88.90	77.80	55.60
Der.fost	7	85.70	85.70	71.40	0.00	0.00	0.00
Der.wewa	13	92.30	92.30	92.30	84.60	69.20	15.40
Neb.croc	1	100.00	100.00	100.00	0.00	0.00	0.00
Neb.baet	57	80.70	52.60	28.10	17.50	8.80	1.80
Aga.neva	2	100.00	100.00	100.00	100.00	100.00	50.00
Aga.pico	2	100.00	100.00	100.00	100.00	50.00	0.00
Ily.dett	9	66.70	66.70	66.70	44.40	44.40	22.20
Hep.hisp	3	100.00	100.00	100.00	100.00	100.00	0.00
Hep.koro	1	100.00	100.00	100.00	100.00	100.00	100.00
Hep.leon	6	83.30	83.30	66.70	50.00	50.00	16.70
Hep.neva	6	100.00	83.30	83.30	83.30	83.30	33.30
Hep.joco	5	100.00	80.00	80.00	60.00	40.00	0.00
Hch.angi	16	68.80	50.00	31.30	31.30	12.50	6.30
Hch.inte	10	100.00	70.00	50.00	40.00	20.00	0.00
Lab.glor	7	100.00	85.70	71.40	14.30	0.00	0.00
Hdn.cata	8	87.50	75.00	62.50	50.00	37.50	0.00
Hdn.gadi	5	80.00	80.00	80.00	80.00	40.00	0.00

Code	N	1%	10%	25%	50%	75%	100%
Hdn.lusi	8	62.50	62.50	62.50	62.50	50.00	25.00
Hdn.madr	1	100.00	100.00	100.00	100.00	100.00	100.00
Hdn.mons	8	100.00	87.50	87.50	87.50	75.00	50.00
Hdn.tati	6	100.00	83.30	66.70	33.30	16.70	0.00
Hdn.zeze	1	100.00	100.00	100.00	100.00	100.00	0.00
Hdn.alba	4	100.00	100.00	100.00	50.00	25.00	0.00
Hdn.alca	4	100.00	50.00	25.00	25.00	25.00	0.00
Hdn.alta	3	66.70	66.70	66.70	66.70	66.70	0.00
Hdn.deli	13	100.00	84.60	69.20	61.50	23.10	0.00
Hdn.isab	3	100.00	100.00	66.70	66.70	66.70	33.30
Hdn.luca	6	100.00	33.30	33.30	33.30	16.70	0.00
Hdn.marc	6	100.00	100.00	100.00	100.00	83.30	33.30
Hdn.mari	5	80.00	80.00	60.00	40.00	40.00	0.00
Hdn.meca	1	100.00	100.00	100.00	100.00	100.00	100.00
Hdn.quet	1	100.00	0.00	0.00	0.00	0.00	0.00
Hdn.serv	18	100.00	83.30	77.80	72.20	66.70	50.00
Lib.hila	3	100.00	100.00	66.70	33.30	33.30	0.00
Lib.igna	10	100.00	90.00	70.00	60.00	40.00	0.00
Lib.mill	2	100.00	100.00	100.00	100.00	100.00	50.00
Lib.mino	2	100.00	100.00	100.00	100.00	0.00	0.00
Lib.monf	1	100.00	100.00	0.00	0.00	0.00	0.00
Lib.mont	13	100.00	92.30	92.30	76.90	76.90	30.80
Lib.nanu	3	100.00	100.00	100.00	66.70	33.30	33.30
Lib.ordu	3	100.00	100.00	100.00	66.70	33.30	33.30
Och.cant	1	100.00	100.00	100.00	100.00	100.00	100.00
Och.ferr	1	100.00	100.00	100.00	100.00	100.00	0.00
Och.iren	5	100.00	20.00	0.00	0.00	0.00	0.00
Och.alba	4	100.00	100.00	100.00	75.00	75.00	75.00
Och.anda	6	83.30	66.70	33.30	33.30	16.70	0.00
Och.caes	6	83.30	66.70	66.70	33.30	16.70	0.00
Och.delg	62	90.30	67.70	35.50	25.80	12.90	6.50
Och.diaz	1	100.00	100.00	100.00	100.00	100.00	100.00
Och.gayo	3	66.70	66.70	66.70	33.30	0.00	0.00
Och.glab	20	65.00	45.00	35.00	15.00	0.00	0.00
Och.javi	1	100.00	100.00	100.00	100.00	0.00	0.00
Och.mont	8	100.00	100.00	50.00	37.50	12.50	0.00

Code	**N**	**1%**	**10%**	**25%**	**50%**	**75%**	**100%**
Och.pedr	2	100.00	100.00	50.00	50.00	0.00	0.00
Och.semo	10	90.00	90.00	80.00	60.00	50.00	30.00
Och.tudm	25	92.00	68.00	28.00	20.00	4.00	4.00
Oul.bert	89	61.80	48.30	37.10	31.50	20.20	6.70
Oul.echi	10	100.00	90.00	60.00	30.00	0.00	0.00
Dry.cham	7	100.00	100.00	85.70	71.40	57.10	14.30
Hotspots	57	89.47	75.44	52.63	38.60	28.07	14.04
Non Hotspot	386	83.16	69.95	57.25	43.78	30.57	14.77
Total	443	83.97	70.65	56.66	43.12	30.25	14.67

If a square is considered protected when at least 25% of its surface area is included, then 56.66% of squares with high priority species, and 52.63% of hotspots are included in the existing Natura 2000 network. If we consider a square as protected only when at least 50% is within Natura 2000, then these percentages are clearly lower, being 43.12% and 38.60% respectively. In fact, 26 of the 57 hotspots are outside the Natura 2000 network, 6 of them completely, even if one uses a 1% overlap as a threshold for inclusion. All of these 'missing' hotspots were in lowland areas, with saline streams or saltpans as their main aquatic ecosystem type (Fig. 3).

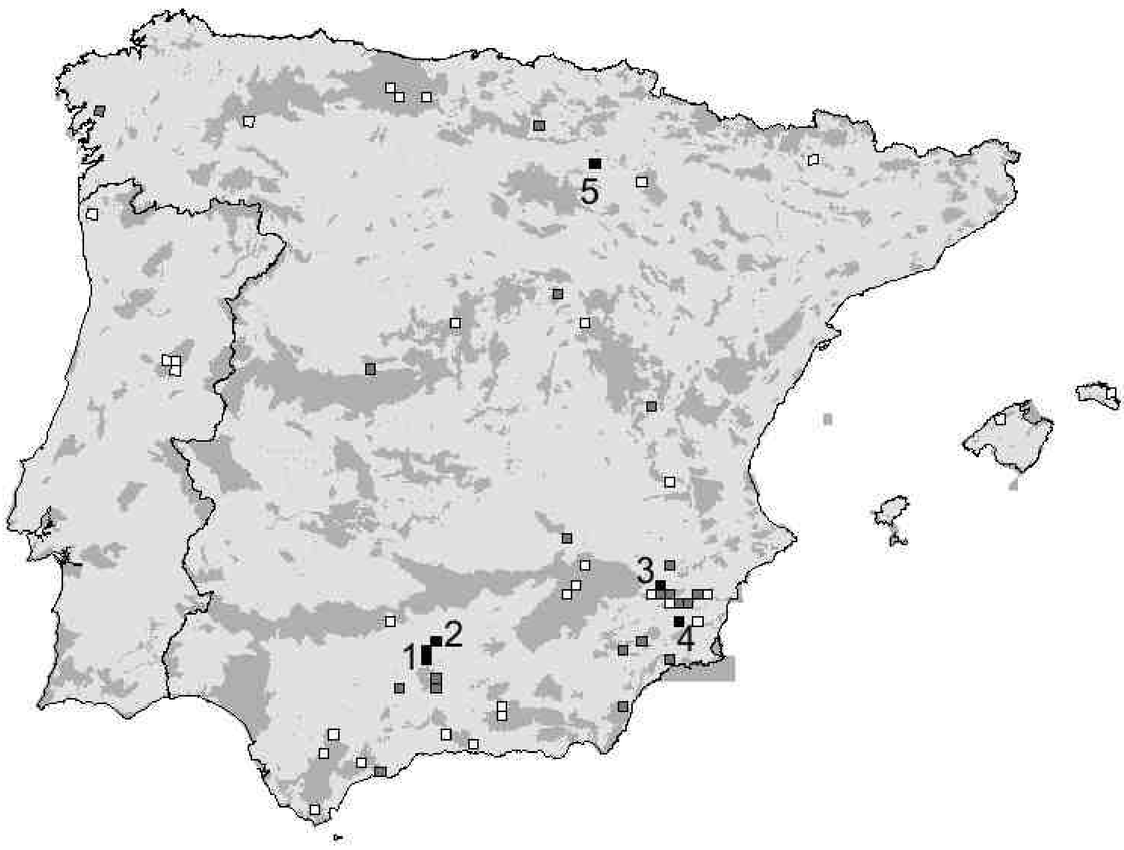

Figure 3. Location of protected hotspots of high-priority species (white); hotspots that are outside the Natura 2000 network (considering 25% surface as threshold for inclusion) (grey) and the 6 squares recognized as hotspots of high-priority species entirely outside the Natura 2000 network (1% of threshold) (black). (1: saltpans and saline streams in La Maturra; 2: saltpans and saline streams in Porcuna; 3: Rambla of Aguamarga; 4: Rambla of Alcantarilla and Rambla of Sangonera; 5: Saline streams in Mendavia). Shaded surface represents the existing Natura 2000 network in the Iberian Peninsula and Balearic Islands.

DISCUSSION

These analyses allow us to prioritise endemic species of Iberian water beetle for conservation status, as well as to assess the effectiveness of the existing Natura 2000 network. In this context it is important to re-iterate that water beetles have been identified as excellent surrogates of inland water biodiversity in general (Bilton et al., 2006; Sánchez-Fernández et al., 2006) and that the results from this study are likely to be reflected in other less well-known groups of freshwater macroinvertebrates, for which we may never have adequate data to conduct the kind of analyses presented here. The key findings of this study are now discussed in turn, starting with potential implications for red list inclusion.

Threatened water beetles and Red Lists

Most of the high-priority endemic species identified appear in headwater streams, illustrating the importance of isolation and speciation in montane lotic systems in generating much of the endemic water beetle diversity in the regions (Ribera and Vogler, 2004). Whilst such species are not usually under obvious proximate threat, their rarity makes them vulnerable, particularly in the face of anthropogenic climate change, which may impact such taxa directly, and through a reduction in the volume of available habitat (Wilson et al., 2005; Calosi et al., 2008). A special case could be the Balearic Islands, in which the increasing demand on water has lead in many cases to the regulation of the headwaters and the disappearance of the upper reach of permanent streams. We also found that a significant number of high priority species were located in saline stream systems. These species point to the importance of such lotic saline systems for speciation (Gómez et al., 2000, 2002; Abellán et al., 2007), and are vulnerable due to their rarity, and the high degree of anthropogenic pressure on their habitats, usually found in more heavily impacted lowland regions (Williams, 2002; Gómez et al., 2005).

Of the 120 species studied, 73 (61.47%) were identified as having high-conservation priority, these comprising 14.3% of all water beetles recorded from the Iberian Peninsula. We propose that these 73 species should be included a number of 'red lists', including the National Catalogue of Threatened Species in Spain and/or Portugal, on Appendix II of the Habitat Directive (Directive 92/43/CEE), and, potentially on the IUCN Red List. This may seem a high number to include in a list of threatened species, and whilst the inclusion of long lists of inconspicuous species in red lists is questionable (Ribera, 2000), most of us feel that it can be justified on the basis of their use in effective habitat protection. Habitats are usually declared as endangered and protected on the basis of an inventory of species, particularly red list species. In this sense, we emphasize that invertebrates red lists, such as that proposed for water beetles on the basis of our analyses, are valuable in the identification and management of protected area networks. This is especially important in freshwater ecosystems, because, until now, species considered for SAC designation are mostly terrestrial vertebrates and very few aquatic invertebrates have been listed in Annex II of the Habitats Directive (Abellán et al., 2006), hampering effective conservation evaluation of such habitats. As discussed in the introduction, the Iberian and Balearic water beetle fauna is well known, and these insects are known to function as effective surrogates of wider inland water biodiversity, making aquatic Coleoptera an ideal group to use in this form.

In the analyses presented here, we focussed on ibero-Balearic endemics, and the degree to which these taxa are protected by existing Natura 2000 networks. As a consequence, we failed to consider some species which are rare at a national, or indeed international level. These include some relatively widespread Palaearctic species, rare in Iberia (e.g. *Gyrinus suffriani* and *Hydaticus seminiger,*); taxa with a predominantly African distribution whose only European outpost is in southern Iberia (e.g. C*ybister vulneratus, Methles cribatellus* and *Trichonectes otini*), and a number of rare or endangered Palaeartic species which are not ibero-balearic endemics (*Acilius duvergeri*, *Potamophilus acuminatus* and *Stenelmis consobrina*). *Acilius duvergeri* is probably the rarest and least known of the larger species of western European aquatic Coleoptera, occurring in well preserved lowland or mountain ponds, always in low numbers. Formerly known from south-western France, where it is now apparently extinct, it is now recorded only from western Iberia and Sardinia, with old records from west Morocco (Bergsten and Miller, 2006). *Potamophilus acuminatus*, although present in Europe and North Africa, is rare throughout its discontinuous geographical range (Horion, 1955), being considered to be on the verge of extinction in central Europe (Kodada, 1991). It requires large, clean, well-oxygenated rivers, with a supply of submerged decaying timber, a threatened and scarce habitat. Finally, *Stenelmis consobrina*, another species of clean, large lowland rivers, is considered to be extinct in central Europe (Ribera, 2000), and is increasingly rare in the south (Olmi, 1976; Rico, 1997).

Habitats and Hotspots

Most of the hotspots identified in the study area represent isolated headwater streams in mountain areas and saline systems mainly from the south-east of the Iberian Peninsula. These saline systems typically support a particular set of stenotopic, high-priority species (*Nebrioporus baeticus*, *Ochthebius andalusicus*, *O. delgadoi*, *O. glaber, O. montesi*, and *O. tudmirensis*), which occupy these habitats in a number of areas of the peninsula. Nevertheless, it is important to point out that, in spite of the apparent geographical homogeneity of these hotspots, independent evolutionary lineages of these saline water taxa may occur in different regions, and these must feature in conservation planning to enable the preservation of the process generating and maintaining the diversity of the species (Gómez et al., 2000; Abellán et al., 2007). On other hand, we also emphasize the importance of the remaining hotspots, largely located in headwater streams or lagoons in mountain areas throughout the study area. These areas have a rich and often highly endemic fauna, in some cases including species whose distributional ranges are limited to individual mountainous systems. Several of these areas are coincident with those highlighted previously for narrow endemic plants (Domínguez-Lozano et al., 2000), mainly Sierra Nevada, Sierra de Alcaráz and Serra da Estrela), suggesting that they could be important centres of endemism in the Iberian Peninsula and Balearic Islands for different groups of organism. Other crucial target sites and habitats for protection are freshwater streams and lagoons located principally in Serra de Arga, streams of NW of Mallorca, Los Alcornocales natural park, Sierra de Guadarrama, Sierra Morena, Pre-Pyrenees, Sierra de Ancares and Picos de Europa in the Cantabrian Mountains.

Hotspots from saline aquatic ecosystems are particularly threatened at present since the lowland and coastal areas where they are located suffer the most intense and frequent changes in land use (Martínez-Fernández et al., 2000), via dredging and stream canalization, drainage, urbanization and other human developments, pollution and loss of salinity (Gómez et al., 2005; Velasco et al., 2006). Whilst hotspots in mountainous areas may require minimal management for conservation, most contemporary extinctions have affected narrow-range taxa or taxa with strict ecological requirements (Fontaine et al., 2007), such as those of montane areas. Furthermore, these species could be most at risk from ongoing climate change (Thomas et al., 2004; Wilson et al., 2005; Calosi et al., 2008), and by the increasingly amount of water pollution generated through the rapid expansion of rural mountain tourism, and relaxation of rural planning restrictions whose effects are already being felt in the region.

Gap analysis and protection from Natura 2000 network

Hotspot gap analysis revealed the importance of peripheral areas of the Natura 2000 network in protecting high-priority species of water beetles, because an important increase in the number of squares protected depending of the threshold considered has been detected. Therefore, rules used to assign reserves to squares will obviously affect estimates of gaps in the representation of species within conservation areas (Araujo, 2004).

Hotspots are actually less protected by the Natura 2000 network than squares containing few species of high priority endemic taxa. This may partly be due to the higher number of hotspots associated with lowland saline systems, as discussed above. At present Natura 2000 fails to protect inland saline water bodies in Iberia, despite their high conservation interest, and their narrow distribution in a global context

(Williams, 1999; Moreno et al., 1997; Gómez et al., 2000; Gómez et al., 2005; Abellán et al., 2007). This failure is probably related to the fact that inland saline habitats are in general socially under-valuated environments, poor in vertebrate species, and because the lowland and coastal areas in which they occur are subject to more intense and frequent changes in land use (Martínez-Fernández et al., 2000; Sánchez-Fernández et al., 2004b).

From species gap analysis, we suggest that special attention should also be focused on four species whose distribution is not currently included in Natura 2000 networks or if included is only present as a minor proportion. It is recommended that the boundaries of the SCI or SPAs closest to the distribution of these species are extended to better include these high-priority species as follows: Sierra Subetica for *Iberoporus cermenius,* Sierra de Picón for *Hydraena quetiae,* Sierra Nevada for *Limnebius monfortei* and Saladares de Cordovilla, Agramón y Laguna de Alboraj, Complejo lagunar de la Charca de Chiprana and Laguna de Pitillas for *Ochthebius irenae*.

Finally, despite the high degree of overlap detected when the distribution maps of species were superimposed on the Natura 2000 network, and the fact that Natura 2000 should, theoretically, provide an appropriate mechanism to avoid deterioration of natural habitats, it is important to point out that the occurrence of a species within a protected area (even with multiple capture records) is not a guarantee of long-term survival. At present the management of SACs and SPAs is focused to protect the habitat and/or species for which the site is designated (usually *only* plants and vertebrates) not the entire biodiversity of a site. Thus, we have a “virtual protection” of the remaining biodiversity in such areas, and no guarantee of success (Sánchez-Fernández et al., 2004b). In particular at present SACs and SPAs often fail to address issues critical for aquatic biodiversity, such as catchment integrity, extra-SAC or SPA catchment land-use, hydrology, and the introduction of non-native species (Lake, 1980; Skelton et al., 1995; Moyle and Randall, 1998). This drawback could be overcome by the identification and declaration of microreserves or areas of special protection for aquatic biodiversity within these extensive areas, and applying specific management measures to protect this aquatic biota. Many activities, such as dam building, water diversion for agriculture, land-use disturbance in the catchments, or the introduction of alien species (Saunders et al., 2002), may occur well outside park boundaries yet still have major negative consequences for freshwater habitats within. Thus, whole-catchment management and natural-flow maintenance are indispensable strategies for freshwater biodiversity conservation (Abellán et al., 2006). Therefore, identifying threatened species and areas along with the above guidelines must be taken into consideration to adequately protect freshwater biodiversity in the future.

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Appendix. (* Species with uncertain endemicity status).

Nº	Family	Species	Code
1	DYTISCIDAE	*Hygrotus fresnedai* (Fery, 1992)	Hyt.fres
2	DYTISCIDAE	*Hydroporus brancoi brancoi* Rocchi,, 1981	Hyd.bran
3	DYTISCIDAE	*Hydroporus brancoi gredensis* Fery, 1999	Hyd.gred
4	DYTISCIDAE	*Hydroporus brancuccii* Fery, 1987	Hyd.brac
5	DYTISCIDAE	*Hydroporus cantabricus* Sharp, 1882	Hyd.cant
6	DYTISCIDAE	*Hydroporus constantini* Hernando and Fresneda, 1996	Hyd.cons
7	DYTISCIDAE	*Hydroporus decipiens** Sharp, 1878	Hyd.deci
8	DYTISCIDAE	*Hydroporus lluci* Fery, 1999	Hyd.lluc
9	DYTISCIDAE	*Hydroporus necopinatus necopinatus* Fery, 1999	Hyd.neco
10	DYTISCIDAE	*Hydroporus nevadensis* Sharp, 1882	Hyd.neva
11	DYTISCIDAE	*Hydroporus normandi alhambrae* Fery, 1999	Hyd.alha
12	DYTISCIDAE	*Hydroporus normandi normandi* Régimbart, 1903	Hyd.norm
13	DYTISCIDAE	*Hydroporus paganettianus* Scholz, 1923	Hyd.paga
14	DYTISCIDAE	*Hydroporus sabaudus sierranevadensis* Shaverdo, 2004	Hyd.sier
15	DYTISCIDAE	*Hydroporus vagepictus* Fairmaire and Laboulbène, 1854	Hyd.vage
16	DYTISCIDAE	*Hydroporus vespertinus* Fery and Heindrich, 1988	Hyd.vesp
17	DYTISCIDAE	*Graptodytes castilianus* Fery, 1995	Grt.cast
18	DYTISCIDAE	*Iberoporus cermenius* Castro and Delgado, 2000	Ibe.cerm
19	DYTISCIDAE	*Rhithrodytes agnus agnus* Foster, 1993	Rhi.agnu
20	DYTISCIDAE	*Rhithrodytes agnus argaensis* Bilton and Fery, 1996	Rhi.arga
21	DYTISCIDAE	*Rhithrodytes bimaculatus* (Dufour, 1852)	Rhi.bima
22	DYTISCIDAE	*Stictonectes occidentalis* Fresneda and Fery, 1990	Stn.occi
23	DYTISCIDAE	*Deronectes algibensis* Fery and Fresneda, 1988	Der.algi
24	DYTISCIDAE	*Deronectes angusi* Fery and Brancucci, 1990	Der.angu
25	DYTISCIDAE	*Deronectes aubei sanfilippoi* Fery and Brancucci, 1997	Der.aube
26	DYTISCIDAE	*Deronectes bicostatus* (Schaum, 1864)	Der.bico
27	DYTISCIDAE	*Deronectes brannanii* (Schaufuss, 1869)	Der.bran
28	DYTISCIDAE	*Deronectes costipennis costipennis* Brancucci, 1983	Der.cost
29	DYTISCIDAE	*Deronectes costipennis gignouxi* Fery and Brancucci, 1989	Der.cosg
30	DYTISCIDAE	*Deronectes delarouzei* (du Val, 1857)	Der.dela
31	DYTISCIDAE	*Deronectes depressicollis* (Rosenhauer, 1856)	Der.depr
32	DYTISCIDAE	*Deronectes ferrugineus* Fery and Brancucci, 1987	Der.ferr
33	DYTISCIDAE	*Deronectes fosteri* Aguilera and Ribera, 1996	Der.fost

Nº	**Family**	**Species**	**Code**
34	DYTISCIDAE	*Deronectes wewalkai* Fery and Fresneda, 1988	Der.wewa
35	DYTISCIDAE	*Stictotarsus bertrandi* Legros, 1956	Stt.bert
36	DYTISCIDAE	*Stictotarsus ibericus** Dutton and Angus, 2007	Stt.iber
37	DYTISCIDAE	*Nebrioporus bucheti cazorlensis* (Lagar, Fresneda and Hernando, 1987)	Neb.buch
38	DYTISCIDAE	*Nebrioporus carinatus* (Aubé, 1836)	Neb.cari
39	DYTISCIDAE	*Nebrioporus croceus* Angus, Fresneda and Fery, 1992	Neb.croc
40	DYTISCIDAE	*Nebrioporus fabressei* (Régimbart, 1901)	Neb.fabr
41	DYTISCIDAE	*Nebrioporus baeticus* (Schaum, 1864)	Neb.baet
42	DYTISCIDAE	*Agabus nevadensis* Lindberg, 1939	Aga.neva
43	DYTISCIDAE	*Agabus picotae* Foster and Bilton, 1997	Aga.pico
44	DYTISCIDAE	*Ilybius dettneri* Fery, 1986	Ily.dett
45	HELOPHORIDAE	*Helophorus hispanicus* (Sharp, 1915)	Hep.hisp
46	HELOPHORIDAE	*Helophorus bameuli* Angus, 1987	Hep.bame
47	HELOPHORIDAE	*Helophorus korotyaevi* Angus, 1985	Hep.koro
48	HELOPHORIDAE	*Helophorus leontis* Angus, 1985	Hep.leon
49	HELOPHORIDAE	*Helophorus nevadensis* Sharp, 1916	Hep.neva
50	HELOPHORIDAE	*Helophorus jocoteroi* Angus and Díaz Pazos, 1991	Hep.joco
51	HELOPHORIDAE	*Helophorus seidlitzii* Kuwert, 1885	Hep.seid
52	HYDROCHIDAE	*Hydrochus angusi* Valladares, 1988	Hch.angi
53	HYDROCHIDAE	*Hydrochus ibericus* Valladares, Díaz-Pazos and Delgado, 1999	Hch.iber
54	HYDROCHIDAE	*Hydrochus interruptus* Heyden, 1870	Hch.inte
55	HYDROCHIDAE	*Hydrochus nooreinus* Henegouven and Sáinz-Cantero, 1992	Hch.noor
56	HYDROPHILIDAE	*Laccobius gloriana* Gentili and Ribera, 1998	Lab.glor
57	HYDRAENIDAE	*Hydraena altamirensis* Díaz Pazos and Garrido, 1993	Hdn.alta
58	HYDRAENIDAE	*Hydraena catalonica* Fresneda, Aguilera and Hernando, 1994	Hdn.cata
59	HYDRAENIDAE	*Hydraena gaditana* Lagar and Fresneda, 1990	Hdn.gadi
60	HYDRAENIDAE	*Hydraena hispanica* Ganglbauer, 1901	Hdn.hisp
61	HYDRAENIDAE	*Hydraena iberica* Orchymont, 1936	Hdn.iber
62	HYDRAENIDAE	*Hydraena lusitana* Berthélemy, 1977	Hdn.lusi
63	HYDRAENIDAE	*Hydraena madronensis* Castro, García and Ferreras, 2000	Hdn.madr
64	HYDRAENIDAE	*Hydraena manfredjaechi* Delgado and Soler, 1991	Hdn.manf
65	HYDRAENIDAE	*Hydraena monstruosipes* Ferro, 1986	Hdn.mons
66	HYDRAENIDAE	*Hydraena tatii* Sainz-Cantero and Alba-Tercedor, 1989	Hdn.tati
67	HYDRAENIDAE	*Hydraena zezerensis* Díaz Pazos and Bilton, 1994	Hdn.zeze
68	HYDRAENIDAE	*Hydraena afussa* Orchymont, 1936	Hdn.afus
69	HYDRAENIDAE	*Hydraena albai* Sáinz-Cantero, 1993	Hdn.alba

Nº	Family	Species	Code
70	HYDRAENIDAE	*Hydraena alcantarana* Ieniesta, 1985	Hdn.alca
71	HYDRAENIDAE	*Hydraena balaerica* d´Orchymont, 1930	Hdn.bale
72	HYDRAENIDAE	*Hydraena bolivari* Orchymont, 1936	Hdn.boli
73	HYDRAENIDAE	*Hydraena corinna* Orchymont, 1936	Hdn.cori
74	HYDRAENIDAE	*Hydraena delia* Balfour-Browne, 1978	Hdn.deli
75	HYDRAENIDAE	*Hydraena gavarrensis* Jäch, Diaz and Martinoy, 2005	Hdn.gava
76	HYDRAENIDAE	*Hydraena isabelae* Castro and Herrera, 2001	Hdn.isab
77	HYDRAENIDAE	*Hydraena lucasi* Lagar, 1984	Hdn.luca
78	HYDRAENIDAE	*Hydraena marcosae* Aguilera, Hernando and Ribera, 1997	Hdn.marc
79	HYDRAENIDAE	*Hydraena marinae* Castro, 2004	Hdn.mari
80	HYDRAENIDAE	*Hydraena mecai* Millán and Aguilera, 2000	Hdn.meca
81	HYDRAENIDAE	*Hydraena quetiae* Castro, 2000	Hdn.quet
82	HYDRAENIDAE	*Hydraena servilia* Orchymont, 1936	Hdn.serv
83	HYDRAENIDAE	*Hydraena sharpi* Rey, 1886	Hdn.shar
84	HYDRAENIDAE	*Hydraena unca* Valladares, 1989	Hdn.unca
85	HYDRAENIDAE	*Limnebius cordobanus* Orchymont, 1938	Lib.cord
86	HYDRAENIDAE	*Limnebius gerhardti* Heyden, 1870	Lib.gerh
87	HYDRAENIDAE	*Limnebius hilaris* Balfour-Browne, 1976	Lib.hila
88	HYDRAENIDAE	*Limnebius hispanicus* Orchymont, 1941	Lib.hisp
89	HYDRAENIDAE	*Limnebius ibericus* Balfour-Browne, 1978	Lib.iber
90	HYDRAENIDAE	*Limnebius ignarus* Balfour-Browne, 1978	Lib.igna
91	HYDRAENIDAE	*Limnebius lusitanus* Balfour-Browne, 1978	Lib.lusi
92	HYDRAENIDAE	*Limnebius millani* Ribera and Hernando, 1998	Lib.mill
93	HYDRAENIDAE	*Limnebius minoricensis* Jäch, Valladares and García-Avilés, 1996	Lib.mino
94	HYDRAENIDAE	*Limnebius monfortei* Fresneda and Ribera, 1998	Lib.monf
95	HYDRAENIDAE	*Limnebius montanus* Balfour-Browne, 1978	Lib.mont
96	HYDRAENIDAE	*Limnebius nanus* Jäch, 1993	Lib.nanu
97	HYDRAENIDAE	*Limnebius ordunyai* Fresneda and Ribera, 1998	Lib.ordu
98	HYDRAENIDAE	*Ochthebius bellieri* Kuwert, 1887	Och.bell
99	HYDRAENIDAE	*Ochthebius cantabricus** Balfour-Browne, 1978	Och.cant
100	HYDRAENIDAE	*Ochthebius ferroi* Fresneda, Lagar and Hernando, 1993	Och.ferr
101	HYDRAENIDAE	*Ochthebius heydeni** Kuwert, 1887	Och.heyd
102	HYDRAENIDAE	*Ochthebius irenae* Ribera and Millán, 1998	Och.iren
103	HYDRAENIDAE	*Ochthebius albacetinus* Ferro, 1984	Och.alba
104	HYDRAENIDAE	*Ochthebius andalusicus* Jäch and Castro, 1999	Och.anda
105	HYDRAENIDAE	*Ochthebius caesaraugustae* Jäch, Ribera and Aguilera, 1998	Och.caes

Nº	Family	Species	Code
106	HYDRAENIDAE	*Ochthebius delgadoi* Jäch, 1994	Och.delg
107	HYDRAENIDAE	*Ochthebius diazi* Jäch, 1999	Och.diaz
108	HYDRAENIDAE	*Ochthebius gayosoi* Jäch, 2001	Och.gayo
109	HYDRAENIDAE	*Ochthebius glaber* Montes and Soler, 1988	Och.glab
110	HYDRAENIDAE	*Ochthebius javieri* Jäch, 2000	Och.javi
111	HYDRAENIDAE	*Ochthebius montesi* Ferro, 1984	Och.mont
112	HYDRAENIDAE	*Ochthebius pedroi* Jäch, 2000	Och.pedr
113	HYDRAENIDAE	*Ochthebius semotus* Jäch, 2001	Och.semo
114	HYDRAENIDAE	*Ochthebius tudmirensis* Jäch, 1997	Och.tudm
115	ELMIDAE	*Oulimnius bertrandi* Berthélemy, 1964	Oul.bert
116	ELMIDAE	*Oulimnius cyneticus* Berthélemy and Terra, 1979	Oul.cyne
117	ELMIDAE	*Oulimnius echinatus* Berthélemy, 1979	Oul.echi
118	ELMIDAE	*Oulimnius tuberculatus perezi* Sharp, 1872	Oul.tubp
119	ELMIDAE	*Limnius perrisi carinatus* Perez-Arcas, 1865	Lin.perc
120	DRYOPIDAE	*Dryops championi* Dodero, 1918	Dry.cham

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