Universidad de León
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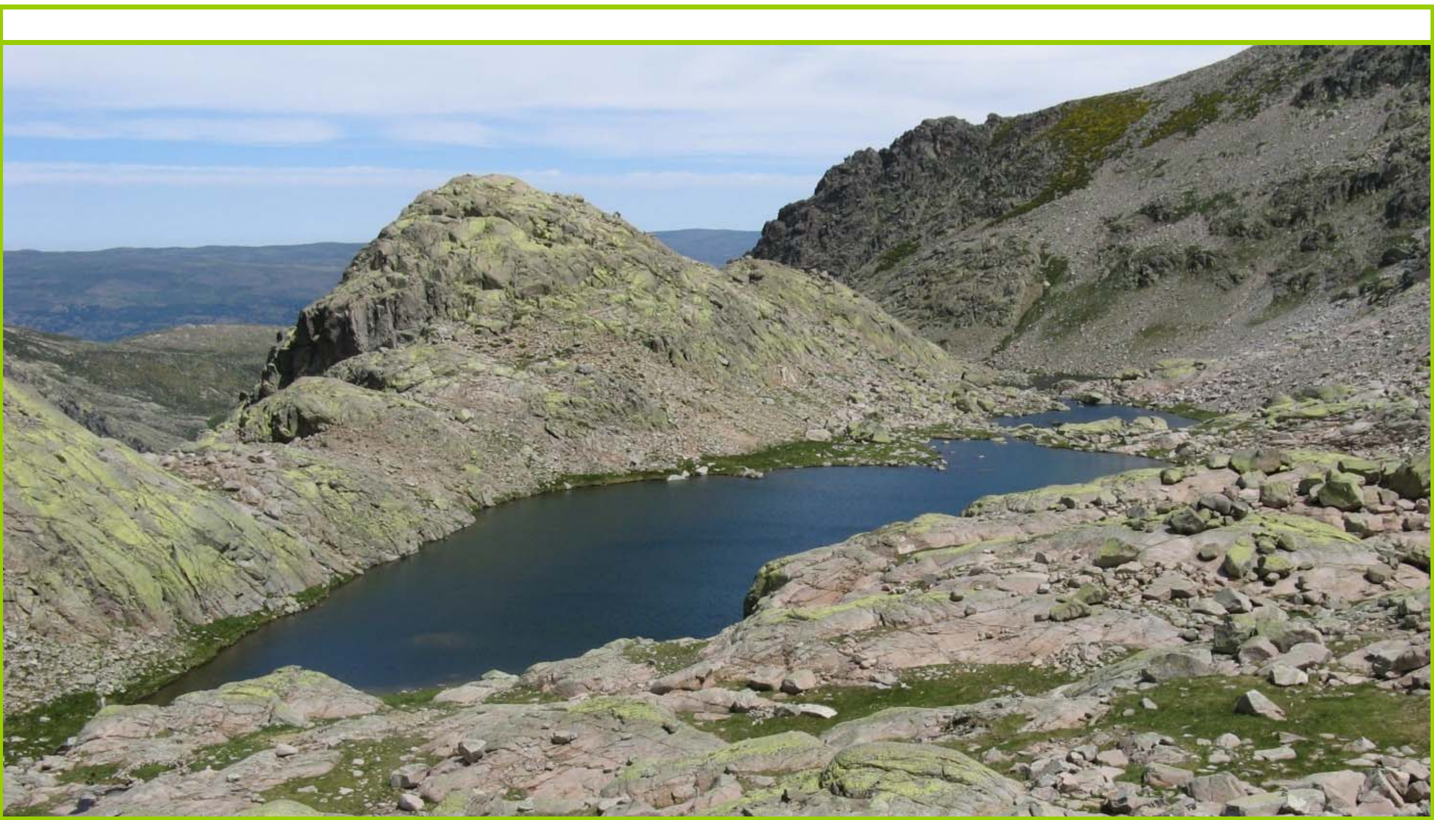
RIQUEZA DE MACROINVERTEBRADOS LITORALES DE LAGUNAS DE MONTAÑA: FACTORES DETERMINANTES Y PATRONES ESPACIALES

LITTORAL MACROINVERTEBRATE RICHNESS IN MOUNTAIN PONDS: DRIVING FORCES AND SPATIAL PATTERNS



Carlos Martínez Sanz
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LITTORAL MACROINVERTEBRATE RICHNESS IN MOUNTAIN PONDS: DRIVING FORCES AND SPATIAL PATTERNS

TESIS DOCTORAL

realizada por

Carlos Martínez Sanz

León 2012

Ilustración de portada:
Fotos realizadas durante un
muestreo en la sierra de
Gredos, Ávila.

*“Nada sustituye un largo
aprendizaje, un cúmulo de
experiencias que se convierte en la
base de la intuición”*

Doug Scout (alpinista)

Universidad de León

Informe del director de la tesis

(Art. 11.3 del R.D. 56/2005)

Dra. Camino Fernández Aláez, Dra. Margarita Fernández Aláez y Dr. Francisco García Criado como directores de la Tesis Doctoral titulada "*Riqueza de macroinvertebrados litorales de lagunas de montaña: factores determinantes y patrones espaciales*" realizada por D. Carlos Martínez Sanz en el Departamento de Biodiversidad y Gestión Ambiental, informan favorablemente el depósito de la misma, dado que reúne las condiciones necesarias para su defensa.

Lo que firman, para dar cumplimiento al art. 11.3 del R.D. 56/2005, en León, a 7 de Febrero de 2012.

Dra. Camino Fernández Aláez　　　　Dra. Margarita Fernández Aláez

Dr. Francisco García Criado

Admisión a trámite del departamento

(Art. 11.3 del R.D. 56/2005)
(Norma 7ª de las Complementarias de la ULE)

El Departamento de Biodiversidad y Gestión Ambiental en su reunión celebrada el día 9 de Febrero de 2012 ha acordado dar su conformidad a la admisión a trámite de lectura de la Tesis Doctoral titulada "*Riqueza de macroinvertebrados litorales de lagunas de montaña: factores determinantes y patrones espaciales*", dirigida por la Dra. Camino Fernández Aláez y la Dra. Margarita Fernández Aláez y el Dr. Francisco García Criado, elaborada por D. Carlos Martínez Sanz y cuyo título en inglés es "*Littoral macroinvertebrate richness in mountain ponds: driving forces and spatial patterns*".

Lo que firman, para dar cumplimiento al art. 11.3 del R.D. 56/2005, en León, a 9 de Febrero de 2012.

El Director del Departamento, La Secretaria,

Dr. Estanislao Luis Calabuig Dña. Raquel Alonso Redondo

Universidad de León

El Real Decreto 99/2011, de 28 de enero (B.O.E. número 35), por el que se regulan las enseñanzas oficiales de doctorado, define la tesis doctoral como un trabajo original de investigación elaborado por el candidato en cualquier campo del conocimiento que capacite al doctorando para el trabajo autónomo en el ámbito de la I+D+i. Las Universidades establecerán el procedimiento para la presentación de la tesis doctoral. La Universidad de León, a través de su consejo de Gobierno, establece que las tesis doctorales podrán ser presentadas por el sistema de compendio de publicaciones científicas.

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Pensaba que esta sería la parte más fácil y rápida de redactar. Quería conseguir que fuera breve y que así realmente se leyera pero, a la vez, que fuera lo suficientemente extenso como para poder mencionar a todas las personas que me han ayudado y que han hecho posible que esta tesis viera la luz. Tras varios intentos, entendí que sintetizar casi siete años de trabajo y agradecimientos en unos pocos párrafos es una tarea un tanto complicada. Por eso, si me he olvidado de alguien, no ha sido mi intención.

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A mi madre

ÍNDICE

RESUMEN - ABSTRACT

Resumen

Esta tesis aborda el estudio de la riqueza biológica de la comunidad de macroinvertebrados en lagunas de montaña. Trata de desvelar aspectos básicos de su conocimiento, como la obtención del mejor método para medir y estimar su valor, explorar los factores de los que depende, o mostrar la importancia que tienen estos sistemas y su contribución a la riqueza de una región. Es el primer estudio global llevado a cabo en Castilla y León sobre macroinvertebrados de sistemas lacustres de montaña y es clave en el marco de la gestión y conservación. Está constituida por cuatro artículos científicos publicados en sendas revistas, cuyas conclusiones dan respuesta a todos los objetivos planteados. El área de estudio de esta tesis es Castilla y León (España), donde fueron muestreadas 55 lagunas de montaña y un gran lago (lago de Sanabria), durante los años 2004 a 2008. Los resultados obtenidos representan un avance en el conocimiento de estos sistemas. El valor global de riqueza regional observada en lagunas de montaña de Castilla y León fue de 143 taxones (la gran mayoría géneros, aunque algunos individuos solo pudieron ser identificados hasta niveles taxonómicos superiores). Conseguir inventarios completos de fauna en un lugar dado, para una comunidad específica o área geográfica determinada, constituye una tarea excesivamente dura e incluso imposible de conseguir, sobre todo cuando se trabaja con invertebrados. En las últimas décadas han surgido un elevado número de técnicas y aproximaciones matemáticas de estimación de riqueza que intentan corregir, subsanar o mitigar, en la medida de lo posible, este problema. En el primer artículo de esta tesis se compararon los estimadores de riqueza más utilizados, obteniéndose un mejor funcionamiento de los estimadores no-paramétricos frente a las técnicas basadas en funciones de acumulación. Jackknife 2 resultó ser el mejor de los métodos no-paramétricos evaluado, seguido por Jackknife 1, Chao 1 y Chao 2. Por lo tanto, Jackknife es propuesto como adecuado para estimar la riqueza biológica de este tipo de comunidad y de hábitat. Un punto crucial en el estudio ecológico de la riqueza es conocer qué factores determinan su valor. Esto se ha llevado a cabo usando una regresión linear múltiple (24 variables medidas en 39 lagunas) para construir un modelo de predicción. Las únicas variables que afectaron significativamente a su valor resultaron ser heterogeneidad (NH) y el grado de la introducción de fauna piscícola (IP). Otras variables tradicionalmente consideradas como importantes (altitud, superficie, pH y permanencia) no mostraron relación con la riqueza de la comunidad de macroinvertebrados. Este modelo, expresado como: $S = 4.922(NH)-2.359(IP)+11.227$, fue testado sobre 12 lagunas de montaña dentro del área de estudio, resultando ser significativamente adecuado. Un resultado con directas implicaciones en el marco de la conservación, fueron los efectos negativos de la introducción de fauna piscícola en lagunas de montaña sobre la riqueza y toda la estructura de la comunidad de macroinvertebrados, siendo estos solo significativos a altas densidades piscícolas. Existe una cuestión adicional relacionada con la riqueza situada en el origen de un viejo debate ecológico: ¿son los conjuntos de pequeñas áreas más útiles en conservación, en términos de riqueza taxonómica, que una sola gran área? Con el objetivo de contribuir y aportar conclusiones sobre este tema, fueron estudiados un número de sistemas cercanos unos de otros (17 pequeñas lagunas de montaña y un gran lago, todos ellos dentro del Parque Natural de Sanabria y alrededores). Los resultados mostraron que la riqueza local de cada laguna de montaña fue mucho menor que la del Lago. Sin embargo, pequeños conjuntos de lagunas resultaron albergar más taxones que un solo sistema lacustre con más superficie que el total del área de este conjunto, mostrando que el tamaño no es la única variable relevante en su riqueza biológica. Estos resultados muestran la relevancia de conjuntos de sistemas lacustres en conservación, actualmente ignorados por la actual legislación Europea.

Abstract

This PhD thesis addresses the study of macroinvertebrate richness in mountain ponds. It intends to disentangle basic issues related to measuring and estimating richness values, factors influencing biodiversity and the contribution of small water bodies to regional richness. This is the first large-scale study carried out in Spain on macroinvertebrates from mountain ponds, as well as a relevant piece of research in the context of ecosystem management and conservation. The thesis consists of four scientific papers answering a number of previously defined objectives. Fifty-five ponds and one lake from Castilla y León (Spain) were sampled (2004 to 2008). Overall observed richness in the study area was 143 taxa (mostly genera, although some specimens were only identified to levels above genus). Richness is usually quite a difficult property to measure. Alternative statistical estimators have been proposed and used to provide an insight into the possible, real richness of a community. Thus, the first paper in this thesis compared the most usual estimators and found out that non-parametric estimators, especially Jackknife 2 (and, to a lesser extent, Jackknife 1, Chao 1 and Chao 2), performed better than techniques based on accumulation curves. Therefore, Jackknife is proposed as an appropriate richness estimator for this type of community and habitat. A crucial investigation target is to get to know which factors are mainly responsible for among-site differences in richness. This was tackled here by using multiple lineal regression (24 variables measured at 39 ponds) to construct a prediction model. Environmental heterogeneity (measured as habitat number, HN) and intensity of fish introductions (FI) proved to be the only variables significantly related to richness. No significant relationship was found between macroinvertebrate richness and variables often considered as relevant (altitude, area, pH or water permanence). The resulting lineal model was expressed as: $S=4.922(NH)-2.359(FI)+11.227$. This model can accurately predict richness in the study area, as shown by testing it on a new set of ponds ($n=12$). An outstanding result of the research, with direct implications in management, is the confirmation of the severe effects of fish introductions on both the community richness and structure, but only at high intensity of fish densities. There is an additional issue related to richness which is in the origin of a long ecological debate: are sets of small areas more useful in conservation, in terms of taxon richness, than single large areas? In order to cast some light on this matter, a number of systems close to each other (seventeen ponds and a large lake, all of them within the limits of Sanabria Natural Park) were studied. The results showed that richness in each individual pond was much lower than in the lake. However, the contribution to regional biodiversity of the set of ponds was higher than that of the lake in spite of their smaller overall area. The potential relevance of sets of water bodies in conservation is ignored by current European legislation, but empirical evidences make it advisable to take them into account.

INTRODUCCIÓN GENERAL

Esta introducción sigue una estructura cronográfica relacionada con el orden en el que aparecen los términos principales que componen su título "*Riqueza, macroinvertebrados* y *lagunas de montaña*", profundizando en cada concepto y presentando los antecedentes científicos referentes a su estudio.

Riqueza biológica y su medición

El término riqueza hace referencia al número de taxones que integran una comunidad biológica. Es una de las características ecológicas fundamentales de cualquier sistema (Foggo *et al.*, 2003) y el concepto más simple e intuitivo para caracterizar su biodiversidad (Gaston, 1996; Magurran, 2004; Chao *et al.*, 2005). Encontrar el mejor método para medir la biodiversidad ha sido una de las cuestiones ecológicas más debatidas en las últimas décadas (Colwell & Coddington, 1994; Gaston, 1996; Gotelli & Colwell, 2001; Magurran, 2004). Históricamente, la biodiversidad ha sido medida a través de un amplio grupo de índices que, a menudo, están integrados por dos componentes: el número de especies (riqueza) y su abundancia (Magurran, 2004). Los índices de diversidad tradicionales dependen tanto del tamaño de la muestra como del patrón de muestreo y, en ocasiones, son difíciles de interpretar. Por otra parte, tanto el carácter complejo como la gran variedad de estos índices hacen difícil la comparación entre estudios científicos (Brose *et al.*, 2003). Recientemente, ambos componentes han sido medidos por separado dando especial importancia a la riqueza, la cual se presenta como el parámetro más utilizado en estudios de biodiversidad (Colwell & Coddington, 1994; Keddy & Drummond, 1996), de ecología de comunidades (Williams & Martínez, 2000), de macroecología (Gaston, 2000; Whittaker *et al.*, 2001) y de conservación (Conroy & Noon, 1996; Kerr, 1997; Biggs *et al.*, 2005). No obstante, y a pesar de su importancia, existen diferentes asuntos científicos referentes a la riqueza biológica todavía pendientes de resolver o con numerosas incógnitas e interrogantes. Cuestiones ecológicas fundamentales como ¿cuál es el mejor modo para medir y estimar la riqueza real de una comunidad biológica?, ¿de qué depende esa riqueza?, ¿cómo afectan o modifican su valor determinadas acciones antrópicas? o ¿cómo podemos utilizar este valor en la gestión de ecosistemas o territorios en el marco de la conservación? Este hecho genera un creciente y obligado interés científico por conocer en profundidad cómo funciona y responde este atributo a diferentes escalas ecológicas y en diferentes ecosistemas o comunidades biológicas naturales.

Para llevar a cabo un estudio de riqueza en una comunidad biológica es necesario conocer su valor, es decir, el número real de taxones diferentes que constituyen esa comunidad. Para ello se requiere, al menos, un conteo de los taxones presentes (Melo &

Froehlich, 2001). No obstante, a menudo conseguir inventarios completos de fauna en un lugar dado, para una comunidad específica o área geográfica determinada, constituye una tarea excesivamente dura e incluso imposible de conseguir (Foggo *et al*., 2003; Hortal *et al*., 2006). Este problema se acentúa cuando trabajamos con el principal componente de la biodiversidad: los invertebrados. El grado de esfuerzo, el tiempo empleado o el tipo de método utilizado en el muestreo de una comunidad biológica puede hacer variar sensiblemente el valor de riqueza observado. De esta manera, el valor de riqueza obtenido en una campaña de muestreo puede estar cerca o, por el contrario, alejarse notablemente del valor de riqueza real de la comunidad estudiada. En cualquier caso, si los inventarios no son completos, la comparación directa de los mismos entre comunidades no es posible, aunque el esfuerzo de muestreo desplegado en cada uno de ellos sea idéntico (Gotelli & Colwell, 2001). En las últimas décadas han surgido un elevado número de técnicas y aproximaciones matemáticas que intentan corregir, subsanar o mitigar, en la medida de lo posible, este problema (Colwell & Coddington, 1994). El uso de este tipo de aproximaciones, como estimadores de riqueza y curvas de acumulación, abunda en numerosos trabajos sobre una gran variedad de taxones y de hábitats (Colwell & Coddington, 1994; Brose *et al*., 2003), pero son escasos los estudios que comparan la eficiencia de los diferentes métodos de estimación de riqueza en lagunas y lagos (Foggo *et al*., 2003) y no existe ningún trabajo que lo haga en lagunas de montaña. Comprobar cuáles son los métodos de estimación de riqueza que mejor funcionan en estos sistemas tan especiales es, sin duda, pieza clave en el estudio de su riqueza.

El conocimiento en profundidad de cualquier característica biológica de una comunidad pasa, ineludiblemente, por conocer qué factores y en qué medida afectan a su valor. En otras palabras, ¿de qué depende esa variable? Por otra parte, y al margen de las razones científicas, el conocimiento de los patrones básicos de distribución de la biodiversidad y, en concreto, de la riqueza biológica, es esencial para desarrollar estrategias de conservación (Gaston, 2000). Es complicado abordar una determinada situación que afecte directamente al valor de la riqueza de una comunidad biológica si desconocemos la medida en que los diferentes factores ambientales operan sobre la misma. Por tanto, conocer la relativa contribución de los procesos locales y regionales que afectan a la riqueza biológica es clave en el conocimiento y mantenimiento de la riqueza y biodiversidad de una comunidad en concreto (Gaston & Spicer, 2004). Existen numerosos trabajos que ponen de manifiesto la relación entre unos pocos factores y la riqueza de macroinvertebrados (Collinson *et al*., 1995; Zimmer *et al*., 2001; Céréghino *et al*., 2008; Oertli *et al*., 2008). Sin embargo, sólo unos pocos lo hacen a la vez con un gran conjunto de variables en lagunas

(Rundle *et al*., 2002) y muy raramente en sistemas alpinos (Hinden *et al*., 2005; Füreder *et al*., 2006; Collado & De Mendoza, 2009). Es evidente que esclarecer esta cuestión en la naturaleza no es tarea fácil, ya que el efecto de muchos de los factores puede verse enmascarado por otras variables, o incluso actuar en sinergia (De Mendoza & Catalan, 2010). Además, es difícil aplicar los patrones de riqueza encontrados en otras regiones, ya que el gran número de variables y la heterogeneidad de las lagunas muestreadas a menudo generan un universo de muestreo extremadamente particular. No obstante, el estudio de las variables que afectan a la riqueza biológica en lagunas de montaña, como ha quedado demostrado, es fundamental tanto desde el punto de vista científico como del práctico.

La necesidad de proteger estos sistemas surge, automáticamente, en el momento en que la propia acción antrópica actúa sobre los mismos y desestabiliza su equilibrio natural, afectando directamente al valor de su riqueza biológica. La introducción de especies alóctonas constituye una de las mayores amenazas para la biodiversidad de cualquier ecosistema (Vitousek *et al*., 1996; Mooney & Cleland, 2001; Davis, 2003), siendo uno de los de los impactos humanos más frecuente, severos y desequilibrante en ecosistema de lagos y lagunas (Kauffman, 1992; Rodríguez *et al.*, 2003; Rodríguez *et al.*, 2005). Es bien conocido que la introducción piscícola en sistemas lacustres afecta negativamente a la riqueza y composición de las comunidades de macroinvertebrados (Diehl & Eklöv, 1995; Kornijów, 1997; Lodge *et al*., 1998), pero en lagunas de montaña de España existen todavía pocos trabajos que evidencien claramente sus efectos (Toro *et al*., 2006). No obstante, en estas lagunas son muy frecuentes este tipo de actuaciones y se producen introducciones piscícolas, muchas veces sin ningún tipo de control ni seguimiento (Martínez-Solano, 2003). De esta manera, esta acción antrópica se presenta como una variable o factor más a la hora de explorar de qué depende la riqueza biológica en estos medios. El estudio en profundidad de cómo afectan los diferentes niveles de introducciones de fauna piscícola en lagunas de montaña es uno de los puntos más importantes para poder comprender cómo responde la riqueza de macroinvertebrados en ecosistemas naturales.

Por otra parte, una de las tendencias en biología de conservación es priorizar ecosistemas en función de su biodiversidad (Gaston & Spicer, 2004; Primack, 2010). Regiones o ecosistemas que albergan un elevado número de taxones son más susceptibles de ser incluidos en planes de conservación. La gran mayoría de los espacios naturales protegidos valoran la gran riqueza y biodiversidad que alberga su territorio y desarrollan sus planes de conservación y recuperación con el objetivo de salvaguardar y preservar su riqueza biológica. Históricamente, desde el punto de vista limnológico, grandes lagos presentan prioridad de protección e inclusión en planes de conservación. Esto es debido, en parte, a la clásica idea de “que cuanto más grandes es el área, más riqueza de taxones alberga”, como postula la relación ecológica básica de especie-área (MacArthur & Wilson, 1967). No obstante, y a pesar de su pequeño tamaño, se ha demostrado que las lagunas y lagos de pequeños tamaño contribuyen significativamente en la riqueza regional de los sistemas de agua dulce continentales (Pond Action, 1994; Linton & Goulder, 2000; Oertli *et al.*, 2002; Nicolet *et al.*, 2004) especialmente en la de macroinvertebrados (Biggs *et al.*, 2005). A pesar de ello, un gran número de pequeñas lagunas han desaparecido en las últimas décadas (Hull, 1997). Existe, por lo tanto, la necesidad de profundizar en el conocimiento de estas pequeñas lagunas y de los sistemas interconectados que forman, como son las lagunas de montaña, y poner de manifiesto su valor en términos de biodiversidad junto con el resto de sistemas limnológicos ya valorados y protegidos.

Riqueza biológica y su medición

Ideas clave

- El estudio de riqueza biológica es útil, adecuado, necesario y, en el caso de lagunas de montaña de España, todavía una rama científica incipiente y poco explorada.
- El punto de partida para cualquier estudio de riqueza es conocer su valor o, al menos, conocer qué técnicas son útiles para estimar su valor en el sistema que queremos estudiar.
- La esencia del conocimiento de la riqueza biológica de una comunidad biológica pasa por conocer de qué variables depende, siendo útil el desarrollo de una herramienta o modelo que muestre la relevancia de los factores que operan sobre la misma.
- Conocer el efecto de los impactos antrópicos sobre la riqueza de macroinvertebrados, es clave si queremos profundizar en su conocimiento científico.
- Es preciso mostrar la importancia de estos sistemas de montaña y su aportación para la biodiversidad de un área, convirtiéndose en pieza clave para la conservación y gestión de espacios naturales.

Macroinvertebrados

Se consideran macroinvertebrados bentónicos a aquellos invertebrados que viven asociados a los distintos sustratos de un ecosistema acuático (bentos) y que por su tamaño pueden ser observados a simple vista y retenidos en redes de luz de malla de entre 250-500 µm. Comprende generalmente aquellos organismos que al final de su desarrollo larval o en su estado adulto alcanzan un tamaño igual o superior a 3 mm. Es una denominación que engloba a un conjunto muy heterogéneo de grupos taxonómicos, desde nematodos y anélidos hasta insectos de diversos órdenes.

El interés por este grupo de organismos ha ido en aumento en toda Europa (Collinson *et al.,* 1995; Brodersen *et al.,* 1998; Heino, 2000; Johnson & Goedkoop, 2002; White & Irving, 2003; Biggs *et al*., 2005; Della Bella *et al*., 2005; Kownacki *et al.*, 2006; Krno *et al*., 2006; Toro *et al*., 2006; Oertli *et al*., 2008; Fjellheim *et al*., 2009). Desde hace mucho tiempo han sido considerados buenos indicadores de la calidad del agua, al menos en medios lóticos (véase una revisión en Reynoldson & Metcalfe-Smith, 1992). A pesar de esto, su estudio en medios leníticos ha recibido poca atención hasta la fecha, aun demostrándose su utilidad como bioindicadores de calidad en lagos europeos (Johnson, 1998; Johnson & Goedkoop, 2002). Este hecho contrasta con la amplia atención dedicada a otros grupos, como el fitoplancton, el zooplancton y los peces. Además, los macroinvertebrados desempeñan un papel fundamental en el funcionamiento de lagos y lagunas, constituyendo una pieza clave en las relaciones tróficas que se desarrollan en el ecosistema (Diehl & Kornijów, 1997). Utilizan diferentes fuentes de alimentación, incluyendo vegetales microscópicos, pequeños animales (zooplancton y otros invertebrados), detrito (alóctonos y autóctonos) y son a su vez consumidos por peces y anfibios. En términos ecológicos, diríamos que constituyen un imprescindible vehículo de transferencia de energía desde los primeros niveles tróficos hasta los vertebrados depredadores.

Por otra parte, el Parlamento Europeo aprobó en el año 2000 la más exigente de las normativas para la gestión de las masas de agua con que ha contado Europa: la Directiva Marco del Agua (DMA) (Council of the European Communities, 2000). En ella se definen, entre otras cosas, las directrices que deben seguir los Estados Miembro para evaluar la calidad (el estado ecológico, según la terminología de la DMA) de ríos, lagos, aguas costeras, de transición y subterráneas. Uno de sus objetivos es lograr que las masas de agua de la Unión Europea alcancen un buen estado ecológico antes del final del 2015. De acuerdo con la DMA, la valoración del estado ecológico de los cuerpos de agua debe determinarse a través de la evaluación de una serie de elementos indicadores de calidad, entre ellos los macroinvertebrados.

Tanto por razones científicas como prácticas, el estudio de este grupo de organismos es clave en el conocimiento ecológico de cualquier sistema acuático. Esta tesis se centra en el estudio de las comunidades de macroinvertebrados litorales de lagunas de montaña, concretamente en la naturaleza de uno de sus atributos: la riqueza.

Breve historia del estudio de lagos y lagunas de montaña

En Europa, las primeras investigaciones limnológicas en áreas de montaña fueron llevadas a cabo a finales del siglo XIX, principalmente durante la década de los años 80. Expediciones y equipos de investigación suizos y franceses obtuvieron los primeros resultados, recopilados más tarde por Zschokke (1900). A principios del siglo XX, numerosos trabajos contribuyeron a caracterizar las comunidades biológicas de lagos y lagunas de montaña constituyendo una, todavía "joven", rama del estudio científico (Zschokke, 1908; Pesta, 1929; Monti, 1903, 1910a, 1910b; Stella, 1931; Moretti, 1938). Un importante avance fue el establecimiento de la primera estación limnológica de alta montaña en el área de Kühtai (Stubai Alps, Tyrol) por O. Steinböck en 1959. Recientemente, la sensibilidad que presentan los sistemas leníticos de alta montaña al cambio global ha generado un incremento de publicaciones científicas (Boggero & Nocentini, 1994; The MOLAR Water Chemistry Group, 1999; Thies *et al*., 2000; Sommaruga & Psenner, 2001; Carrera *et al*., 2002; Curtis *et al*., 2002; Mosello *et al*., 2002; Thies *et al*., 2002; Marchetto & Rogora, 2004). Por otra parte, la implementación de la Directiva Marco del Agua (Council of the European Communities, 2000) también ha suscitado un interés creciente por el funcionamiento ecológico estos sistemas y de su importancia en el conjunto global de la biodiversidad (Oertli, 1995; Biggs *et al*., 2000; Oertli *et al*., 2001; Oertli *et al*., 2002; Biggs *et al*., 2005; Oertli *et al*., 2005; Céréghino *et al*., 2008; Oertli *et al*., 2008).

En España, a finales del siglo XIX las grandes cumbres comenzaban a suscitar interés por viajeros, naturalistas e incluso monarcas (Alfonso XII llegó hasta el pico y la laguna de Peñalara en el Sistema Central). Bajo una marcada influencia europea se estaba gestando el reconocimiento de estos medios en nuestro país. En 1911 el Museo de Ciencias Naturales crea la Estación Alpina de Biología (centro auxiliar para facilitar las investigaciones en Guadarrama) y en 1912, año en el que nace la limnología en España (Casado y Montes, 1992), tienen lugar las primeras expediciones e investigaciones en el lago de Sanabria (Ciria y Vinent, 1913; Halbfass, 1913). Estos trabajos se centraban en la morfología, geología, hidrografía (Aragón, 1913) y el fitoplancton (Bachmann, 1913). Más tarde, con estas mismas muestras, Burckhardt (1921) publicó un trabajo sobre zooplancton. Los especialistas españoles en estas materias todavía eran escasos, años después González Guerrero (1928) aprovecharía estas muestras en sus investigaciones sobre algas continentales. Aragón completa sus estudios con otras dos lagunas incluidas en esta tesis, La Baña y Truchillas. En la misma época se llevó a cabo un estudio mucho más próximo al concepto de limnología sobre el lago Sanabria (Taboada Tundidor, 1913). Sería este el primer naturalista español en abordar este campo de la investigación. En 1914 se concluye el primer Laboratorio de Hidrobiología en España, pero habría que esperar más tiempo para la aparición de trabajos sobre el verdadero funcionamiento de estos medios (Arévalo, 1929; Pardo, 1932; Margalef, 1949; Margalef, 1975). En las últimas décadas, el interés por estos sistemas se ha centralizado sobre todo en el área de los Pirineos (Ej: Catalán *et al* 1992; Rieradevall *et al*., 1999; Catalán *et al*., 2002; Catalán *et al*., 2009a, 2009b; Collado & De Mendoza, 2009; De Mendoza & Catalán, 2010), Sierra Nevada (Ej.: Sánchez-Castillo *et al*., 1989; Morales-Baquero *et al*., 1992; Cruz-Pizarro *et al*., 1996), parte del Sistema Central (Ej.: Toro y Montes 1995; Granados & Toro 2000a, 2000b, 2001) y Sanabria (Ej.: Aldasoro *et al*., 1992; De Hoyos, 1996; Negro *et al*., 2000; Real *et al*., 2000), siendo escasos los trabajos en el resto de la comunidad de Castila y León (Ej.: Martínez-Solano, 2003; Negro *et al*., 2003). Esta tesis constituye la primera investigación limnológica en el conjunto de lagunas de montaña de esta región.

Objetivos y estructura de la tesis

Objetivos

La pretensión de esta tesis es esclarecer diversas cuestiones científicas, todavía no resueltas o poco estudiadas, relacionadas con el estudio de riqueza de macroinvertebrados de lagunas de montaña.

Las tres grandes líneas de trabajo u objetivos principales son los siguientes:

1. Encontrar el mejor método para medir y estimar la riqueza de macroinvertebrados en lagunas de montaña.

2. Desvelar de qué factores depende la riqueza de macroinvertebrados en lagunas de montaña.

3. Mostrar la importancia de estos sistemas lagunares en la contribución a la riqueza de una región y su aplicación en el ámbito de la conservación.

Dentro del estudio de estas tres grandes líneas de trabajo aparecen otra serie de objetivos secundarios. Diversas cuestiones cuya consecución también representan un avance importante en el conocimiento de la limnología en general y de la riqueza, en particular, de estos sistemas tan especiales y poco estudiados:

4. Desarrollar un modelo concreto de predicción en lagunas de montaña que muestre los factores que afectan a la riqueza biológica de macroinvertebrados y que permita obtener una primera aproximación de su valor conociendo una serie de variables concretas.

5. Poner de manifiesto los efectos de la introducción de salmónidos sobre la composición de la comunidad de macroinvertebrados de lagunas de montaña.

6. Caracterización de la comunidad de macroinvertebrados de lagunas de montaña de Castilla y León.

Estructura de la tesis

Esta tesis está formada por cuatro artículos científicos ya publicados en sendas revistas (figura 1). Estos cuatro artículos constituyen el núcleo central de la tesis y a través de ellos se abordan los objetivos expuestos en el apartado anterior. Esta introducción general da unidad y sentido a lo desarrollado por cada trabajo publicado. La parte final de la misma versa sobre los objetivos perseguidos en esta tesis y expone, en este mismo apartado, la estructuración general y su relación con la consecución de estos objetivos (tabla 1). En el área de estudio se presenta de manera global las lagunas estudiadas, sus características, su origen, estado de conservación y descripción geológica de sus cubetas. No obstante, cada artículo contiene su área de estudio particular, que especifica el subconjunto del total utilizado. Del mismo modo, la metodología empleada en cada artículo es desarrollada ampliamente en el mismo, por esta razón no se ha incorporado un apartado general de metodología, ya que resultaría reiterativo (no lo es en el caso del área de estudio, puesto que en este apartado se proporciona información adicional que no aparecen el los diferentes artículos). Una discusión final trata de enlazar y desentrañar todas las cuestiones comunes abordadas en cada artículo, así como de presentar las futuras y necesarias líneas de investigación. El siguiente apartado expone las conclusiones generales. Finalmente, se han incorporado dos anexos. El Anexo I muestra un listado global de los taxones recogidos en las lagunas estudiadas, cumpliendo el objetivo número 6 planteado en esta investigación. Esta relación de taxones no aparece específicamente en ninguno de los artículos, ya que se obtiene del estudio global de la tesis. El Anexo II es una recopilación fotográfica de las lagunas muestreadas, junto con un cuadro explicativo de algunas de sus características. El fin de este último anexo es el de generar al lector una primera idea de cómo es cada sistema lacustre estudiado.

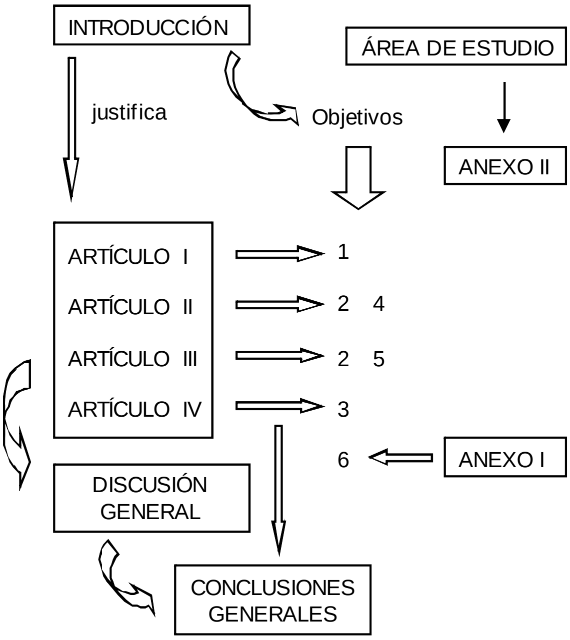

Figura 1. Esquema de la organización de la tesis

Tabla 1. Relación de los artículos que integran esta tesis y los objetivos tratados en cada uno.

Artículos	Objetivos
I. Martínez-Sanz, C., F. García-Criado, C. Fernández-Aláez & M. Fernández-Aláez, 2010. Assessment of richness estimation methods on macroinvertebrate communities of mountain ponds in Castilla y León (Spain). *Annales de Limnologie-International Journal of Limnology*, 46: 101–110.	**1**
II. Martínez-Sanz, C., C. Fernández-Aláez & F. García-Criado, 2012. Richness of littoral macroinvertebrate communities in mountain ponds from NW Spain: what factors does it depend on? *Journal of Limnology*, 71(1): 154-163.	**2** **4**
III. Martínez-Sanz, C., F. García-Criado & C. Fernández-Aláez, 2010. Effects of introduced salmonids on macroinvertebrate communities of mountain ponds in the Iberian system of Spain. *Limnetica*, 29 (2): 221-232.	**2** **5**
IV. Martínez-Sanz, C., C.S.S. Cenzano, M. Fernández-Aláez & F. García-Criado, 2012. Relative contribution of small mountain ponds to regional richness of littoral macroinvertebrates and the implications for conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems*, (in press)	**3**

Epítomes de los artículos

I. El primer artículo de esta tesis trata sobre la estimación de riqueza de comunidades de macroinvertebrados en lagunas de montaña. Aparece la primera aproximación al conocimiento de cuál o cuáles de los estimadores de riqueza desarrollados funcionan mejor en este tipo de sistemas. Recoger el número real de especies, e incluso géneros y familias, de macroinvertebrados presentes un sistema lacustre de montaña es, con los medios actuales de muestreo, una tarea prácticamente imposible. Por lo tanto, es recomendable usar estas técnicas cuando se aborda esta área de la investigación, especialmente si se van a efectuar comparaciones entre diferentes comunidades o localidades.

II. En el segundo artículo de esta tesis se intenta poner de manifiesto los factores o variables que afectan al valor de la riqueza de macroinvertebrados de lagunas de montaña. Mediante un modelo de predicción se establece un gradiente de relevancia significativa de los factores que realmente operan sobre esta variable. Junto con las variables ambientales estudiadas, se han añadido factores de origen antrópico, configurando de esta manera un profundo conocimiento sobre este parámetro y brindando la posibilidad de efectuar una primera predicción de su valor en comunidades biológicas similares.

III. En el tercer artículo se estudia el efecto de uno de los impactos antrópicos más frecuentes en lagunas de montaña, la introducción de fauna piscícola. En el anterior artículo se muestra cómo esta variable afecta al valor de la riqueza, pero no se estudia la composición de la comunidad, es decir, qué grupos de organismos se ven afectados y en qué medida. En otras palabras ¿quién está implicado en la variación de la riqueza? En este artículo trabajamos con la estructura y composición de la comunidad y su relación con el máximo nivel de impacto piscícola detectado en el área de estudio.

IV. El cuarto artículo da sentido práctico al estudio de riqueza en general y al llevado a cabo en los otros tres artículos. Intenta cubrir una parte “olvidada u obviada” en el estudio de patrones de biodiversidad en lagunas de montaña. La vigente legislación Europea promueve la conservación de habitas singulares (Directiva Habitas) y la implementación de medidas para preservar y mejorar la calidad ecológica (Directiva Marco de Agua). Sin embargo, no tiene en cuenta el hecho de que sistemas de lagunas pueden tener una gran importancia en la contribución de riqueza regional, a pesar de su pequeño tamaño. En este trabajo se muestra el valor en términos de biodiversidad de un grupo de pequeñas lagunas de montaña del Parque Natural de Sanabria y alrededores y su relación con un gran lago, el lago de Sanabria.

ÁREA DE ESTUDIO

Las lagunas estudiadas

Todos los datos utilizados en esta tesis fueron obtenidos de 55 lagunas de montaña (más el lago de Sanabria), muestreadas durante los años 2004 a 2008. La gran mayoría fueron muestreadas solo un año, pero para alguna de ellas se han obtenido series temporales de hasta 3 años seguidos (ver área de estudio particular de los artículos). Todas pertenecen a la comunidad autonómica de Castilla y León, la cual constituye el ámbito geográfico de la tesis. Castilla y León con una superficie de 94.223 km^2, a grandes rasgos, es una basta plataforma central (de 400 a 1.000 m.s.n.m.) rodeada de sistemas montañosos por todos sus puntos cardinales, con una sola salida al mar en su parte más occidental por el río Duero. La cota más alta se encuentra en Picos de Europa (Torre Cerredo, 2.648 m.s.n.m.). A lo largo de todos estos sistemas montañosos se localizan las lagunas de montaña objeto de este estudio (figura 2).

Selección de las lagunas. En primer lugar, se elaboró una lista de lagunas de montaña existentes partiendo del Catálogo de Zonas Húmedas de Castilla y León y la Ampliación del Catálogo. Esta relación fue completada con otras serie de lagunas de cierta entidad (mayor de 0,3 hectáreas) conocidas por el equipo de investigación o encontradas en bibliografía divulgativa. Este inventario de partida incluía más de 100 sistemas. Se escogieron las de mayor entidad y aquellas que formaban grupos concretos y bien definidos. Se pretendía disponer de un conjunto de lagunas heterogéneo pero con suficientes "réplicas" de cada tipo (lagunas temporales, sin impactos, con impactos, a diferentes altitudes...). El lago de Sanabria fue utilizado para efectuar comparaciones de riqueza con otras lagunas de montaña del Parque Natural de Sanabria y alrededores, cubriendo las necesidades requeridas por los objetivos planteados en esta tesis.

A continuación se presentan todas las lagunas muestreadas por zonas de muestreo, lo que nos proporciona la posibilidad de mostrar información adicional y de importancia de las propias lagunas y de las regiones en las que se encuentran (estado de protección, características geográficas y origen específico). Además, el Anexo II contiene fotos de los sistemas lacustres considerados, así como de algunas de sus características físicas, morfométricas y una relación de las presiones de origen antrópico a los que están sometidos.

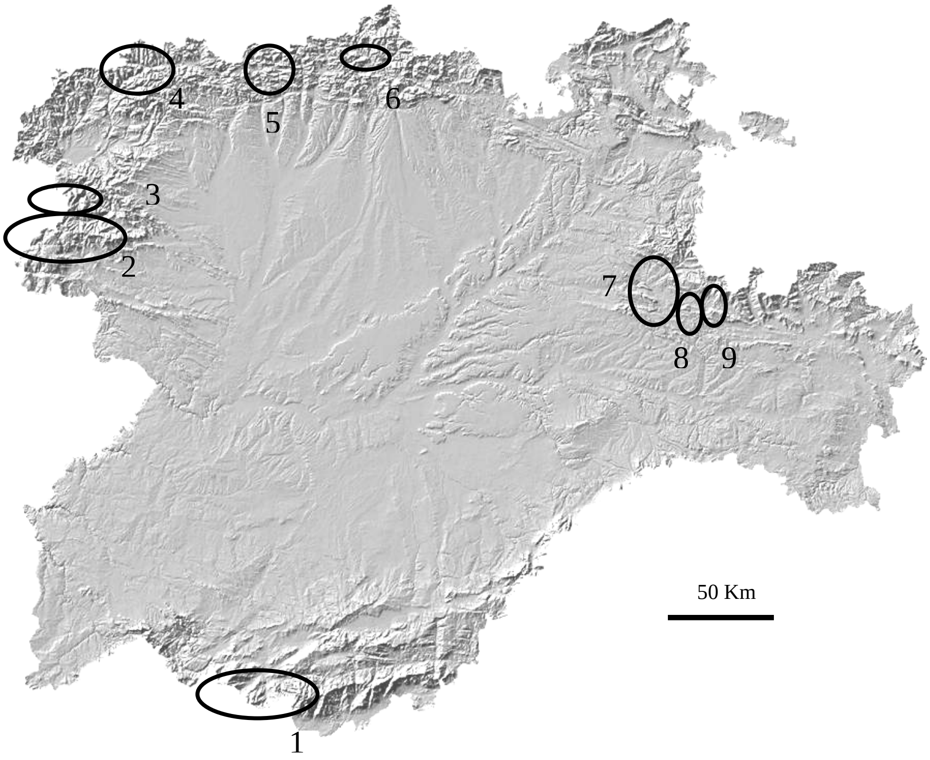

Figura 2. Zonas muestreadas. 1. Gredos, 2. Sanabria, 3. La Cabrera, 4. Babia, 5. San Isidro, 6. Fuentes Carrionas, 7. Demanda, 8. Urbión y 9. Cebollera.

Presentación de las lagunas por zonas de muestreo

(Las fotos de las lagunas se encuentran en el Anexo II)

1. Gredos

La sierra de Gredos se localiza en el centro del Sistema Central constituyendo su núcleo principal. Delimita Castilla y León en su parte más meridional (Ávila) y alcanza cotas de hasta 2.592 m.s.n.m. (pico del Moro Almanzor). Es un espacio natural relativamente amplio (86.236 hectáreas) y se encuentra protegido bajo la figura de parque regional (declarado en 1996). Los glaciares pleistocénicos jugaron en esta área un importante papel erosivo, originando tras su retirada diferentes lagunas de montaña. De entre las muestreadas, cuatro de ellas se encuentran en el centro de este sistema (Cimera, foto 1; Mediana, foto2; Cervunal, foto 3 y Grande de Gredos, foto 4), tres más hacia el oeste (Cuadrada, foto 5; Caballeros, foto 6 y El Barco, foto 7) y la octava en la parte más occidental de Gredos (El Trampal, foto 8).

2. Sanabria

El Parque Natural del Lago de Sanabria y alrededores (declarado en 1978) se sitúa en el extremo occidental de Castilla y León. Comprende una superficie de 22.365 hectáreas y altitudes máximas de hasta 2127 m. (Peña Trevinca). El lago de Sanabria (fotos 9 y 10), también incluido en esta tesis, es lo que se denomina un lago glaciar frontal. Sus aguas se hallan contenidas por varios arcos morrénicos (5, 6 y hasta 7 según los autores) que depositó la lengua glaciar al final de su recorrido. Las otras 17 lagunas estudiadas se encuentran en la parte más alta del Parque, 15 de ellas al oeste del lago, sobre una paleo-superficie horadada por la acción de un gran casquete glaciar, cuyo hielo sobreexcavó las zonas de roca más débil, creando pequeñas cubetas en las que, al retirarse el hielo, se formaron lagunas (Camposagrado I; Camposagrado II, foto 11; El Payón, foto 12; Lacillo, fotos 13 y 14; Pies Juntos; El Cuadro, foto 15; Clara Grande, foto 16; Clara Pequeña, foto 17; Roya Grande, foto 18; Roya Pequeña, foto 19; Pedrina, foto 20; Castromil, foto 21; Aguas Cernidas, foto 22; Mancas, foto 23 y Sotillo, foto 24) y dos de ellas al norte del lago de Sanabria, sobre la misma superficie morfoestructural aunque corresponde a dos cabeceras diferentes de un mismo valle (La Yegua, foto 25 y Los Peces, foto 26).

3. La Cabrera

En la sierra de la Cabrera (León) se han estudiado otras dos lagunas, ambas bajo la figura de protección de monumentos naturales (declaradas en 1990): lago de Truchillas (foto 27), laguna típica de circo glaciar y el lago de La Baña (foto 28) que, a pesar de encontrarse ubicada dentro de un valle glaciar, su formación es atribuida a un gran deslizamiento de ladera, el cual cerró la salida de las aguas del arroyo del lago.

4. Babia

La comarca leonesa de Babia (espacio natural del Valle de San Emiliano, 55.200 hectáreas, declarada Reserva de la Biosfera por la UNESCO, 2004) funciona como límite natural entre Asturias y León. Destaca la presencia de la gran mole calcárea de Peña Ubiña (2.417 m), poniendo de manifiesto la naturaleza básica de la litología predominante de la zona. Se muestrearon 5 lagunas de montaña de origen glaciar. Laguna Grande de Babia (Foto 29), originada por sobre-excavación del surco de pizarras por la lengua de hielo que se alimentó de una transfluencia glaciar; laguna de Las Verdes (foto 30); lagunas de La Mata, de las cuales se han muestreado dos (fotos 31 y 32), originadas por yuxtaposición glaciar; y la laguna de Riolago de Babia (Foto 33). Las dos Matas y la laguna Grande de Babia se reconocen como LIG (lugar de interés geológico).

5. San Isidro

Aproximadamente a 60 Km en dirección este, dentro de esta gran Cordillera Cantábrica y todavía en la provincia de León, encontramos otro núcleo de muestreo incluido en esta tesis, es la cuenca del alto Porma (puerto de San Isidro). Aquí se visitaron 4 lagunas de origen glaciar: lago Ausente (foto 34) y lago de Isoba (foto 35); y otros dos sistemas lacustres temporales menos conocidos: laguna de Robledo (foto 36) y laguna de Tronisco (foto 37).

6. Fuentes Carrionas

El resto de lagunas de montaña muestreadas dentro de la Cordillera Cantábrica se encuentran más hacia el este, en el límite entre las provincias de León y Palencia. Es la antesala del Parque Regional y Nacional de Picos de Europa. Se trata del Parque Natural de Fuentes Carrionas y Fuente Cobre – Montaña Palentina (78.360 hectáreas, declarado en 2000), entorno donde destacan grandes cumbres como Peña Prieta (2539), Mojón de las Tres Provincias (2499), Curavacas (2524) y Espigüete (2450). Las 5 lagunas de montaña muestreadas están ubicadas en las cabeceras de los valles glaciares y, como sucedía en numerosas lagunas de Gredos, muy alejadas de la influencia antrópica. Se ha muestreado el Pozo Curavacas (foto 38), el Pozo de Las Lomas (foto 39), Hoyos de Vargas I (foto 40), Hoyos de Vargas II (foto 41) y laguna de Fuentes Carrionas (foto 42).

Justo entre las provincias de Burgos, Soria y La Rioja, nos encontramos con cuatro áreas de muestreo relativamente cercanas donde se muestrearon 14 lagunas de montaña de origen glaciar: sierra de La Demanda (donde también se incluye la sierra de Neila), Picos de Urbión y sierra de Cebollera.

7. Demanda

Se muestreó la laguna glaciar Pozo Negro (foto 43), Muñalba (foto 44) y las lagunas glaciares de Neila, que constituyen la esencia del Parque Natural de Las Lagunas de Neila (6.290 hectáreas): Los Patos (foto 45), Brava (foto 46), Las Pardillas I y II (fotos 47 y 48), Negra (foto 49) y Haedillo I y II (foto 50).

8. Urbión

En el área de los Picos de Urbión, Soria (Pico de Urbión, 2228 m), las lagunas muestreadas confieren nuevamente el valor a una región natural y otorgan la categoría de Parque Natural de la Laguna Negra y Circos Glaciares de Urbión (4.617 hectáreas): laguna Negra de Urbión (Foto 51); Helada (foto 52) y Larga (foto 53).

9. Cebollera

Finalmente, en dirección este y en el corazón de la sierra de Cebollera (Soria) otras dos lagunas glaciares completan el área de estudio de esta tesis: laguna Verde (foto 54) y laguna de Cebollera (foto 55).

Marco Geológico

Litología

Las características litológicas de los materiales sobre los que se encuentran emplazadas las lagunas determinan algunas de sus propiedades fisicoquímicas. El pH, la alcalinidad, la conductividad e incluso la concentración de diferentes metales son algunos ejemplos de variables vinculadas directamente con las características geológicas del área. No existe ninguna barrera entre el agua de las lagunas y los materiales sobre los que se asientan, por lo que parece lógico dedicar un apartado de esta tesis a describir las características geológicas del las áreas de estudio.

Todas las lagunas muestreadas se encuentran ubicadas en el Macizo Ibérico (Paleozoico) y en una parte del Sistema Ibérico (al este del área de estudio) donde podemos encontrar materiales pertenecen al Mesozoico (concretamente del Cretácico). Desde un punto de vista geológico, las lagunas estudiadas se localizan en tres zonas diferentes: 1. Zona Cantábrica, 2. Zona Centroibérica y 3. Sistema Ibérico. (Se presentan estas zonas según el orden geológico lógico).

1. Zona Cantábrica.

Existen una serie de características comunes que definen a esta zona geológica como una gran unidad. Todas las rocas presentes son sedimentarias excepto ocasionales intrusiones plutónicas en el área de Fuentes Carrionas. La mayoría son sedimentarias marinas (desde el Cámbrico hasta el Carbonífero inferior), el resto (Carbonífero medio-superior) son continentales. Existe una marcada alternancia de rocas carbonatadas y siliciclásticas, predominando unas u otras según el área en la que nos encontremos. Toda la Zona Cantábrica se originó durante la Orogenia Varisca, viéndose también afectada posteriormente por la Orogenia Alpina.

1.1. Comarca de Babia.

Es el área donde podemos encontrar las lagunas más occidentales de la Zona Cantábrica. En la alternancia de los materiales sedimentarios que constituyen el área, existe un fuerte predominio de los carbonatados (calizas y dolomías) sobre los siliclásticos. Las rocas carbonatadas son fácilmente meteorizadas por el agua mediante los procesos de karstificación. La existencia de lagunas en estas zonas tan permeables principalmente se debe, en este caso concreto, a la existencia de materiales más impermeables (principalmente lutitas y areniscas) que se encuentran intercalados entre las calizas. La laguna Grande de

Babia y la laguna de las Verdes se encuentran emplazadas sobre el Grupo La Vid (Devónico, dividido formalmente en cuatro formaciones). En concreto, ambos lagos se asientan sobre una estrecha franja de lutitas de la Formación Valporquero, pero se encuentran rodeados por las calizas del Devónico Inferior-Medio de la Formación Santa Lucía. Las lagunas de La Mata se encuentran ubicadas sobre areniscas y pizarras del Carbonífero inferior (Formación San Emiliano) y Riolago de Babia sobre pizarras negras y areniscas del Silúrico (Formación Formigoso).

1.2. Puerto de San Isidro

Las rocas sedimentarias predominantes son siliclásticas, aunque algunas de las lagunas se encuentran ampliamente rodeadas por rocas carbonatadas (Ej.: Isoba). El lago Ausente se encuentra sobre cuarcitas del Ordovícico (Formación Barrios). La laguna de Tronisco sobre areniscas y lutitas del Ordovícico Inferior (Formación Oville). La laguna de Robledo sobre lutitas del Carbonífero (Formación Fresnedo). El lago Isoba, aunque emplazado sobre una estrecha franja de lutitas y areniscas (Formación Beleño), está rodeado por calizas tableadas del Carbonífero (Formación Barcaliente).

1.3. Macizo de Fuentes Carrionas

Nuevamente existe una alternancia de litologías, pero predominan claramente las rocas siliclásticas. Es en esta zona donde el ambiente sedimentario de formación de las rocas posee un carácter más continental que marino. Conglomerados cuarcíticos (Formación Curavacas): Pozo Curavacas, Pozo de las Lomas y Hoyos de Vargas I. Areniscas y lutitas (Formación Lechada) del Carbonífero: Hoyos de Vargas II y laguna de Fuentes Carrionas. Destaca la presencia de una intrusión (rareza en el ámbito cantábrico) de rocas ígneas (granodiorita biotítica) de edad Varisca en las cuencas de recepción de Pozo de las Lomas y la laguna de Fuentes Carrionas.

2. Zona Centroibérica.

Está constituida por rocas paleozoicas tectonizadas durante la Orogenia Varisca, pero a diferencia de la Zona Cantábrica, existe un predominio de rocas ígneas intrusivas (granitos) y rocas metamórficas de diferente grado (pizarras y gneises), abundando más unas u otras según la zona.

2.1. Cabrera

Abundan las pizarras metamórficas. El lago de La Baña se encuentra emplazado y rodeado por pizarras, cuarcitas y areniscas metamórficas del

Ordovícico. El lago de Truchillas lo hace también sobre materiales del Ordovícico pero ligeramente más antiguos, son las cuarcitas blancas metamórficas del Ordovícico Inferior.

2.2. Sanabria

Abundan los gneises, rocas de naturaleza siliclástica. Granodioritas y gneis glandulares de la unidad Ollo de Sapo, que suelen dar las zonas más desgastadas por la erosión (facies micácea).

2.3. Gredos

Formado por rocas ígneas intrusivas, principalmente granitos, granodioritas y granitos bióticos porfídicos.

3. “Sistema Ibérico”

Dentro de este heterogéneo sistema, solo una parte de la sierra de la Demanda es la única que pertenece al Macizo Ibérico (rocas sedimentarias, paleozoicas y originadas durante la Orogénia Varisca). En este ámbito, el Pozo Negro se encuentra emplazado sobre alternancias de metareniscas y pizarras del Cámbrico Superior de la Formación Najerilla.

El resto del Sistema Ibérico está constituido por rocas originadas en plataformas Cretácicas de la Era Mesozoica. La laguna de Muñalba se ubicada sobre areniscas del Cretácico. Las lagunas glaciares de Neila están emplazadas sobre materiales cretácicos de areniscas conglomeráticas y arcillas. Por último, tanto las lagunas de la sierra de Urbión como las de Cebollera se encuentran sobre litologías siliclásticas cretácicas (calcarenitas, limonitas, conglomerados silíceos y cuarzoarenitas).

Geomorfología

Origen general de las lagunas estudiadas. Todas las lagunas incluidas en esta tesis, como ya se ha mencionado anteriormente, son de origen glaciar. Es decir, su formación está asociada a la acción de los hielos glaciares durante el Cuaternario (Pleistoceno). Las lagunas glaciares de montaña surgen debido a la obstrucción del drenaje superficial que supone el propio hielo glaciar y, una vez retirado (hace 16000-18000 años), a la obstrucción realizada por sus morrenas, a deslizamientos postglaciares y a las cubetas de sobreexcavación que genera (Korup & Tweed, 2007). En este contexto, la litología juega un papel importante. Así, la presencia de litologías más deleznables es la causa de la escasez de lagunas glaciares en la Cordillera Cantábrica respecto a otros sistemas montañosos, dónde están mucho mejor representadas, incluso a pesar de que en algunos de ellos la acción glaciar fuera menos intensa (Santos González, 2011). Tanto en montañas graníticas (sierra de Gredos) como en otras integradas por materiales resistentes (sierra de Neila, Picos de Urbión) son relativamente abundantes; mientras que son muy escasas y de reducidas dimensiones en macizos de litologías más deleznables y que sufrieron una glaciación similar (sierra de la Demanda). Dentro de la Cordillera Cantábrica, la única zona con mayor densidad de lagunas coincide con la presencia de conglomerados resistentes y granodioritas (Fuentes Carrionas).

Por otra parte, es preciso aclarar y definir una tipología de lagunas de origen glaciar y explicar su *génesis*. Básicamente, podemos encontrarnos tres situaciones geomorfológicas:

1. Lagunas que ocupan el fondo de una cubeta de sobre-excavación glaciar. Estando localizada en un circo, en una artesa, en un collado, en una altiplanicie... (Ej.: Pozo las Lomas)
2. Lagunas cuyas aguas se hallan contenidas por morrenas, ya sean frontales, laterales o fronto-laterales (Ej.: lago de Sanabria)
3. Se dan ambos casos a la vez, siendo esta variante lo más habitual en la formación de las lagunas de esta tesis. La laguna se represa por acumulaciones morrénicas pero, a la vez, ocupa una pequeña cubeta de sobre-excavación (Ej.: lago Ausente).

Adicionalmente, hay casos especiales ligados a deslizamientos de rocas (Ej.: lago La Baña) o lagunas yuxtaglaciares, que en realidad son casi siempre un caso especial de las cerradas por morrenas de tipo lateral (Ej.: Las Matas I y II).

ARTÍCULOS

ARTÍCULO I

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Assessment of richness estimation methods on macroinvertebrate communities of mountain ponds in Castilla y León (Spain)

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Abstract – Complete inventories of the fauna at a given place, for a specific community or geographical area are often exceedingly hard to get. In recent years a number of estimation techniques have emerged that can be used to extrapolate from these samples to the true number of species in an area. These estimation models are based on different mathematical approaches and can be classified as either species accumulation curves or nonparametric estimators (Brose *et al.*, 2003, *Ecology*, *84*, 2364–2377). In this paper, we have tested the performance of some of the richness estimators on nineteen mountain ponds in Castilla y León (Spain) in order to provide guidance on their potential use in future researches. We collected benthic macroinvertebrate of these ponds from the littoral zone with a pond net by kicking and sweeping. Ten-second samples were collected in each pond up to a total time of 3 to 5 minutes per pond, depending on the pond size. In addition, two of the ponds, were sampled in 2004, 2006 and 2007 providing a three-year time series. The results of this study showed that Jackknife 2 was the best of the evaluated methods based on all chosen criteria and also performed well across all studied ponds. Jackknife 1, Chao 1 and Chao 2 also presented good results and they were inferior to Jackknife 2 mainly because of the requirement for larger sub-sample sizes.

Key words: Richness / estimators / macroinvertebrate / mountain ponds / accumulation curves / non-parametric estimators

Introduction

Wetlands and small water bodies such as many mountain ponds have frequently been degraded or destroyed by human alteration (Chapin *et al.*, 2000; Schindler *et al.*, 2001). Shallow aquatic habitats are particularly vulnerable to impacts from anthropogenic inputs (Karakoç *et al.*, 2003; Schippers *et al.*, 2006; Søndergaard and Jeppesen, 2007). This poses a serious threat to the biodiversity of systems which, in the case of ponds, are considered to support a high richness of organisms, particularly macroinvertebrates (Oertli *et al.*, 2002; Williams *et al.*, 2004), both on a local and regional basis (Toro *et al.*, 2006). Nobody questions the need to preserve such systems and their diversity although only recently research programs have focused on them (Biggs *et al.*, 2005; Oertli *et al.*, 2005; Bilton *et al.*, 2009).

Taxon richness, especially species richness, is the simplest and the most intuitive concept for characterizing biodiversity (Gaston, 1996; Chao *et al.*, 2005). It is currently the most used parameter, not only in biodiversity studies (Magurran, 1988; Colwell and Coddington, 1994; Flather, 1996; Keddy and Drummond, 1996) but also in community and trophic ecology (Martinez *et al.*, 1999; Williams and Martinez, 2000) for conservation (Prendergast *et al.*, 1993; Pressey *et al.*, 1993; Conroy and Noon, 1996; Kerr, 1997) and macroecology (Gaston, 2000; Whittaker *et al.*, 2001). Therefore, richness measures are becoming valuable tools for a number of scientific applications as well as a means to assess environmental degradation. The assessment of richness in a given area requires a count of observed species or taxa (Melo and Froehlich, 2001) but, unfortunately, in biological and ecological sciences, the compilation of complete species census and inventories is costly or even impossible (Foggo *et al.*, 2003; Hortal *et al.*, 2006). In addition, biodiversity data suffer from heterogeneity in sampling strategies and/or

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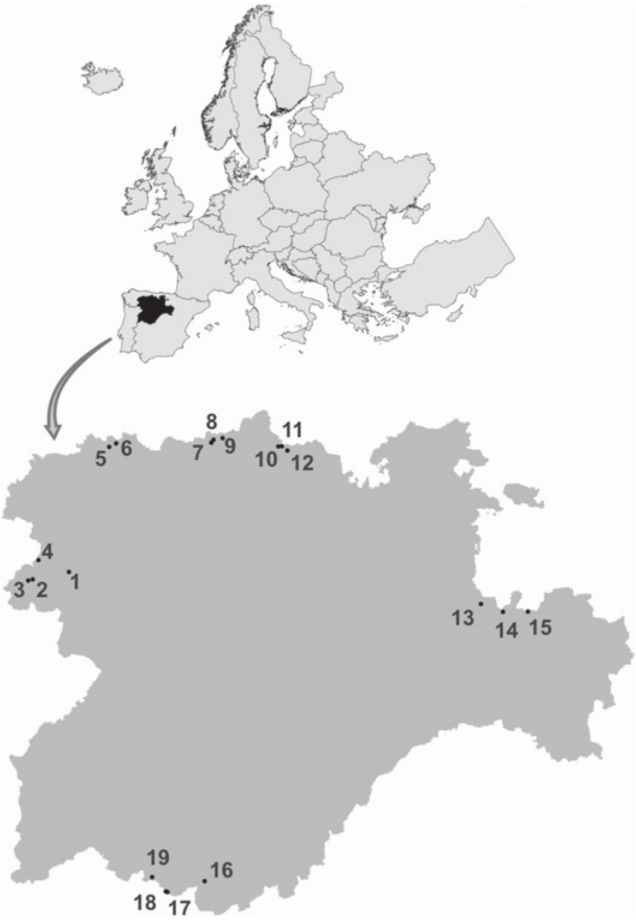


Fig. 1. Study area and mountain ponds: 1. Truchillas (León); 2. Roya (Zamora); 3. Camposagrado (Zamora); 4. Baña (León); 5. Grande de Babia (León); 6. Verdes (León); 7. Robledo (León); 8. Isoba (León); 9. Tronisco (León); 10. Hoyos de Vargas (León); 11. Fuentescarrionas (Palencia); 12. Curavacas (Palencia); 13. Pardillas (Soria); 14. Helada (Soria); 15. Cebollera (Soria); 16. Cervunal (Ávila); 17. Caballeros (Ávila); 18. Cuadrada (Ávila); 19. Trampal (Ávila).

sample size: the larger the sampling effort, the larger will be the number of observed taxon (Walther *et al.*, 1995).

To circumvent this problem, it is possible to use estimation methods (Burnham and Overton, 1979; Smith and van Belle, 1984; Gotelli and Colwell, 2001). Taxon richness estimation techniques have been developed, mainly in the past 20 years, and are emerging as a powerful tool for providing a cost-effective method of assessing richness in an area without the need for full inventories. These estimation methods have been the subject of a growing body of literature (Colwell and Coddington, 1994; Hammond, 1994; Gotelli and Colwell, 2001), with validation of the methods for a variety of taxa and habitats remaining a priority (Colwell and Coddington, 1994; Melo and Froehlich, 2001; Brose *et al.*, 2003). Richness estimation models are based on different mathematical approaches and can be classified as either species accumulation curves or nonparametric estimators (Gotelli and Colwell, 2001; Brose *et al.*, 2003). Taxon accumulation curves extrapolate species richness *vs.* sample size data to an asymptote of total richness (Soberón and Llorente, 1993; Colwell and Coddington, 1994). The most often used accumulation curves are the exponential equation (Holdridge *et al.*, 1971) and the Michaelis-Menten model (Michaelis and Menten, 1913). Nonparametric estimators are sampling theoretic extrapolation methods that only require the number of samples in which each taxon is found rather than any parametric information about their abundance.

The performance of several of these estimators have been carried out and compared in a number of studies (Chazdon *et al.*, 1998; Keating *et al.*, 1998; Peterson and Slade, 1998; Walther and Morand, 1998; Chiarucci *et al.*, 2001; Walther and Martin, 2001; Brose, 2002; Longino *et al.*, 2002; Borges and Brown, 2003; Chiarucci *et al.*, 2003; Melo *et al.*, 2003; Brose and Martinez, 2004; see review in Walther and Moore, 2005). However, in freshwaters their use has mostly been restricted to stream invertebrates (Melo and Froehlich, 2001) and lake zooplankton (Dumont and Segers, 1996; Arnott *et al.*, 1998). Few researches of this kind have been undertaken in ponds (Foggo *et al.*, 2003), but none in mountain ponds. Current and future research conducted in these ecosystems would be aided by knowing which of the estimators can preferably be chosen for a reliable assessment of true richness. It would be particularly useful to know whether estimators based on single samples can provide such information. In this study we have addressed this issue for macroinvertebrates in mountain ponds from a large Spanish region: Castilla y León. We have tested the performance of some of the richness estimators on nineteen mountain ponds in Castilla y León (Spain) in order to provide guidance on their potential use in future researches.

Materials and methods

Study area

Castilla y León (north Spain) is a vast region (94 223 km^2) consisting of a large central plateau (700–1000 m a.s.l.) surrounded by a set of mountain ranges (altitudes up to 2600 m a.s.l.). Nineteen mountain ponds were selected for this study (Fig. 1). The pond selection was intended to record a wide gradient of environmental conditions of altitude (from 1400 to 2200 m a.s.l.), area (between 0.5 and 8 ha), depth (maximum depths between 0.3 and 14 m), and water permanece (temporary and permanent systems). Furthermore, most of the shallow, usually temporary, systems supported dense stands of vegetation (either submerged, emergent or both) whereas littoral zone in deep ponds was mostly sandy to stony and was poorly vegetated except for *Isoetes*, a species present in most of the ponds.

Sampling and sorting of macroinvertebrates

All ponds were sampled once in June/July 2006 or 2007. Benthic macroinvertebrates were collected from

Table 1. Summary of the eleven evaluated estimators. NP: non-parametric estimators, ESAC: estimators based on the extrapolation of species accumulation curves.

Abbreviation	Estimator	Type	References
ACE	Abundance-based coverage estimator of species richness	NP	Chao and Lee (1992); Chao *et al.* (2000); Chazdon *et al.* (1998) in Colwell (2004)
ICE	Incidence-based coverage estimator of species richness	NP	Lee and Chao (1994); Chao *et al.* (2000); Chazdon *et al.* (1998) in Colwell (2004)
Chao 1	Abundance-based estimator of species richness	NP	Chao (1984) in Colwell (2004)
Chao 2	Incidence-based estimator of species richness	NP	Chao (1984, 1987); Colwell (2004)
Jackknife 1	First-order Jackknife richness estimator	NP	Burnham and Overton (1979); Heltshe and Forrester (1983) in Colwell (2004)
Jackknife 2	Second-order Jackknife richness estimator	NP	Smith and van Belle (1984) in Colwell (2004)
Bootstrap	Bootstrap richness estimator	NP	Smith and van Belle (1984) in Colwell (2004)
MMRuns	Transformation of Michaelis-Menten hyperbole by Raaijmakers. Estimate curves averaged over randomizations (runs)	ESAC	Raaijmakers (1987); Colwell (1997)
MMMean	Transformation of Michaelis-Menten hyperbole by Raaijmakers. Estimate curve computed once for mean species	ESAC	Raaijmakers (1987); Colwell (1997)
Clench	Estimation of Michaelis-Menten function asymptote	ESAC	Clench (1979) in Soberón and Llorente (1993)
Exp Neg	Estimation of negative exponential function asymptote	ESAC	Miller and Wiegert (1989) in Soberón and Llorente (1993)

the littoral zone with a pond net (FBA standard, mesh size 500 μm) by kicking and sweeping. Ten-second samples were collected in each pond up to a total time of 3 (18 samples), 4 or 5 (30 samples) minutes per pond depending on the size (< 1 ha, 1–5 ha or > 5 ha, respectively). Total sampling time was proportionally distributed among the main habitats according to their surface in the pond. Macroinvertebrates were separated from the plant material and counted under a binocular microscope (10×). The specimens were identified to genus (often the lowest attainable taxonomic level) excepting Diptera (to subfamily) and Oligochaeta (class).

Selection and calculation of richness estimations methods

The performances of 11 different richness estimators (Table 1) were compared. Nine of them, ACE, ICE, Chao 1, Chao 2, Jackknife 1, Jackknife 2, Bootstrap, MMRuns and MMMeans were calculated with the software EstimateS version 7.0 (Colwell, 1997). They have all been widely used and studied (Chazdon *et al.*, 1998; Brose *et al.*, 2003; Chiarucci *et al.*, 2003). Chao 1 and Jackknife 1 are designed to estimate richness from single samples while the rest require several samples. In addition, we evaluated the performance of two asymptotic accumulation functions, Clench and Negative Exponential functions. In these functions, richness is calculated as the asymptote value of the function fitted to the smoothed taxon accumulation curve provided by EstimateS version 7.0 (100 randomizations; Colwell, 2004). This ideal curve represents an unbiased description of the sampling process, where possible effects due to the order by which the samples have been taken or listed are removed by randomizing their order of entrance in the curve. We used the software Statistica 6.0 to fit each function to the data and calculate the asymptote value from the obtained parameters (Soberón and Llorente, 1993; Hortal *et al.*, 2004). These accumulation functions are able to predict estimate richness when they are close to the asymptote. They are all standard, widely used methods that have been previously tested and discussed by a number of authors under different circumstances (*e.g.* Palmer, 1990; Colwell and Coddington, 1994; Coddington *et al.*, 1996; Condit *et al.*, 1996; Carlton and Robison, 1998; Chazdon *et al.*, 1998; Walther and Morand, 1998; Gotelli and Colwell, 2001; Walther and Martin, 2001; Petersen and Meier, 2003).

Evaluation of estimator performance

The concepts of bias, precision and accuracy (see a review of Bruno and Joslin, 2005) are frequently used to assess the performance of richness estimation methods (Brose *et al.*, 2003; Foggo *et al.*, 2003; Walther and Moore, 2005). These approaches include measures of bias and accuracy of the estimated richness in relation to the true richness using an *a priori* chosen sub-sample size. However, the estimated richness is strongly dependent on sample size (Colwell and Coddington, 1994; Melo and Froehlich, 2001; Petersen and Meier, 2003) and different sub-samples sizes will produce different bias and accuracy values (Hellmann and Fowler, 1999). In addition, these approaches require data for maximum species numbers for their calculation, so they cannot be used here. Instead, we used some criteria we argue are more practical and functional (see also in Melo and Froehlich, 2001). Mere comparisons of the differences between estimated and observed richness are quite useful: estimators whose final estimation values do not even reach the observed richness (richness measured by sampling) cannot be considered good estimators because they obviously underestimate true richness. The behaviour of the curve shape in each pond (erratic or non-erratic) and the similarity in the curve shapes across ponds (measured as a simple scale: 1, low; 2, intermediate; 3, high) give information about the reliability and constancy of the estimators. The minimum number of samples required to attain the observed richness and the constancy of this number, measured as standard deviation (SD), are an indication of how great the sampling effort (number of samples) must be to obtain a reliable estimation. Other features being equal, an estimator requiring low sampling effort should be preferred.

No doubt, the best way to assess the performance of the estimators would be comparing the estimations with true richness values. Unfortunately, such values are difficult to obtain and are not available for any of the ponds in the study area. We have tried to partially solve this shortcoming by using information from additional samplings when these were available. Two of the ponds, Grande de Babia and Helada, were sampled in summer 2004, 2006 and 2007 (whether as twenty-four 10-second samples or as a single 4-minute one, depending on the year). Pooling data from three visits to each pond provided a more comprehensive (although probably not complete yet) taxon list. We have taken the global richness over these three years as the best available approximation to true richness of these ponds. We refer to this value as "*true richness*" in the text. This made it possible to compare estimated richness with this assumed "*true richness*". This comparison only has been used here as an additional criterion.

Results

The nine ponds differed in macroinvertebrate abundance, observed richness and number of samples collected (Table 2). Despite the differences in the sampled assemblages, the performance of the estimators to all criteria was similar through all ponds (*e.g.* Figs. 2A–2F).

MMMeans, MMRuns and bootstrap were the estimators provided by the EstimateS software requiring highest number of samples to attain observed richness, with average values of 70%, 55%, and 66%, respectively, of the total number of samples collected. Moreover, these average values were highly variables in MMMeans and MMRuns, showing standard deviations of 6.84 and 7.43, respectively (Table 3). Bootstrap, however, showed consistent patterns, absence of erratic behaviours and high similarity curves shapes across ponds (see Figs. 2A–2K). In contrast, the dissimilarity of curve shapes and the erratic behaviours of MMRuns were evident (see Figs. 2G–2K).

Jackknife 2 performed satisfactorily in all the criteria. It only required 30% of total number of samples to attain the observed richness. Similar values were obtained for Chao 2 and ICE (31% and 37% respectively), but Jackknife 2 displayed higher similarity in curve shapes (see Figs. 2A–2K). In addition, ICE was quite erratic and the curve shapes were less similar across ponds. The response of Jackknife 1 was almost as good as that of Jackknife 2 in minimum sample number and constancy (SD) and it did not display erratic behaviours either. Finally, the performance of ACE estimator was intermediate between the previous estimators for all the criteria.

Table 2. Summary of observed richness (*S*), number of ten-second samples (*n*) and total number of individuals of the ponds included in the study.

Ponds	*S*	*n*	Total individuals
Baña	27	24	1418
Caballeros	26	24	3354
Camposagrado	38	18	2083
Cebollera	25	19	3110
Cuadrada	23	18	2318
Curavacas	21	24	1549
Cervunal	22	18	3480
Fuentes Carrionas	18	24	611
Grande de Babia	12	24	1589
Helada	26	24	3823
Hoyos de Vargas	22	18	2888
Isoba	24	24	694
Verdes	28	18	31 719
Pardillas	30	18	2565
Robledo	18	24	664
Roya	42	24	1101
Trampal	30	24	4546
Tronisco	14	18	1171
Truchillas	26	30	3130

Asymptotic accumulation functions required a high number of samples to attain observed richness and these values had low constancy (Table 3). Expo, in particular, would never reach such value while Clench always needs more that 100% of samples collected to assess observed richness. However, these functions provided curves similar across ponds and with little erratic behaviours (see *e.g.* Fig. 3).

Comparison with "true richness"

Total richness found through three years of sampling at Helada and Grande was considerably higher than the observed richness measured in a single year: 35 against 26 in Helada, 19 against 16 in Grande (Table 4). If we assume this accumulated richness to be a better approach to true richness, as exposed above, we may test which estimators assess richness more precisely. Two of them, Jackknife 2 and Chao 2, provided richness estimations very close to this assumed true richness (Table 4). Estimations by Jackknife 1 are quite close to "true richness" (Table 4) and are only slightly lower than those obtained by Jackknife 2. Chao 1, in contrast, shows a heterogeneous response, with values well below "true richness" (Helada) or above it (Grande de Babia). The relationship between Chao 1 and Chao 2 is not consistent either (Table 4). MMRuns, MMMeans, Bootstrap and accumulation curves provided estimations well below these values. All the richness estimators, excepting species accumulation curves, provided values closer to three-year accumulated richness than to richness measured in a single year (Table 4).

Discussion

There is a general agreement on the advisability of using richness estimators as a better approach to richness than mere lists of observed taxa (Palmer, 1990; Colwell and Coddington, 1994; Bruno and Joslin, 2005; Walther and Moore, 2005). The real debate is about choosing the best estimator for a particular study, taxonomical group or data set. This is a controversial issue, still under study, because different authors reach different conclusions on which is the best estimator, as shown by contrasting results obtained in their studies by, for example, Colwell and Coddington (1994); Walther and Morand (1998); Chiarucci *et al.* (2003); Foggo *et al.* (2003) and Hortal *et al.* (2006). Therefore, until more conclusive information is available, checking the suitability of several estimators seems a convenient starting point when studying little known communities.

Overall, non-parametric methods performed better than accumulation curves in our study. There is a general consensus on the advantages of using nonparametric estimators. They are usually less biased and more precise than accumulation curves, as reported by Brose *et al.* (2003) from a study of landscape simulation under three sampling intensities. Curve-fitting models have been extensively tested and usually perform worse than non-parametric estimators (Melo and Froehlich, 2001; Walther and Moore, 2005). The reason for the usually superior performance of non-parametric estimators might be due to the fact that they, unlike curve models, have been developed from several and underlying models of detection probability (Cam *et al.*, 2002).

In spite of the use of different approaches and different data sets by different authors to evaluate estimation methods, there is some congruence in these results. Expo was the worst estimator found by Peterson and Slade (1998) on data sets derived from state automobile license plates observed in Mexico City and Lawrence, Kansas. These data sets had the advantage of providing known "communities" to be sampled. MMClench was also considered inadequate by Keating *et al.* (1998) on simulated and real data sets. In Melo and Froehlich (2001) the worst of the evaluated methods were MMRuns, MMMeans and Expo and, just as in our study, MMRuns produced erratic behaviour at small sub-samples sizes. In accordance with these studies, MMRuns, MMMeans and the two asymptotic accumulation functions (negative exponential function and Clench function), cannot be recommended for richness estimations on mountain ponds like those in our study area.

The ACE, ICE and Bootstrap methods performed at an intermediate level in our study. In relation to these estimators, the literature is extremely varied and it could be explained from the differences in their sample coverage: different sampling intensity, community evenness, and consequently different sample coverage yield different results concerning estimator ranking (Brose *et al.*, 2003). Bootstrap was a poor estimator in the studies of Colwell and Coddington (1994) and Chazdon *et al.* (1998). This estimator also needed an unacceptably large subsample (65.3%) to estimate sample richness in the study by Melo and Froehlich (2001). Bootstrap and ACE perform poorly at low sampling efforts, which is the portion of the effort curve of greatest interest in practical terms (Foggo *et al.*, 2003). However, Bootstrap has been considered a good estimator in studies with few rare species, such as parasite species richness (Walther and Morand, 1998), or for intensely sampled assemblages (Smith and van Belle, 1984). Hortal *et al.* (2006) showed that although Bootstrap estimates were highly precise, they clearly underestimated species richness (also reported by Chiarucci *et al.*, 2003). ICE and ACE methods have also been found inadequate for species-poor assemblages (Walther and Morand, 1998) but very useful when richness is high (Chazdon *et al.*, 1998). We have not found any relation between the behaviour of the different richness estimators and any of the characteristics of the studied ecosystems (altitude, latitude, temporality, nature of the substrate, depth and vegetal cover). Neither we have found any evidence of a variable behaviours of those richness estimators based on the nature of data set (total abundance; observe richness and percentage of rare taxa). Therefore, with the obtained results in our study, we can not make a solid discussion about the why of the obtained disparity of the conclusions by different authors in relation to these estimators. In the future, we recommend carry out a study more methodical, concrete and specialized that clarifies the true utility of these estimators.

On the other hand, Jackknife 2 was the best of the evaluated methods based on all criteria and also performed well across all studied ponds. Jackknife 1, Chao 1 and Chao 2 also presented good results and they were inferior to Jackknife 2 mainly because of the requirement for larger sub-sample sizes. Similar results have been obtained in previous studies. In Melo and Froehlich (2001) the range of subsample sizes of Jackknife 2, Jackknife 1 and Boot estimators needed to estimate richness in total samples were, respectively, 22.4–26.7, 35.6–41.3 and 63.6–66.7% of total samples. In a similar study, Hellmann and Fowler (1999) found that for Jackknife 1, Jackknife 2 and Boot, the sub-sample size needed to estimate richness in the total sample were, respectively, 22.6–29.1, 36.8–43.9 and 63.1–69.0% of total samples. In both cases, these values are very close to those found in our study. Melo and Froehlich (2001) also concluded that Jackknife 2 was the best estimator methods based on four criteria: (1) the smallest sub-sample size required to estimate the observed richness in the total sample; (2) constancy of the sub-sample size needed to estimate the observed richness in the total sample, measured as 1 standard deviation (SD) of the previous criterion; (3) lack of erratic behaviour in curve

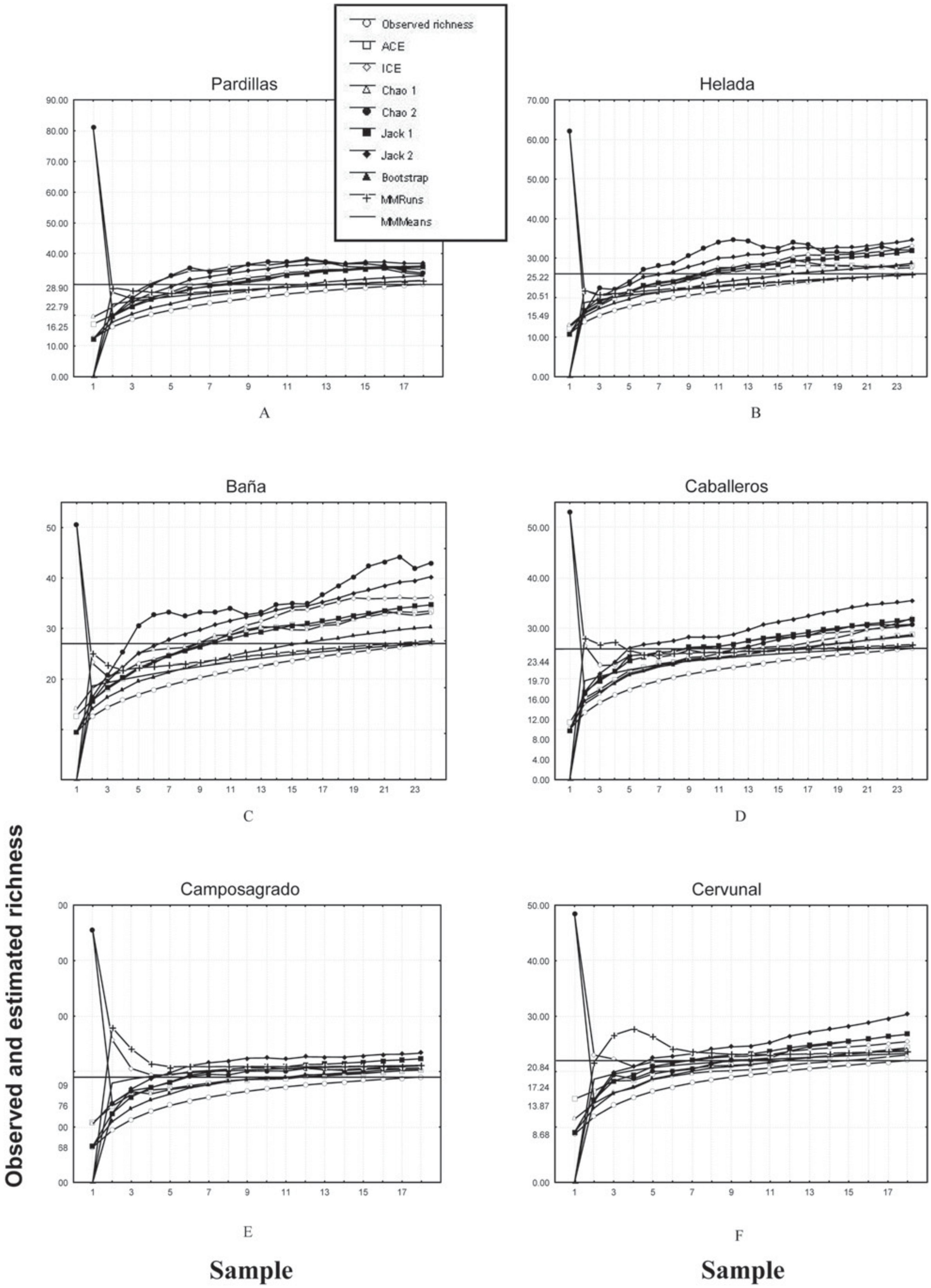
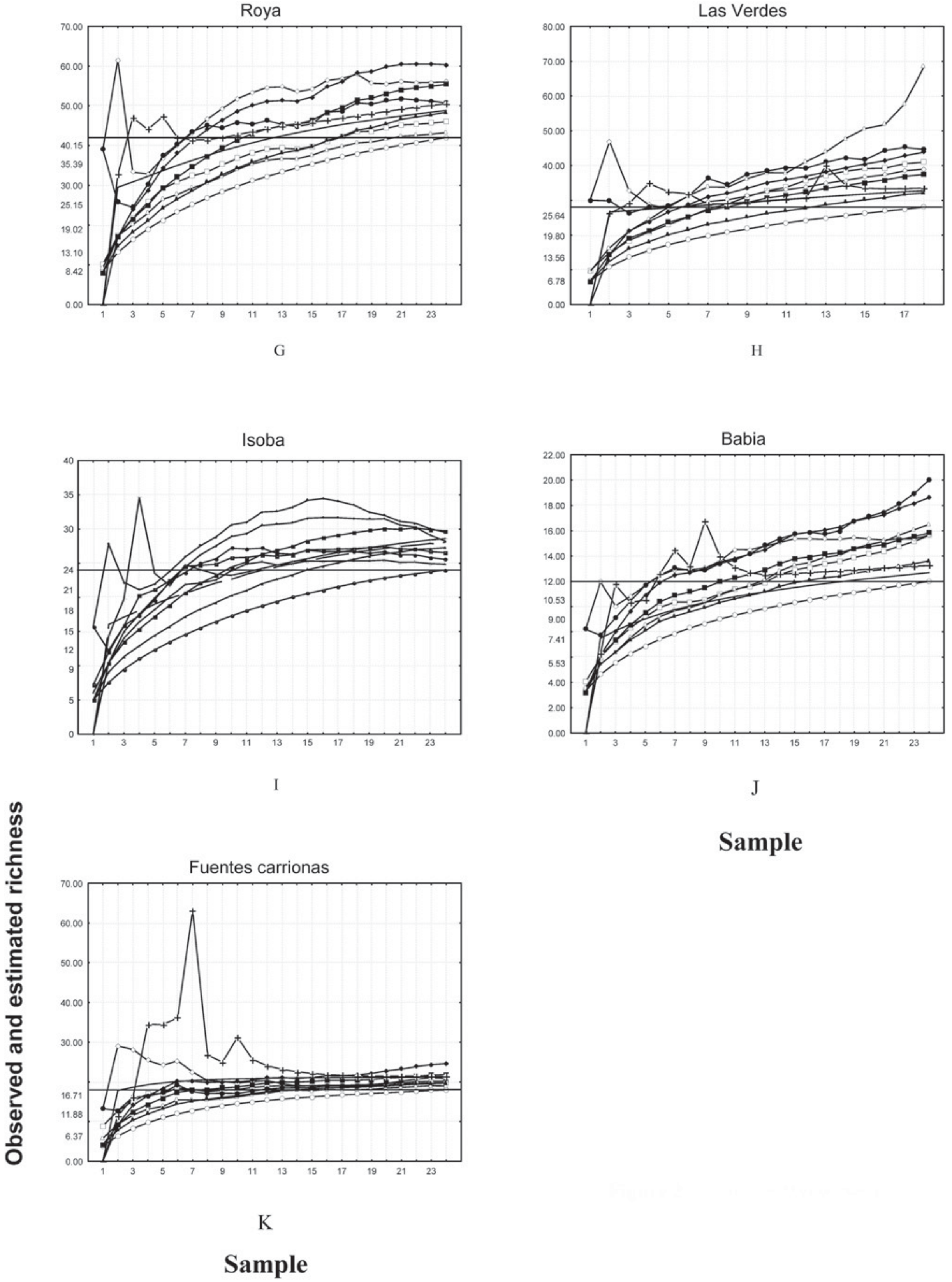


Fig. 2. Curves of accumulation of observed richness and estimates for ponds. The horizontal line indicates the richness observed in the pond. See Table 1 for definitions of the estimators.



G H

I J

K

Fig. 2. (Continued.)

Table 3. Performance of the estimators scored by principal criteria. Mean of minimum sample number (*x*) required to estimate observed richness in all ponds and percentage in relation to the total number of samples collected (%); constancy of minimum sample number across the ponds (*SD*); erratic behaviour (*EB*) and similarity in the curve shape (1, low; 2, intermediate; 3, high).

Estimator	*x*	%	*SD*	*EB*	Similarity
ACE	10	47	3.7	without	2
ICE	9	37	3.2	with	2
Chao 1	10	45	4.5	without	2
Chao 2	7	31	2.6	without	2
Jack 1	9	42	2.1	without	3
Jack 2	6	30	1.3	without	3
Bootstrap	14	66	2.6	without	3
MMRuns	14	55	7.4	with	1
MMMeans	15	70	6.8	without	2
Clench	32	+100	11.2	without	3
Expo	-	-	-	without	3

Table 4. Observed richness on Helada and Grande de Babia through three years, in 2006 and values of methods estimation in 2006.

Estimator	Helada	Babia
True temporal richness (2004, 2006, 2007)	35	19
Observed richness (2006)	26	16
ACE (2006)	28	16
ICE (2006)	33	16
Chao 1 (2006)	27	20
Chao 2 (2006)	32	19
Jack 1 (2006)	32	16
Jack 2 (2006)	35	19
Bootstrap (2006)	29	14
MMRuns (2006)	26	13
MMMeans (2006)	26	13
Clench (2006)	27	14
Expo (2006)	24	11

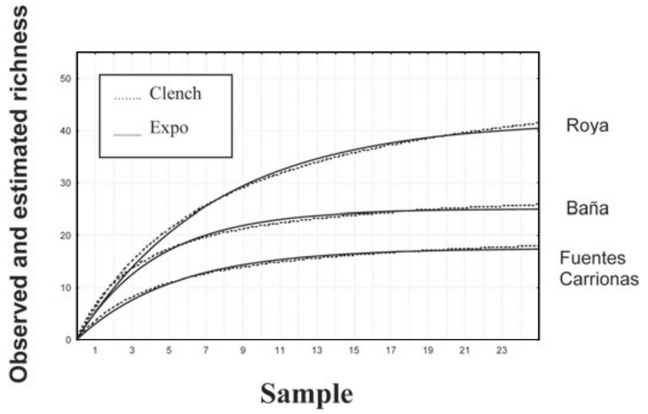


Fig. 3. Asymptotic accumulation functions, Clench and Negative Exponential, of three ponds: Roya, Baña and Fuentes Carrionas.

shape, specially large variations of estimates for closely similar sub-sample sizes; and (4) similarity in curve shape through the six sample data sets. In Petersen and Meier (2003), first and second order Jackknife methods also gave the most accurate estimate of the species richness of the collectable Danish Asilidae using museum label data. They found that first and second order Jackknife and Chao 2 perform better than the other estimation methods because they require less than 50% of the full sample to give stable estimated species richness values. Colwell and Coddington (1994) argued in favour of Chao 2 and Jackknife 2 in a study in which they evaluated the performance of eight methods on a seed-bank data set. Palmer (1990) concluded that Jackknife 1 is the least biased estimator. Chazdon *et al.* (1998) in a study of seedling and sapling diversity found that ICE and Chao 2 were robust to sample size and patchiness. Walther and Morand (1998) evaluation of parasites per host data sets recommended the use of Chao 2 and Jackknife 1. Peterson and Slade (1998) found the Chao 2 method was one of the best (they did not evaluate Jackknife 1, Jackknife 2 and Chao 1). Using bias and precision as criteria, the two Chao estimators had the best overall performance, followed by the Jackknife 1 and Jackknife 2 estimators, in the study by Walther and Martin (2001). Of course, even Chao and Jackknife estimators may have sometimes a bad perform, the variation of those results depend in many factors that change the structure of the data used on the calculus of the estimators. Total species richness, sample size, and variables that change the aggregation of individuals within samples (Walther and Moore, 2005). In our study these variables are similar and stables in all ponds, this could explain the homogeneity and the constancy of the good behaviour of those two groups of richness estimators in all the studied ecosystems.

An ideal situation for evaluating richness estimators is to compare the estimated value to total species richness in an area. We do not know the total number of taxa of the ponds included in this study, neither have we an expert assessment of species richness, although such expert assessments often are likely to be higher than an estimate based on a collection gathered by non-specialists (Coddington *et al.*, 1996; Longino *et al.*, 2002; Petersen and Meier, 2003). Therefore, we estimated "*true richness*" by pooling the results obtained in three sampling years. Jackknife 2 and Chao 2 were also the best estimators methods. Their values were very close to this assumed true richness. On the other hand, the worst evaluated methods were MMRuns, MMMeans bootstrap and asymptotic accumulation functions. The result based on this variable supports our previous results. The information here provided suggests that estimation of richness in Spanish mountain ponds should probably be based on Jackknife 2 (and Chao 2?) whenever a high number of samples is available. Otherwise, Jackknife 1 (and Chao 1?) may be an acceptable option.

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ARTÍCULO II

Título:
Richness of littoral macroinvertebrate communities in mountain ponds from NW Spain: what factors does it depend on?

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Richness of littoral macroinvertebrate communities in mountain ponds from NW Spain: what factors does it depend on?

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ABSTRACT

Recent researches have started to provide useful information on the littoral macroinvertebrates living in European mountain ponds. However, there is still uncertainty on the factors really shaping their communities. Understanding patterns of biodiversity in these systems is essential for conservation and management purposes. In this paper, we sampled littoral macroinvertebrates at 51 mountain ponds from a wide Spanish region (Castilla y León) in order to define which of a set of environmental variables were responsible for differences in richness (genus level or above). One macroinvertebrate sample was collected at each pond (in late June or early July between 2004 and 2008) by kicking and sweeping following a multihabitat time-limited sampling. Twenty-four variables measured at 39 ponds were used to generate a predictive model by multiple linear regression. This model revealed number of habitats and fish stocking as the only significant variables, showing their relative importance against variables traditionally considered to influence richness in mountain ponds and lakes (for example, altitude and pond size). Furthermore, this model accurately predicted richness when tested on a new set of twelve ponds. Additional data analyses proved that neither altitude nor habitat type significantly influenced macroinvertebrate richness, while water permanence had a slight effect (the number of taxa was slightly lower in temporary than in permanent ponds).

Key words: richness, littoral macroinvertebrates, mountain ponds, predictive model, environmental variables.

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INTRODUCTION

Mountain ponds of glacial origin are one of the most remote and undisturbed aquatic environments in Europe (Čiamporová-Zaťovičová et al. 2010). These high alpine ponds are particularly sensitive to global change, especially to climate warming, and constitute ideal systems for long-term monitoring of global changes (Gurung 2005; Oertli et al. 2008). Among the biological communities present in these lakes, benthic invertebrates are excellent indicators of local as well as global temperature changes (Fjellheim et al. 2000) and they are one of the most common groups of organisms used to assess the health of aquatic ecosystems (Sharma, and Rawat 2009).

On the other hand, these small aquatic systems are rich and diverse habitats and play a key role in safeguarding aquatic biodiversity (Biggs et al. 2005; Shieh, and Chi 2010). These systems are considered to support a high richness of organisms, particularly macroinvertebrates (Oertli et al. 2002; Williams et al. 2004), both on a local and regional scale (Toro et al. 2006). Understanding patterns of biodiversity distribution is essential to conservation strategies (Gaston 2000), and resolving the relative contributions of local and regional processes might provide a key to understanding global patterns of biodiversity (Gaston, and Spicer 2004). Therefore, it is crucial to achieve a good understanding of the richness patterns in these systems, especially the environmental factors influencing their diversity.

Several studies document clear relationships between composition or richness of macroinvertebrate communities and a variety of ecologically relevant gradients in ponds, such as hydroperiod (Collinson et al. 1995), surface area (Céréghino et al. 2008), water chemistry (Friday 1987; Boix et al. 2008), pond connectivity (Gascón et al. 2008; Oertli et al. 2008), habitat heterogeneity (Della Bella et al. 2005), presence of large predators (Zimmer et al. 2001) and altitude (Lin et al. 2006). However, only a few of them have specifically addressed the issue of which of a large set of factors really determine richness of ponds (see Rundle et al. 2002), and only rarely in alpine systems (Hinden et al. 2005; Füreder et al. 2006; Collado, and De Mendoza 2009). Ecological researches on littoral macroinvertebrates from Spanish mountain ponds have only recently been undertaken. Good examples are the contributions by Catalan et al. (2009) and De Mendoza, and Catalan (2010) in Pyrenean lakes, as well as those by Granados, and Toro (2000) and Toro et al. (2006) in the Central mountain system.

The effects of ecological factors on macroinvertebrate communities (and on their richness in particular) are complex and difficult to disentangle, partially because richness–environment relationships are typically masked by other physical or geometric constraints (De Mendoza, and Catalan 2010). We might expect richness to be depressed

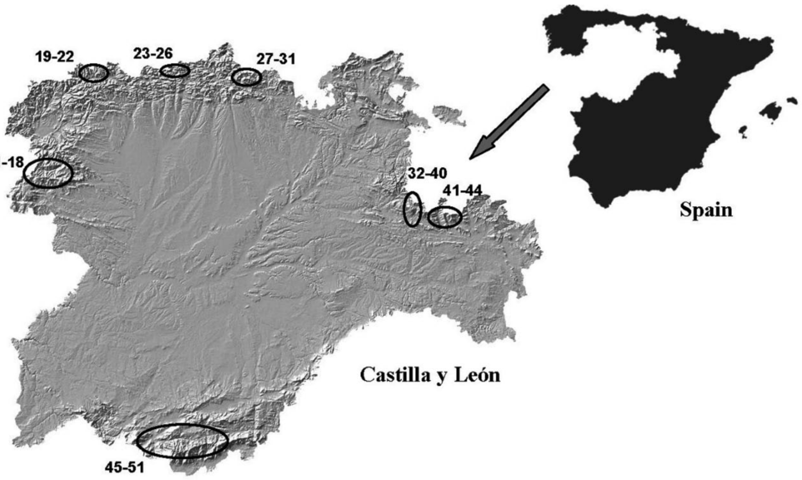

Fig. 1. Geographical situation of the ponds included in the study. 1-18: Truchillas, La Baña, El Payón, Lacillo, Pies Juntos, El Cuadro, Clara grande, Clara pequeña, Roya grande, Roya pequeña, Pedrina, La Yegua, Los Peces, Camposagrado, Castromil, Aguas Cernidas, Mancas, Sotillo; 19-22: Las Verdes, La Mata I, La Mata II, Grande de Babia; 23-26: Ausente, Isoba, Tronisco, Robledo; 27-31: Curavacas, Fuentes Carrionas, Pozo Lomas, Hoyos de Vargas I, Hoyos de Vargas II; 32-40: Pardillas I, Pardillas II, Los Patos, Brava, Negra de Neila, Haedillo I, Haedillo II, Pozo Negro, Muñalba; 41-44: Negra de Urbión, Larga, Helada, Cebollera; 45-51: Cimera, Laguna Grande, Trampal, El Barco, Caballeros, Cuadrada, Cervunal.

in harsh environments, such as high-altitude (Hoffman et al. 1996), ultraoligotrophic (Wetzel 2001) or acid ecosystems (Schell, and Kereks 1989), as well as in heavily impacted systems, as those subjected to salmonid introductions (Carlisle, and Hawkins 1998; Martínez-Sanz et al. 2010). In contrast, it should be high in heterogeneous ponds supporting a high habitat diversity. The characteristics of the habitat (substrate type, vegetation cover, etc.) are also likely to influence richness, although in unknown ways. All these interactive factors give a complex scene making richness prediction a difficult task.

A good understanding of how the combined action of factors determines richness of mountain ponds would aid in management of ponds and biodiversity preservation. It would be helpful to know which environmental factors may be relevant and which secondary for a particular system. In particular, we must gain knowledge on the effect of human activities compared to natural factors. In this study, we aim to generate, by means of a prediction model, a gradient of factor relevance on macroinvertebrate richness in mountain ponds from a relatively wide region. We have also individually analysed the hypothetical influence on richness of several factors traditionally considered relevant for pond macroinvertebrates.

METHODS

Study area

Fifty-one mountain ponds of quaternary glacial origin were selected for this study (Fig. 1). All of them are located in Castilla y León, a wide Spanish region consisting of a vast central plateau (from 600 to 1000 m a.s.l.) surrounded by mountains (altitudes up to 2600 m a.s.l.). The pond selection was intended to record a wide gradient of environmental conditions of altitude (from 1360 to 2195 m a.s.l.), area (between 3000 m^2 and 0.12 km^2), depth (maximum depths between 0.3 and 15 m), littoral slope, habitat type (substrate and type and coverage of aquatic vegetation), and water permanence (temporary and permanent systems).

Macroinvertebrate sampling

All the ponds were sampled once in late June or early July between 2004 and 2008. Benthic macroinvertebrate samples were collected from the littoral zone (at depths below 1 m) with a pond net (FBA standard, mesh size 500 μm) following a multihabitat time-limited sampling (Collinson et al. 1995; Biggs et al. 2000; Briers, and Biggs 2005). Each pond was sampled during three (ponds below 0.01 km^2), four (0.01-0.05 km^2) or five minutes (>0.05 km^2). Total sampling time was proportionally distributed among the main habitats according to their surface in the pond. Macroinvertebrates were separated from the plant material, counted under a binocular microscope (10×) and identified to genus except for Oligochaeta (identified to class); Dolichopodidae, Empididae, Ephidridae, Sciomyzidae, Simuliidae, Tabanidae, Tipulidae (to family); Limnephilidae, Ceratopogonidae, Chironomidae and Limoniidae (to sub-family).

Environmental variables

Twenty-four environmental variables were measured in this study. Several of these environmental variables were directly measured in each pond (maximum depth,

Tab. 1. Environmental characteristics of the 51 mountain ponds included in the study. Mean values, range and several percentiles are shown for the variables included in the MLR model. Average richness across ponds (dependent variable in the model) is also shown. Cont.: Continuous; Discr.: Discrete; TN: total nitrogen; TP: total phosphorus; S fish: fish richness; FI: fish impact; NH (number of habitats in each pond).

	Richness	Longitude (º)	Altitude (m a.s.l.)	Surface (km^2)	Depth (m)	Alkalinity (mg L^{-1} CaCO$_3$)	pH	Nitrate (mg L^{-1})	NT (mg L^{-1})	PT µg L^{-1})	S Fish	FI	NH
Variables type	Cont.	Cont.	Cont.	Cont.	Cont.	Cont.	Cont.	Cont.	Cont.	Cont.	Cont.	Discr.	Discr.
Mean	18.8	5.3	1779.51	0.0314	4.79	0.22	6.56	0.046	1.59	44.81	0.88	5 Classes	4 Classes
DV	6.59	1.51	204.22	0.0321	4.19	0.53	0.81	0.07	6.76	46.78	1.06		
Minimum	6	2.65	1360	0.003	0.3	0	4.92	0	0	5.51	0	0 (n = 21)	1 (n = 12)
1*st* quartile	14	3.9	1640	0.01	1.4	0.035	6.23	0.01	0.28	17.6	0	1 (n = 6)	2 (n =17)
Median	18	5.58	1770	0.021	3.8	0.058	6.51	0.02	0.52	29.05	1	2 (n = 5)	3 (n = 20)
3rd quartile	23.5	6.8	1880	0.035	7.3	0.1	6.9	0.03	1.0	49.3	1.3	3 (n = 7)	4 (n = 2)
Maximum	35	6.83	2195	0.12	15	2.77	8.97	0.367	48.82	190.41	4	4 (n = 3)	

surface, conductivity, pH and Secchi disk). The granulometric nature of the substrate was estimated as percentage of stones, gravel or fine substrate in the sampled area. Land use was accounted for by measuring percentage of the basin covered by bushes or wood. Other variables considered were altitude, longitude, percentage of macrophyte cover, concentration of chlorophyll, alkalinity and nutrients in the water column (nitrate, total nitrogen, soluble reactive phosphorus and total phosphorus), and slope. Water persistence was expressed as one of two classes (temporary or permanent) and littoral slope was qualitatively estimated by assigning a value between 1 (nearly plain bottom) and 5 (steep shores).

In addition, we generated a discrete variable to asses the impact of introduced fishes (from 0, no fish, to 4, maximum impact). Each pond was assigned to a fish impact class on the basis of previous information on fish stocking in some ponds (Granados, and Toro 2000a; Martínez-Solano et al. 2003), expert knowledge of local anglers, personal observations during the survey and the results of a fyke-net sampling carried out during summer 2009 to obtain rough estimates of species abundances. Fish density as inferred from fyke-net sampling and personal observations during the survey and number of fish species present (also used as a continuous variable in the analysis) have been key criteria to assign a pond to an impact class. The highest impact value (4) was attributed to ponds with high intensity of salmonid stocking or extremely high densities of other species. These population levels are always kept by repeated introductions. Ponds with no fish were assigned to class 0. The remaining ponds were ranged between these two extremes according to the data provided by the fyke-net sampling.

There is one more variable which could prove critical in richness prediction: habitat heterogeneity. We addressed this issue by identifying five possible meso-habitat types in the study area study (hydrophytes, helophytes, stones without vegetation, sand/gravel without vegetation and fine substrate without vegetation) and counting how many of them were present in a given pond.

This variable set accounts for natural among-pond differences as well as for the main impacts in the study area (fish stocking and changes in land use). Eutrophication, which can be consequence of these and other environmental pressures, is also considered. Of all these variables, only a subset was used for further analysis. Many variables were ruled out because they were highly intercorrelated (significant Pearson or Spearman's correlation, p=0.05). Several variables were surrogates for others. For example, pH was correlated with conductivity. Other variables as surface and depth were correlated with littoral slope, water persistence and percentage of macrophyte. Some evident relationships among total nitrogen and total phosphorus with soluble reactive phosphorus, chlorophyll and Secchi depth were also detected. Twelve variables were finally included in the model: longitude, altitude, pond area, depth, alkalinity, pH, nitrate, total nitrogen, total phosphorus, fish richness, fish impact, and number of mesohabitats (Tab. 1). Altitude was a surrogate for substrate type and land use, as also shown by de Mendoza, and Catalan (2010), while pond area and depth were significantly correlated with littoral slope and macrophyte cover. Maximum depth, on the other hand, is a good measure of the hydroperiod length with the additional advantage on water permanence of being measured as a continuous variable (and not just as one of two values, temporary or permanent).

Data analysis

General prediction model. We performed multiple linear regression (MLR) analysis generated by a step-wise selection technique to quantify the magnitude, direction and significance of the relations (Robinson et al. 2004). Thirty-nine ponds were randomly selected for MLR analysis while the remaining ones were kept as an independent data set to test the model.

The linear regression model assumes a linear relationship between the dependent variable and each predictor. We examined a scatterplot of richness by each independent variable to determine whether a linear model is reasonable for these variables. However, in practice, this assumption can virtually never be confirmed; fortunately, multiple regression procedures are not greatly affected by minor deviations from this assumption. Linear regression model involves several additional assumptions. Firstly, the error term has a normal distribution with a mean of 0. Again, even though most tests (specifically the *F*-test) are quite robust with regard to violations of this assumption, it is always necessary, before drawing final conclusions, to review the distributions of the major variables of interest. We tested this assumption by using the Kolmogorov-Smirnov test over the error term. Secondly, the variance of the error term is constant across cases and independent of the variables in the model. This homoscedasticity of the error term was tested by a residual scatterplot in the analysis. Thirdly, the value of the error term for a given case is independent of the values of the variables in the model and of the values of the error term for other cases. We tested autocorrelation among error terms by Durbin-Watson test. Besides, we removed from the regression analysis intercorrelated variables (Spearman's test, p=0.05). The statistical processing was performed with the SPSS Statistic 17.0 package.

We tested the MLR model by applying it to an independent data set (12 ponds of the study area) and comparing predicted with observed richness values by means of a one way analysis of variance (ANOVA) of repeated measures and Scheffé test.

Finally, we used a generalized additive model (GAM) to explore the graphical response of richness to the variables that were significant in the linear regression model (Schimek 2000; Guisan et al. 2002). Through a cubic spline scatterplot smoother (along with the 95% confidence bands) generated by the model, we can evaluate the nature of the relationship between the predictor and the residualized (adjusted) dependent variable values, and hence the nature of the influence of the respective predictor in the overall model. Computational details regarding the cubic spline smoother, and comparisons to other smoothing algorithms, can be found in Hastie, and Tibshirani (1990) and Schimek (2000). The statistical processing was performed with the STATISTICA 8.0 package.

Particular prediction models. We generated an additional multiple regression model on ponds of the study area without human impact (fish impact: 0 or 1; 37 ponds) to explore the possible relation between observed richness and other natural predictors undetected in the general model. Multiple linear regression was also applied to additional data subsets in order to remove the effect of both habitat heterogeneity and fish impact. Two models were generated, one on temporary (fishless) ponds (n=11) and another on ponds with one single habitat (stones) and low (0-1) fish impact (n=8)

Influence of selected variables. On the same pond set, we explored by means of box-plots and one–way analysis of variance (ANOVA) hypothetical relation-ships between three variables (altitude, habitat type and water permanence) and richness.

RESULTS

General prediction model

The MLR model included two variables as the best predictors of richness: number of habitats (NH) and impact by fish introductions (FI) (R^2=0.579, $F_{2.29}$=19.913, p<0.001). The relationship between richness and the predictors was described by the following equation: S=4.922(NH) – 2.359(FI)+11.227, being number of habitats the first variable selected by the model. The significance value of the *F* statistic is less than 0.01, which means that the variation explained by the model is not due to chance. The regression and residual sums of squares were approximately equal (863 and 628, respectively), which indicates that about half of the variation in observed richness is explained by the model. The large value of the multiple correlation coefficient (R=0.761) indicates a strong relationship between the observed and modelpredicted values of the dependent variable and error of estimate is considerably low, about 4.656. The tolerance collinearity statistic (T), percentage of the variance in a given predictor that cannot be explained by the other predictors, was very near to one (0.982), and variance inflation factor was below two (1.018). Therefore, there were not multicollinearity effects between predictors and the standard error of the regression coefficients will not be inflated. On the other hand, about assumptions of MLR, the error terms of this regression had a normal distribution (Kolmogorov-Smirnov test); they were constant across cases and independent of the variables in the model (there were not heteroscedastic) and Durbin-Watson showed not autocorrelation between them (1.719). Therefore, all the assumptions of the multiple linear regression model were fulfilled.

The model performance was tested on a set of 12 new ponds. ANOVA showed no significant difference between observed richness and richness predicted by the MLR model (p=0.525) in this new data set.

The graphs generated by GAM give an insight into the response of richness to the two variables in the MLR. Richness and number of mesohabitats showed a positive relation, but not all along the gradient. In ponds with only one and two habitats, apparently the richness was the same (Fig. 2). In contrast, richness was inversely related to fish impact (Fig. 3), although this relationship was only evident for high impact values (3 or 4).

Particular prediction models

In order to analyse only the influence on richness of natural variables, we carried out another multiple linear regression on a subset of the available samples: those with no or low fish impact (0 or 1). In the model generated, only the number of habitats significantly explained the response of richness (S=4.533(NH) + 10.699). This model was separately applied to two groups of samples: ponds with no or little fish impact (impact value 0 or 1) and ponds with high fish impact (3-4). Differences between observed and predicted richness values were significant for ponds with high fish impact (*p*=0.006) but not for low fish impact (*p*=0.99). This result helps to show that negative effect of fish is only evident at high impact level (Fig. 3).

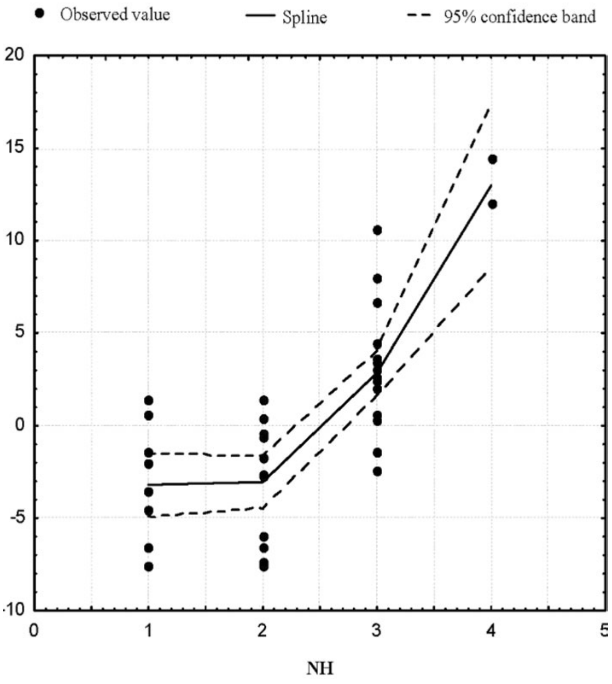

Fig. 2. Response functions for observed richness (PR, partial residual) and numbers of habitats (NH). The dashed lines are approximate 95% confidence intervals around the smooth function.

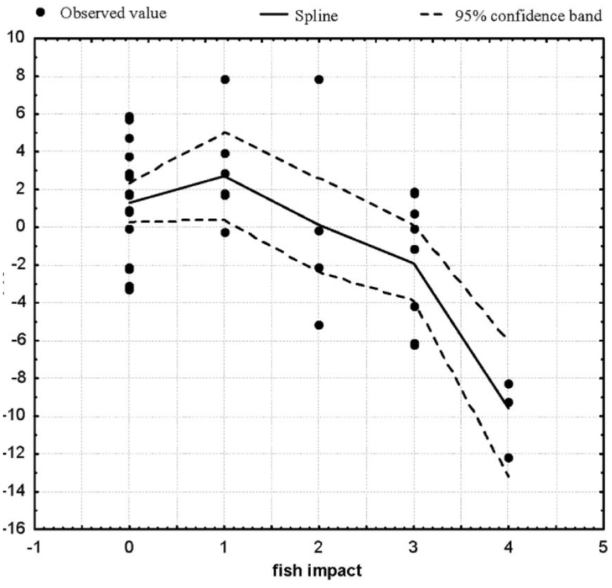

Fig. 3. Response functions for observed richness (PR, partial residual) and fish impact. The dashed lines are approximate 95% confidence intervals around the smooth function.

Multiple linear regression was also applied to additional data subsets in order to remove the effect of both habitat and fish impact. The model applied to permanent ponds with stony substrate and low fish impact proved that no variable significantly explained richness values. When applied to temporary, vegetated ponds with low fish impact, a significant contribution of depth and, secondly, total nitrogen to the model was observed, with *F* significant value of 0.02. This result seems to indicate a direct relationship between hydroperiod length (as indicated by depth) and richness.

Influence of selected variables

Finally, we explored by means of box-plots and ANOVA hypothetical relationships between three variables (altitude, habitat type and water permanence) and richness. Only ponds with low fish impact (impact level 0 or 1) were considered. The results are shown in Figs 4 to 6. No significant differences were detected except for water permanence (*p*=0.031): richness proved to be slightly lower in temporary ponds.

These results might be conditioned to some extent by the effect of habitat heterogeneity in the case of permanent ponds. Therefore, we tried to cast some light on the effect of altitude on richness by selecting a small but homogeneous data subset: nine permanent ponds with just one mesohabitat. The selected ponds were otherwise heterogeneous in altitude (1480 to 2195 m a.s.l.) and richness (from 13 to 22 taxa). The Pearson correlation coefficient showed no significant relationship between altitude and richness (R^2=0.1589).

DISCUSSION

The only relevant variables influencing macroinvertebrate richness in the study area were habitat heterogeneity (measured as number of mesohabitats in a pond) and intensity of fish impact. The relationship between these variables and richness is well known and its ecological basis well understood. The habitat heterogeneity classic hypothesis proposes that an increase in the number of habitats leads to an increase in species diversity in a landscape (MacArthur, and MacArthur 1961) because of an expansion in the number of partitionable niche dimensions (Willig 2000). On the other hand, several researches in Europe, North America and Oceania have reported negative consequences of fish introductions on macroinver-

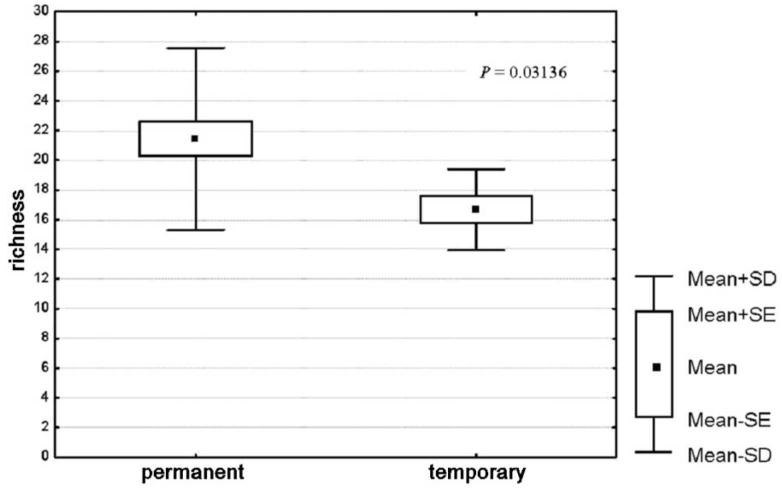

Fig. 4. Richness of macroinvertebrate community in permanent and temporary ponds.

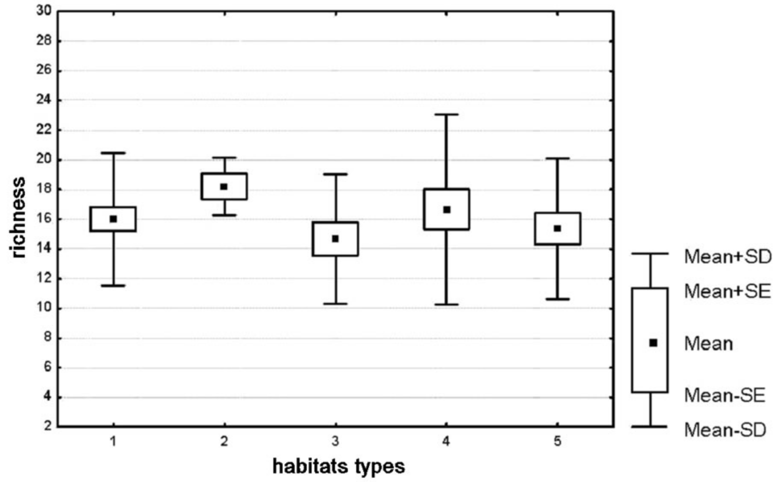

Fig. 5. Richness of macroinvertebrate community in each type of habitat. 1. stones without vegetation, 2. gravel without vegetation, 3. fine substrate without vegetation, 4. helophyte zone and 5. hydrophyte zone.

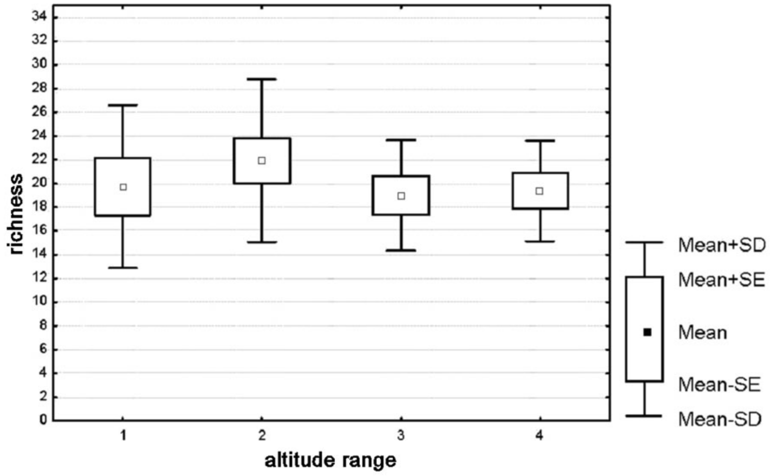

Fig. 6. Richness of macroinvertebrate community of different altitude range. 1 (1400-1600 m a.s.l.); 2 (1600-1800 m a.s.l.); 3 (1800-2000 m a.s.l.); 4 (2000-2200 m a.s.l.).

tebrate communities, whether on their composition or on richness (Crowder, and Cooper 1982; Carlisle, and Hawkins 1998; Oertli et al. 2001; Knapp et al. 2001; Zimmer et al. 2001; Tate, and Hershey 2003). Similar results have been reported in Spanish lakes and ponds after the introduction of lake trout (*Salvelinus fontinalis*) in Peñalara Lake (Toro et al. 2006) or of rainbow trout (*Onchorhynchus mykiss*) in mountain ponds of the Iberian System (Martínez-Sanz et al. 2010).

The result is relevant because it stresses the predominant influence of heterogeneity and man-induced impacts over that of variables traditionally considered to influence richness in mountain ponds and lakes. Some of these factors, apart from heterogeneity and fish stocking, are altitude (Hinden et al. 2005; Čiamporová-Zaťovičová et al. 2010; Mendoza, and Catalan 2010), pH (Schell, and Kerekes 1989; Dumnicka, and Galas 2002; Mousavi 2002), and water permanence (Wissinger et al. 2009). Different habitat types (stone, gravel, cobbles, sand, silt, aquatic plants, or woody debris) are also known to support macroinvertebrate communities differing in size structure and species composition (Oertli 1995; Gardner et al. 2001; Tolonen et al. 2001; Weatherhead, and James 2001). Of these variables, pH was not expected to influence richness in our study. Only pH values below 5.0 seem to have an appreciable effect on lake macroinvertebrate richness or community composition (Mossberg, and Nyberg 1979; Harve, and McArdle 1986; Schell, and Kereks 1989). Sometimes, the acidification threshold causing richness depletion is even much lower than 5.0 (Crisman et al. 1980). Therefore, it is hardly surprising that pH did not proved to be relevant in our study area, where pH values were always above 4.9 and usually above 6.0.

Neither was pond size expected to play a significant role. The assumption of increasing richness with increasing habitat size dates back to the island biogeograpical theory proposed by MacArthur, and Wilson (see Rosenzweig 1995 for a review) and has often been invoked to explain richness patterns. However, the relation between macroinvertebrate richness and pond size is far from evident. Several studies have failed to find such relationship or found it only for selected taxa (Gee et al. 1997; Oertli 2002). Of course, this does not fully refute the richness-size hypothesis. We should state, instead, that size effect is often overruled by other factors, such as vegetation and fish presence (as proposed by Scheffer et al. 2005) or heterogeneity (as suggested by Heino 2000, Céréghino et al. 2008, or by the results shown in this study). In a sense, our results do not completely oppose the theory of island biogeography, which predicts an effect of size on richness because larger islands have more habitats, as well as larger populations (see Rosenzweig 1995). Nevertheless, the possibility of a detectable effect of pond size itself cannot be ruled out provided that the range of areas is wide enough, as supported by the data provided by (Aho 1978) whose study comprised ponds and lakes between 0.03 and 147.3 km^2.

The reason why some other variables (altitude and water permanence) were not significant in our MLR model was not so easily explained and required an additional data processing to check whether they might play a secondary role (unravelled in the general model) or not. Besides, habitat type could not be included in the MLR model but deserved consideration too.

Water permanence has sometimes considered to shape macroinvertebrate richness in a wide range of pond types (Collinson et al. 1995; Della Bella et al. 2005; Waterkeyn et al. 2008), but not always (Boix et al. 2008). We did find temporary ponds to harbour less taxa than permanent ones, although the relationship was weak (ANOVA), and, in any case, superseded by heterogeneity and fish impact (as shown by the global regression model). Nevertheless, no much importance must be attached to this result. It might perfectly be a simple consequence of the low habitat heterogeneity in temporary ponds. In fact, when they are compared with permanent ponds having similar number of environments (one or two) and fish impact (0-1), differences disappear (richness values between 13 and 21 in temporary ponds, and between 14 and 22 in permanent ones, except for one pond with 27 taxa).

There is no doubt that different habitats usually harbour different macroinvertebrate taxa (Weatherhead, and James 2001; Della Bella et al. 2005). However, the effect of habitat type on richness is less known. This is a relevant issue because of the implications when interpreting variable-richness relationships. If there were substantial among-habitat differences in richness, trends attributed to environmental gradients (altitude, for example) might partly be a consequence of varying environments or pond type (vegetation cover, type of substrate, etc.) making it difficult to draw sound conclusions. Vegetated environments have sometimes been reported to have higher richness values than unvegetated ones (Wetzel 2001) and, on the other hand, sediments environments frequently have low taxonomic richness (Oertli et al. 2005). No significant among-habitat differences in richness were found in this study in spite of the wide range of habitat types involved (stones, gravel, silt, helophytes, hydrophytes). Within-habitat variability is very high but average values are similar across types. We must assume, therefore, that factors other than habitat type are responsible for differences in richness.

Altitude has often been considered the most important driver force of macroinvertebrate biodiversity in mountain ponds (*e.g.*, Oertli et al. 2000). Why was not altitude relevant in our study? Certainly, the altitudinal range available (1360-2195 m a.s.l.) is not as large as those in the Alps or the Pyrenees; however, it should be enough to detect changes if richness decreased gradually along the altitude gradient. One possible explanation is that richness drop is only apparent above an altitude threshold. The conclusions obtained by Mendoza, and Catalan (2010) and Čiamporová-Zaťovičová et al. (2010) are in this line. Mendoza, and Catalan (2010) showed that the inverse relationship between the number of macroinvertebrate groups relative to altitude was expressed best by a simple linear regression model (p<0.001) when considering only lakes above 2500 m. Čiamporová-Zaťovičová et al. (2010) found that the strongest response of macroinvertebrate fauna will likely occur in shallow alpine lakes above 2000 m a.s.l., when the duration of ice cover shortens below the 190-day threshold. Similarly, Lin et al. (2006) found only 4.6 taxa per pond in eight alpine ponds above 3000 m a.s.l. in central Taiwan, indicating that taxon richness in Taiwan ponds was related to altitude, with increasing altitude being associated with a reduction in species richness In addition, Hinden et al. (2005) showed an altitude-related decline in taxon richness in ponds of Switzerland (1860-2757 m a.s.l.). However, an analysis of their results show no richness decline when only ponds below 2500 m a.s.l. are selected. In the light of these data, it is quite possible that effects on richness are only evident above 2000 or 2500 m a.s.l., near or above the upper altitude limit in our study. Most authors explain this relationship as a consequence of harsh conditions in high altitude ponds and the specific adaptations required to survive in such extreme conditions (Čiamporová-Zaťovičová et al. 2010; Mendoza, and Catalan 2010). The results obtained in our study might suggest another possible explanation. High altitude ponds might support a lower number of habitats (see Hoffman et al. 1996). It is likely that many ponds from high altitude zones are dominated by a single meso-habitat (probably stones or sand) while lower lakes may incorporate other mesohabitats (silty sedimentation areas, vegetation of different types, etc.). As shown in our study, littoral macroinvertebrate richness may be closely related to number of habitats (but not to habitat type). In our study area there is not a trend towards habitat impoverishment at high altitudes. This fact might be responsible for the lack of altitude-richness relationship if heterogeneity (but not altitude) was the real determinant factor. Habitat homogeneity and harsh conditions might be complementary explanations of low richness in high-altitude ponds.

For a correct pond management, we must gain knowledge on the simultaneous effects of factors and their relative importance for richness. Furthermore, in areas where there are few (if any) pristine systems left, such as most of Europe, it is necessary to define to which extent the effect of man-induced impacts can overcome that of natural factors. As deduced from literature, macroinvertebrate richness in mountain ponds can be expected to depend on a number of interacting variables, such as altitude, heterogeneity, habitat type (substrate, vegetation), water permanence, pond size and, when extreme values happen, pH. However, heterogeneity is likely to be an essential factor relegating the remaining ones to a background position. Apart from these natural variables, richness can greatly depend on man-induced impacts (Wilcove et al. 1998; Steinman et al. 2003; Martínez-Sanz et al. 2010). The role played by fish in determining macroinvertebrate richness is particularly outstanding for mountain pond management. As deduced from the present study, several relevant evidences arise. Firstly, fish introductions may become a severe impact for littoral macrobenthic communities, even more than any other disturbance like land use changes. Secondly, low density fish communities, even though artificially introduced, seem to have no evident effect on macroinvertebrate richness (lower than other factors, in any case, and, thus, undetectable). In contrast, high densities caused by fish stocking can turn fish into an essential factor overcoming most other environmental variables. Nevertheless, sound conclusions on this issue require further, detailed analyses taking into account fish community composition and density.

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ARTÍCULO III

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Effects of introduced salmonids on macroinvertebrate communities of mountain ponds in the Iberian system of Spain

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ABSTRACT

Effects of introduced salmonids on macroinvertebrate communities of mountain ponds in the Iberian system of Spain

This study aimed to assess the impact of salmonid stocking on macrobenthic communities in Spanish mountain ponds. Macroinvertebrates were collected with a hand net following a multihabitat, time-limited sampling in eight ponds (four of them stocked with salmonids and four non-stocked) in the Iberian system. A number of macroinvertebrate-based metrics, as well as several physical and chemical variables, were measured and compared between stocked and non-stocked sites. We hypothesised that stocked ponds would have lower abundances and richness of large and mobile taxa and higher amounts of phosphorus in the water column than non-stocked ones. The results proved that macroinvertebrates were adversely affected by salmonid introductions and might be appropriate indicators of this type of impact. Total richness (both measured and estimated) and diversity were significantly lower in disturbed ponds. Coleoptera, Trichoptera and Heteroptera, large and mobile taxa, were particularly sensitive to the impact. No significant differences in nutrients were detected, although total phosphorus concentrations were usually higher in stocked ponds.

Key words: Mountain ponds, salmonid introduction, macroinvertebrates, non-parametric estimators.

RESUMEN

Efectos de la introducción de salmónidos sobre la comunidad de macroinvertebrados de lagunas de montaña del sistema ibérico, España

El objetivo de este estudio fue testar el impacto de la introducción de salmónidos sobre las comunidades de macroinvertebrados de un conjunto de lagunas de montaña en el noreste de la Península Ibérica. Los macroinvertebrados fueron colectados con una red de mano siguiendo un muestreo de tipo multihábitat, con un determinado tiempo limitado de muestreo en ocho lagunas (cuatro de ellas con introducción de salmónidos y otras cuatro sin dicho impacto) del sistema ibérico. Se compararon características físicas, variables químicas y atributos de la comunidad de macroinvertebrados entre los dos grupos de lagunas (con introducción y sin introducción de salmónidos). Partimos de la hipótesis de que en aquellas lagunas en las que se habían introducido salmónidos nos encontraríamos con baja abundancia y riqueza de taxones de macroinvertebrados móviles y de gran tamaño, así como de altas concentraciones de fósforo en la columna de agua. Los resultados mostraron que la comunidad de macroivertebrados se vio seriamente afectada por la introducción de salmónidos y que podrían ser indicadores apropiados de ese tipo de impacto. Riqueza total (tanto medida como estimada) y diversidad de macroinvertebrados fueron significativamente más bajas en lagunas en las que se llevó a cabo las introducciones de salmónidos. Coleoptera, Trichoptera y Heteroptera, taxones grandes y móviles, fueron particularmente sensibles a dicho impacto. La concentración de fósforo fue usualmente más elevada en aquellas lagunas que habían sufrido la introducción de salmónidos aunque las diferencias no fueron estadísticamente significativas.

Palabras clave: *Lagunas de montaña, introducción de salmónidos, macroinvertebrados, estimadores no paramétricos.*

INTRODUCTION

One of the most extensive human influences on ecosystems is the introduction of non-native species (Wilcove *et al*., 1998), as this perturbation is a major threat to native biodiversity (Vitousek *et al*., 1996; Davis, 2003). This impact is more severe when top predators are introduced into spatially restricted environments such as lakes (Kauffman, 1992). In particular, a number of studies in Europe, North America and Oceania have reported negative consequences of fish introductions on macroinvertebrate populations (Leppä *et al*., 2003) and communities (Crowder & Cooper, 1982; Morin, 1984; Mitelbach, 1988; Allan & Flecker, 1993; Diehl & Eklöv, 1995; Kornijów, 1997; Lodge *et al*., 1998). Salmonids are size-selective predators, and their introduction into previously fishless alpine lakes in North America and Europe has resulted in the local decline or elimination of large-bodied macroinvertebrates (Wurtsbaugh, Brocksen & Goldman, 1975; Angradi & Griffith, 1990; Larson *et al*., 1992; Liss *et al*., 1995; Carlisle & Hawkins, 1998; Drake & Naiman, 2000). Moreover, they also may have strong effects on ecosystem function (Eby *et al*., 2006; Simon & Townsend, 2003), enhancing, for example, phosphorus recycling from the littoral to the pelagic zone (Leavitt *et al*., 1994; Schindler *et al*., 2001).

Stocking with salmonids has also been a common practice in Spanish mountain lakes for several decades. However, the consequences of these introductions on the ecosystem have seldom been studied. There is some evidence of the effects on amphibians (Martínez-Solano *et al*., 2003), but none regarding the response of the macroinvertebrate community.

The aim of this study was to compare the littoral macroinvertebrate community and the values of some chemical variables in salmonid-stocked and non-stocked mountain ponds in the Iberian System. We predicted that ponds stocked with salmonids would have lower abundances and richness of large and mobile macroinvertebrates and higher amounts of phosphorus in the water column than non-stocked ponds. The importance of studies like this is strengthened by the current need to implement the Water Framework Directive (European Council, 2000). Water bodies are to be classified on the basis of their ecological status using quality elements, including benthic invertebrates. In this context, it is essential to know the effect of salmonid introductions, which are one of the most widespread impacts in North Spanish mountain lakes.

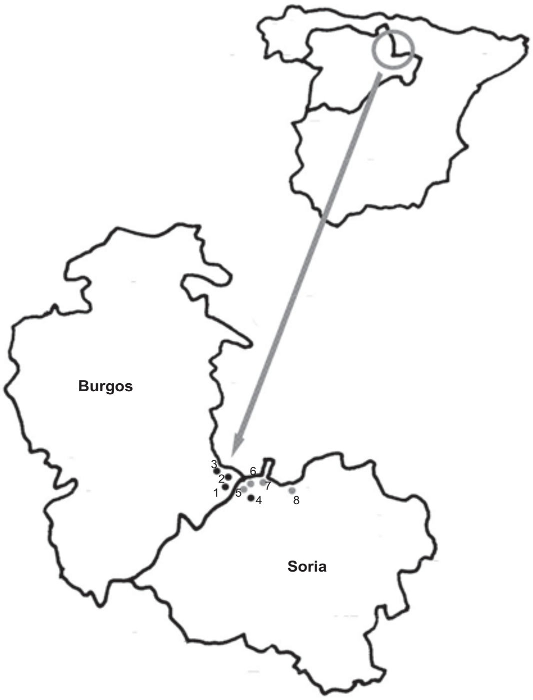

Figure 1. Study area. Black points represent stocked ponds and white points represent non-stocked ponds. Ponds: 1. Patos, 2. Brava, 3. Negra de Neila, 4. Negra de Urbión, 5. Helada, 6. Larga, 7. Verde and 8. Cebollera. *Área de estudio. Puntos negros: lagunas "impactadas" (S) y puntos blancos: lagunas "sin impactar" (NS). Lagunas: 1. Patos, 2. Brava, 3. Negra de Neila, 4. Negra de Urbión, 5. Helada, 6. Larga, 7. Verde y 8. Cebollera.*

MATERIALS AND METHODS

Study area

Eight permanent ponds located in the Iberian System (north-central Spain) were included in this study (Fig. 1). They are all similar in origin (glacial), catchment geology (siliceous), substratum (mostly sandy or stony) and altitude (Ta-

Table 1. Some characteristics of the ponds included in the study. Chlorophyll a concentration (Chl. a), total nitrogen (TN), soluble reactive phosphorus (SRP) and total phosphorus (TP). *Características de las lagunas incluidas en este estudio. Concentración de clorofila a (Chl. a), nitrógeno total (TN), ortofosfato (SRP) y fósforo total (TP).*

Ponds	Stocked	Altitude (m.a.s.l.)	Surface (ha)	Max. depth (m)	hydrophytes (%)	Chl. a	Secchi	Conductivity (µS/cm)	pH	Nitrate (mg/l)	NT (mg/l)	PRS (µg/l)	PT (µg/l)
Los Patos	Yes	1870	2.9	2	1	3.6	120	9.5	6.2	0.12	0.33	10.46	115.77
Brava	Yes	1860	1.3	5.5	1	0	280	6.3	6.01	0.004	0.32	4.53	7.41
Negra Neila	Yes	1900	11.8	4.7	3	3.2	210	8.7	6.5	0.17	0.25	9.90	36.05
Negra Urbión	Yes	1750	3.3	9	3	5	200	18	6.53	0.002	0.31	18.97	46.55
Verde	No	1910	1.6	2	1	0	200	18.3	6.7	0.002	0.21	4.81	4.81
Larga	No	2010	1.0	2	15	20.92	200	4.17	5.44	0.16	0.22	7.86	13.18
Helada	No	2000	5.1	3	10	3.1	150	6.11	6.07	0.009	0.49	4.35	11.53
Cebollera	No	1850	3.31	1.2	15	10.33	120	13	6.1	0.009	0.48	12.93	50.01

ble 1). Only some differences in size and depth were found between the ponds. Negra de Neila pond is 11.8 hectares, a slightly higher area than the other (on average 2.7 hectares), and is 9 meters deep, which is also slightly higher than the rest of study ponds (on average 2.9 meters). Table 1 also shows some morphometrics, chemical and physical characteristics of study ponds, as well as visual estimation of the percentage of coverage of aquatic hydrophytes.

According to the regional administration, four of the ponds (Patos, Brava, Negra de Neila, Negra de Urbión) were repeatedly stocked between 1976 and 1995 with brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) to support recreational angling (Martínez-Solano, 2003). There are not precise data available about the numbers or biomass of fish introduced per pond and year. As an illustrative example, two of these ponds (Patos & Brava) were stocked during that period with a total of 69000 kg of salmonids (3450 kg/yr on average). In the absence of more accurate information, we have divided the studied ponds into two categories: 1) ponds that have not been stocked, or "non-stocked ponds" (Cebollera, Helada, Larga and Verde) and 2) ponds that have been stocked at least once, or "stocked ponds" (Brava, Patos, Negra de Neila, Negra de Urbión).

Sampling

All of the ponds were sampled once in June 2004 (except Cebollera, which was sampled in June 2006). Three ponds, Patos, Brava and Helada, were sampled again in either 2005 or 2006. These additional samplings have been used here to check for interannual variability, but not to assess the impact of fish stocking. Secchi depth was measured in the lakes and water samples were taken for analyses in the laboratory of total phosphorus (TP) and soluble reactive phosphorus (SRP).

Benthic macroinvertebrate samples were collected from the littoral zone (from the shore up to 1 meter in depth) with a pond net (FBA standard, mesh size 500 µm) following a multihabitat, time-limited sampling (Collinson *et al.*, 1995; Briers & Biggs, 2005). Each pond was sampled for three to five minutes depending on the area. Total sampling time was proportionally distributed among the main habitats according to their surface in the pond. To allow for among-site comparisons, abundance data were standardised to one minute. Macroinvertebrates were separated from the plant material and counted under a binocular microscope (10×) and identified by genus except for Oligochaeta (identified to class); Glossiphoniidae, Sphaeriidae, Leptophlebiidae, Aeshnidae (to family), Diptera (to subfamily) and Limnephilidae (identified to tribe).

Metric selection and data analysis

We used a diverse selection of metrics to assess the effects of salmonid introductions on mountain ponds of the study area. We calculated the total abundance and richness of the community as well as the total and percentage abundance and richness of the most relevant taxa. Diversity was estimated using the Shannon Wiener diversity index. The taxa were assigned to a trophic group following Tachet *et al*. (2002), and the percentages of trophic groups were calculated. The list of metrics tested in this study is available in Table 2.

Although species richness is a natural measure of diversity, it is an elusive quantity to measure properly (May, 1988). Only an exhaustive sampling inventory can directly appraise true richness. Nevertheless, in practice, such sampling inventory can rarely be managed due to the limitations of time and money. For this reason, we used non-parametric estimations of species richness (chao 1 and jackknife 1) (Chao, 1987; Heltshe & Forester, 1983) together with the original measured richness. Non-parametric estimators are sampling theoretic extrapolation methods that only require the number of samples in which each species is found, rather than any parametric information about their abundance. Chao 1 and jackknife 1 are designed to estimate richness from single samples, whereas all other non-parametric estimators require several samples. All non-parametric estimators were calculated over 50 randomised iterations of the species accumulation using *EstimateS ver* 8.

The responses of the indices to the impact were graphically explored by means of box-plots showing mean value, standard error and standard deviation of the metrics within each pond category (stocked and non-stocked). One-way analysis of variance (ANOVA) was applied to detect significant differences ($p < 0.05$). The assumptions of normality and variance homogeneity were tested using the Kolmogorov-Smirnov and Levene's tests, respectively. Metrics that did not fulfil the assumptions of ANOVA were either log-transformed (absolute abundance and richness measures) or were transformed using the formula $\sin^{-1}(x/100)^{\frac{1}{2}}$. The statistical processing was performed with the STATISTICA 6.0 package.

Table 2. Values of the metrics measured in each pond. Only metrics presented in box-plots are included. *Valores de las variables medidas en cada laguna. Sólo son incluidas aquellas representadas en los box-plots.*

	Patos	Brava	Negra Neila	Negra Urbión	Verde	Larga	Helada	Cebollera
Measured richness	6	8	7	10	16	21	23	22
Chao 1 richness	6	8	7.5	11	18	24	26.33	29
Jackknife 1 richness	6	7	7	10	16	21	23	22
Total abundance	787	102	182	2016	282	1245	2373	1015
Shannon-Wiener	1.36	2.18	1.19	1.49	3.25	3.02	2.70	1.63
Richness of Odonata	0	0	0	0	1	1	0	5
Richness of Heteroptera	0	0	0	2	2	2	4	1
Richness of Coleoptera	1	1	1	1	4	8	6	5
Richness of Trichoptera	1	2	1	1	2	3	4	2
Richness of Chironomidae	2	3	3	3	3	3	3	3
Absolute abundance of Odonata	0	0	0	0	12	19	0	5.3
Absolute abundance of Heteroptera	0	0	0	17.5	10	13	56	10.3
Absolute abundance of *Sialis*	0	0	0.6	0	36	136	27.3	6.3
Absolute abundance of Coleoptera	5.9	4.3	0.6	3.7	40	465	326.7	22.7
Absolute abundance of Trichoptera	1.5	7.7	1.2	0.9	18.0	122.0	67.3	0.7
Absolute abundance of Chironomidae	723	82	146	1985	144	441	489	956
Percentage of Odonata	0	0	0	0	4.2	1.5	0	0.7
Percentage of Heteroptera	0	0	0	0.87	3.5	1.0	2.3	1.0
Percentage of *Sialis*	0	0	0.3	0	12.8	10.9	1.1	0.6
Percentage of Coleoptera	0.7	4.2	0.3	0.2	14.2	37.3	13.8	2.2
Percentage of Trichoptera	0.2	7.5	0.6	0.1	6.4	9.8	2.8	0.1
Percentage of Chironomidae	91.9	80.7	80.3	98.4	51.1	35.4	20.6	94.1
Percentage of collector-filterers	38.1	45.4	73.7	31.9	16.3	1.8	42.1	8.1
Percentage of shredders	0.3	8.4	0.9	0.2	12.8	11.2	2.9	0.2
Percentage of scrapers	53.8	8.4	4.6	54.9	19.9	47.9	21.7	64.9
Percentage of predators	1.5	31.1	2.3	12.9	50.3	36.9	8.6	26.0
Total phosphorus (µg/l)	115.8	7.4	36.1	46.5	4.8	13.2	11.5	50.0
Soluble reactive phosphorus (µg/l)	10.5	4.5	9.9	18.0	4.8	7.9	4.3	12.9

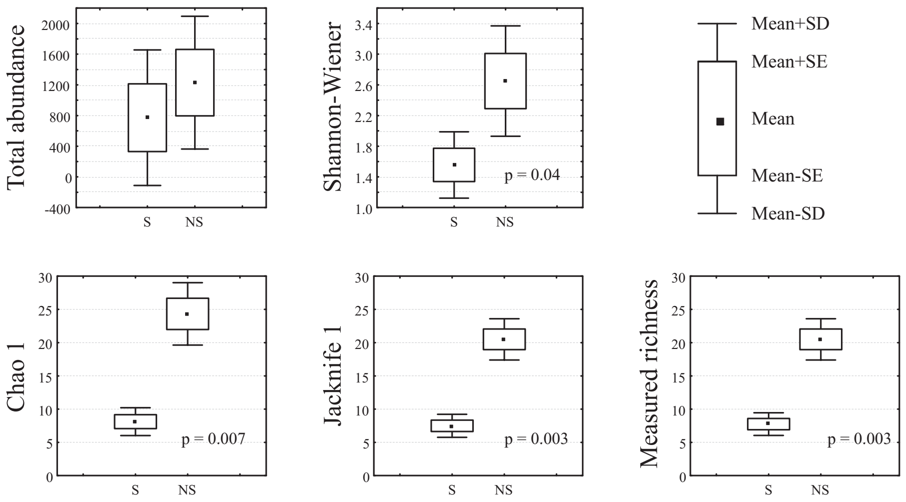

Figure 2. Total abundance, Shannon-Wiener index and richness (both measured and estimated) in stocked (S) and non-stocked (NS) ponds. *Abundancia total, índice Shannon-Wiener y Riqueza (tanto medida como estimada) en lagunas "impactadas" (S) y lagunas "sin impactar" (NS).*

It would be desirable to check the consistency of the results by repeating the previous analyses in different years, but only three ponds were sampled twice. Instead, we have used the available information to assess the within-lake, interannual variability in the metric values by calculating coefficients of variation. If the variability in the community is low, we may assume that the differences between stocked and non-stocked ponds will essentially remain unchanged.

RESULTS

Response of the metrics

Figures 2-5 show the differences between stocked and non-stocked ponds. Taxa and indices significantly differing between pond types are represented. In addition, attributes showing non-significant differences have been included in order to keep potentially useful information. The values of these indices in each pond are presented in Table 2.

The macroinvertebrate community as a whole was negatively affected by salmonid introductions, as deduced from the comparative richness (both measured and estimated) and Shannon index in stocked ponds. Total abundance also showed low values in stocked ponds but differences were only statistically significant for the Shannon index ($p = 0.04$) and richness (Fig. 2). In fact, total richness was the metric most severely affected by the impact. The values of measured richness in non-stocked ponds ranged from 16 to 22 taxa (Table 2) while stocked ponds supported impoverished communities (from 6 to 10 taxa). The estimation provided by chao 1 and jackknife 1 seemed to indicate that the actual richness was very close to the measured values in most of the sites. Jackknife 1 provided estimated richness values equal to those measured while chao 1 estimations were equal (two ponds) or slightly higher (five ponds) than the measured richness. Only in Cebollera were the values notably divergent (22 against 29) (Table 2). In all the cases (measured richness, chao 1 and jackknife 1), differences between stocked and non-stocked ponds were statistically significant ($p = 0.003$, $p = 0.0007$, and $p = 0.003$, respectively).

Coleoptera, Heteroptera and Trichoptera proved to be sensitive to salmonid stocking, as revealed by the low values of abundance (individuals/minute sampling), percentage abundance and richness (Fig. 3). The differences shown by the box-plots were statistically significant (Coleoptera richness ($p = 0.001$), % He-

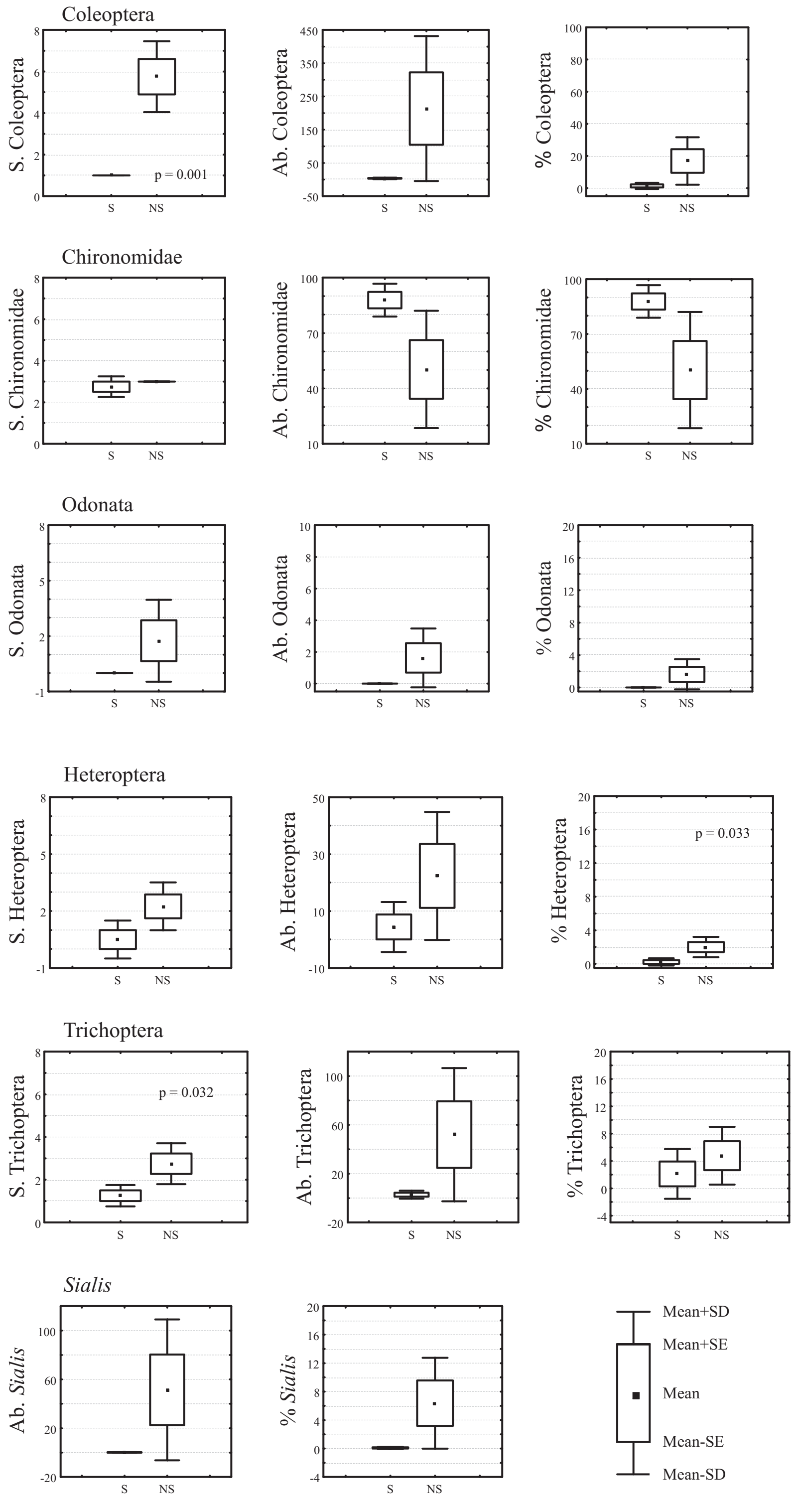

Figure 3. Richness (S), absolute abundance (Ab.) and percentage abundance (%) of the taxa affected by salmonid introductions. (S) stocked; (NS) non-stocked. *Riqueza (S), abundancia absoluta (Ab.) y porcentaje de abundancia (%) de los taxones afectados por la introducción de salmónidos. Lagunas "impactadas" (S) y lagunas "sin impactar" (NS).*

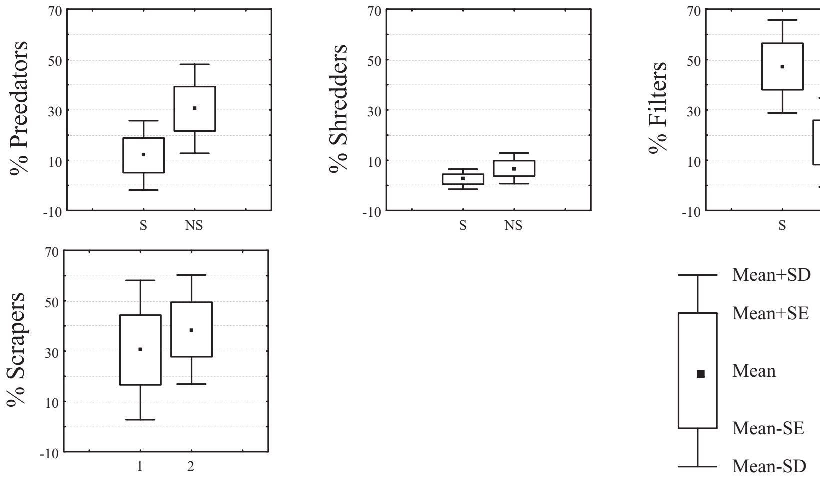

Figure 4. Percentages (%) of trophic groups in stocked (S) and non-stocked (NS) ponds. *Porcentajes (%) de los grupos tróficos en lagunas "impactadas" (S) y lagunas "sin impactar" (NS).*

teroptera ($p = 0.033$) and Trichoptera richness ($p = 0.032$). In contrast, Chironomidae were apparently unaffected and occurred in higher percentages in stocked ponds. On the other hand, the differences were not significant on macroinvertebrate trophic strategy (Fig. 4).

Orthophosphate and total phosphorus did not differ between stocked and non-stocked ponds (Fig. 5), although higher means were observed in stocked ponds.

Interannual variability

In general, there were only minor temporal/interannual qualitative changes in the community structure and composition, as revealed by the low CV values of the richness and diversity measures, most of them below 25 % (Table 3). The CV values of the remaining metrics of the community (absolute and percentage abundance measures) were a bit higher, although usually below 100 % and mostly below 50 %, indicating that quantitative changes were also not high. Only rare taxa showed relatively high interannual fluctuations (CV above 100 %) in relative abundances, but this can be considered an expected consequence of the low number of captures.

DISCUSSION

These results highlight the importance of salmonid stocking as an impact shaping the littoral macroinvertebrate community of Spanish mountain lakes. The predatory activity of salmonids, particularly intense on large and mo-

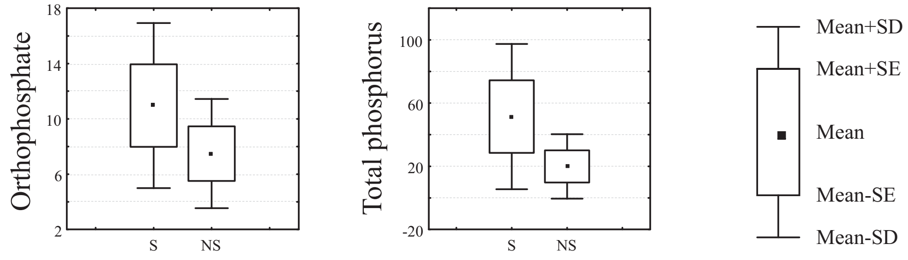

Figure 5. Orthophosphate (μ g/l) and total phosphate (μ g/l) in stocked (S) and non-stocked (NS) ponds. *Ortofosfato (μ g/l) y fósforo total (μ g/l) tróficos en lagunas "impactadas" (S) y lagunas "sin impactar" (NS).*

Table 3. Interannual variability (coefficient of variation) of the metrics in the ponds sampled for two years. *Variabilidad interanual (% coeficiente de variación) de variables en las lagunas muestreadas dos años.*

Metric	Patos	Brava	Helada
Measured richness	0	22.3	3.0
Chao 1 richness	0	22.3	3.7
Jackknife 1 richness	0	22.3	3.0
Total abundance	74.9	24.9	70.2
Shannon-Wiener	14.9	141.1	141.1
Richness of Odonata	0	141.4	0
Richness of Heteroptera	0	0	0
Richness of Coleoptera	141.4	0	20.2
Richness of Trichoptera	47.1	28.3	20.2
Richness of Chironomidae	0	0	0
Absolute abundance of Odonata	0	141.4	
Absolute abundance of Heteroptera	0	0	89.8
Absolute abundance of *Sialis*	0	0	73.7
Absolute abundance of Coleoptera	141.4	68.3	81.5
Absolute abundance of Trichoptera	64.3	61.4	135.3
Absolute abundance of Chironomidae	83.0	16.6	3.6
Percentage of Odonata	0	141.4	0
Percentage of Heteroptera	0	0	25.4
Percentage of *Sialis*	0	0	115.5
Percentage of Coleoptera	141.4	87.0	16.0
Percentage of Trichoptera	110.0	39.9	122.6
Percentage of Chironomidae	11.8	8.4	72.9
Percentage Filterers	34.5	13.3	29.9
Percentage Shredders	125.4	23.8	22.6
Percentage Scrapers	0.9	56.6	37.4
Percentage Predators	141.4	9.3	78.7
Total phosphorus (TP)	65.7	84.2	47.6
Ortophosphates (RSP)	68.1	50.5	24.7

bile taxa, resulted in modifications of the community structure, as previously demonstrated by a number of studies (Carlisle & Hawkins, 1998; Drake & Naiman, 2000; Knapp *et al.*, 2001). In the ponds included in the present study, Heteroptera, Coleoptera and Trichoptera were the groups most negatively affected. This selective effect entailed changes in the metrics based on these taxa (reduction of their richness or percentage abundances in stocked ponds), which extended to the whole community, resulting in relatively low values of total richness and diversity (Shannon index). Abundance in stocked ponds was not significantly lower because the dominant taxon in the study area, Chironomidae, was not affected by salmonid predation.

The results obtained could not be tested by repeating the sampling in a different year. However, the low inter-annual variability in the metrics detected in three of the ponds (see Kashian & Burton, 2000 and Trigal *et al.*, 2006 for a comparison of the CV values) point to probable stability in the community composition among years in both stocked and non-stocked ponds.

The results obtained are not surprising and are in accordance with expectations. However, the consequences of the introductions of salmonids are not always so evident (Wissinger *et al.*, 2006), and several studies have revealed either moderate or no effects of fish predation (Hanson & Leggett, 1986; Cobb & Watzin, 1998). There are at least three possible factors that might explain the effects observed in the study area: habitat structure, density of salmonids and the non-native nature of the predator.

Aquatic vegetation often plays an important role in regulating the predator-prey interaction (Jeppesen *et al.*, 1998). The predation effect of benthivorous fish is often stabilized by structural complexity, and the incidence on the abundance, body size, diversity and community structure of macroinvertebrates are less pronounced within vegetation stands than in open water (Crowder & Cooper, 1982; Gilinsky, 1984; Diehl, 1992; Diehl & Eklöv, 1995; Diehl & Kornijów, 1997). In addition, numerous laboratory experiments have demonstrated that the rates at which most fish species encounter and attack prey decline with increased density of artificial or natural hydrophytes (Diehl, 1988; Nelson & Bonsdorff, 1990). Wissinger *et al*. (2006) compared the benthic invertebrate communities of New Zealand lakes with and without introduced rainbow and brown trout, and all of the large-bodied benthic taxa were present in all the lakes. They hypothesised that the beds of submerged vegetation provided refuge for large macroinvertebrates, resulting in a minimal impact on the community. The ponds in the study, especially stocked ponds, lack submerged vegetation with the exception of sparse shoots of hydrophytes (*Ranunculus*, *Callitriche*) (Table 1). The absence of refuge is a reliable explanation for the high intensity of the impact on large bodied macroinvertebrates in the present study.

It is important to consider the density of salmonids after introduction. Knapp *et al*. (2005) observed that the ratio of the observed number of taxa to that expected to occur in the absence of introduction were inversely related to trout density, presumably because predation pressure increased with fish density (Pierce and Hinrichs, 1997, Leppa *et al*., 2003). We do not have precise data on salmonid density in the studied ponds; however, the little information available on stocked biomass (around 3500 kg/year in Brava and Patos together) and the extremely high number of trout observed near the shores (personal observation) make it reasonable to assume an extremely high fish density existed, at least in those two ponds. The absence of density data per pond makes it impossible to check whether there is an inverse correlation between salmonid density and the abundance or richness of macroinvertebrate taxa. Nevertheless, high fish density might be a relevant factor explaining the severe impact observed. Salmonids are not native predators in the studied ponds (J. C. Pena and B. Fuertes, personal communication), and their effects on native prey may be strong. This idea is supported by several studies that have shown that introduced predators may have particularly strong effects on native prey that do not recognize new predators and thus do not show appropriate avoidance behaviours (Shave *et al*., 1994, Kiesecker & Blaustein 1997), or if the introduced predators have foraging strategies that differ from those of native predators (McIntosh & Townsend, 1996).

The influence of stocking on the chemical characteristics of the water column was less evident. We expected an increase in nutrient concentrations as a consequence of nutrient recycling enhancement (Vannis, 1996; Tátrai *et al*., 2003) and of the repeated additions of food made by the regional authority to support fish populations. Contrary to expectations, no significant differences were found in phosphorus concentrations between stocked and non-stocked ponds (Fig. 5).

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ARTÍCULO IV

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Relative contribution of small mountain ponds to regional richness of littoral macroinvertebrates and the implications for conservation

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Relative contribution of small mountain ponds to regional richness of littoral macroinvertebrates and the implications for conservation

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ABSTRACT

1. Biodiversity is a central concept in conservation programme design. Until recently, ponds were neglected habitats probably owing to their small size and to the ignorance of their real conservation value. The classical theory of species-area relationship (SAR) might apparently support such a view by predicting low richness values in small habitat patches. SAR theory does not take into account the fact that groups of small habitat patches can significantly contribute to regional richness, regardless of their overall small area. This work intends to contribute to the SLOSS (single large or several small) debate with data on littoral macroinvertebrates from mountain ponds. Do groups of small ponds support communities with higher biodiversity than a single large lake?

2. Littoral macroinvertebrate richness, both local and regional, were measured in 17 ponds and one large lake from Sanabria Natural Park (NW Spain). In order to guarantee valid comparisons among systems, observed and estimated richness, as well as rarefaction methods were used.

3. Although local richness in the lake was much higher than in any single pond, regional richness of ponds widely exceeded the value measured in the lake regardless of their small overall area. Six to seven ponds were enough to obtain an accumulated average richness equivalent to that in the lake.

4. This pattern may be caused partly by increased habitat heterogeneity as proposed by the niche theory. Metacommunity theory might help to explain the high regional richness measured in the group of ponds in the study area.

5. Whatever the explanation, it is evident that groups of mountain ponds strongly contribute to regional richness, a conclusion that should be taken into account by management programmes. There is a gap in this respect in European legislation (the EC Habitat Directive and Water Framework Directive), which fails to include groups of ponds as an additional habitat category.

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KEY WORDS: richness; littoral macroinvertebrates; mountain ponds; Sanabria Lake; conservation

INTRODUCTION

One of the trends in conservation biology is to prioritize sites on the basis of their biodiversity (Gaston and Spicer, 2004). Despite their small surface area, ponds and small lakes are potentially rich and diverse habitats, and contribute significantly to regional freshwater biodiversity (Pond Action, 1994; Linton and Goulder, 2000; Oertli *et al*., 2002; Nicolet *et al*., 2004) especially macroinvertebrates (Biggs *et al*., 2005). However, a large number of small ponds (up to 40–90% for some European countries, Hull, 1997) have disappeared during the last century.

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Protecting water bodies for conservation requires an understanding of regional as well as local diversity. Certainly, large lakes might suppport higher number of species, as postulated by the well-known species-area relationship (SAR) (MacArthur and Wilson, 1967), one of the cornerstones of modern ecological science and conservation biology (Rosenzweig, 1995). According to this principle, large areas should be selected preferably as conservation targets. This rule, however, has provoked an intense debate (Simberloff and Abele, 1981), named 'SLOSS' (single large or several small), on whether protecting one large area is better than many small ones. This debate also applies to pond and lakes. Many authors have failed to find a relationship between size and diversity in ponds (Biggs *et al*., 1994; Gee *et al*., 1997; Scheffer *et al*., 2006, Martínez-Sanz *et al*., unpublished data), and the number of sites has even been proposed to be more relevant than their size in determining pond macroinvertebrate biodiversity (Oertli *et al*. 2002). However, such studies often refer to limited size ranges, and differences might indeed appear when research is extended to include large lakes, at least for some organisms (Søndergaard *et al*., 2005). The niche theory (NT), which has long competed with the SAR, could explain why many small ponds provide higher richness values than a few large ones. It suggests that habitat heterogeneity and niche partitioning among species are the most important drivers of species richness. From this, high richness could be expected in complex areas harbouring a number of ponds with varying environmental characteristics.

Which of these theories best explains patterns of macroinvertebrate biodiversity in mountain ponds and lakes? Heterogeneity, but not size, has already been shown to be a crucial driving force of alpha richness in Spanish mountain ponds (Martínez-Sanz *et al*., unpublished data). It is still to be shown whether richness in groups of ponds is greater than in single large lakes.

In this study, genus richness was used to test the relative contribution of small ponds and a large lake to regional richness in Sanabria Natural Park (north-west Spain). The objectives were to provide data from mountain ponds and lakes to help in management and conservation by contributing to the SLOSS debate. We hypothesize that a large lake will support higher macroinvertebrate biodiversity than any single pond but a group of ponds connected by dispersal will have a larger number of taxa, regardless of their small area.

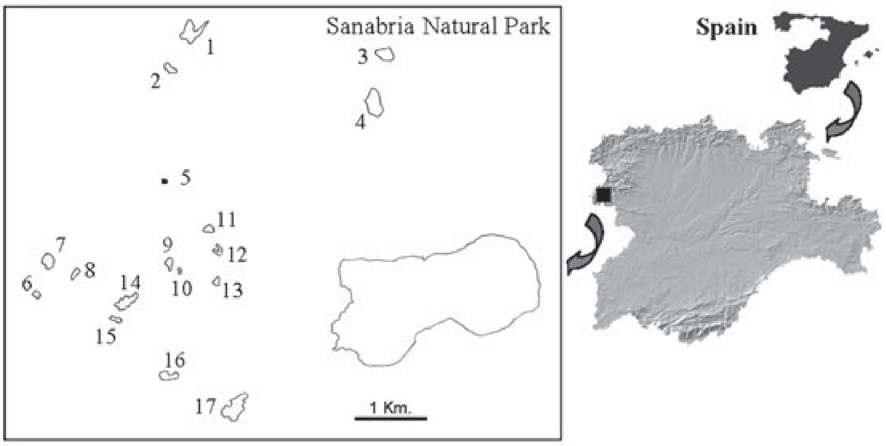
MATERIALS AND METHODS

Study area

The data were collected in Sanabria Natural Park, a 223.65 km² protected area including the largest glacial lake in Spain (Sanabria Lake, 3.19 km²) and around 30 ponds of sizes up to 0.12 km², most of them permanent, as well as a number of small temporary pools (see map in Figure 1). The short distance between ponds and the absence of mountain barriers allow for macroinvertebrate dispersal among them, creating a system of interconnected patches. The degree of connection is variable within the study area. There is a central core of ponds very close to each other (distance to the nearest water body between 30 and 900 m), some of them directly connected during flooding, and several ponds located further apart from this core (distance to the nearest pond up to 2500 m). The lake and 17 ponds were sampled in this study. Sanabria Lake (997 m.a.s.l.) is 50 m deep. The littoral zone is characterized by coarse substrate (stones, gravel and occasionally sand), mostly devoid of vegetation (except for *Isoetes* in some areas). Other mesohabitats present in this lake are: (1) a small area with emerging macrophytes (*Juncus*, *Eleocharis palustris*, *Equisetum fluviatile*, *Antinoria agrostidea*) on soft sediment (near the river inflow); and (2) artificial sandy beaches. It harbours a fish community dominated by *Salmo trutta*, *Barbus hocagei*, *Cobitis calderoni*, and *Leuciscus cephalus*. The ponds selected for the study were located at a glacial peneplain at 1580–1800 m.a.s.l and comprised different types. Some of them (see Table 1) were shallow, temporary ponds (maximum depth in summer below 40 cm) with soft sediment and dense vegetation stands of submerged (*Potamogeton natans*, *Ranunculus*) and emergent (*Juncus*, *Antinoria agrostidea*, *Glyceria fluitans*) plants. Some others were relatively deep, permanent ponds (maximum summer depths between 1 and 15 m) with coarse substrate (stones, gravel and sand) with or without *Isoetes*; occasionally with partial belts of emergent macrophytes (*Carex*, *Juncus*) or, more often, sparse stands of helophytes (*Antinoria, Glyceria*, *Carex*, *Alopecurus*, *Galium*, *Elocharis* or *Littorella*). Submerged vegetation other than *Isoetes* (*Callitriche*, *Potamogeton*, *Luronium*) is usually rare and sparse in permanent ponds and only a few of them harbour small areas of dense submerged macrophytes (*Myriophyllum*, *Ranunculus*). All the ponds are

F1

T1



1: Lacillo; **2**: Aguas cernidas; **3**: La Yegua; **4**: Los Peces; **5**: Pies juntos; **6**: Camposagrado I; **7**: Camposagrado II; **8**: Castromil; **9**: Roya grande; **10**: Roya pequeña; **11**: El Cuadro; **12**: Mancas; **13**: El Payón; **14**: Clara grande; **15**: Clara pequeña; **16**: Pedrina; **17**: Sotillo

Figure 1. Location of the 17 study ponds and Sanabria Lake.

historically fishless but, except for the temporary ponds, have been stocked in recent decades mostly with *Salmo trutta* and *Chondrostoma arcasii*. Further information on these ponds and on the lake can be found in Table 1.

Macroinvertebrate sampling

The ponds were sampled once in June or July between 2004 and 2008. Sanabria Lake was sampled once in June 2009. Benthic macroinvertebrate samples were collected from the littoral zone (at depths below 1 m) with a pond net (FBA standard, mesh size 500 μm) following a multihabitat time-limited sampling (Collinson *et al*., 1995; Biggs *et al*., 2000; Briers and Biggs, 2005). Each aquatic system was sampled for 3 min (ponds below 0.01 km²), 4 min (0.01–0.05 km²), 5 min (>0.05 km²) or 15 min (Sanabria Lake). Total sampling time was proportionally distributed among the main habitats according to their surface area in the pond or lake. Samples from each mesohabitat were pooled to obtain a single, integrated sample per pond. Macroinvertebrates were counted under a binocular microscope (10×) and, with a few exceptions, identified to genus (Appendix). Genus level has been taken as a feasible, reliable surrogate of species richness (Balmford *et al*., 1996; Lee, 1997; Magurran, 2004). The main identification keys used were Alba (1982), Conesa (1985), Jansson (1986), Tachet *et al*. (2002), and Tierno de Figueroa *et al*. (2003).

Measures of diversity (richness)

Local diversity (alpha richness) was calculated as the number of taxa observed in each pond and lake. Gamma diversity (regional richness) was the total number of taxa found in the 17 ponds or in the whole study area (ponds plus lake). Richness, especially species richness, is the simplest and the most intuitive concept for characterizing biodiversity (Gaston, 1996; Chao *et al*., 2005) but the compilation of a complete species census is costly or even impossible (Foggo *et al*., 2003; Hortal *et al*., 2006). Besides, biodiversity data suffer from heterogeneity in sampling strategies and/or sample size: the larger the sampling effort, the larger will be the number of observed taxa (Walther *et al*., 1995). Therefore, in addition to observed richness (Sobs), measures of estimated richness were calculated using Chao 1 (SChao) and Jackknife 2 (SJack) (Chao, 1984; Foggo *et al*., 2003), the non-parametric estimators which performed best in a previous study on mountain ponds (Martínez-Sanz *et al*., 2010). In the present study, each pond (or the lake) was taken as a sample. Chao 1 was used to estimate richness when only one sample was available (Sanabria Lake) while Jackknife 2 was used to estimate regional richness from a number of samples. Calculations were made with the EstimateS software (Colwell, 2004) and Statistica 6.0 was used to build each curve. Observed alpha richness and richness estimated by Chao 1 in each pond and lake were used to test whether Sanabria Lake had more taxa than any single pond. Jack 2 estimations are merely used in this paper as information to complement observed richness.

Testing whether the group of ponds supported higher diversity values than Sanabria Lake

Table 1. Some characteristics of Sanabria Lake and of the 17 mountain ponds included in the study

	Altitude (m a.s.l.)	Surface (km²)	Depth max (m)	Persistence	Conductivity (μS cm⁻¹)	pH	PT (μg L⁻¹)	NT (mg L⁻¹)	NH
Sanabria Lake	997	3.1875	50	P	15	6.5	15	0.6	4
Lacillo	1700	0.111	4.8	P	17	6.63	23	0.8	3
Aguas cernidas	1805	0.022	1.9	P	18	6.92	48	0.8	2
La Yegua	1790	0.042	6.5	P	10	6.74	20	0.4	3
Los Peces	1700	0.1	2.5	P	14	7.35	24	1.2	3
Pies Juntos	1670	0.0003	1	P	12	6.37	50	48.8	3
Camposagrado I	1700	0.0015	0.4	T	11	5.98	39	0.6	2
Camposagrado II	1660	0.015	2.5	P	14	6.56	25	0.5	4
Castromil	1685	0.018	0.3	T	7.3	6.22	101	0.9	1
Roya grande	1620	0.021	6.5	P	17	6.70	32	0.6	3
Roya pequeña	1620	0.01	2	P	12	6.33	57	1.1	2
El Cuadro	1680	0.025	8	P	15	6.60	9	0.3	3
Mancas	1600	0.028	8	P	24	7.11	13	0.3	4
El Payón	1590	0.016	6..5	P	10	6.72	56	0.5	3
Clara grande	1600	0.057	12	P	6	6.30	10	0.2	3
Clara pequeña	1600	0.015	3.5	P	43	5.19	25	1.4	3
Pedrina	1730	0.077	5.6	P	7.5	6.24	20	1.9	2
Sotillo	1580	0.12	5.6	P	27	7.10	23	0.5	2

NH, number of mesohabitats in each pond (the mesohabitats considered were hydrophytes, helophytes, stones without vegetation, sand/gravel without vegetation and fine substrate without vegetation); P, permanent and T, temporary

required a different statistical approach. The different sampling effort made on the lake (15 min sampling) and on the whole of the ponds (55 min) jeopardizes richness comparisons. Although raw data have been compared, rarefaction has been used as an additional way to confirm differences in richness (Gotelli and Colwell, 2001). Rarefaction is a technique providing the number of taxa expected in a subsample of a given size (Sanders, 1968). The software Species Diversity and Richness 3.03 has been used to calculate estimated richness for several subsample sizes. For a given subsample size, the software creates several combinations of subsamples (taken from the overall sample set) and calculates the average estimated richness and the standard error. These values have been used to construct the corresponding rarefaction curves.

RESULTS

As shown in Table 2, between 14 and 58 genera (Sobs) were collected in each single pond (mean = 33). The estimations provided by Chao 1 were only slightly higher, between 15.5 and 63.5 (Table 2). Local richness in the Sanabria Lake, both observed and estimated, was higher (81 and 110.5 genera, respectively). The rarefaction curve for Sanabria Lake (Figure 2) showed, for subsamples of 250 to 2000 individuals (the approximate abundance range in the ponds), expected richness values from nearly 40 to 70 genera, higher than alpha richness in most of the ponds. The Appendix includes a list of the taxa collected in ponds and in the lake.

Regional observed richness in the 17 ponds was 111 taxa, and the estimated richness (Jackknife 2)

Table 2. Values of estimated (Chao 1) and observed alpha richness

	Chao 1	Observed
Sanabria Lake	110.5	82
Lacillo	49.7	36
Aguas Cernidas	35	35
La Yegua	24.2	24
Los Peces	42.2	34
Pies juntos	25	22
Camposagrado I	15.5	14
Camposagrado II	53	47
Castromil	26.2	26
Roya grande	63.5	58
Roya pequeña	28	27
El cuadro	42.5	39
Mancas	49.5	45
El Payón	37.5	30
Clara Grande	51.2	42
Clara pequeña	31.5	25
Pedrina	37.7	36
Sotillo	22.3	19

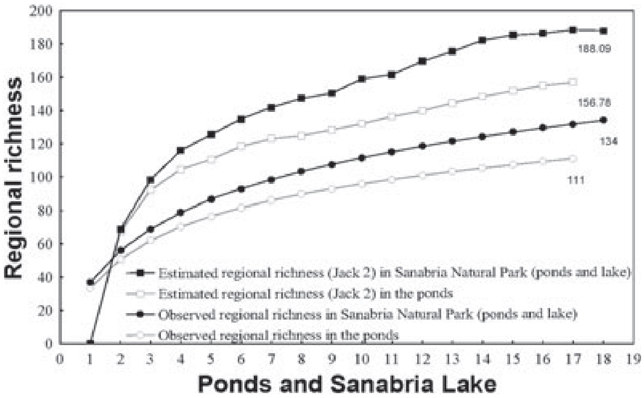


Figure 2. Rarefaction curves and standard error bars for the group of study ponds (above) and for Sanabria Lake (below). The curve for the ponds is only partly shown. The full curve is shown in the lower-right corner of the graph.

157. The regional richness measured in the natural park (17 ponds together with the lake) was 134, while estimated richness was 188.1 (Figure 3). Out of these 134 genera, 39.6% were collected only in the ponds and 17.2% only in the lake. The remaining 43.2% were common to both lake and ponds. A comparative analysis helps to highlight the contribution of ponds to regional richness in relation to the lake. Nearly 83% of the 134 taxa collected in the study (Sobs) were present in the ponds (only 60% in the lake), a large proportion in spite of the small overall area of the 17 ponds ($0.68\,km^2$). Calculations based on estimated richness (SJack) provided similar contributions to regional richness, 83% for ponds and 70% for the lake. The rarefaction curves constructed for the whole set of ponds and for the lake (Figure 2) confirmed that richness in the set of ponds was higher than in the lake, even after standardization for the same sampling effort (same number of individuals), since there was no overlapping between the curves or the standard error bars.

Another relevant result is the minimum number of ponds necessary to attain the richness value observed in Sanabria Lake. Such information can be obtained from the accumulation curve shown in Figure 3, where it is easy to check that only around six or seven ponds, on average, are necessary to record as many taxa as in the lake.

DISCUSSION

Littoral macroinvertebrate richness in Sanabria Natural Park was high: 134 taxa recorded across the 18 water bodies studied. Nevertheless, the variety of ecosystem types (ponds and a lake) included in the study impairs global comparisons with other studies unless data from both types are split.

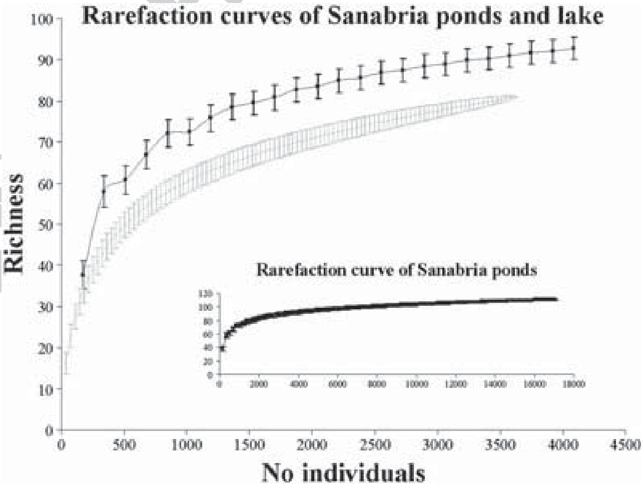


Figure 3. Accumulation curves of observed and estimated richness (regional richness) for the group of ponds and for the whole study area (ponds and the lake).

The number of taxa per pond (alpha richness) recorded in this study (between 14 and 58, with an average of 33) reveals a high biodiversity in relation to previous data from European mountain ponds, even when compared with studies with finer taxonomic resolution. Species richness per pond in the Tatra mountains (Slovakia, Poland) was found to be between 3 and 27 in studies by Krno *et al*. (2006) and Fjellheim *et al*. (2009), with average values of 11.5 and 15, respectively. In the Rila and Retezat mountains (Bulgaria and Romania, respectively), Fjellheim *et al*. (2009) found between 8 and 24 species, values similar to those reported by this author for two ponds in Central Norway (17 species per pond) and in the Julian Alps district (4–15 species, mean = 11). In a Swiss National Park, Oertli *et al* (2008) found a mean of 11.3 species per pond (range 6–24).

The outcome is similar for regional richness. The 17 ponds sampled in Sanabria (excluding the lake to make comparisons easier) provided 111 taxa. Regional richness of littoral macroinvertebrates reported for European mountain ponds is usually much lower. Fjellheim *et al*. (2009) found 29 species in the Julian Alps (six ponds), 37 species in the Italian Alps (three ponds), 30 species in the Retezat mountains (three ponds), 27 in the Rila mountains (four ponds) and 36 species in the Tatra mountains (six ponds). Krno *et al* (2006) found 93 taxa (species, except some genera) in 45 alpine lakes. Some other examples are the 42 genera identified by Kownacki *et al*. (2006) in eight small mountain ponds from southern Poland, the 47 species collected by Oertli *et al*. (2008) in 25 alpine ponds (Swiss National Park) or the 61 genera reported by Solimini *et al*. (2007) for 31 ponds in the Apennines. Similarly, Raddum and Fjellheim (2002) studied three Norwegian alpine catchments and only recorded 35, 30 and 16 species in each one (32 genera among the three regions). Toro *et al*. (2006) collected 90 macroinvertebrate species in 22 mountain ponds in central Spain (Gredos).

It is outside the scope of this research to explain the reasons for this comparatively high richness in Spanish mountain systems and, particularly, in the Sanabria Natural Park. Latitudinal patterns have often been invoked to explain biodiversity in large-scale studies of lake communities (Heino, 2002; Kernan *et al*., 2009) and might also be involved here to some extent.

Several ecological theories could help to explain the richness observed in this study. Alpha richness in Sanabria Lake was higher than in any single pond in the study (observed and estimated), in accordance with the predictions made by the species-area relationship (SAR). This is so even after applying rarefaction curve techniques in order to estimate richness for a sample size (number of individuals) similar to that collected in ponds. Therefore, lake size might affect richness, although many authors have often failed to find a relationship between macroinvertebrate richness and pond size (Oertli *et al*., 2002, Martínez-Sanz *et al*., in press). It is likely that the size effect is only apparent when differences in area are large. As for the ponds, and following SAR predictions, taxon richness should increase as new ones are sampled merely as a consequence of the increased sampling area. However, this increase in richness was so high that only an average of six or seven ponds provided as many genera as the whole lake, regardless of their comparatively low accumulated area. Factors other than size must also be involved.

Heterogeneity is a strong alpha richness driver (Martínez-Sanz *et al*., in press). We could easily assume that increasing the number of ponds means higher heterogeneity as new, different ponds are incorporated. Therefore, as proposed by the niche theory, beta diversity and thus regional richness will be higher. It is difficult to assess the extent to which this theory explains the biodiversity in the study area. Certainly, there were differences among ponds, particularly depth and water permanence, but most of them were similar in many aspects, such as altitude, and their physical and chemical characteristics. Habitat composition was not identical across ponds. However, the whole study area has a limited number of habitat types, many of them present in most of the ponds. Therefore, within-pond heterogeneity is relatively high compared with among-pond heterogeneity. So, in spite of the fact that heterogeneity (among-pond differences in habitat) may play a role, it is unlikely that it is fully responsible for high regional richness in these ponds.

Seemingly, SAR and niche theories can partly explain richness patterns in the study area, but not completely. Given the special distribution of water bodies in Sanabria Natural Park, metacommunity theory may provide a complementary explanation of the high regional richness (Leibold *et al*., 2004). The study area consists of a group of ponds relatively close to each other and without mountain barriers separating them. Macroinvertebrate dispersal must be common, although colonization rates will vary according to the degree of isolation of each particular pond. This, together with local (within-pond) extinctions could help to maintain high regional richness through beta diversity.

The most noteworthy result in the context of conservation was the high regional richness in a group of small ponds when compared with richness in a single, large lake. This supports previous observations made by Oertli *et al*. (2002), who found that a group of small ponds had more species and had a higher conservation value than a single large pond of the same total area. This high conservation value of the small ponds was reinforced by the data on the relative contribution to total regional richness in Sanabria Natural Park. The 17 ponds represented a comparatively small area (17% of the total), but contributed 82% of the genera recorded in the study. Moreover, 39.6% of all the taxa were collected only in the ponds. Certainly, the lake provided several genera not collected in any of the ponds, but in a smaller proportion (17% of the 134 taxa identified in the study). One outstanding conclusion arising from this information is that size is not the only relevant factor for richness. In fact, the presence of a number of ponds relatively close to each other and interconnected by dispersal can be particularly important in terms of biodiversity. Most European countries now have river, stream and sometimes lake monitoring programmes. However, routine monitoring of ponds is seldom undertaken. Furthermore, the current legislation does not take into consideration the potential value for biodiversity of groups of nearby ponds closely related by dispersal. The Habitats Directive (Council of the European Communities, 1992) was intended as a tool to preserve and enhance natural habitats and wild fauna and flora. Annex I is a list of natural habitat types of community interest whose conservation requires the designation of special areas of conservation. Several wetland types are included (although none of them refers to mountain water bodies), but no reference is made to groups of ponds. The Water Framework Directive, WFD (Council of the European Communities, 2000) is another crucial document for maintaining and restoring quality in aquatic systems. It does not address the conservation value of ecosystems, but that of preservation and enhancement of 'ecological status'. However, both concepts are closely related (Boon and Lee, 2005), since the implementation of measures to improve ecological status of aquatic systems should help to maintain their conservation value. The lower size threshold proposed for lakes by the WFD (0.5 km^2), excludes small lake ecosystems, turning its implementation for ponds into an optional decision. Results such as those presented here lead to the notion that groups of ponds must be given greater emphasis in national and international legislation.

ACKNOWLEDGEMENTS

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APPENDIX A

Macroinvertebrate taxa and total number of individuals collected in the Sanabria Natural Park. Data for the lake and for the groups of ponds are shown separately.

	Lake	Ponds
Oligochaeta	703	1184
Hirudinea		
Erpobdella	16	139
Helobdella	12	71
Hirudidae	0	6
Gastropoda		
Ancylus	14	1
Radix	6	153
Pisidium	8	135
Insecta		
Ephemeroptera		
Cloeon / Procloeon	138	141
Caenis	418	829
Eurylophella	13	258
Ephemera	4	0
Habrophlebia	128	202
Thraulus	168	98
Leptophlebiidae	0	330
Siphlonurus	0	37
Odonata		
Coenagrion/Ischnura	3	4
Erythromma lindeni	16	0
Enallagma	0	233
Lestes	2	15
Boyeria irene	4	0
Cordulegaster	6	0
Gomphus	4	0
Onychogomphus cf. *forcipatus*	2	0
Sympetrum	0	15
Plecoptera		
Nemoura	1	0
Leuctra	1	0
Heteroptera		
Gerris	7	2
Hydrometra stagnorum	8	0
Notonecta	0	188
Plea	3	21
Ilyocoris	0	1
Naucoris maculatus	80	0
Glaenocorisa	0	38
Arctocorisa	0	1
Corixa	0	3
Hesperocorixa	7	1582
Sigara	51	1650
Micronecta	70	27
Microvelia	0	2
Lepidopera		
Pyralidae	0	74
Coleoptera		
Acilius	0	6
Agabus	1	7
Bidessus	11	12
Dytiscus	0	13
Ilybius	1	39
Laccophilus	1	495
Rhantus	0	66
Graptodytes	0	280
Hydroporus	0	89
Hygrotus	1	203
Nebrioporus	0	28
Stictotarsus	1	5
Dryops	4	30
Orectochilus villosus	1	0
Gyrinus	0	3
Haliplus	0	120
Oulimnius	526	1095
Stenelmis canaliculata	5	0
Helophorus	0	12
Hydraena	0	1
Limnebius	0	4
Hydrochus	0	1
Anacaena	0	1
Berosus	0	13
Enochrus	0	16
Helochares	17	214
Trichoptera		
Ecnomus	14	0
Agraylea	3	0
Hydrobius	0	7
Laccobius	0	111
Athripsodes	0	71
Mystacides	171	83
Limnephilini	0	137
Stenophylacini	0	35
Plectrocnemia	18	44
Pseudoneureclipsis	8	0
Agrypnia	0	46
Tinodes	19	15
Diptera		
Ceratopogoninae	107	79
Chaoborus	1	1
Paduniella vandeli	1	0
Ablabesmyia	127	493
Dasyhelea	0	2
Procladius	5	209
Tanypus	1	3
Acricotopus	5	478
Corynoneura	75	1177
Cricotopus	58	145
Heterotrissocladius	5	55
Heterotanytarsus	2	0
Nanocladius	8	1
Paralimnophyes	1	33
Parakiefferiella	0	173
Paratrissocladius	11	0
Psectrocladius	1	229
Orthocladius	0	8
Cladopelma	2	127
Apedillum	0	8
Chironomus	0	17
Cryptochironomus	0	11
Demicryptochironomus	17	0
Dicrotendipes	20	819
Endochironomus	14	277
Glyptotendipes	0	81
Microtendipes	1	1
Parachironomus	1	26
Pagastiella	20	0
Paratendipes	4	25
Phaenopsectra	204	6
Polypedilum	18	166
Stictochironomus	1	30
Tribelos	0	23
Stictochironomus	28	0
Cladotanytarsus	31	228
Micropsectra	0	12
Paratanytarsus	17	882
Neozavrelia	0	21
Stempellina	15	2
Tanytarsus	25	349
Virgatanytarsus	93	1
Stempellinella	0	4

(Continued)

(*Continues*)

(Continued)

	Lake	Ponds
Macropelopia	0	23
Paramerina	0	2
Telopelopia	0	16
Prodiamesa	0	3
Zavrelia	2	4
Phalacrocera	0	1
Dolichopodidae	1	1
Hexatomini	0	2
Simuliidae	0	2
Tabanidae	0	2
Empididae	1	0
Ephydridae	1	0
Diptera n.i.	0	35

DISCUSIÓN GENERAL

Esta sección trata de exponer y unificar cuestiones abordadas en los artículos que componen esta tesis. Cada uno posee su propia discusión, por lo aquí nos limitamos a desarrollar un organizado compendio de ideas comúnmente tratadas. De esta manera, se da unidad al conjunto de la investigación y se establecen puntos de partida para futuros estudios. Con el fin de dar una mayor fluidez al texto, se ha intentado reducir el número de citas bibliográficas, siendo numerosas las alusiones a los artículos donde se trata cada asunto.

Hasta la fecha, el trabajo de mayor ámbito geográfico sobre macroinvertebrados de lagunas de montaña en Castilla y León era el de Granados y Toro (2001), limitado al Parque Regional de la Sierra de Gredos. Los 143 taxones registrados a lo largo del desarrollo de esta tesis (127 de ellos, géneros) constituyen, por consiguiente, la primera aproximación al valor global de riqueza regional en Castilla y León. En el Anexo I se recoge un listado general de los taxones encontrados en el área de estudio, información que no aparece reflejada en ninguno de los artículos precedentes. Es conveniente recordar que los quironómidos solo se han identificado hasta género en el área de Sanabria. De contar con esta resolución taxonómica en el resto de regiones, la riqueza sería indudablemente mayor. Esta información constituye uno de los objetivos planteados en la tesis y únicamente se ha utilizado para caracterizar la biodiversidad global del área de estudio. No se han efectuado comparaciones con otras áreas, solo un breve esbozo en el artículo IV destaca la gran biodiversidad de una zona concreta (Sanabria) con respecto a otras áreas de montaña de Europa. Sería interesante que futuros estudio se centraran en la comparación de riquezas regionales de diferentes áreas de montaña a lo largo de Europa, pudiéndose establecer (si lo hubiese) un gradiente latitudinal o longitudinal a una escala espacial superior a la tratada en esta tesis. Este enfoque es útil como aproximación preliminar al estudio del efecto del cambio climático (Roots, 1989; Beniston *et al*., 1997) y es clave en el conocimiento de los patrones globales que condicionan la riqueza (Hawkins *et al*., 2003; Willig *et al*., 2003).

Este parámetro, la riqueza observada, es una variable ampliamente utilizada. Proporciona una primera visión de la biodiversidad de un sistema o área (ver artículo IV y III) y se obtiene de forma inmediata en cualquier investigación (Colwell & Coddington, 1994; Magurran, 2004). Es fundamental en numerosos estudios ecológicos, ya que, a menudo, los trabajos de investigación no tienen como objetivo determinar la riqueza, sino conocer la comunidad. Sin duda, lo ideal sería “observar” toda la riqueza de una comunidad, pero en nuestro campo de estudio (lagunas e invertebrados), ninguna investigación es capaz de recoger toda la biodiversidad de un enclave (al menos, no con certeza). En estos casos hay que optar por utilizar un valor de riqueza observada (tratando de estandarizar el muestreo en la medida de lo posible, lo cual es muy difícil si no imposible) o, si la comparación de valores de riqueza es un objetivo clave en sí mismo, se puede recurrir a procedimientos estadísticos: rarefacción, por ejemplo (ver artículo IV). En ocasiones, el objetivo de una investigación es conocer cuál es la riqueza total de la comunidad, aunque no se pueda recoger todos los taxones. Es un dato de enorme interés si se quiere caracterizar una compleja comunidad en un enclave protegido o emblemático, efectuar comparaciones directas entre comunidades y, sobre todo, si se pretende obtener una noción aproximada de cómo de completo es nuestro inventario (el conocimiento de la biodiversidad, por lo tanto) en una localidad o región (véase un ejemplo cercano en Valladares *et al.*, 2010). En estos últimos casos necesitamos el uso de estimadores.

En el artículo I obtuvimos una serie de resultados que sugerían el uso de unos determinados estimadores de riqueza frente a otros. Jack 1, Jack 2, Chao 1 y Chao 2 fueron los mejor posicionados, siendo su uso recomendado dependiendo del número de muestras. En este punto, se plantea un interrogante frecuente ante un resultado de este tipo ¿es esta conclusión una realidad en la práctica científica? En los sucesivos artículos se utilizaron algunos de estos estimadores como apoyo metodológico. En el artículo III, Jack 1 y Chao 1 funcionaron adecuadamente para realizar comparaciones entre comunidades de macroinvertebrados partiendo de una sola muestra. Parte de las comparaciones entre comunidades de macroinvertebrados que llevamos a cabo en la zona de Sanabria (artículo IV) fuero realizadas con Jack 2 (con numerosas muestras) y con Chao 1 (solo una muestra). Chao 1 fue seleccionado por la robustez que presentó tanto en el artículo I como en el III y Jack 2 por resultar el mejor de los estimadores cuando disponemos de numerosas muestras en nuestra investigación (artículo I) y en la de otros autores (Hellmann & Fowler, 1999; Melo & Froehlich, 2001). En ambos caso el resultado fue adecuado. El que solo hayamos utilizado, en los artículos que constituyen esta tesis, los estimadores que resultaron mejores en estos sistemas (grupos de Chao y Jackknife) no invalida el uso de otros que ofrecieron un resultado intermedio en nuestro trabajo (ACE, ICE y Bootstrap) y que han resultado adecuados en otras investigaciones (Smith & van Belle, 1984; Chanzon *et al.*, 1998; Walther & Morand, 1998; Hortal *et al.*, 2006). Sin embargo, los estimadores basados en funciones de acumulación no son recomendados en esta tesis para estimar riqueza en lagunas de montaña y efectuar posteriores comparaciones entre comunidades. No obstante, estas últimas técnicas pueden ser útiles para calcular relaciones de esfuerzos de muestreo (Jiménez-Valderde & Hortal, 2000).

Por otra parte, se ha demostrado que el estudio ecológico de la comunidad de macroinvertebrados es incompleto si no se analizan o, al menos, se tienen en cuenta los demás compartimentos biológicos del sistema lacustre estudiado. El modelo de predicción de riqueza desarrollado pone de manifiesto, entre otras cuestiones (ver artículo II), la importancia de las comunidades piscícolas y la heterogeneidad de la propia lagunas para la riqueza de macroinvertebrados. En el artículo II se muestra de que forma el impacto de la introducción de fauna piscícola en general, afecta negativamente al valor de la riqueza de la comunidad de macroinvertebrados de lagunas de montaña. No obstante, como ya se menciona en el propio artículo, a presiones de impacto leves (IP = 1 y 2) los efectos no parecen ser negativamente significativos para la riqueza, lo cual también fue apoyado por los resultados del modelo de predicción de riqueza particular que se desarrolló sin (o con bajo) impacto de peces. En el artículo III acotamos tanto el tipo de fauna piscícola (salmónidos) como el grado de impacto (impacto severo), obteniendo la misma conclusión que en el artículo II. Por lo tanto, podemos concluir, de manera global, que la introducción (severa) de fauna piscícola en general y de salmónidos, en particular, afecta negativamente a la riqueza. Además, en el artículo III se demostró que estos efectos negativos no solo afectaban a la riqueza, sino que lo hacían sobre toda la estructura de la comunidad. En relación a este asunto, la DMA establece la necesidad de clasificar las masas de agua en cinco categorías de estado ecológico diferentes (alto, bueno, moderado, pobre y malo). La evaluación de dichas masas de agua y su clasificación en diferentes categorías de estado ecológico debe hacerse atendiendo a un conjunto de elementos indicadores de calidad, entre los que se encuentran los macroinvertebrados. El estado ecológico alto es equivalente a las condiciones de referencia, es decir, a las de aquellos sistemas mínimamente alterados por las actividades humanas. Atendiendo a los resultados obtenidos en esta tesis, aquellas lagunas en las que el único impacto de origen antrópico sea la presencia de bajas densidades de fauna piscícola, pueden considerarse sistemas poco alterados, en lo que se refiere a la comunidad de macroinvertebrados y, por tanto, considerarse sistemas en condiciones de referencia de un determinado tipo de lagunas. La importancia de esta conclusión radica en el hecho de que existen pocos sistemas lacustres de montaña sin algún tipo de impacto antrópico y el establecimiento de las condiciones biológicas de referencia sería prácticamente imposible. Una vez logrado este primer paso en el conocimiento de estos sistemas, se propone la evaluación de los efectos de este y otros impactos antrópicos, no solo sobre la riqueza de la comunidad de macroinvertebrados, también sobre todas las demás variables de este y otros compartimentos del ecosistema de lagunas de montaña.

La otra variable que resultó ser significativa en el modelo desarrollado en el artículo II es la heterogeneidad, en este caso medida como número de mesohábitats (hidrófitos, helófitos, cantos sin vegetación, grava y arena sin vegetación y sustrato fino sin vegetación). El modelo de predicción mostró un efecto directamente proporcional entre esta variable y el valor de la riqueza de la comunidad de macroinvertebrados. Se predice un aumento de especies debido a que, a grandes rasgos, a mayor nº de hábitats mayor nº de nichos disponibles (MacArthur & Wilson, 1967). Esta relación, bien conocida por la comunidad científica, puede ser extendida a sistemas de lagunas. Un asunto de notable trascendencia es la contribución de conjuntos de lagunas a la biodiversidad de una región (por ejemplo, de un espacio natural protegido), comparada con la riqueza local (la de cada ecosistema por separado) o con la de un ecosistema de gran superficie (un gran lago). El artículo IV ha permitido comprobar qué conjuntos de lagunas de pequeña superficie, aun teniendo valores de riqueza relativamente bajos, proporcionan globalmente la mayor parte de los taxones de la región, superando ampliamente a un gran lago. Dados los resultados del artículo II, que apoyaban una realidad bien contrastada (Willig, 2000), era inevitable presuponer la heterogeneidad como causa de la elevada riqueza regional en esos sistemas lagunares. No obstante, como se discute en el artículo IV, no parece que en este caso sea razón suficiente para explicar la enorme diferencia entre riquezas local y regional. Esto lleva a plantear interesantes propuestas en el contexto de la teoría de metacomunidades, en auge en los últimos años (Allen *et al*., 2011), cuya comprobación empírica deberá ser objeto de futuras investigaciones. En este mismo contexto, podemos extrapolar los resultados del artículo II, relativos a número de hábitats en una laguna, para aplicarlos a una escala espacial todavía mayor y hablar ahora de diferentes tipos de ecosistemas/lagunas dentro de una gran área. Esta tesis aborda el estudio de un conjunto de sistemas repartidos sobre una región amplia (94.223 km^2) que, lógicamente, incorpora una mayor variedad de tipos de ecosistema que un parque natural concreto. Cabe esperar que este incremento de la heterogeneidad física sea responsable, al menos parcialmente, de la elevada riqueza en el conjunto de Castilla y León. Por supuesto, esto no es más que una exposición tentativa; no hay que olvidar que los factores que operan a escalas espaciales grandes no son los mismos. Sería interesante dilucidar cuál es el factor responsable de esta elevada riqueza regional observada en Castilla y León, sirviendo como hipótesis de partida la ya mencionada incorporación de diferentes ecosistemas y aumento de heterogeneidad. Esta misma idea podría ser invocada en futuros estudios, como posible explicación al (ya mencionadas al principio de esta discusión) gradiente latitudinal de riqueza, complementando sendas y clásicas propuestas e hipótesis para tratar de explicar este patrón: respuesta de las especies a gradientes de intensidad de las interacciones ecológicas, a la estabilidad y predictibilidad ambiental, productividad y energía, área disponible y procesos de colonización (Rohde, 1999; Kaspari *et al.*, 2003).

El hecho de que la heterogeneidad sea un factor preponderante para la riqueza posee, fundamentalmente, dos importantes implicaciones prácticas relacionadas con la DMA.: 1. Evaluación del estado ecológico. Para evaluar el estado ecológico de una serie de lagunas muy diferentes entre sí, la riqueza no se presenta como un buen parámetro indicador; esto es debido a que la propia heterogeneidad podría enmascarar otros efectos, en este caso, de degradación. No obstante, es posible tratar de que las lagunas incluidas dentro de un tipo particular varíen poco en número de ambientes. En este sentido, el problema (las diferencias en riqueza) solo se produciría con las lagunas con muy pocos ambientes (ver artículo II). Por ejemplo, las lagunas de elevada altitud y dominadas por cantos (un único ambiente) podrían constituir por sí mismas un tipo, separado de otras lagunas con variedad de ambiente. Esto es solo un primer esbozo o idea, ya que no es objeto de esta tesis, para futuras investigaciones; 2. Consideración de conjuntos de ecosistemas como unidades de gestión. Las autoridades competentes pueden considerar como masa de agua lagunas conectadas entre si físicamente. A la vista de nuestros resultados, es posible considerar que un sistema de lagunas no unidas físicamente pero sí interrelacionadas por dispersión puedan tener características biológicas peculiares, ya que las comunidades biológicas de una laguna dada no dependen solo de la laguna en sí (y su tipo de hábitats) sino de la fauna existente en la región.

Finalmente, en diferentes partes de esta tesis se ha destacado la importancia que tienen los sistemas lacustres de montaña, las comunidades de macroinvertebrados que albergan y el conocimiento de su riqueza biológica. Tanto por razones científicas como prácticas (gestión y conservación), el estudio de esta variable se nos presenta como un hecho obligado, básico e imprescindible en cualquier investigación ecológica. Al margen del asunto de la medición de la riqueza biológica (riqueza observada o estimada) anteriormente tratado, sería deseable poder anticipar la riqueza de una laguna (o sistemas de lagunas) dada en función de sus características ambientales. Tal capacidad de predicción tiene inmediatas aplicaciones a la gestión, como la estimación de la riqueza que le corresponde a una laguna y la comparación con la riqueza observada (o estimada) para deducir desviaciones de las condiciones esperadas (tal vez, por presencia de impactos). Esta tarea ha sido abordada de forma muy preliminar en el artículo II, que proporciona un modelo con la capacidad arriba mencionada. Además, este se presenta como una posible herramienta para evaluar estado ecológico conforme a las exigencias de la DMA, que contempla los modelos de predicción, ya sean de riqueza, de composición de la comunidad o de otras variables biológicas (Ej: RIVPACS: modelo de predicción aplicado a la evaluación de calidad biológica, y ahora a la del estado ecológico; Clarke *et al*., 2003). En este punto, es importante recordar que este modelo de predicción de riqueza fue planteado desde una perspectiva meramente académica para ver qué factores eran los más importantes en la riqueza. Por lo tanto, únicamente debe ser interpretado como apoyo o primera aproximación al valor real de la riqueza y, sobre todo, solo debe utilizarse en lagunas de montaña de características similares a las incluidas en el artículo II; es decir, con los rangos de valores presentes en la tabla 1. Surgen así una serie de nuevas ideas y mejoras del modelo propuesto: 1) ampliar el rango de valores de las variables utilizadas. En concreto, variables tradicionalmente reconocidas como importantes como altitud (más de 2500 m a.s.l.), tamaño (más de 0.5 Km^2), pH (valores extremos) o profundidad (más de 5 m) y que en nuestro modelo no fueron significativas (ver artículo II) posiblemente por poseer de un estrecho rango de valores; 2) extensión del modelo a sistemas o conjuntos de lagunas de montaña (como el conjunto de lagunas estudiadas en el artículo IV); 3) comprobar respuestas no lineales de riqueza, es decir, profundizar en la obtención de modelos con mayor resolución o detalle; 4) elaboración de otro modelo solo a partir de variables no afectadas por la actividad humana. Esto ayudaría a predecir la riqueza que le corresponde a una laguna y, por comparación con la riqueza observada o estimada, a intuir la presencia de degradación ambiental.

CONCLUSIONES GENERALES

- Los métodos no-paramétricos de estimación de riqueza biológica son más adecuados que las técnicas basadas en funciones de acumulación para estimar el número de taxones que integran la comunidad de macroinvertebrados en lagunas de montaña.

- Jackknife 2 es el mejor de los métodos no-paramétricos evaluado, seguido por Jackknife 1, Chao 1 y Chao 2. Se recomienda el uso de Jackknife 1 y Chao 1 cuando se dispone de una sola muestra y de Jackknife 2 y Chao 2 cuando se dispone de múltiples muestras.

- Son muchos los factores que pueden condicionar la riqueza de una laguna de montaña; sin embargo, es indudable que la heterogeneidad del ambiente y determinados impactos (como las introducciones piscícolas intensivas) pueden desempeñar un papel primordial, aun siendo factores frecuentemente excluidos en las investigaciones. Su preponderancia sobre otras variables es una realidad que debe tenerse en cuenta en la gestión y conservación de lagunas de montaña.

- El modelo de predicción de riqueza generado en esta tesis brinda la oportunidad de estimar el número de taxones de macroinvertebrados esperado en una laguna de montaña dada. Aunque preliminar, es una herramienta que puede mostrarse útil en el marco de la gestión de este tipo de ecosistemas.

- La introducción de peces en lagunas de montaña afecta negativamente a la comunidad de macroinvertebrados, no solo en su riqueza sino también en su composición taxonómica. Los efectos únicamente son significativos a altas densidades piscícolas. En ausencia de otros impactos destacados, los sistemas lacustres con bajas densidades piscícolas pueden asimilarse a localidades de referencia, según entiende este concepto la Directiva Marco del Agua.

- Los sistemas lagunares (agrupaciones de lagunas en espacios geográficos reducidos) tienen un papel muy destacado en conservación, ya que albergan una elevada riqueza regional de macroinvertebrados, muy superior a ecosistemas individuales de superficie equivalente.

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ANEXOS

A continuación se incluye el listado de taxones recogidos en las lagunas estudiadas. Los géneros de la familia Chironomidae sólo son utilizados en el estudio comparativo del Parque Natural de Sanabria que se lleva a cabo en el capítulo IV. En el resto de la tesis únicamente se ha utilizado el dato de las tres subfamilias de Chironomidae. Se ha seguido la ordenación de taxones propuesta por el proyecto Fauna Ibérica (Museo Nacional de Ciencias Naturales, CSIC): http://www.fauna-iberica.mncn.csic.es/

- F. Mollusca
  - Cl. Gastropoda
    - SubCl. Pulmonata
      - O. Basommatophora
        - Fam. Physidae
          - *Physa*
        - Fam. Lymnaeidae
          - *Radix*
          - *Lymnaea*
        - Fam. Planorbidae
          - *Gyraulus*
        - Fam. Ancylidae
          - *Ancylus*
  - Cl. Bivalvia
    - SubCl. Heterodonta
      - O. Veneroida
        - Fam. Sphaeriidae
          - *Pisidium*

- F. Annelida
  - Cl. Hirudinea
    - O. Arhynchobdellida
      - Fam. Erpobdellidae
        - *Erpobdella*
      - Fam. Hirudinidae
    - O. Rhynchobdellida
      - Fam. Glossiphoniidae
        - *Glossiphonia*
        - *Helobdella*
  - Cl. Oligochaeta.

- F. Arthropoda
  - SuperCl. Crustacea
    - Cl. Branchiopoda
      - O. Anostraca
        - Fam. Chirocephalidae
          - *Chirocephalus*
    - Cl. Malacostraca
      - O.Amphipoda
        - Fam. Gammaridae
          - *Gammarus*
      - O. Decapoda
        - Fam. Astacidae
          - *Pacifastacus*

- SuperCl. Insecta
  - Cl. Euentomata
    - O. Odonata
      - Fam. Lestidae
        - *Lestes*
      - Fam. Coenagrionidae
        - *Coenagrion/Ischnura*
        - *Erythromma lindeni*
        - *Enallagma*
        - *Pyrrhosoma*
      - Fam. Aeshnidae
      - Fam. Libellulidae
        - *Libellula*
        - *Sympetrum*
    - O. Ephemeroptera
      - Fam. Baetidae
        - *Cloeon*
        - *Procloeon*
      - Fam. Caenidae
        - *Caenis*
      - Fam. Ephemerellidae
        - *Eurylophella*
      - Fam. Heptagenidae
        - *Ecdyonurus*
      - Fam. Leptophlebiidae
        - *Habrophlebia*
        - *Thraulus*
      - Fam. Siphlonuridae
        - *Siphlonurus*
    - O. Plecoptera
      - Fam. Perlodidae
      - Fam. Perlidae
        - *Dinocras*
      - Fam. Nemouridae
        - *Nemoura*
      - Fam. Leuctridae
        - *Leuctra*
    - O. Hemiptera
      - Fam. Gerridae
        - *Gerris*
      - Fam. Hydrometridae
        - *Hydrometra*
      - Fam. Veliidae
        - *Microvelia*
      - Fam. Notonectidae
        - *Notonecta*
      - Fam. Pleidae
        - *Plea*
      - Fam. Naucoridae
        - *Naucoris*
      - Fam. Corixidae
        - *Glaenocorisa*

- O. Megaloptera
    - Fam. Sialidae
        - *Sialis*
- O. Coleoptera
    - SubO. Adephaga
        - Fam. Gyrinidae
            - *Gyrinus*
        - Fam. Haliplidae
            - *Brychius*
            - *Haliplus*
        - Fam. Noteridae
            - *Noterus*
        - Fam. Hygrobiidae
            - *Hygrobia*
        - Fam. Dytiscidae
            - Subfam. Laccophilinae
                - *Laccophilus*
            - Subfam. Hydroporinae
                - *Bidessus*
                - *Hydroglyphus*
                - *Hygrotus*
                - *Hydroporus*
                - *Graptodytes*
                - *Scarodytes*
                - *Stictonectes*
                - *Deronectes*
                - *Stictotarsus*
                - *Nebrioporus*
            - Subfam. Colymbetinae
                - *Platambus*
                - *Agabus*
                - *Ilybius*
                - *Rhantus*
            - Subfam. Dytiscinae
                - *Acilius*
                - *Dytiscus*
    - SubO. Polyphaga
        - Fam. Hydrophilidae
            - Subfam. Helophorinae
                - *Helophorus*
            - Subfam. Hydrochinae
                - *Hydrochus*
            - Subfam. Hydrophilinae
                - *Berosus*
                - *Anacaena*
                - *Laccobius*
                - *Helochares*
                - *Enochrus*
                - *Hydrobius/Limnoxenus*
        - Fam. Hydraenidae

        - *Hydraena*
        - *Limnebius*
    - Fam. Elmidae
        - *Elmis*
        - *Oulimnius*
    - Fam. Dryopidae
        - *Dryops*

- O. Diptera
  - SubO. Nematocera
    - Superfam. Tipuloidea
      - Fam. Tipulidae
      - Fam. Cylindrotomidae
          - *Phalacrocera*
      - Fam. Limoniidae
          - Tribu Hexatomini
    - Superfam. Culicoidea
      - Fam. Chaoboridae
          - *Chaoborus*
      - Fam. Culicidae
          - *Anophelinae*
        - Subfam. Culicinae
    - Superfam. Chironomoidea
      - Fam. Ceratopogonidae
        - Subfam. Ceratopogoninae
      - Fam. Chironomidae
        - Subfam. Tanypodinae
          - *Tanypus*
          - *Procladius*
          - *Macropelopia*
          - *Ablabesmyia*
          - *Paramerina*
          - *Telopelopia*
        - Subfam. Orthocladiinae
          - *Acricotopus*
          - *Corynoneura*
          - *Cricotopus*
          - *Heterotrissocladius*
          - *Nanocladius*
          - *Parakiefferiella*
          - *Orthocladius*
          - *Psectrocladius*
          - *Paralimnophyes*
        - Subfam. Chironominae
          - *Chironomus*
          - *Cladopelma*
          - *Apedillum*
          - *Cryptochironomus*
          - *Dicrotendipes*
          - *Endochironomus*
          - *Glyptotendipes*
          - *Microtendipes*
          - *Parachironomus*
          - *Paratendipes*
          - *Phaenopsectra*

- *Polypedilum*
- *Stictochironomus*
- *Tribelos*
- *Cladotanytarsus*
- *Micropsectra*
- *Neozavrelia*
- *Paratanytarsus*
- *Stempellina*
- *Stempellinella*
- *Tanytarsus*
- *Virgatanytarsus*
- *Zavreliella*
- *Prodiamesa*

SubO. Brachycera
    Fam. Tabanidae
    Fam. Empididae
    Fam. Dolichopodidae

SubO. Cyclorrhapha
    Fam. Sciomyzidae
    Fam. Ephydridae

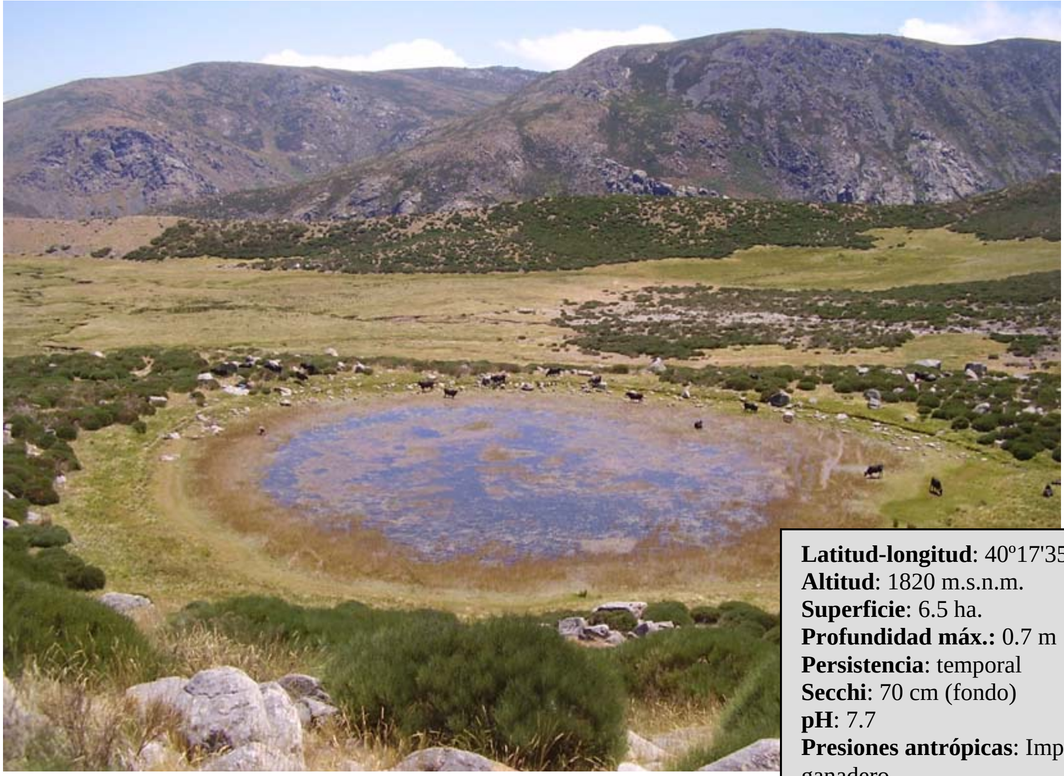
O. Trichoptera
    Fam. Polycentropodidae
        Plectrocnemia/Cyrnus
        Polycentropus
    Fam. Psychomyiidae
        Tinodes
    Fam. Phryganeidae
        Agrypnia
    Fam. Limnephilidae
      Subfam. Limnephilinae
        Tribu Limnephilini
        Tribu Stenophylacini / Chaetopterygini
    Fam. Uenoidae
        Thremma
    Fam. Leptoceridae
        Athripsodes
        Mystacides
    Fam. Sericostomatidae
        Seriscotoma

Latitud-longitud: 40º15'47"-5º18'20"
Altitud: 2140 m.s.n.m.
Superficie: 5.7 ha.
Profundidad máx.: 9.8 m
Persistencia: permanente
Secchi: 950 cm
pH: 6.5
Presiones antrópicas: Introducción piscícola

Foto 1. Laguna Cimera

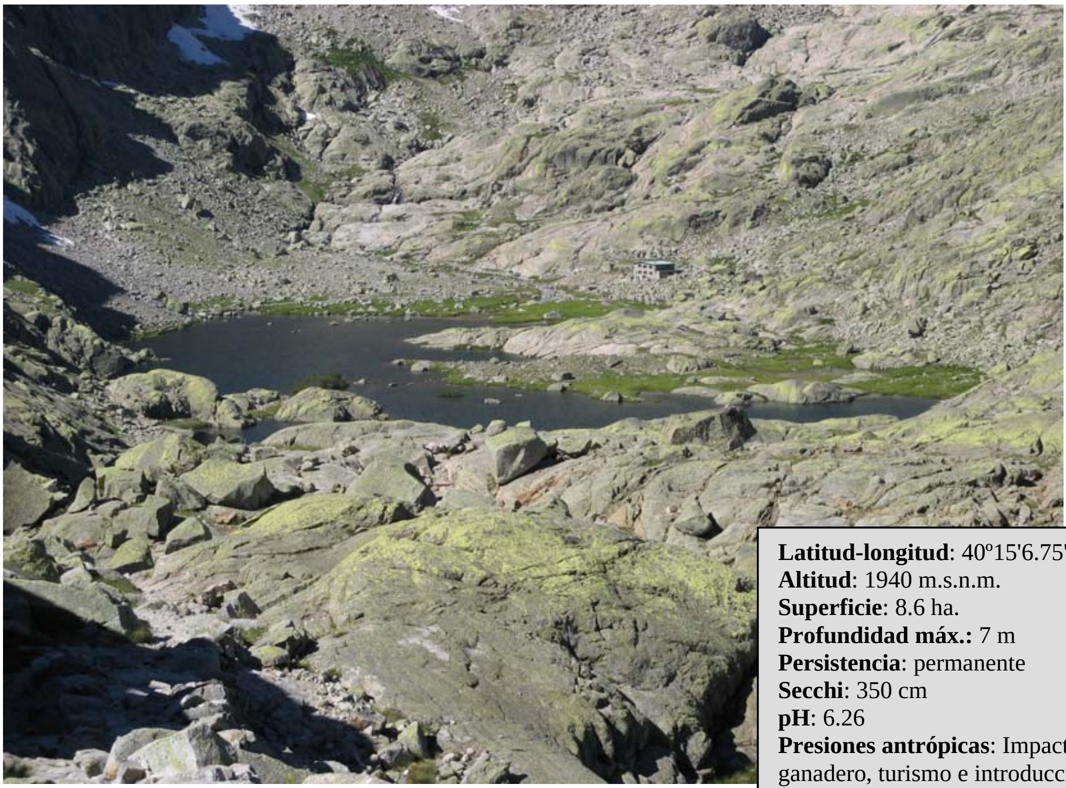
Latitud-longitud: 40º16'5"-5º18'6"
Altitud: 2090 m.s.n.m.
Superficie: 0.5 ha.
Profundidad máx.: 2.5 m
Persistencia: permanente
Secchi: 200 cm
pH: 6.29
Presiones antrópicas: introducción piscícola

Foto 2. Laguna mediana



Latitud-longitud: 40º17'35"-5º17'23"
Altitud: 1820 m.s.n.m.
Superficie: 6.5 ha.
Profundidad máx.: 0.7 m
Persistencia: temporal
Secchi: 70 cm (fondo)
pH: 7.7
Presiones antrópicas: Impacto ganadero

Foto 3. Cervunal



Latitud-longitud: 40º15'6.75"-5º16'39"
Altitud: 1940 m.s.n.m.
Superficie: 8.6 ha.
Profundidad máx.: 7 m
Persistencia: permanente
Secchi: 350 cm
pH: 6.26
Presiones antrópicas: Impacto ganadero, turismo e introducción piscícola

Foto 4. Grande de Gredos

Foto 5. Laguna Cuadrada

Latitud-longitud: 40º13'16"-5º36'6"
Altitud: 2080 m.s.n.m.
Superficie: 0.7 ha.
Profundidad máx.: 3.8 m
Persistencia: permanente
Secchi: 380 cm (fondo)
pH: 6.11
Presiones antrópicas: 0

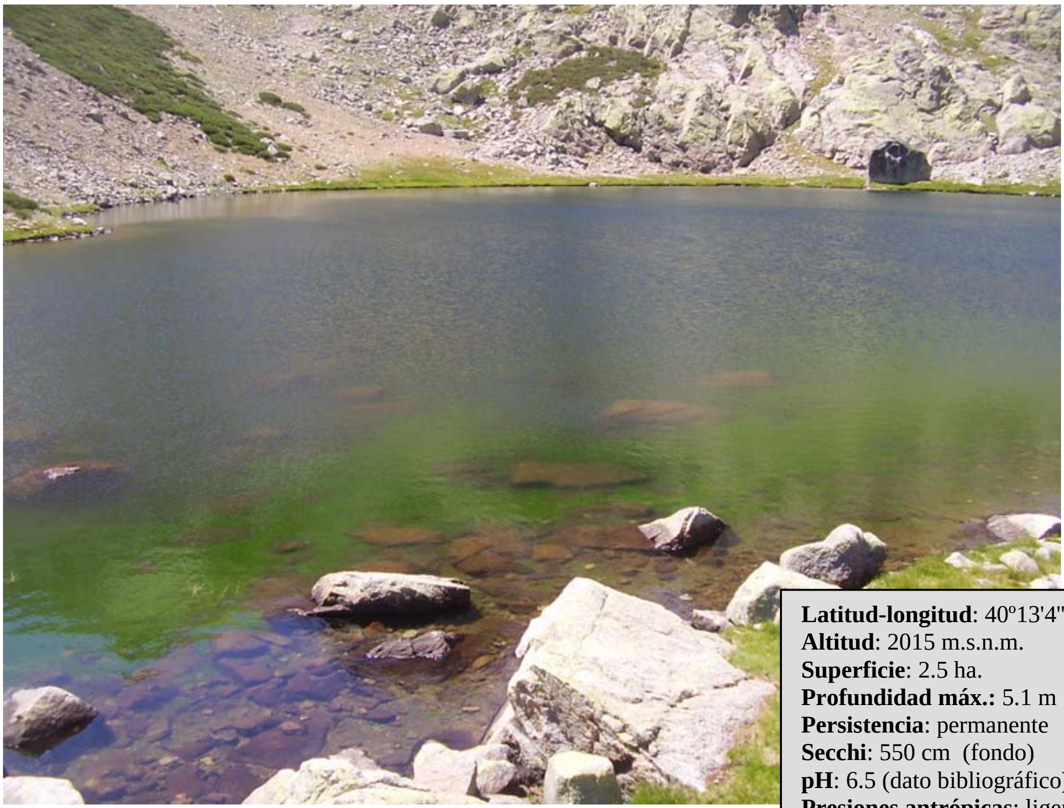
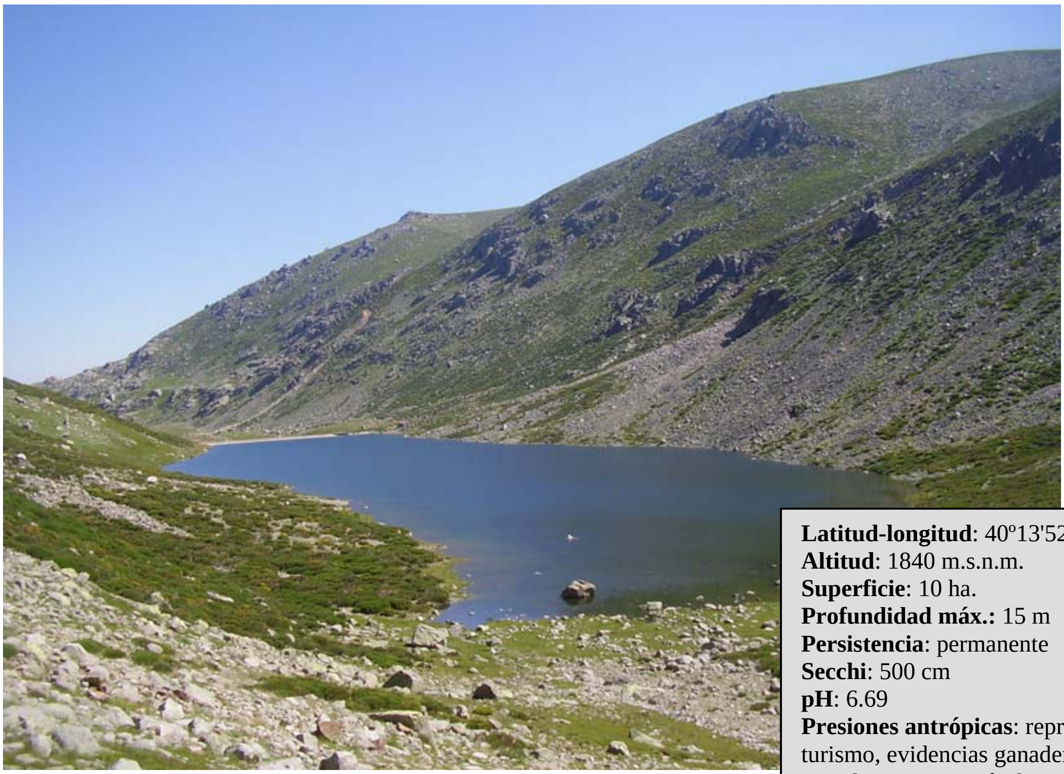


Foto 6. Laguna de los Caballeros

Latitud-longitud: 40º13'4"-5º35'18"
Altitud: 2015 m.s.n.m.
Superficie: 2.5 ha.
Profundidad máx.: 5.1 m
Persistencia: permanente
Secchi: 550 cm (fondo)
pH: 6.5 (dato bibliográfico)
Presiones antrópicas: ligeras evidencias ganaderas, ¿peces?



Latitud-longitud: 40º13'52"-5º36'14"
Altitud: 1840 m.s.n.m.
Superficie: 10 ha.
Profundidad máx.: 15 m
Persistencia: permanente
Secchi: 500 cm
pH: 6.69
Presiones antrópicas: represada, turismo, evidencias ganaderas e introducciones piscícolas

Foto 7. Laguna el Barco

Latitud-longitud: 40º18'32"-5º43'1"
Altitud: 2120 m.s.n.m.
Superficie: 1.3 ha.
Profundidad máx.: 4.1 m
Persistencia: permanente
Secchi: 410 cm (fondo)
pH: 6.69
Presiones antrópicas: evidencias ganaderas e introducciones piscícolas

Foto 8. Laguna El Trampal

Latitud-longitud: 42º7'22"-6º43'17"
Altitud: 997 m.s.n.m.
Superficie: 319 ha.
Profundidad máx.: 50 m
Persistencia: permanente
Secchi: 650 cm
pH: 6.5
Presiones antrópicas: Turismo e introducciones piscícolas

Foto 9. Lago de Sanabria

Foto 10. Lago de Sanabria

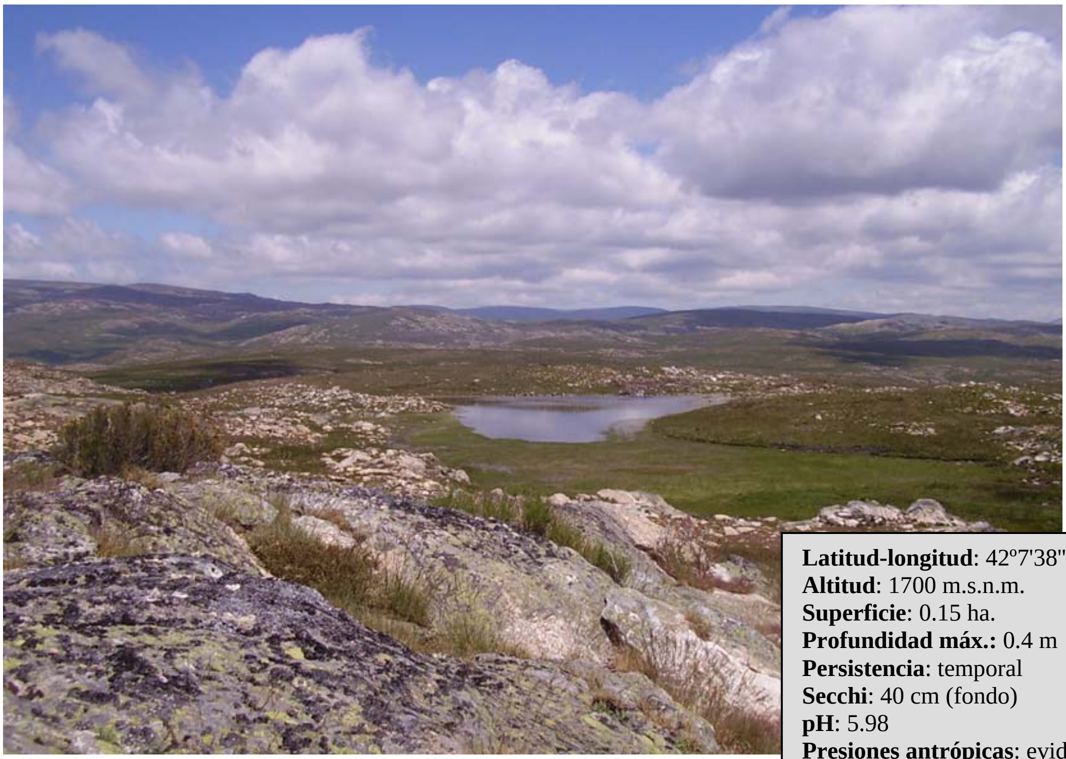


Foto 11. Camposagrado II

Latitud-longitud: 42º7'38"-6º49'48"
Altitud: 1700 m.s.n.m.
Superficie: 0.15 ha.
Profundidad máx.: 0.4 m
Persistencia: temporal
Secchi: 40 cm (fondo)
pH: 5.98
Presiones antrópicas: evidencias ganaderas, ¿peces?

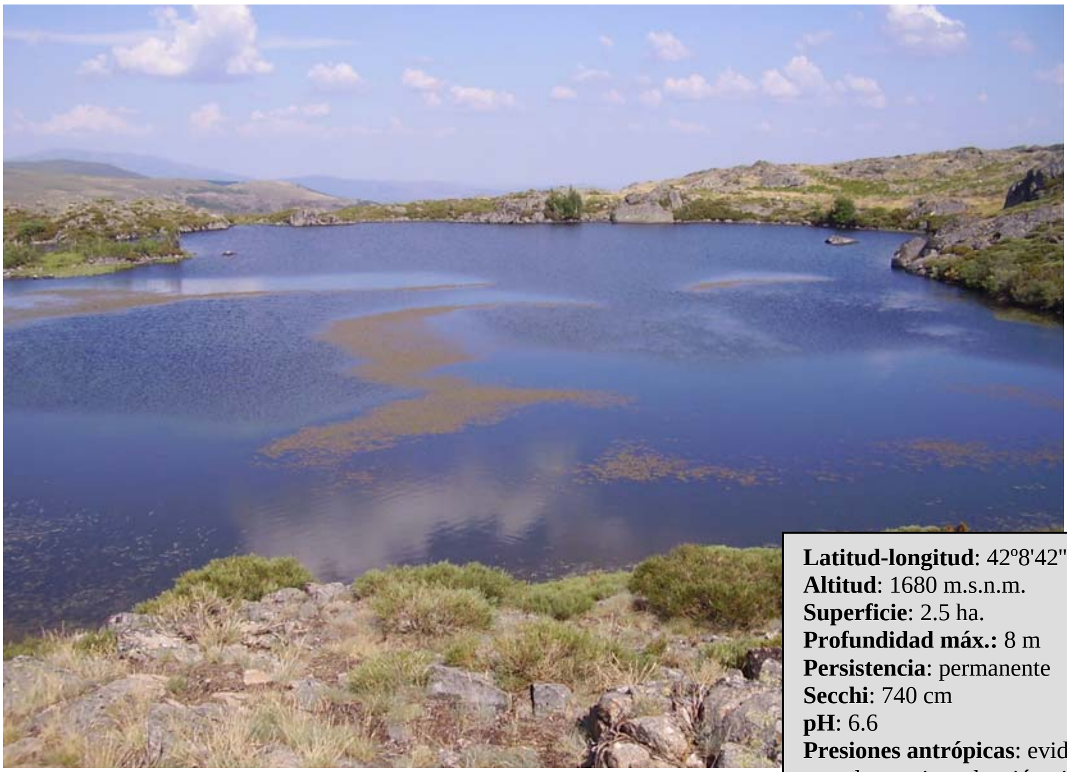
Foto 12. El Payón

Latitud-longitud: 42º7'56"-6º46'35"
Altitud: 1590 m.s.n.m.
Superficie: 1.6 ha.
Profundidad máx.: 6.5 m
Persistencia: permanente
Secchi: 150 cm
pH: 6.72
Presiones antrópicas: evidencias ganaderas, ¿peces?

Latitud-longitud: 42º11'51"-6º47'30"
Altitud: 1700 m.s.n.m.
Superficie: 11.1 ha.
Profundidad máx.: 4.8 m
Persistencia: permanente
Secchi: 480 cm (fondo)
pH: 6.63
Presiones antrópicas: evidencias ganaderas e introducción piscícola

Foto 13. Lacillo

Foto 14. Lacillo



Latitud-longitud: 42º8'42"-6º46'45"
Altitud: 1680 m.s.n.m.
Superficie: 2.5 ha.
Profundidad máx.: 8 m
Persistencia: permanente
Secchi: 740 cm
pH: 6.6
Presiones antrópicas: evidencias ganaderas e introducción piscícola

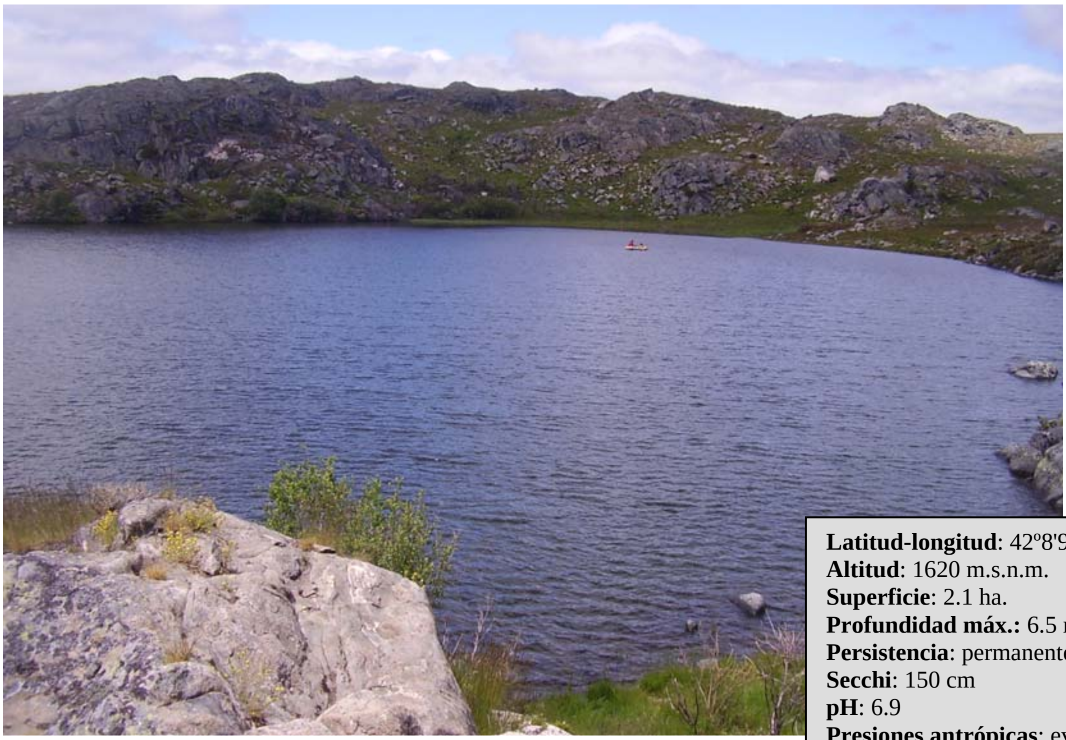
Foto 15. El Cuadro

Latitud-longitud: 42º7'10"-6º48'32
Altitud: 1600 m.s.n.m.
Superficie: 5.7 ha.
Profundidad máx.: 12 m
Persistencia: permanente
Secchi: 1000 cm
pH: 6.3
Presiones antrópicas: evidencias ganaderas e introducción piscícola

Foto 16. Clara grande

Latitud-longitud: 42º7'1"-6º48'36"
Altitud: 1600 m.s.n.m.
Superficie: 1.5 ha.
Profundidad máx.: 3.5 m
Persistencia: permanente
Secchi: 350 cm (fondo)
pH: 6.02
Presiones antrópicas: evidencias ganaderas e introducción piscícola

Foto 17. Clara pequeña



Latitud-longitud: 42º8'9"-6º47'39
Altitud: 1620 m.s.n.m.
Superficie: 2.1 ha.
Profundidad máx.: 6.5 m
Persistencia: permanente
Secchi: 150 cm
pH: 6.9
Presiones antrópicas: evidencias ganaderas e introducción piscícola

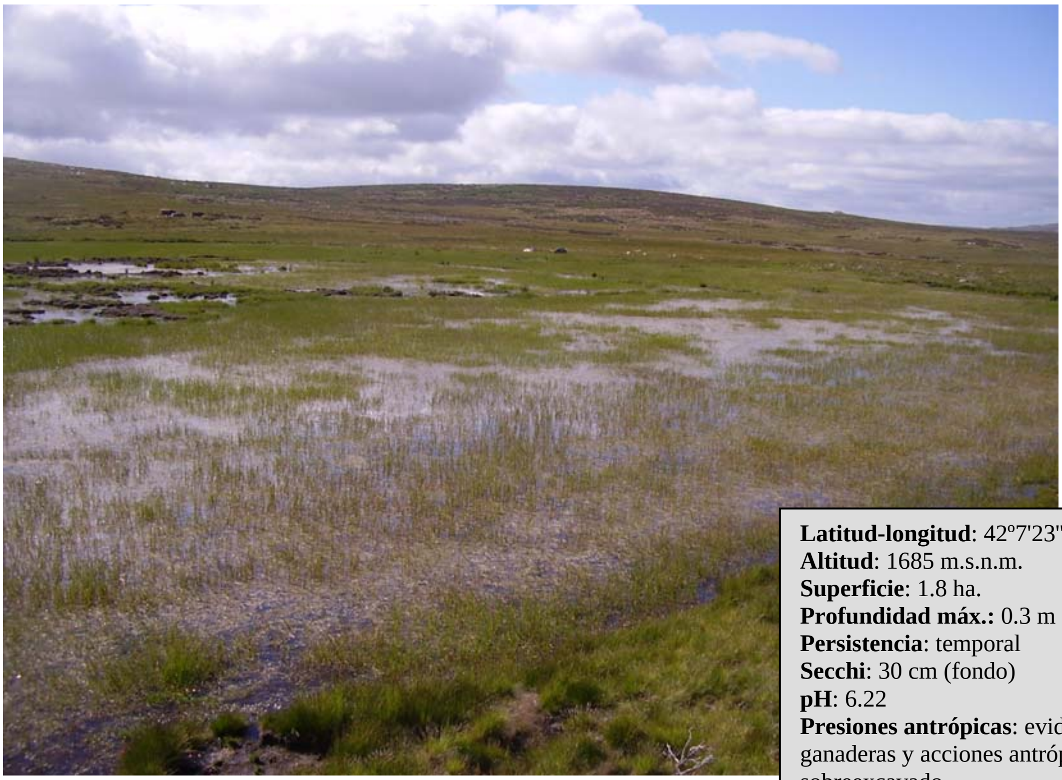
Foto 18. Roya grande

Latitud-longitud: 42º8'5"-6º47'31"
Altitud: 1620 m.s.n.m.
Superficie: 1 ha.
Profundidad máx.: 2 m
Persistencia: permanente
Secchi: 200 cm (fondo)
pH: 6.33
Presiones antrópicas: evidencias ganaderas e introducción piscícola

Foto 19. Roya pequeña

Latitud-longitud: 42º6'20"-6º48'6"
Altitud: 1730 m.s.n.m.
Superficie: 7.7 ha.
Profundidad máx.: 5.6 m
Persistencia: permanente
Secchi: 275 cm
pH: 6.24
Presiones antrópicas: evidencias ganaderas e introducción piscícola

Foto 20. Pedrina



Latitud-longitud: 42º7'23"-6º50'20"
Altitud: 1685 m.s.n.m.
Superficie: 1.8 ha.
Profundidad máx.: 0.3 m
Persistencia: temporal
Secchi: 30 cm (fondo)
pH: 6.22
Presiones antrópicas: evidencias ganaderas y acciones antrópicas de sobreexcavado

Foto 21. Castromil

Latitud-longitud: 42º11'7"-6º47'50"
Altitud: 1805 m.s.n.m.
Superficie: 2.2 ha.
Profundidad máx.: 1.9 m
Persistencia: permanente
Secchi: 100 cm
pH: 6.92
Presiones antrópicas: evidencias ganaderas e introducciones piscícolas

Foto 22. Aguas Cernidas

Latitud-longitud: 42º8'34"-6º46'30"
Altitud: 1600 m.s.n.m.
Superficie: 2.8 ha.
Profundidad máx.: 8 m
Persistencia: permanente
Secchi: 200 cm
pH: 7.11
Presiones antrópicas: evidencias ganaderas, represamiento e introducciones piscícolas

Foto 23. Mancas

Latitud-longitud: 42º5'53"-6º46'40"
Altitud: 1580 m.s.n.m.
Superficie: 12 ha.
Profundidad máx.: 5.6 m (dato bibliográfico
Persistencia: permanente
Secchi: 250 cm
pH: 7.1
Presiones antrópicas: evidencias ganaderas, represamiento e introducciones piscícolas

Foto 24. Sotillo

Foto 25. La Yegua

Latitud-longitud: 42º11'22"-6º43'35"
Altitud: 1790 m.s.n.m.
Superficie: 4.2 ha.
Profundidad máx.: 6.5 m
Persistencia: permanente
Secchi: 400 cm
pH: 6.72
Presiones antrópicas: evidencias ganaderas, turismo e introducciones piscícolas

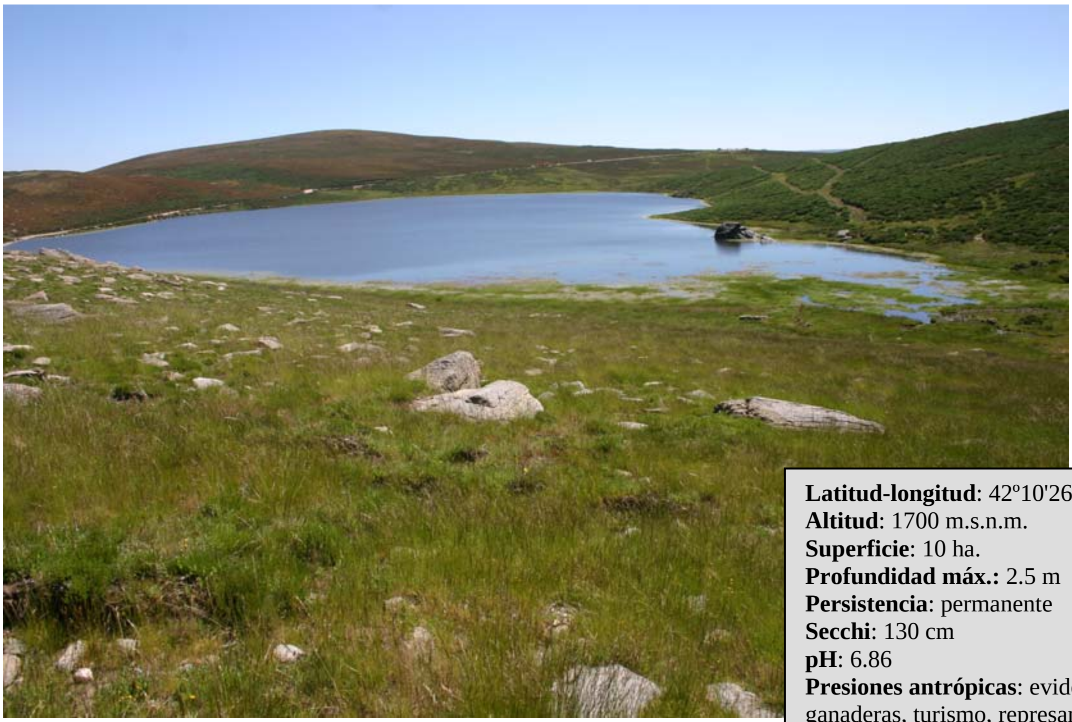
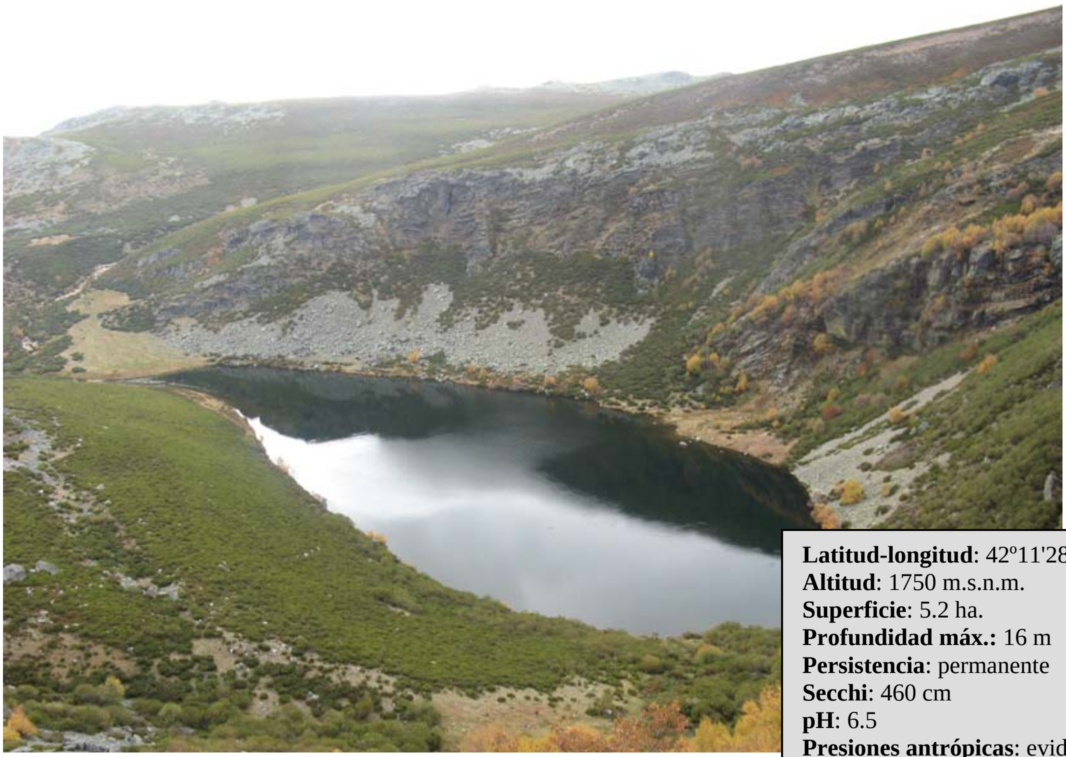


Foto 26. Los Peces

Latitud-longitud: 42º10'26"-6º43'55"
Altitud: 1700 m.s.n.m.
Superficie: 10 ha.
Profundidad máx.: 2.5 m
Persistencia: permanente
Secchi: 130 cm
pH: 6.86
Presiones antrópicas: evidencias ganaderas, turismo, represamiento e introducciones piscícolas



Latitud-longitud: 42º11'28"-6º29'40"
Altitud: 1750 m.s.n.m.
Superficie: 5.2 ha.
Profundidad máx.: 16 m
Persistencia: permanente
Secchi: 460 cm
pH: 6.5
Presiones antrópicas: evidencias ganaderas e introducciones piscícolas

Foto 27. Truchillas

Latitud-longitud: 42º15'23"-6º45'8"
Altitud: 1400 m.s.n.m.
Superficie: 2.5 ha.
Profundidad máx.: 2 m
Persistencia: permanente
Secchi: 200 cm
pH: 6.2
Presiones antrópicas: evidencias ganaderas, manipulación antrópica de la cuenca e introducciones piscícolas

Foto 28. La Baña

Latitud-longitud: 42º58'42"-6º11'46"
Altitud: 1430 m.s.n.m.
Superficie: 3.4 ha.
Profundidad máx.: 3.6 m
Persistencia: permanente
Secchi: 120 cm
pH: 9
Presiones antrópicas: evidencias ganaderas, turismo e introducciones piscícolas

Foto 29. Laguna Grande de Babia

Latitud-longitud: 43º0'12"-6º8'20"
Altitud: 1720 m.s.n.m.
Superficie: 1 ha.
Profundidad máx.: 2.5 m
Persistencia: permanente
Secchi: 250 cm (fondo)
pH: 7.8
Presiones antrópicas: evidencias ganaderas e introducciones

Foto 30. Las Verdes

Latitud-longitud: 42º58'16''-6º13'37''
Altitud: 1500 m.s.n.m.
Superficie: 0.4 ha.
Profundidad máx.: 0.5 m
Persistencia: temporal
Secchi: 50 cm (fondo)
pH: 4.7
Presiones antrópicas: evidencias ganaderas

Foto 31. La Mata I

Latitud-longitud: 42º58'5''-6º13'32''
Altitud: 1480 m.s.n.m.
Superficie: 0.3 ha.
Profundidad máx.: 0.5 m
Persistencia: temporal
Secchi: 50 cm (fondo)
pH: 5.3
Presiones antrópicas: evidencias ganaderas

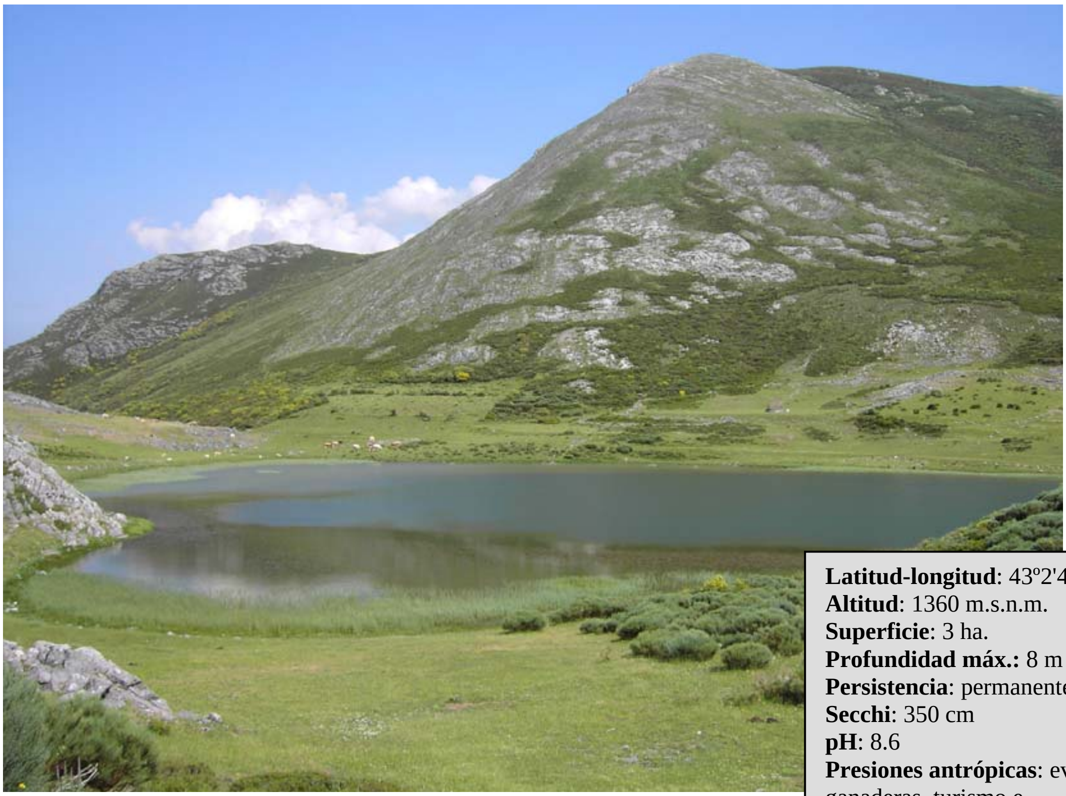
Foto 32. La Mata II

Latitud-longitud: 42º54'15"-6º4'53"
Altitud: 1750 m.s.n.m.
Superficie: 0.5 ha.
Profundidad máx.: 4 m
Persistencia: permanente
Secchi: 80 cm (fondo)
pH:
Presiones antrópicas: evidencias ganaderas, ¿peces?

Foto 33. Riolago de Babia.

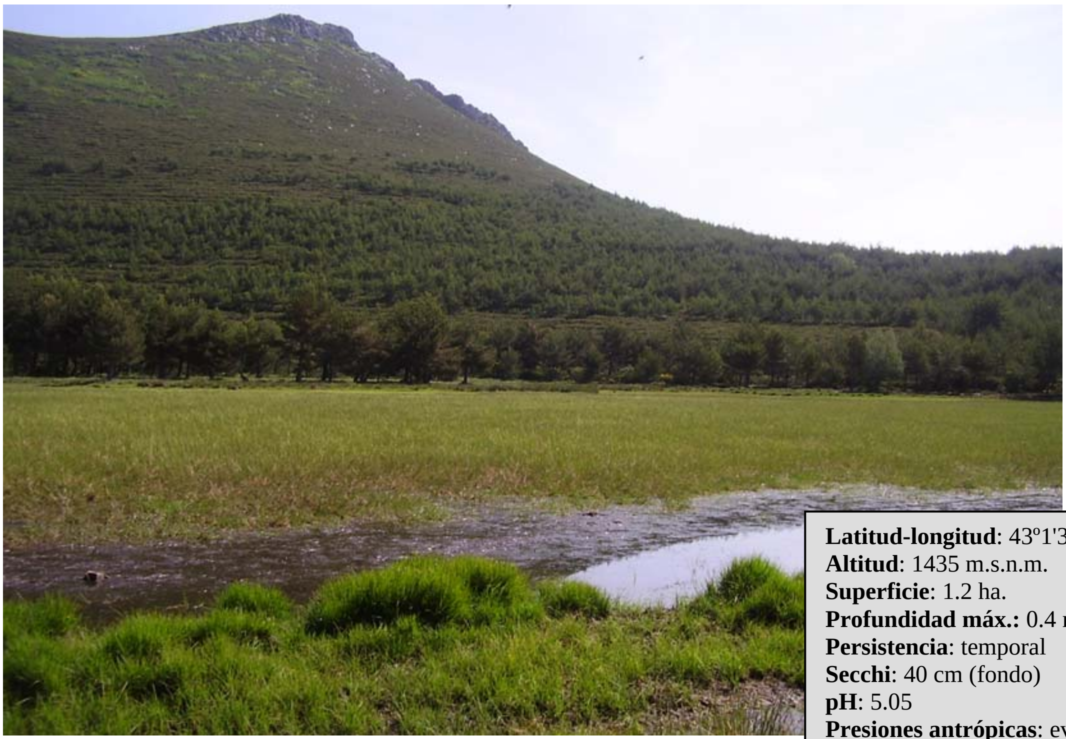
Latitud-longitud: 43º2'38"-5º21'15"
Altitud: 1750 m.s.n.m.
Superficie: 3.6 ha.
Profundidad máx.: 14 m
Persistencia: permanente
Secchi: 760 cm
pH: 5
Presiones antrópicas: evidencias ganaderas

Foto 34. Lago Ausente



Latitud-longitud: 43º2'46"-5º18'54
Altitud: 1360 m.s.n.m.
Superficie: 3 ha.
Profundidad máx.: 8 m
Persistencia: permanente
Secchi: 350 cm
pH: 8.6
Presiones antrópicas: evidencias ganaderas, turismo e introducciones piscícolas

Foto 35. Lago de Isoba



Latitud-longitud: 43º1'38"-5º19'56
Altitud: 1435 m.s.n.m.
Superficie: 1.2 ha.
Profundidad máx.: 0.4 m
Persistencia: temporal
Secchi: 40 cm (fondo)
pH: 5.05
Presiones antrópicas: evidencias ganaderas.

Foto 36. Laguna de Robledo

Latitud-longitud: 43º3'29"-5º14'18"
Altitud: 1770 m.s.n.m.
Superficie: 1.2 ha.
Profundidad máx.: 0.3 m
Persistencia: temporal
Secchi: 30 cm (fondo)
pH: 7
Presiones antrópicas: evidencias ganaderas

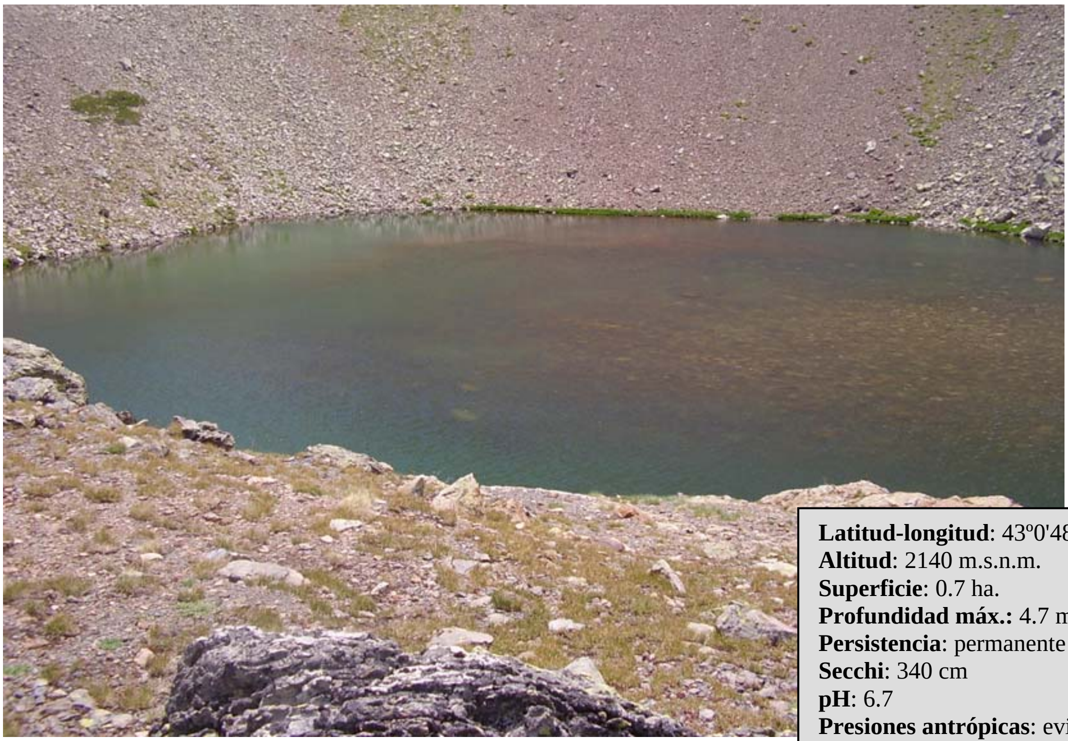
Foto 37. Laguna de Tronisco

Latitud-longitud: 42º59'24"-4º41'10"
Altitud: 1790 m.s.n.m.
Superficie: 2.7 ha.
Profundidad máx.: 8.5 m
Persistencia: permanente
Secchi: 590 cm
pH: 6.34
Presiones antrópicas: evidencias ganaderas e introducciones piscícolas

Foto 38. Pozo Curavacas

Latitud-longitud: 43º0'39"-4º44'42"
Altitud: 2060 m.s.n.m.
Superficie: 2.3 ha.
Profundidad máx.: 7.6 m
Persistencia: permanente
Secchi: 760 cm (fondo)
pH: 6.86
Presiones antrópicas: evidencias ganaderas

Foto 39. Pozo Las Lomas

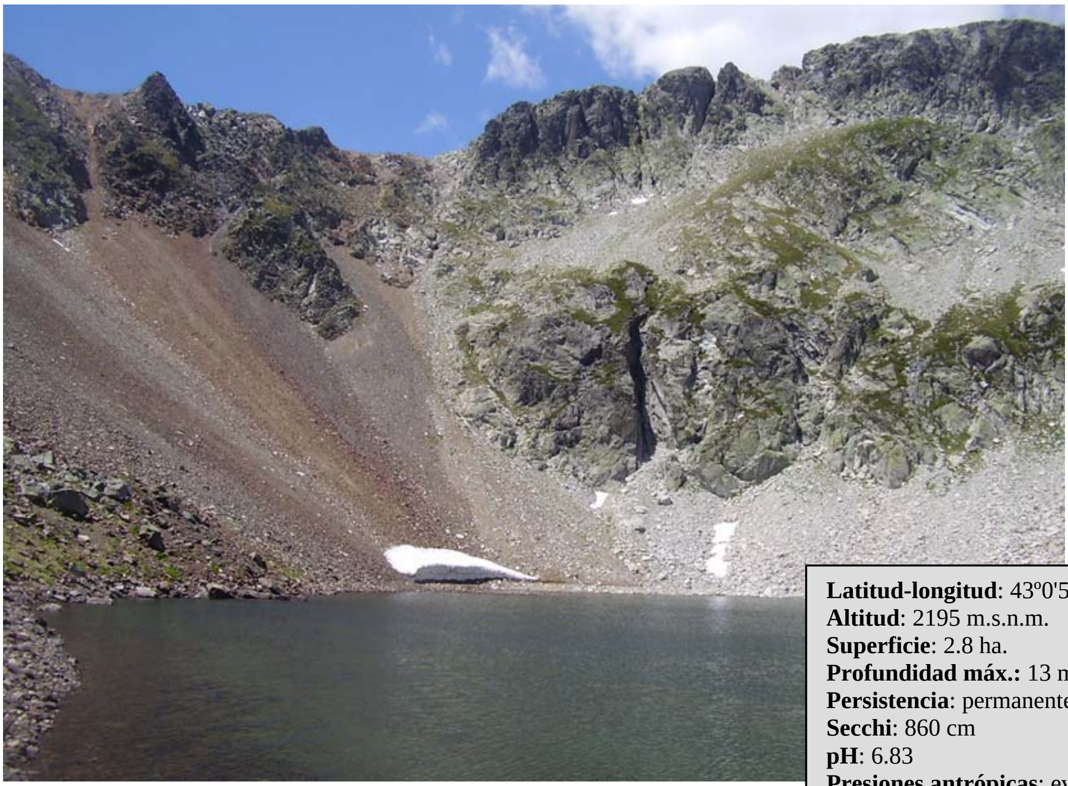


Latitud-longitud: 43º0'48"-4º45'50"
Altitud: 2140 m.s.n.m.
Superficie: 0.7 ha.
Profundidad máx.: 4.7 m
Persistencia: permanente
Secchi: 340 cm
pH: 6.7
Presiones antrópicas: evidencias ganaderas

Foto 40. Hoyos de Vargas I

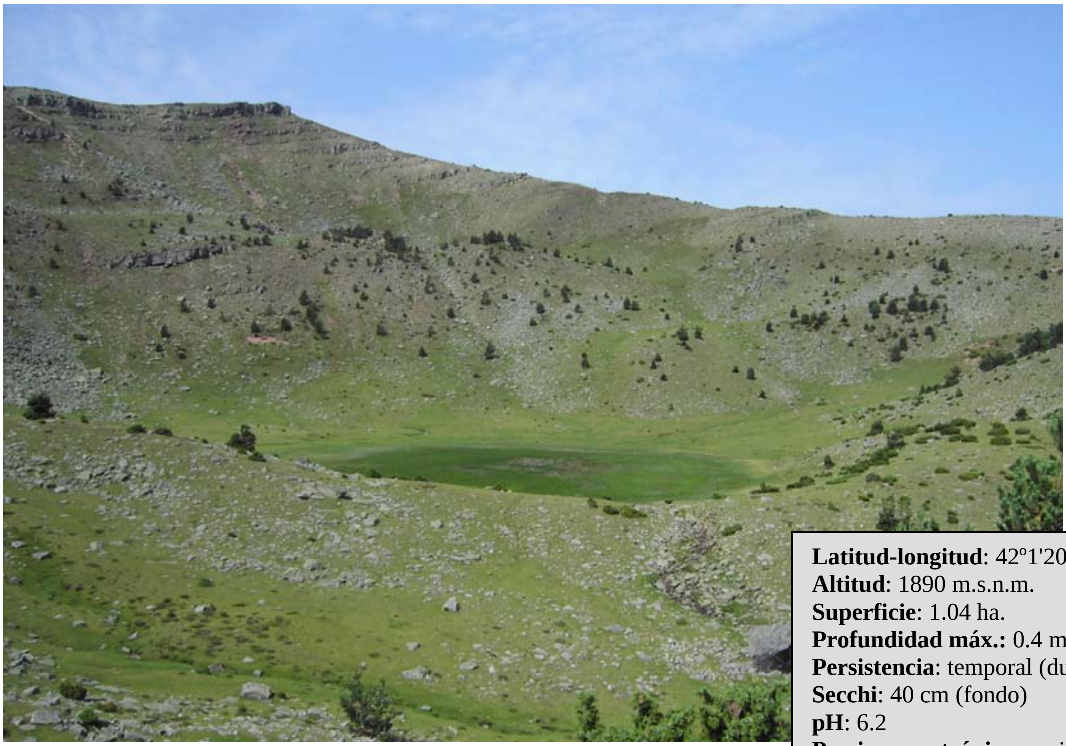
Latitud-longitud: 43º0'51"-4º45'59"
Altitud: 2120 m.s.n.m.
Superficie: 0.6 ha.
Profundidad máx.: 3.8 m
Persistencia: permanente
Secchi: 220 cm
pH: 6.6
Presiones antrópicas: evidencias ganaderas

Foto 41. Lagunas de Hoyo de Vargas II



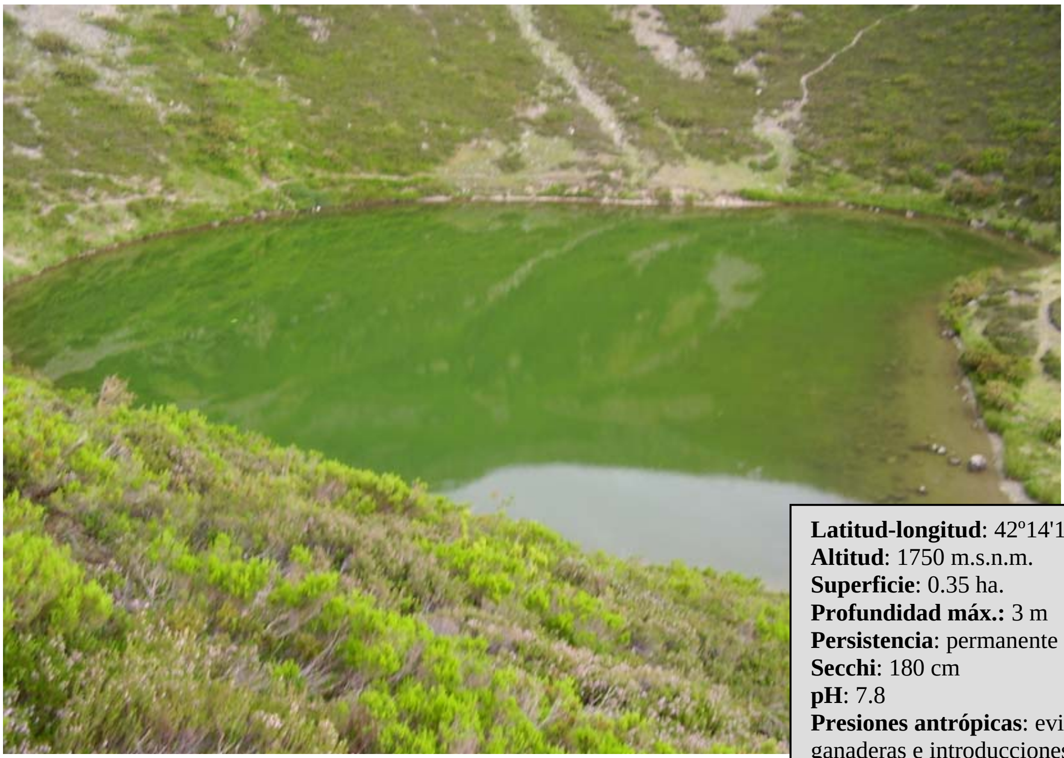
Latitud-longitud: 43º0'56"-4º44'1"
Altitud: 2195 m.s.n.m.
Superficie: 2.8 ha.
Profundidad máx.: 13 m
Persistencia: permanente
Secchi: 860 cm
pH: 6.83
Presiones antrópicas: evidencias ganaderas

Foto 42. Fuentes Carrionas



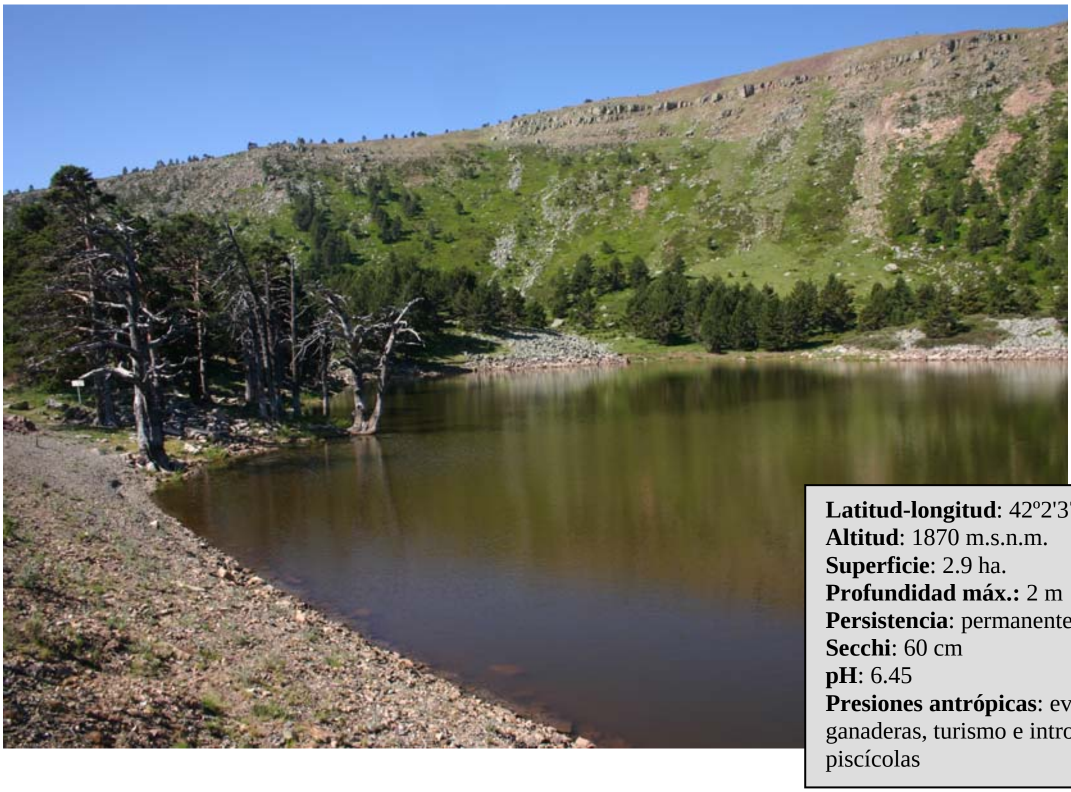
Latitud-longitud: 42º1'20''-2º55'29''
Altitud: 1890 m.s.n.m.
Superficie: 1.04 ha.
Profundidad máx.: 0.4 m
Persistencia: temporal (dudoso)
Secchi: 40 cm (fondo)
pH: 6.2
Presiones antrópicas: evidencias ganaderas

Foto 43. Laguna de Muñalba



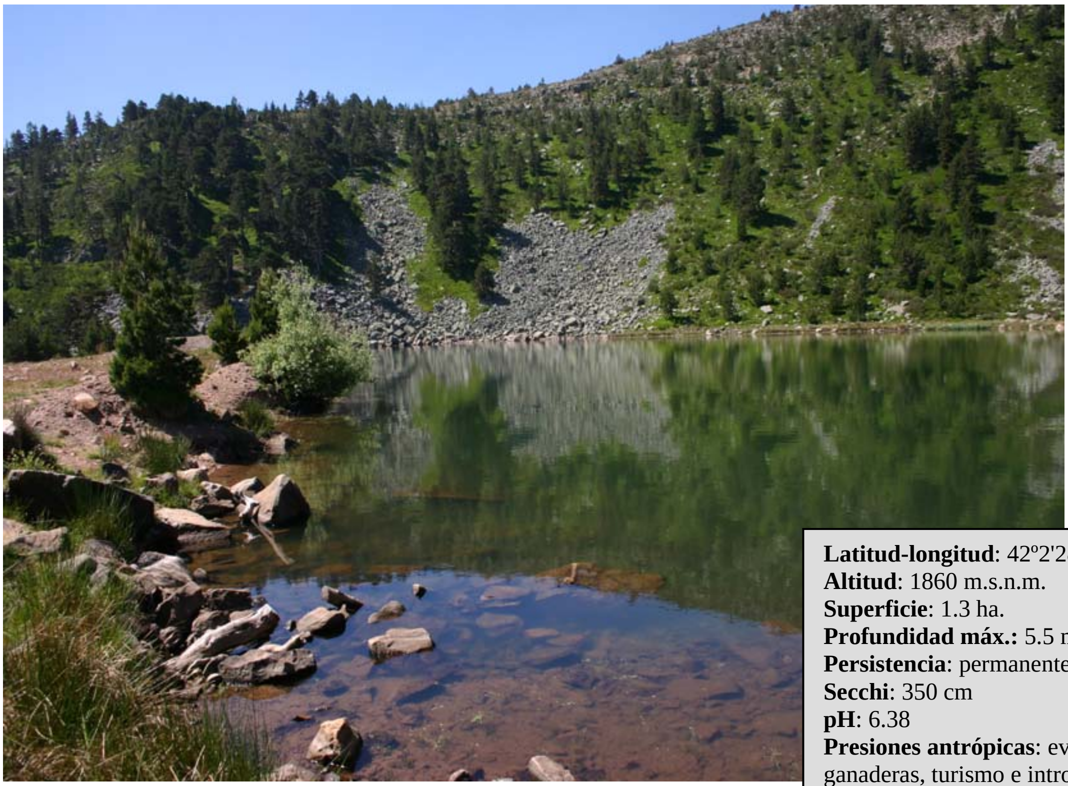
Latitud-longitud: 42º14'10''-3º6'26''
Altitud: 1750 m.s.n.m.
Superficie: 0.35 ha.
Profundidad máx.: 3 m
Persistencia: permanente
Secchi: 180 cm
pH: 7.8
Presiones antrópicas: evidencias ganaderas e introducciones piscícolas

Foto 44. Pozo Negro



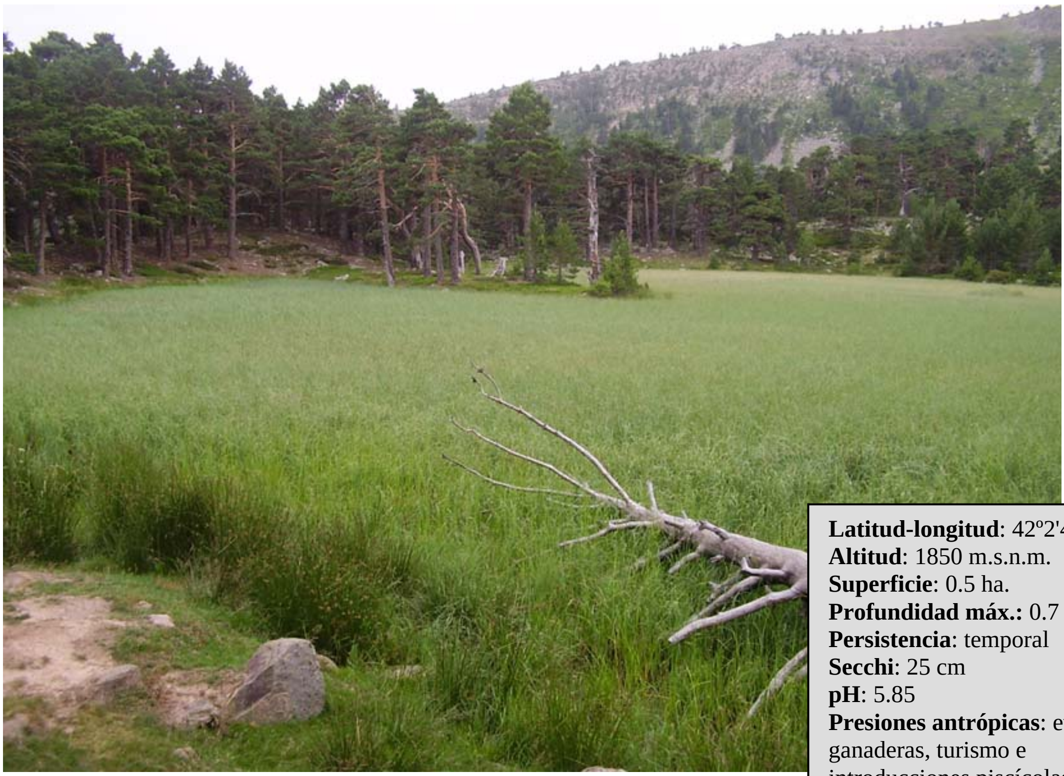
Latitud-longitud: 42º2'37"-3º2'47"
Altitud: 1870 m.s.n.m.
Superficie: 2.9 ha.
Profundidad máx.: 2 m
Persistencia: permanente
Secchi: 60 cm
pH: 6.45
Presiones antrópicas: evidencias ganaderas, turismo e introducciones piscícolas

Foto 45. Laguna de Los Patos



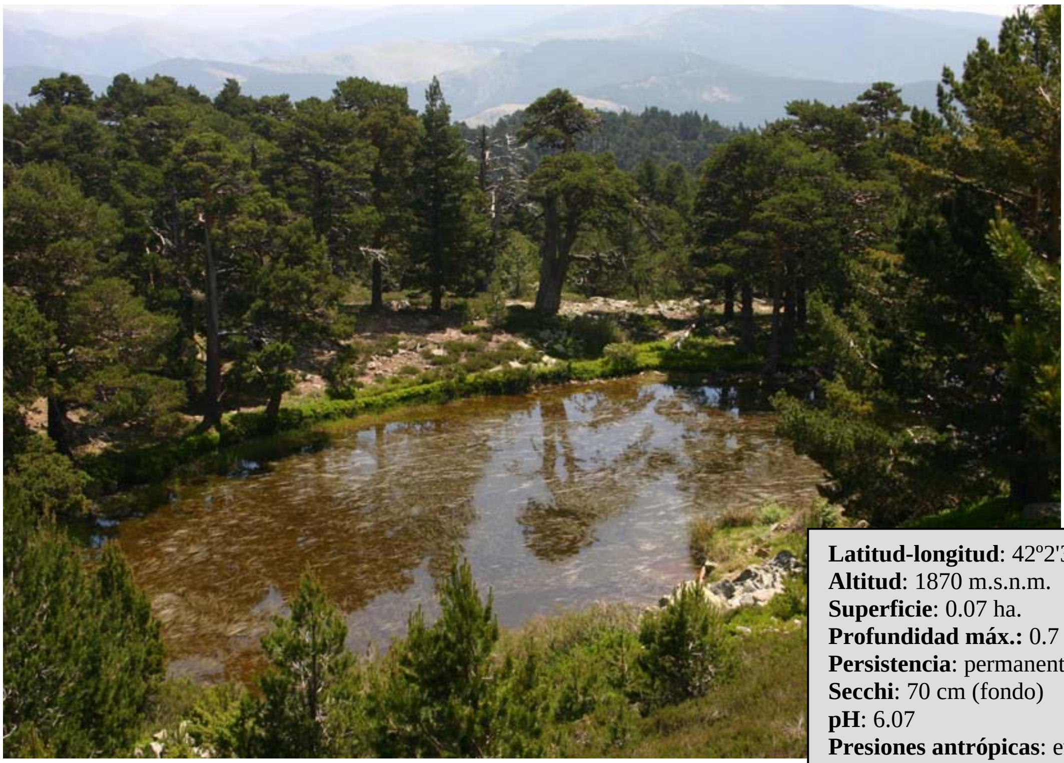
Latitud-longitud: 42º2'28"-3º2'36"
Altitud: 1860 m.s.n.m.
Superficie: 1.3 ha.
Profundidad máx.: 5.5 m
Persistencia: permanente
Secchi: 350 cm
pH: 6.38
Presiones antrópicas: evidencias ganaderas, turismo e introducciones piscícolas

Foto 46. Laguna Brava



Latitud-longitud: 42º2'42"-3º2'37"
Altitud: 1850 m.s.n.m.
Superficie: 0.5 ha.
Profundidad máx.: 0.7 m
Persistencia: temporal
Secchi: 25 cm
pH: 5.85
Presiones antrópicas: evidencias ganaderas, turismo e introducciones piscícolas

Foto 47. Las Pardillas I

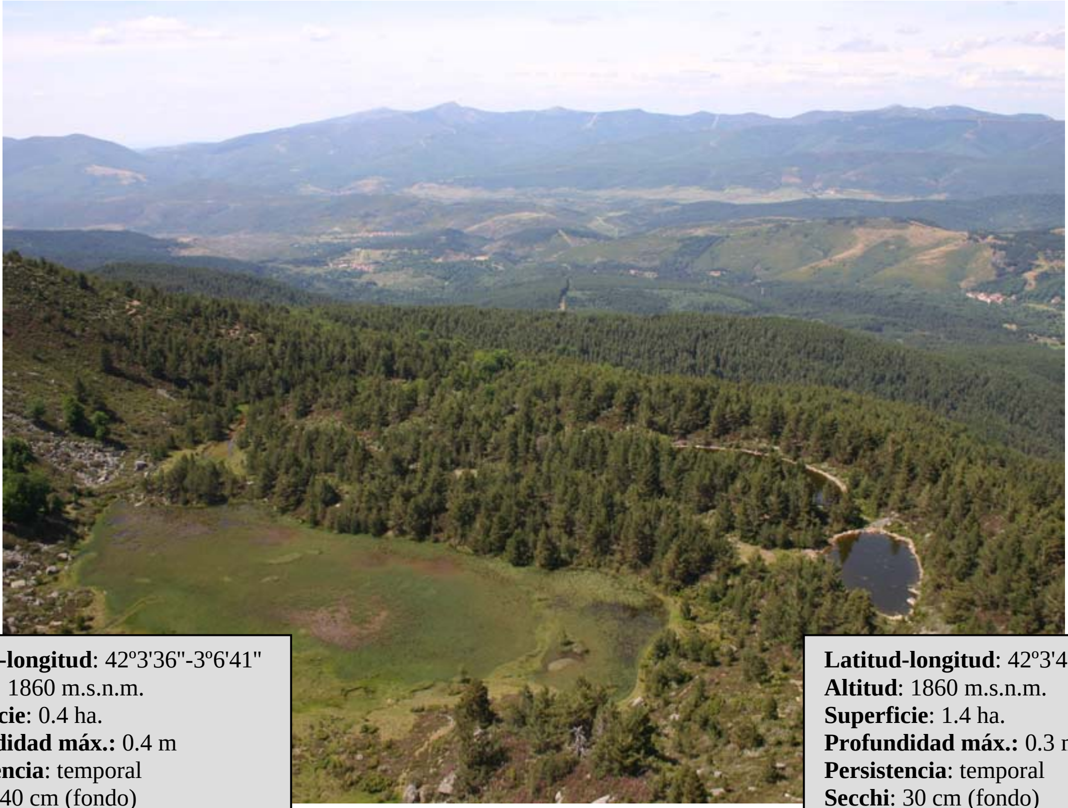


Latitud-longitud: 42º2'38"-3º2'39"
Altitud: 1870 m.s.n.m.
Superficie: 0.07 ha.
Profundidad máx.: 0.7 m
Persistencia: permanente
Secchi: 70 cm (fondo)
pH: 6.07
Presiones antrópicas: evidencias ganaderas, turismo e introducciones piscícolas

Foto 48. Las Pardillas II

Latitud-longitud: 42º2'55''-3º3'46''
Altitud: 1900 m.s.n.m.
Superficie: 11.8 ha.
Profundidad máx.: 15 m
Persistencia: permanente
Secchi: 400 cm
pH: 6.98
Presiones antrópicas: evidencias ganaderas, turismo e introducciones piscícolas

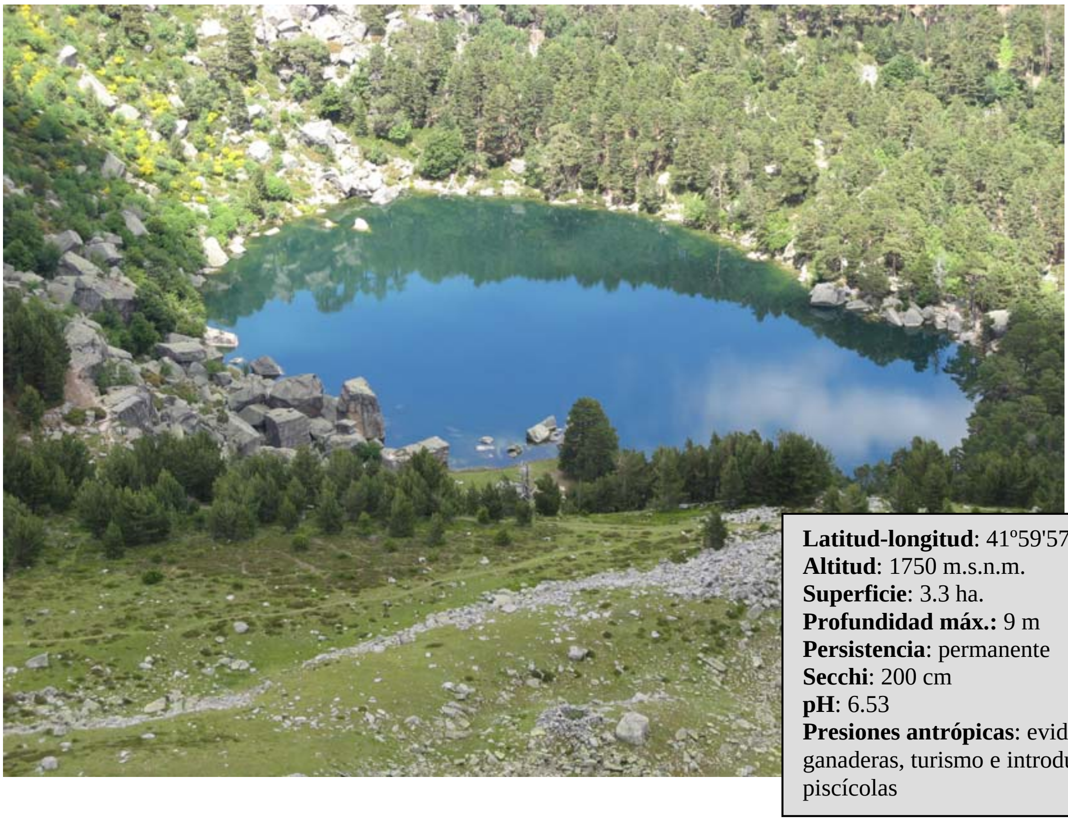
Foto 49. Laguna Negra de Neila



Latitud-longitud: 42º3'36''-3º6'41''
Altitud: 1860 m.s.n.m.
Superficie: 0.4 ha.
Profundidad máx.: 0.4 m
Persistencia: temporal
Secchi: 40 cm (fondo)
pH: 5.13
Presiones antrópicas: evidencias ganaderas

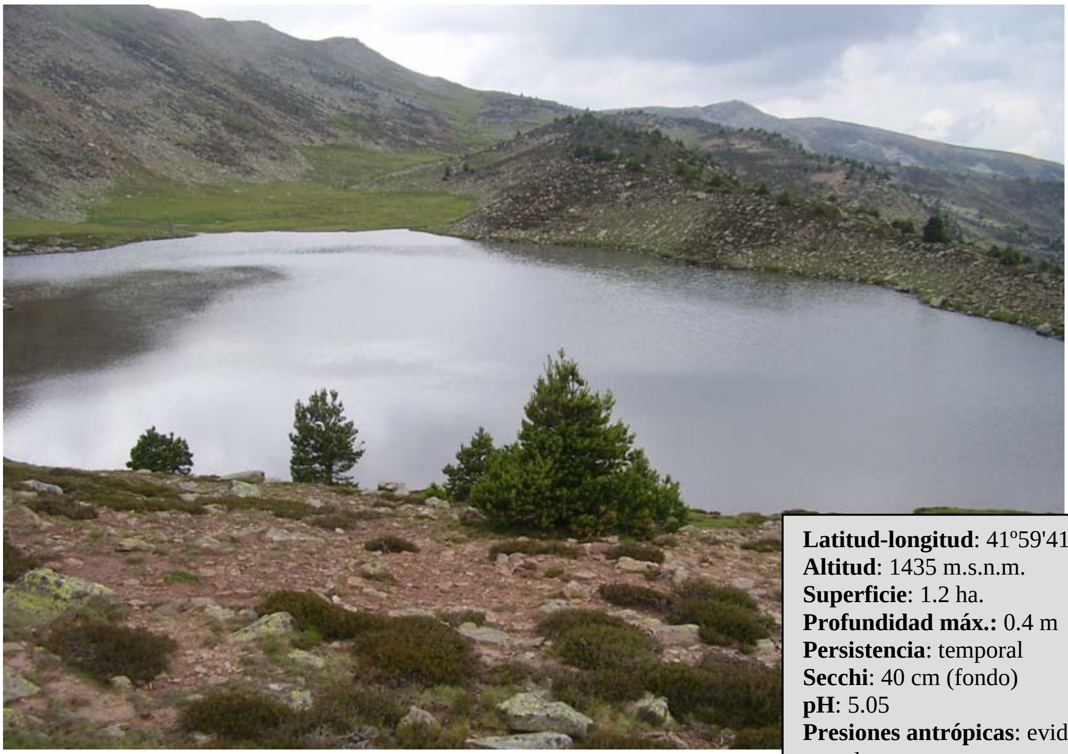
Latitud-longitud: 42º3'40''-3º6'40''
Altitud: 1860 m.s.n.m.
Superficie: 1.4 ha.
Profundidad máx.: 0.3 m
Persistencia: temporal
Secchi: 30 cm (fondo)
pH: 6.26
Presiones antrópicas: evidencias ganaderas

Foto 50. Haedillo I y II



Latitud-longitud: 41º59'57"-2º50'51"
Altitud: 1750 m.s.n.m.
Superficie: 3.3 ha.
Profundidad máx.: 9 m
Persistencia: permanente
Secchi: 200 cm
pH: 6.53
Presiones antrópicas: evidencias ganaderas, turismo e introducciones piscícolas

Foto 51. Laguna Negra de Urbión

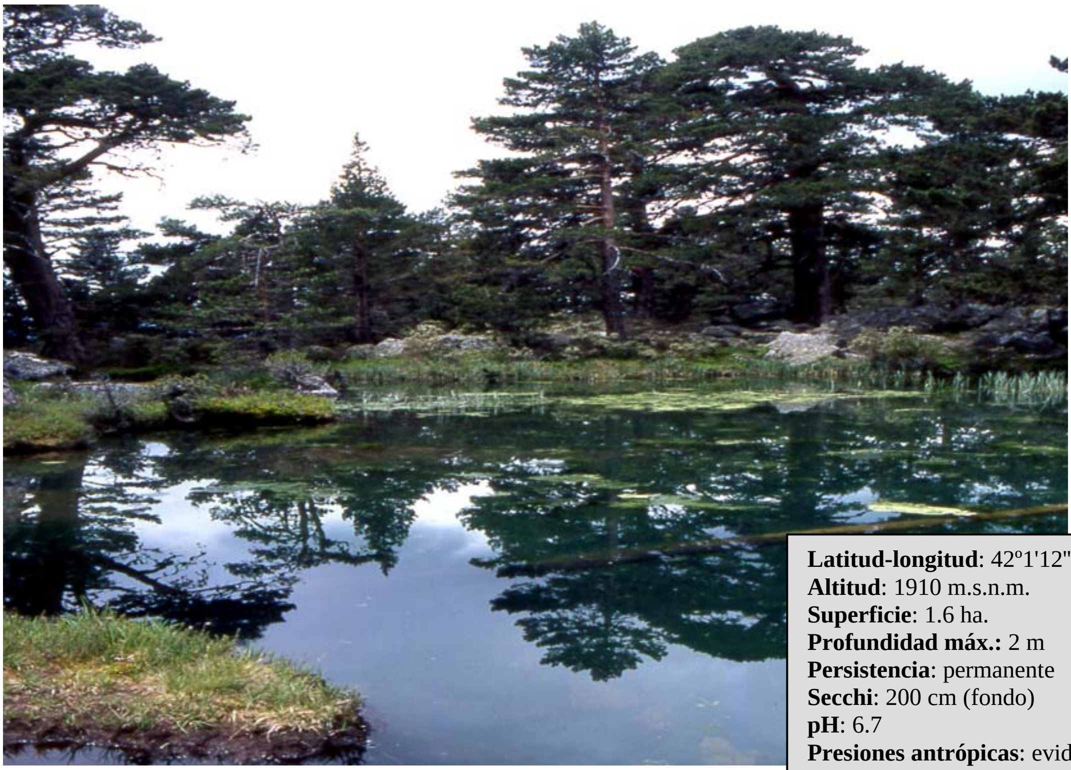


Latitud-longitud: 41º59'41"-2º51'37"
Altitud: 1435 m.s.n.m.
Superficie: 1.2 ha.
Profundidad máx.: 0.4 m
Persistencia: temporal
Secchi: 40 cm (fondo)
pH: 5.05
Presiones antrópicas: evidencias ganaderas.

Foto 52. Laguna Helada

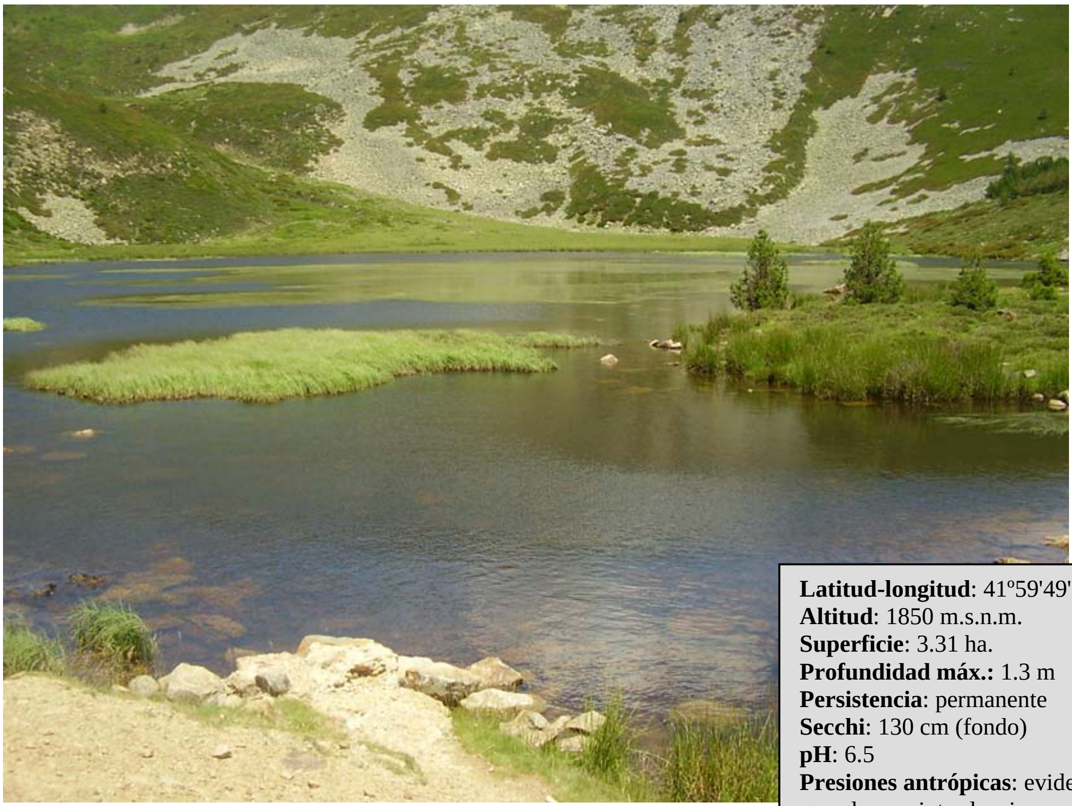
Latitud-longitud: 42º0'20"-2º52'1"
Altitud: 2010 m.s.n.m.
Superficie: 1 ha.
Profundidad máx.: 1.9 m
Persistencia: permanente
Secchi: 190 cm (fondo)
pH: 6.05
Presiones antrópicas: evidencias ganaderas

Foto 53. Laguna Larga



Latitud-longitud: 42º1'12"-2º44'45"
Altitud: 1910 m.s.n.m.
Superficie: 1.6 ha.
Profundidad máx.: 2 m
Persistencia: permanente
Secchi: 200 cm (fondo)
pH: 6.7
Presiones antrópicas: evidencias ganaderas.

Foto 54. Laguna Verde de Soria



Latitud-longitud: 41º59'49"-2º39'10"
Altitud: 1850 m.s.n.m.
Superficie: 3.31 ha.
Profundidad máx.: 1.3 m
Persistencia: permanente
Secchi: 130 cm (fondo)
pH: 6.5
Presiones antrópicas: evidencias ganaderas e introducciones piscícolas

Foto 55. Laguna de Cebollera

Se adjuntan dos cuadros con las características de las lagunas de las que no se poseen fotos propias:

Camposagrado I

Latitud-longitud: 42º7'41"-6º51'48"
Altitud: 1700 m.s.n.m.
Superficie: 0.15 ha.
Profundidad máx.: 0.4 m
Persistencia: temporal
Secchi: 40 cm (fondo)
pH: 5.98
Presiones antrópicas: evidencias ganaderas

Pies Juntos

Latitud-longitud: 42º11'27"-6º48'20"
Altitud: 1670 m.s.n.m.
Superficie: 0.3 ha.
Profundidad máx.: 0.9 m
Persistencia: permanente
Secchi: 90 cm (fondo)
pH: 6.37
Presiones antrópicas: evidencias ganaderas e introducción piscícola