Dinámica espacio temporal de la comunidad de macroinvertebrados de las lagunas temporales de Doñana

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INTRODUCCIÓN:

Las lagunas temporales y sus comunidades de macroinvertebrados

Las lagunas temporales son medios acuáticos de pequeño o mediano tamaño que sufren una fase de desecación recurrente de longitud variable y que suele ser impredecible en su forma y duración (Williams 1997). El periodo de inundación de los medios acuáticos temporales puede definirse por un conjunto de características que algunos autores engloban en lo que denominan "hidrorégimen" (ver Wissinger 1999; Hulsmans *et al.* 2008; Vanschoenwinkel *et al.* 2009): 1) *La regularidad de la inundación* es la frecuencia anual de la inundación-desecación; 2) *La previsibilidad de la inundación* es la probabilidad de que la inundación se produzca en el mismo momento cada año así como la probabilidad de que un cuerpo de agua se inunde todos los años; 3) *El hidroperiodo* es la duración del periodo de inundación desde la formación hasta la desecación de las lagunas.

La principal fuente de variabilidad del "hidrorégimen" viene determinada por la gran variabilidad del hidroperiodo (Hulsmans *et al.* 2008). En un área se puede detectar una amplia variabilidad en hidroperiodo entre las diferentes lagunas que componen un sistema. A su vez, el hidroperiodo de estas lagunas puede variar con la estacionalidad de la abundancia y distribución de las precipitaciones y de la evaporación, y según la cantidad de la precipitación anual. Esta complejidad hace que algunos autores hayan definido el hidroperiodo como eventos separados a lo largo de un mismo año (Boix *et al.* 2004) o como la duración media entre diferentes años (Schneider 1999), en vez de considerar la duración total de la inundación en el ciclo anual (Florencio *et al.* 2009).

Las lagunas pueden considerarse permanentes, semipermanentes o temporales (Wissinger 1999) en función de la longitud del hidroperiodo. Pero dentro de las lagunas temporales existe un amplísimo gradiente de hidroperiodo que abarca desde las lagunas de duración más efímera, que se inundan de manera esporádica, hasta las lagunas semipermanentes que sólo se secan en años de sequía extrema (Grillas *et al.* 2004; Williams 2006). La longitud del hidroperiodo constituye un factor importante para determinar la composición faunística que albergan las lagunas temporales (Wiggins *et al.* 1980; Grillas *et al.* 2004; Williams 2006).

Las especies de macroinvertebrados que principalmente se encuentran en las lagunas temporales son dípteros, odonatos, coleópteros, heterópteros, efemerópteros, branquiópodos, anélidos y gasterópodos (Taylor *et al.* 1999). Esta fauna adaptada a las lagunas temporales se beneficia de la ausencia de los depredadores típicos de los medios permanentes, como los peces (Wellborn *et al.* 1996). Sin embargo, la presión de depredación incrementa a lo largo del hidroperiodo ya que muchas especies de coleópteros acuáticos y de larvas de odonatos son importantes depredadores que aparecen en lagunas de más largo hidroperiodo (Schneider & Frost 1996; Spencer *et al.* 1999; Bilton *et al.* 2001a). La aparición de estos depredadores puede limitar la riqueza de algunos grupos como ocurre por ejemplo con el zooplancton, que a pesar de que incrementa su riqueza a lo largo del gradiente de hidroperiodo (Serrano & Fahd 2005), esta riqueza está limitada en las lagunas de más largo hidroperiodo (Frisch *et al.* 2006; Fahd *et al.* 2000).

La adaptación de las especies a los medios acuáticos temporales consiste en una serie de estrategias de supervivencia con las que se enfrentan a la desecación y a la imprevisibilidad de la inundación y desecación. La principal estrategia de supervivencia es la sincronización de sus ciclos de vida con la duración del periodo de inundación (Wiggins *et al.* 1980). Muchas de estas especies, además, presentan ciclos de vida anfibios en los que generalmente alternan fases de vida acuática larvaria con fases adultas de vida terrestre. Este es el caso de los odonatos y de coleópteros, particularmente de algunas especies de la familia Scirtidae. La dispersión hacia medios más permanentes y la formación de estructuras de resistencia son las dos estrategias básicas para afrontar la desecación (Wiggins *et al.* 1980; Batzer & Wissinger 1996; Williams 2006). Estas estrategias están descritas de forma detallada en la Tabla 1, que considera los principales grupos que aparecen en los medios acuáticos temporales. Según estas estrategias, podemos considerar las especies como residentes, si permanecen en las lagunas temporales después de su desecación mediante estructuras de resistencia, o no residentes si abandonan las lagunas de forma activa (dispersión activa) durante la desecación. Por otra parte, muchas especies de insectos acuáticos adaptados a las lagunas temporales presentan estrategias intermedias con formas residentes de resistencia y formas no residentes de dispersión (Batzer & Wissinger 1996). Un ejemplo de estas estrategias intermedias sería *Berosus signaticollis* (Charpentier, 1825), una especie que cuenta con adultos dispersores que además se entierran en el sedimento como estrategia para resistir el periodo seco de las lagunas (Boix *et al.* 2001).

Dentro de la fauna residente se consideran los dispersores pasivos sin capacidad de vuelo cuya dispersión es dependiente de los agentes externos. En la fauna residente, algunas especies forma estructuras de resistencia frente a la desecación que se dividen en huevos, efipios y quistes (platelmintos, oligoquetos, nematodos, anostráceos, concostráceos, notostráceos, cladóceros, copépodos, ostrácodos, rotíferos, colémbolos, efemerópteros, tricópteros y dípteros, Tabla 1) así como en otras especies, los estados adultos y larvarios pueden sobrevivir enterradas en el sedimento (Tabla 1): platelmintos, nematodos, oligoquetos, bivalvos, gasterópodos, hirudíneos, cladóceros, copépodos, ostrácodos, anfípodos, isópodos, decápodos, plecópteros, odonatos, coleópteros, tricópteros y dípteros (Wiggins *et al.* 1980; Higgins & Merrit 1999; Bilton *et al.* 2001b; Williams 2006). Entre los distintos vectores que favorecen la dispersión pasiva destacan a) el viento (Bilton *et al.* 2001b; Cáceres & Soluk 2002); b) las conexiones de agua entre lagunas (Van de Meuter *et al.* 2006); c) la dispersión animal mediante endozoocoria (transporte interno) y ectozoocoria (transporte externo) de aves acuáticas (Figerola & Green 2002; Green & Sánchez 2005; Frish *et al.* 2007), los anfibios (Bohonak & Whiteman 1999), mamíferos (Vaschoenwinkel *et al.* 2008) e insectos acuáticos (Van de Meuter *et al.* 2008) –la dispersión pasiva mediante endozoocoria requiere de la viabilidad de las formas de resistencia tras la digestión estomacal mientras que mediante ectozoocoria requiere del transporte de las especies adheridas a las estructuras externas del tegumento (Bilton *et al.* 2001b)–; d) la dispersión humana que puede ser mediada incluso por los instrumentos de muestreo (Waterkeyn *et al.* 2010).

La proporción de fauna residente que estiva en el sedimento aumenta en los medios con más larga duración del hidroperiodo (Dietz-Brantley *et al.* 2002). Sin embargo, Anderson & Smith (2004) observaron experimentalmente que en medios acuáticos temporales el 70% de las especies de invertebrados que componían las comunidades alcanzaron los cuerpos de agua mediante dispersión activa y no como fauna residente. La dispersión activa requiere de la capacidad de vuelo de las especies y estas habilidades dispersivas varían entre distintos grupos taxonómicos (Bilton *et al.* 2001b). La dispersión constituye la principal estrategia frente a la desecación para las especies de coleópteros y heterópteros acuáticos, que poseen excelentes capacidades dispersivas (Wiggins *et al.* 1980; Bilton *et al.* 2001b, Tabla 1). La dispersión no sólo les permite abandonar las lagunas durante la desecación de las lagunas y desplazarse hacia medios más permanentes, sino que además permite la recolonización de estas lagunas con el comienzo del periodo de inundación (Wiggins *et al.* 1980; Higgins & Merrit, 1999; Bilton *et al.* 2001b, Williams 2006). El coste energético que supone la dispersión se ha mantenido evolutivamente a través de un equilibrio compensado entre reproducción y dispersión (Bilton *et al.* 2001b). Dentro de las adaptaciones fisiológicas que asumen este coste energético se encuentra la autolisis de los músculos de vuelo descrito por Johnson (1969) en Bilton *et al.* (2001b), con la que se obtiene energía para la reproducción una vez que ya se ha producido la dispersión. Otra adaptación fisiológica a la temporalidad consiste en el desarrollo de individuos sin capacidad funcional de vuelo o de formas ápteras junto a los individuos dispersantes de heterópteros y coleópteros acuáticos; estas formas no dispersantes presentan una alta fecundidad que asegura la reproducción en los medios temporales sin el coste adicional de la dispersión (Bilton *et al.* 2001b).

Tabla 1: Estrategias de supervivencia de la principal fauna característica de los medios acuáticos temporales (adaptación de la tabla de Williams 2006 p.138).

Grupo faunístico	**Estrategia de desecación y estado de huevo, juveniles, larvas o adultos**
Algas/ flagelados/ protozoos	Células vegetativas modificadas con paredes engrosadas; vainas mucilaginosas; acumulación de aceite en las células; quistes asexuales resistentes a la desecación
Esponjas	Reducción del tamaño corporal; gemación
Platelmintos	Dormancia de huevos; quistes de resistencia para los estadios jóvenes, adultos, fragmentos del animal y capullos.
Rotíferos	Supervivencia como organismos deshidratados; algunos secretan quistes de protección.
Nematodos	Huevos; larvas; adultos
Bivalvos	Estados juveniles y adultos
Gasterópodos	Adultos con una capa protectora de mucus seco en la abertura del caparazón; adultos y juveniles sobreviven en el aire húmedo y bajo algas secas de la cubeta de la laguna.
Oligoquetos	Dormancia de huevos; quistes de resistencia para los estadios jóvenes, adultos y fragmentos del animal.
Hirudíneos	Sobreviven como individuos deshidratados; algunas especies construyen pequeñas líneas de células con mucus
Tardígrados	Estado de resistencia
Ácaros	Posibles larvas de resistencia; en la mayoría de especies, las larvas se adhieren a insectos dispersantes como hospedadores que abandonan el hábitat hacia medios más permanentes. Las larvas se mantienen adheridas al hospedador en estos medios permanentes.
Anostráceos	Huevos de resistencia
Notostráceos	Huevos de resistencia
Concostráceos	Huevos de resistencia
Cladóceros	Efipios; adultos sobreviven en el sedimento húmedo.
Copépodods	Huevos en diapausa; copepoditos grandes y adultos enquistados
Ostrácodos	Huevos de resistencia; próximos al estado adulto en el sedimento húmedo.
Anfípodos/	Inmaduros próximos a la lámina de agua subterránea

isópodos	
Decápodos	Juveniles y adultos en túneles donde llega la lámina de agua subterránea
Colémbolos	Huevos de resistencia
Plecópteros	Diapausa en los estadios tempranos
Efemerópteros	Huevos de resistencia
Odonatos	Larvas resistentes; adultos dispersantes
Hemípteros (Heterópteros)	Adultos dispersantes
Coleópteros	Pupas semiterrestres; adultos que se entierran en el sedimento; adultos dispersantes; huevos de resistencia.
Tricópteros	Huevos en diapausa; masas de huevos de resistencia gelatinosos; pupas terrestres; adultos dispersantes; larvas enterradas en el sedimento.
Quironómidos	Estadios larvarios tardíos resistentes; algunas veces en capullos de seda y/o mucus; adultos dispersantes; posibles huevos de resistencia.
Mosquitos	Huevos de resistencia; estadios larvarios tardíos y pupas resistentes.
Otros dípteros	Huevos de resistencia; larvas y pupas
Peces	Adultos dispersantes; dormancia de adultos en el sustrato; huevos de resistencia.
Anfibios	Adultos dispersantes; dormancia de adultos en el substrato.

Las lagunas temporales: Una ventana hacia la conservación

A pesar de que los medios permanentes en general albergan un mayor número de especies (Bazzanti *et al.* 1996; Brooks 2000; Della Bella *et al.* 2005), los medios acuáticos temporales presentan una fauna singular compuesta por especies exclusivas o poco abundantes en los medios permanentes (Collinson *et al.* 1995; Williams 1997; Céréghino *et al.* 2008). Sin embargo, la fauna frecuente en medios permanentes suele estar poco representada en las lagunas temporales, ya que requieren de adaptaciones específicas frente a la desecación y no disponen del tiempo suficiente para completar sus ciclos de vida.

A pesar de la fauna singular que albergan, las lagunas temporales han sido excluidas de los principales programas de conservación durante décadas debido a su reducido tamaño y su carácter temporal en comparación con otros medios acuáticos (Williams *et al.* 2001; Grillas *et al.* 2004; Williams 2006; Zacharias *et al.* 2007; Céréghino *et al.* 2008). Es cierto que las lagunas de mayor tamaño albergan un mayor número de especies pero sin embargo no existe una clara relación entre el área y la riqueza de especies para las lagunas temporales (Friday 1987; Bilton *et al.* 2001a; Oertli *et al.* 2002). De hecho, las pequeñas lagunas temporales pueden albergar una mayor densidad de especies por unidad de superficie que aquellas lagunas de mayor tamaño

(Oertli *et al.* 2002). Estas lagunas de reducido tamaño tienen un papel fundamental en el mantenimiento de la biodiversidad en los sistemas de lagunas no fragmentados, tal y como Gibbs (1993) detectó para las especies de galápagos, pequeñas aves y mamíferos, anuros y urodelos. Las lagunas temporales además contribuyen a mantener la heterogeneidad ambiental en estos sistemas, algo esencial para promover su biodiversidad (Williams 1997; Urban 2004; Biggs *et al.* 2005; Jeffries 2005).

Los medios acuáticos temporales son particularmente abundantes en las zonas más áridas del globo terráqueo formándose en un número extraordinario de ambientes distintos (tropicales, templados e incluso marítimos) (Williams 1996), aunque pueden localizarse en cualquier región (Williams *et al.* 2001; Williams 1996). Según su origen podemos encontrar medios temporales no sólo en zonas áridas y semiáridas (como por ejemplo salinas temporales, lagunas en desiertos), sino también en regiones húmedas tropicales, donde se forman lagunas incluso sobre troncos de árboles o conchas de caracoles; en las regiones templadas, que engloban una gran diversidad de lagunas temporales con distintas denominaciones; en zonas marítimas donde se forman las marismas o las lagunas litorales y en las regiones ártica y antártica, donde aparecen ríos glaciares y lagunas del deshielo. En la Tabla 2 se exponen algunos términos usados para denominar las lagunas temporales por todo el mundo.

Tabla 2: Términos locales que muestran un ejemplo de la diversa denominación de las lagunas temporales en las distintas regiones. Se indica el área donde el término está en uso y notas sobre las características de estas lagunas (adaptación de la tabla de Williams *et al.* 2001, p.8).

Término	**Área de uso**	**Notas**
Turlochs	Irlanda	En zonas calcáreas tales como "the Burren".
Billabongs	Australia	Llanuras fluviales de inundación, casi todas temporales.
Gnammas	Oeste de Australia	Lagunas temporales en los afloramientos de granito
Vernal pools	Norteamérica	Se inundan en primavera
Autumnal ponds	Norteamérica	Se inundan en otoño
Playas	África, Europa	Lagunas temporales o lagos muy extensos y someros característicos de zonas áridas.
Rain pools	África, Australia	Pequeñas lagunas temporales generalmente sobre rocas.
Tinajas	Norteamérica	Sistemas de charcos inundados sobre rocas
Dayas	África del Norte	Lagunas temporales sobre sustratos arenosos

Vleis	Sudáfrica	Lagunas temporales sobre estratos arcillosos o afloramientos de rocas
Seasonal ponds	Ubicuo	
Ephemeral ponds	Ubicuo	

Se han llevado a cabo numerosos estudios en distintos continentes que ponen de manifiesto la singularidad de la fauna de medios temporales en comparación con la mayor diversidad de medios permanentes: por ejemplo humedales en Norteamérica (Brooks 2000; Tarr *et al.* 2005; Jocqué *et al.* 2007a), llanuras inundadas y “turloughts” en Australia (Hillman & Quinn 2002; Porst & Irvine 2009), lagunas Mediterráneas europeas (Bazzanti *et al.* 1996; Della Bella *et al.* 2005) e incluso en la India, y en el Himalaya (Sharma & Rawat 2009). Se han evaluado diferentes aspectos sobre la estructura de las comunidades de macroinvertebrados de distintos sistemas acuáticos temporales. El efecto de la estacionalidad se ha evaluado en arroyos intermitentes de Australia (Boulton & Lake 1992) y lagunas temporales de Europa (Boix *et al.* 2004; Culioli *et al.* 2006; Florencio *et al.* 2009); el papel de los hábitats efímeros en la biodiversidad se evaluó en lagunas efímeras formadas en los márgenes de arroyos de Arizona (Graham 2002) o en los “rock pools” de Sudáfrica (Jocqué *et al.* 2007b); o por ejemplo el efecto de la variabilidad ambiental se ha analizado en “seasonal ponds” en Minnesota (Batzer *et al.* 2004). A pesar de las grandes diferencias geográficas y climáticas entre Norteamérica, Europa y Australia, las lagunas temporales presentan una fauna similar en los distintos continentes, en las que es típica una alta diversidad de hemípteros (heterópteros) y coleópteros (Williams 1997).

En Europa, la presencia de especies singulares, la riqueza específica y la estructura de las comunidades de macroinvertebrados se han utilizado para evaluar algunos aspectos relacionados con la conservación de los sistemas de lagunas temporales. La conectividad (i.e. la conexión entre lagunas bien de forma física o bien con una proximidad tal que facilita la movilidad de la fauna entre lagunas) en estos sistemas de lagunas es esencial para mantener la diversidad de especies ya que favorecen la dispersión (Van de Meutter *et al.* 2006; Oertli *et al.* 2008) y genera comunidades particulares en lagunas más aisladas, que están prácticamente formadas por excelentes dispersores (Briers & Biggs 2005; Sanderson *et al.* 2005). La emergencia de la fauna residente durante la inundación, que contribuye en gran medida a la diversidad del sistema, se produce bajo condiciones ambientales particulares determinadas por variables como el pH y la conductividad (Brendonck 1996; Brendonck *et al.* 1998). La temperatura y la salinidad son algunos de estos condicionantes ambientales cuyos parámetros son de interés para la conservación (Waterkeyn *et al.* 2009). En particular, las características ambientales que favorecen la diversidad de macroinvertebrados en los sistemas de lagunas naturales en países como Francia, Inglaterra ó Finlandia por ejemplo, pueden ser de interés para la conservación de los medios temporales y fluctuantes en otras regiones (Heino 2000; Nicolet *et al.* 2004; Sanderson *et al.* 2005; Waterkeyn *et al.* 2008; Bilton *et al.* 2009). Estos estudios determinan las variables que generalmente afectan a la estructura de las comunidades de macroinvertebrados, aunque los valores de dichas variables son particulares para las diferentes regiones. En la Península Ibérica, donde son frecuentes las lagunas temporales de ámbito Mediterráneo, se han desarrollado aproximaciones a la conservación a través de los condicionantes ambientales naturales que determinan una alta riqueza de macroinvertebrados (Trigal *et al.* 2007; Gascón *et al.* 2008; Ruhí *et al.* 2009) así como a través de las rarezas, endemismos y distribuciones restringidas de dichas especies (Ribera *et al.* 1996; Ribera *et al.* 1998; Millán *et al.* 2005; Garrido & Munilla 2008).

La alta diversidad de lagunas de la Península Ibérica es un reflejo del alto contraste litológico y climático que confiere a la Península un alto interés limnológico por este tipo de sistemas acuáticos (Alonso 1998). Alonso (1998) clasifica las lagunas peninsulares según su mineralización, turbiedad y temporalidad: 1) según su grado de mineralización en base a los iones mayoritarios se clasifican en dulces (<10 meq totales), de mineralización intermedia (10-100 meq totales) y otra de mayor mineralización (> 14000 meq totales); 2) la turbiedad es debida a los sólidos inorgánicos en suspensión, generalmente las arcillas, pudiendo diferenciar dos grandes grupos que son altamente variables, de aguas claras y de aguas turbias; 3) la temporalidad de las lagunas depende en gran medida de su profundidad, apareciendo lagunas permanentes generalmente a partir de 2 m de profundidad. Esta alta variabilidad se traduce en la aparición de una gran diversidad de lagunas temporales en España, de las cuales destacan las de Doñana y Menorca por su alto grado de conservación (Ewald *et al.* 2010). En concreto, el Parque Nacional Doñana es un enclave natural que sustenta uno de los humedales naturales con mayor estatus de conservación de toda Europa. Este humedal engloba una gran extensión inundada que compone la marisma así como un sistema de lagunas que consta de más de 3000 cuerpos de agua en periodos de máxima inundación (Gómez-Rodríguez 2009). El área de Doñana está incluida en la convención RAMSAR desde el año 1982 y ha sido posteriormente designada como "World Heritage Site" por la UNESCO en 1995. La contribución singular de la flora y fauna de las lagunas temporales de Doñana a las lagunas europeas mediterráneas y alpinas (IAPs) se muestra en la Fig.1 (Díaz-Paniagua *et al. under review*).

Los invertebrados y los macroinvertebrados acuáticos constituyen las grandes ausencias en las Listas Rojas de la IUCN (IUCN 2010). Las especies acuáticas mejor mejor estudiadas pertenecen a los grupos de anfibios, odonatos y plantas acuáticas, que presentan un elevado número de especies con categorías de amenaza en los Libros Rojos (Oertli *et al.* 2005; IUCN 2010). Sin embargo, las especies de grupos menos estudiados de las lagunas temporales tales como crustáceos, coleópteros y heterópteros están infravalorados en sus categorías de conservación a pesar de que muchos de ellos podrían presentar tendencias similares a las especies de los grupos más estudiados (Oertli *et al.* 2005).

Fig.1: Parte de la ficha que muestra la importancia que tienen las lagunas temporales de Doñana para la conservación en las regiones Mediterránea y alpinas europeas (Tomada de Díaz-Paniagua *et al. under review*).

DOÑANA TEMPORARY PONDS

MED - SP

Administrative region	Spain (Huelva , Andalucía)
Coordinates (latitude/longitude)	37°00´N, 06°38'W.
Altitude	0-32 m
Area	54000 ha
Selection criteria#	A (habitat), B (species), C (density)

- **Site description**

More than 3000 temporary ponds flood after heavy autumn or winter rains in Doñana National Park (SW Spain). This Park includes three main landscapes: (i) a silty-clay floodplain with a seasonally flooded marshland formed at the mouth of the Guadalquivir River, (ii) a mobile dune system running parallel to the Atlantic coast line with several trains of fronts and dune slacks, and (ii) a stable dune area originated by earlier dune generations with smooth depressions which constitute the basins of most temporary ponds. Mediterranean scrub is the typical vegetation of this area. It is mainly composed of *Halimium halimifolium* and *Stauracanthus genistoides* with isolated cork oak trees (*Quercus suber*) and thickets of pines (*Pinus pinea*).

Freshwater ponds are filled (with rainfall, runoff and/or groundwater discharge) in smooth basins on sandy soils with superficial horizons of silty texture or on soils with an argilic horizon of semipermeable sediment which favours the accumulation of water. Ponds present a wide variation in size, length of the aquatic phase, and limnological features, partly depending on their location: in large or small depressions on the stable dunes, in wet sand dune slacks, or in transitional areas towards the marshland. The number of ponds and their characteristics also vary among successive years, which also implies a significant interannual variation in species richness in each pond.

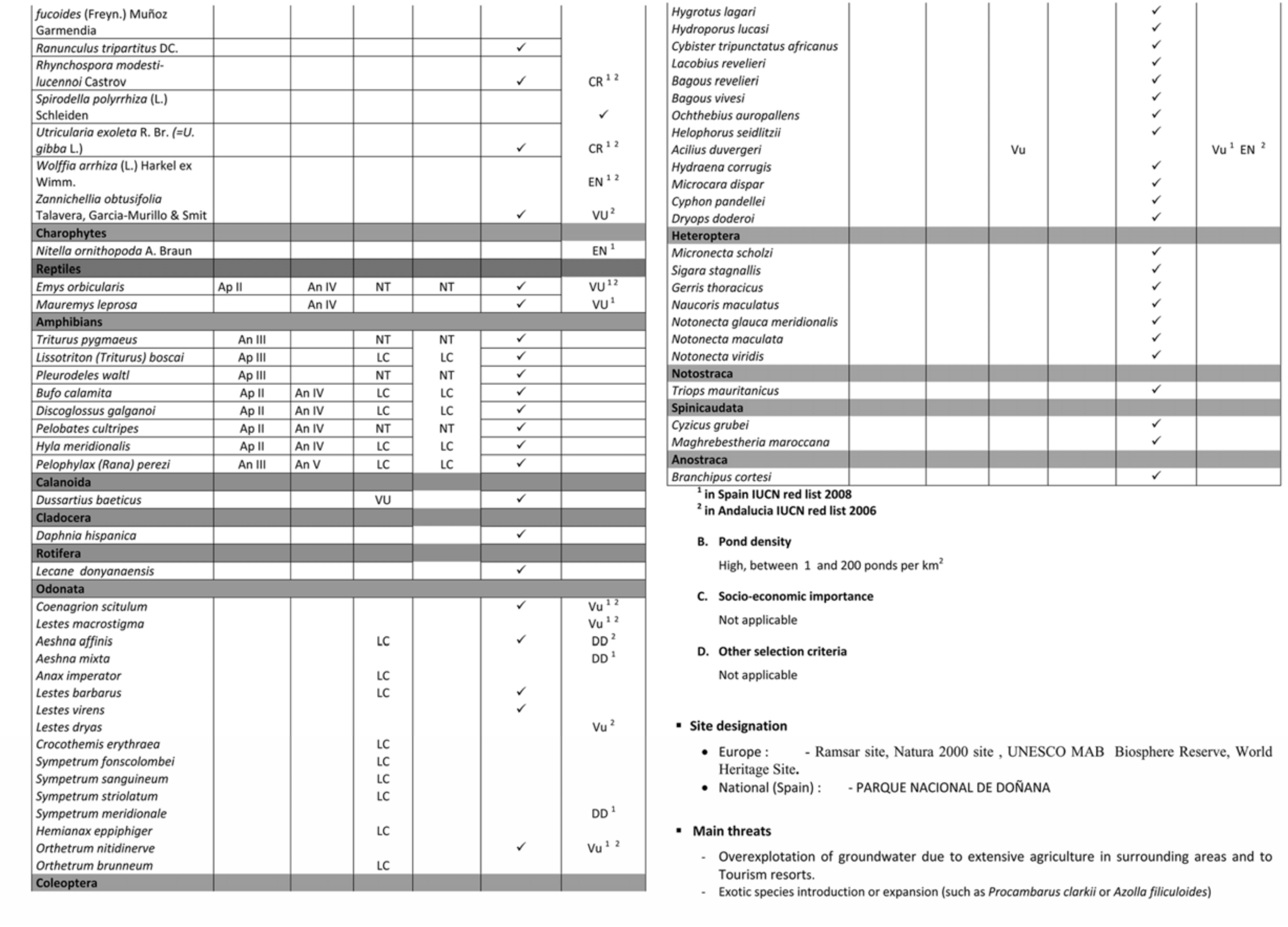
Selection criteria

A. **Habitats of European importance (Annex I of Habitat Directive)**

- 3170 :Mediterranean Temporary Ponds
- 3160 Natural dystrophic lakes and ponds
- 3130 Oligotrophic to mesotrophic standing waters with vegetation of the *Littorelletea uniflorae* and/or the *Isoeto-Nanojuncetea*
- 3140 Hard oligo-mesotrophic waters with benthic vegetation of *Chara spp.*

Species of European conservation importance

Scientific name	Protection		International Red List		Endemism to Europe	Other status
	Berne Convention B1.I#	Habitat Directive B1.II#	IUCN B2.I#	Europe B2.II#	B3#	B4#
Aquatic plants						
Apium inundatum L.					✓	
Avellara fistulosa (Brot.) Blanca & C. Díaz					✓	CR $^{1\ 2}$
Bulbostylis cicoiana (Savi) K. Lye (=*Fimbristilis cycoiana* Savi)					✓	EN ^{1}CR ^{2}
Callitriche lusitanica Schotsman					✓	DD^{1}EN^{2}
Callitriche platycarpa sensu Castroviejo et al., 1980 (=*C. obtusangula* Le Gall)					✓	
Caropsis verticillaoinundata (*Thorella*) Rauschert		Ann II			✓	CR $^{1\ 2}$
Elatine alsinastrum L.					✓	
Eryngium corniculatum Lam.					✓	VU ^{2}
Eryngium galioides Lam.					✓	VU ^{2}
Fuirena pubescens (Poir.) Kunth					✓	VU ^{2}
Glyceria fluitans (L.) R. Br.						NT ^{2}
Gratiola linifolia Vahl					✓	NT ^{2}
Hydrocharis morsus-ranae L.					✓	CR $^{1\ 2}$
Isolepis fluitans (L.) R. Br. (*Scirpus fluitans* L.)					✓	
Juncus x donyanae Fernández Carvajal					✓	
Juncus enmanuelis A. Fernandes & Garcia					✓	
Juncus heterophyllus Dufour					✓	
Lemna trisulca L.						CR ^{1}DD ^{2}
Marsilea strigosa Willd		Ann II			✓	VU ^{2}
Micropiropsis tuberosa Romero Zarco & Cabezudo		Ann II			✓	EN $^{1\ 2}$
Potamogeton lucens L.					✓	
Potamogeton natans L.					✓	✓
Potamogeton polygonifolius Pourret					✓	
Ranunculus peltatus subsp.					✓	



fucoides (Freyn.) Muñoz Garmendia						
Ranunculus tripartitus DC.					✓	
Rhynchospora modesti-lucennoi Castrov					✓	CR $^{1\ 2}$
Spirodella polyrrhiza (L.) Schleiden						✓
Utricularia exoleta R. Br. *(=U. gibba* L.)					✓	CR $^{1\ 2}$
Wolffia arrhiza (L.) Harkel ex Wimm.						EN $^{1\ 2}$
Zannichellia obtusifolia Talavera, Garcia-Murillo & Smit					✓	VU ^{2}
Charophytes						
Nitella ornithopoda A. Braun						EN ^{1}
Reptiles						
Emys orbicularis	Ap II	An IV	NT	NT	✓	VU $^{1\ 2}$
Mauremys leprosa		An IV			✓	VU ^{1}
Amphibians						
Triturus pygmaeus	An III		NT	NT	✓	
Lissotriton (Triturus) boscai	Ap III		LC	LC	✓	
Pleurodeles waltl	Ap III		NT	NT	✓	
Bufo calamita	Ap II	An IV	LC	LC	✓	
Discoglossus galganoi	Ap II	An IV	LC	LC	✓	
Pelobates cultripes	Ap II	An IV	NT	NT	✓	
Hyla meridionalis	Ap II	An IV	LC	LC	✓	
Pelophylax (Rana) perezi	An III	An V	LC	LC	✓	
Calanoida						
Dussartius baeticus			VU		✓	
Cladocera						
Daphnia hispanica					✓	
Rotifera						
Lecane donyanaensis					✓	
Odonata						
Coenagrion scitulum					✓	Vu $^{1\ 2}$
Lestes macrostigma						Vu $^{1\ 2}$
Aeshna affinis			LC		✓	DD ^{2}
Aeshna mixta						DD ^{1}
Anax imperator			LC			
Lestes barbarus			LC		✓	
Lestes virens					✓	
Lestes dryas						Vu ^{2}
Crocothemis erythraea			LC			
Sympetrum fonscolombei			LC			
Sympetrum sanguineum			LC			
Sympetrum striolatum			LC			
Sympetrum meridionale						DD ^{1}
Hemianax eppiphiger			LC			
Orthetrum nitidinerve					✓	Vu $^{1\ 2}$
Orthetrum brunneum			LC			
Coleoptera						
Hygrotus lagari					✓	
Hydroporus lucasi					✓	
Cybister tripunctatus africanus					✓	
Lacobius revelieri					✓	
Bagous revelieri					✓	
Bagous vivesi					✓	
Ochthebius auropallens					✓	
Helophorus seidlitzii					✓	
Acilius duvergeri			Vu			Vu ^{1} EN ^{2}
Hydraena corrugis					✓	
Microcara dispar					✓	
Cyphon pandellei					✓	
Dryops doderoi					✓	
Heteroptera						
Micronecta scholzi					✓	
Sigara stagnallis					✓	
Gerris thoracicus					✓	
Naucoris maculatus					✓	
Notonecta glauca meridionalis					✓	
Notonecta maculata					✓	
Notonecta viridis					✓	
Notostraca						
Triops mauritanicus					✓	
Spinicaudata						
Cyzicus grubei					✓	
Maghrebestheria maroccana					✓	
Anostraca						
Branchipus cortesi					✓	

[1] in Spain IUCN red list 2008

[2] in Andalucia IUCN red list 2006

B. Pond density

High, between 1 and 200 ponds per km^{2}

C. Socio-economic importance

Not applicable

D. Other selection criteria

Not applicable

- **Site designation**
  - Europe : - Ramsar site, Natura 2000 site , UNESCO MAB Biosphere Reserve, World Heritage Site.
  - National (Spain) : - PARQUE NACIONAL DE DOÑANA

- **Main threats**
  - Overexplotation of groundwater due to extensive agriculture in surrounding areas and to Tourism resorts.
  - Exotic species introduction or expansion (such as *Procambarus clarkii* or *Azolla filiculoides*)

Categorías de conservación para la flora y fauna de lagunas temporales:

Los sistemas acuáticos se consideran los ecosistemas más amenazados por su vulnerabilidad ante las perturbaciones (IUCN 2010). Dentro de éstos, las lagunas son ecosistemas acuáticos altamente amenazados por la actividad humana, donde principalmente destacan problemas de conservación asociados a la acidificación de sus aguas, la acumulación de nutrientes, la contaminación e invasión de especies exóticas, que han provocado la pérdida y deterioro de un gran número de estos medios acuáticos en los últimos años (Oertli *et al.* 2005). Sin embargo, en comparación con medios acuáticos como ríos y lagos, la fauna y flora de las lagunas presenta una alta diversidad (Biggs *et al.* 2005).

En particular, las lagunas temporales se caracterizan por su alta fragilidad frente a las perturbaciones externas. Dada la singularidad de las especies que albergan y su alta biodiversidad, estos medios han suscitado recientemente un gran interés para la conservación y para conocer los parámetros que los condicionan (Williams *et al.* 2001; Grillas *et al.* 2004; Zacharias *et al.* 2007; Céréghino *et al.* 2008). Las lagunas temporales han sido reconocidas dentro de la Convención Ramsar para Humedales (Resolución Ramsar VIII.33), y en particular, las lagunas temporales mediterráneas han sido incluidas como Hábitats Prioritarios de conservación por la Unión Europea en la ya mencionada Directiva Habitat (Natura code 3170, CEE, May 21st 1992, según Ruíz 2008).

Las lagunas temporales, algunas incluso datan de hace miles de años, se distribuyen por toda la geografía Europea (Williams *et al.* 2001). El declive actual de estas lagunas se debe a las perturbaciones humanas principalmente relacionadas con su régimen temporal como el incremento de la inundación o la profundización artificial de sus cubetas (Williams *et al.* 2001; Grillas *et al.* 2004), con el empobrecimiento de especies en lagunas de reducido tamaño por contaminación (Williams *et al.* 2001; Grillas *et al.* 2004; Biggs *et al.* 2005), la erosión y los incendios que afectan al régimen hidrológico natural de las lagunas (Grillas *et al.* 2004), la destrucción por usos ganaderos y agrícolas (Williams 1997; Grillas *et al.* 2004), el depósito de sedimentos que termina colmatando las lagunas (Grillas *et al.* 2004) y las alteraciones en el aporte freático por el uso agrícola y urbano de las aguas subterráneas, una de las principales amenazas del sistema de lagunas temporales de Doñana (Serrano & Serrano 1996).

La singularidad de la fauna macroinvertebrada de Doñana no sólo está determinada por su adaptación a los medios temporales, sino además por su localización geográfica particular en el sur de la Península Ibérica. El Parque Nacional de Doñana presenta la localización más septentrional de los humedales de Europa lo que determina una fauna macroinvertebrada más similar al Norte de África que al resto peninsular. Particularmente, la fauna de coleópteros acuáticos presenta una alta similitud con las especies del Norte de África más que con las especies peninsulares, donde la Ribera del Ebro constituye la principal barrera biogeográfica (Ribera et al. 1996; Ribera 2000).

La mayoría de las especies que se han encontrado en Doñana hasta la fecha presentan una amplia distribución en Europa. Sin embargo destaca el coleóptero acuático *Canthydrus diophthalmus* (Reiche & Saulcy 1855), una especie considerada extinta en Europa continental sólo citada previamente en un humedal degradado de Almería Millán *et al.* (2005). De las 110 especies de coleópteros acuáticos citados por Millán *et al.* (2005) en el área de Doñana, sólo cuatro resultaron endémicos de la Península Ibérica, tres de amplia distribución peninsular, *Helophorus seidlitzi* Kuwert 1885, *Hydraena corrugis* d'Orchymont 1934 y *Cyphon pandellei* Bourgeois 1884, y una especie de distribución más restringida *Microcara dispar* Seidlitz 1872. Por su rareza en el territorio Ibérico destacan *Acilius duvergeri* Gobert 1874, *Rhantus hispanicus* Sharp 1882, *Cymbiodita marginella* (Fabricius 1792), *Paracymus phalacroides* (Wollaston 1867) y *Dryops doderoi* Bollow 1936. Sin embargo, *R. hispanicus* es una especie particularmente abundante en las lagunas del área de Doñana (Montes *et al.* 1982). *A. duvergeri* es un ditíscido de tamaño mediano catalogado como vulnerable en las distintas Listas Rojas de invertebrados (IUCN 2010). Esta especie, que está considerada relicta con una distribución altamente fragmentada (Ribera 2000), no ha sido recolectado en las últimas prospecciones en Doñana (Millán *et al.* 2005). En estas prospecciones se hace hincapié en las especies de coleópteros de las familias Scirtidae, Heteroceridae, Georissidae, Limnichidae y Bagoini, cuyo conocimiento en el territorio peninsular es escaso (Millán *et al.* 2005).

Sigara stagnalis (Leach 1817) es la especie de heteróptero citada con mayor abundancia en el área de Doñana (Montes *et al.* 1982) y con una distribución más restringida (distribución Atlántica-Mediterránea desde el Norte de Marruecos hasta el sur de Escandinavia) según Jansson (1986) and Nieser *et al.* (1994).

Entre la fauna de odonatos del Parque Nacional de Doñana descrita por Agüesse (1962) y Montes *et al.* (1982) destacan *Lestes macrostigma* (Eversmann, 1836), *Orthetrum nitidinerve* (Selys, 1841) y *Coenagrion scitulum* (Rambur, 1842) que están catalogadas como vulnerables en las Listas Rojas (Fig.1). *L. macrostigma* actualmente se considera una especie extinta en el área de Doñana a pesar de que solía aparecer como especie frecuente y abundante en el área (Ferreras-Romero *et al.* 2005). Entre las especies descritas para el área de Doñana (Agüesse 1962; Montes *et al.* 1982), *Aeshna mixta* Vander Linden, 1823, *Crocothemis erythrarea* (Brullé, 1832), *Lestes barbarus* (Fabr., 1798), *Sympetrum fonscolombei* (Selys, 1841), *Sympetrum striotalum* (Charpentier, 1840), *Sympetrum sanguineum* (Müller, 1764) y *Anax imperator* Leach, 1815 se encuentran incluídas en las Listas Rojas (Fig.1).

El sistema de lagunas de Doñana constituye un enclave ideal para estudiar el estado de conservación de las poblaciones de macroinvertebrados acuáticos, que se componen principalmente por especies de coleópteros, odonatos y heterópteros acuáticos (Fig.1).

La importancia del método de muestreo

La caracterización de las comunidades de macroinvertebrados de los sistemas acuáticos puede depender fuertemente de la técnica de muestreo utilizada (Turner & Trexler 1997; Hyvönen & Nummi 2000; O'Connor *et al.* 2004). Según el objetivo del muestreo, unas técnicas pueden ser eficientes para capturar organismos activos mientras que otras lo son para capturar un número representativo del total de especies que habitan un medio acuático determinado (Cellot 1989; Rincón & Lobón-Cerviá 1997). Para capturar una fauna representativa del sistema no son apropiadas las trampas dirigidas a capturar exclusivamente organismos activos. Estás trampas muestran diferencias en la composición de especies y número de individuos que capturan en función de la hora a la que se realiza el muestreo, debido a los ritmos internos de actividad diaria de los propios organismos (Rincón & Lobón-Cerviá 1997; Céréghino & Lavandier 1998; Hampton & Duggan 2003). La manga es el método más habitualmente usado para muestrear las comunidades de macroinvertebrados de lagunas temporales (ver por ejemplo: Maciolek 1989; Collinson *et al.* 1995; Nicolet *et al.* 2004; Bilton *et al.* 2006). Su eficiencia en lagunas temporales es controvertida, existiendo estudios a favor (Cheal *et al.* 1993; García-Criado & Trigal 2005) y en contra de su uso eficiente en estos medios (Muzaffar & Colbo 2002; O'Connor *et al.* 2004).

En los estudios de macroinvertebrados es frecuente el sacrificio de todos los individuos muestreados para su posterior identificación y cuantificación en el laboratorio. Este tipo de muestreos con extracción, además de suponer el sacrificio de un gran número de ejemplares, incluyendo especies vulnerables y/ó protegidas, puede suponer un importante sesgo en muestreos posteriores sobre todo en aquellos estudios que evalúan variaciones temporales de las poblaciones a corto plazo. Por ello, en este estudio queremos resaltar la necesidad de realizar muestreos no invasivos, intentando afectar lo mínimo posible a la riqueza y diversidad de estos medios acuáticos temporales que son altamente vulnerables y que merecen preservar sus especies singulares. Para la elaboración de esta tesis doctoral, los muestreos se han realizado mediante la identificación y conteo de los individuos *in situ* y su devolución inmediata al medio natural, salvo excepciones de difícil identificación que sí se sacrificaron para su detallada identificación en el laboratorio.

Las especies exóticas: una amenaza para la fauna acuática autóctona de Doñana

La llegada de especies exóticas es una de las grandes amenazas para la fauna nativa (Witte *et al.* 2000) ya que pueden desequilibrar el funcionamiento de los ecosistemas mediante el establecimiento de sus poblaciones (Ricciardi *et al.* 1997, Maezono & Miyashita 2003). En el Parque Nacional de Doñana se han detectado diversas especies acuáticas exóticas con graves consecuencias para el funcionamiento de las comunidades nativas como son el copépodo *Acarthia tonsa* (Frisch *et al.* 2006), el cangrejo rojo americano *Procambarus clakii* (Geiger *et al.* 2005), el gasterópodo *Potamopyrgus antipodarum* (Rodríguez-Pérez 2006), el galápago de Florida *Trachemys scripta elegans* (Pérez-Santigosa *et al.* 2006), los peces *Gambusia affinis* y *Lepomis gibbosa* (García-Berthou *et al.* 2007), o el helecho acuático *Azolla filiculoides* que constituye una auténtica amenaza para la fauna de macrófitos autóctonos (García-Murillo *et al.* 2007). El coríxido exótico *Trichocorixa verticalis verticalis*, que es nativo de la costa Atlántica de Norte América y de las islas del Caribe, es la única especie de heteróptero acuático detectado en Europa como especie invasora (Rabitsch 2008). Ha sido citado recientemente en las próximidades de la desembocadura del Guadalquivir (Günther 2004, Millán *et al.* 2005) y en la costa SW de Portugal (Sala & Boix 2005), ambas localizaciones próximas a Doñana. La alta tolerancia que esta especie tiene a la salinidad así como el amplio gradiente ambiental en la que puede ser localizada (Van de Meutter *et al.* 2010) convierte a esta especie en una posible amenaza para las poblaciones de coríxidos acuáticos autóctonos de Doñana así como para la fauna que compone las comunidades de macroinvertebrados.

La variabilidad ambiental de las lagunas temporales y su papel en la estructura de las comunidades de macroinvertebrados

Características ambientales particulares pueden afectar la estructura de las comunidades de macroinvertebrados en las lagunas temporales (Williams 1997), que se encuentran resumidas en la Fig.2. El hidroperiodo, la salinidad, la concentración de nutrientes, las coberturas vegetales, la composición de los principales iones, la superficie y la profundidad de las lagunas son algunos ejemplos de variables ambientales que afectan a la estructura de las comunidades de invertebrados y macrófitos en las lagunas (ver por ejemplo: Boix *et al.* 2008; Della Bella *et al.* 2008; Waterkeyn *et al.* 2008; Bilton *et al.* 2009).

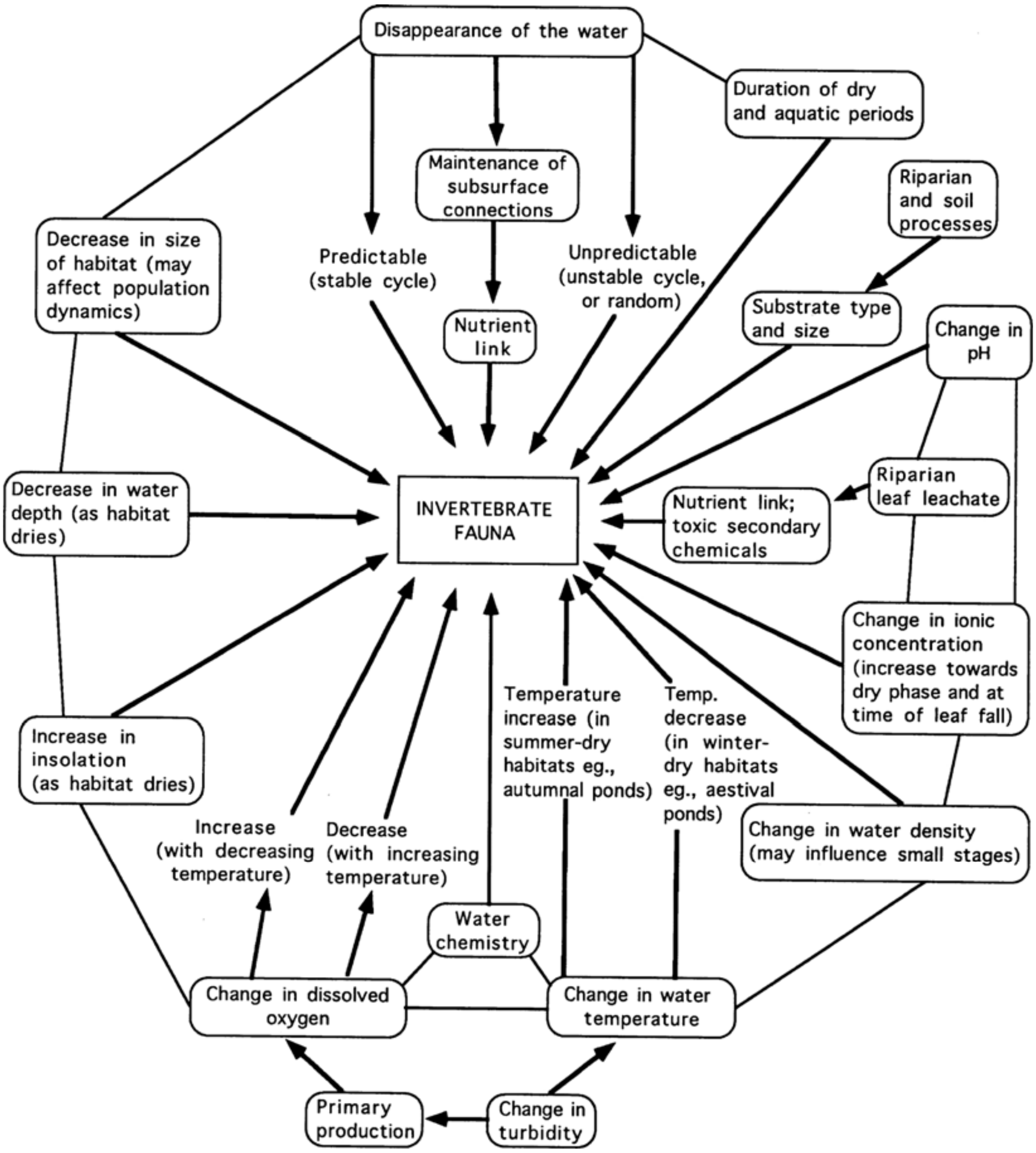


Fig.2: Variables químicas y físicas que influyen en la estructura de las comunidades de invertebrados de las lagunas temporales (tomado de Williams 1997). Las flechas indican los posibles efectos de los factores abióticos sobre la fauna invertebrada, a veces de

forma indirecta a través de otros factores; y las distintas conexiones determinan las interacciones entre factores.

Las lagunas temporales se caracterizan por una alta variabilidad en sus características ambientales tanto a nivel estacional como a nivel interanual (Williams 2006). Acorde a esta alta variabilidad ambiental, las comunidades de macroinvertebrados sufren también altas variaciones interanuales y estacionales (Boulton & Lake 1992; Bazzanti *et al.* 1996; Angélibert *et al.* 2004; Boix *et al.* 2004; Culioli *et al.* 2006). En los sistema de lagunas temporales, la conectividad o la posibilidad de intercambio de individuos entre lagunas, junto con la heterogeneidad de los medios acuáticos (amplio gradiente de hidroperiodo) son dos factores esenciales que posibilitan la conservación de una alta diversidad de macroinvertebrados acuáticos (Urban 2004).

Las estacionalidad anual de las lagunas temporales constituye un proceso cambiante desde el inicio de la inundación, tras las intensas precipitaciones, hasta el siguiente verano, cuando ya las lagunas se han secado. Durante este periodo, el sistema de lagunas se puede considerar dividido en distintas fases en las que además de las características de la propias lagunas (como por ejemplo profundidad, superficie, etc.) cambia también el número de hábitats (lagunas) disponibles para las especies de macroinvertebrados. En la primera fase, se produce la inundación de las lagunas, en la que, cuando las precipitaciones acumuladas acaban produciendo la inundación de la mayoría de las lagunas temporales. Posteriormente suele seguir una fase más estable en cuanto a número de medios inundados, en las que las lagunas cumplen su papel de hábitats acuáticos para muchas especies, hasta que empiece la desecación. Dentro de un área, la fase de desecación es un proceso gradual que comienza con la desecación de la primera laguna temporal, y su longitud es mayor cuanto mayor es el gradiente de hidroperiodo de las lagunas. En esta fase, el número de hábitats acuáticos disponibles va reduciéndose de forma gradual. A lo largo de este proceso, también las comunidades de macroinvertebrados, así como las características ambientales de sus hábitats, sufren importantes cambios, que se resumen en tres fases principales desde la inundación hasta la desecación de las lagunas (Bazzanti *et al.* 1996; Boix *et al.* 2004; Culioli *et al.* 2006; Florencio *et al.* 2009): 1) Durante la fase de inundación de las lagunas, las especies dispersantes colonizan las lagunas temporales y se produce la emergencia de la fauna residente; 2) Durante la fase acuática intermedia, las poblaciones de macroinvertebrados se encuentran ya establecidas y las especies desarrollan su ciclo de vida siendo frecuente el desarrollo de los estadios larvarios; 3) Al principio de la fase de desecación, en la que las lagunas de menor hidroperiodo son las primeras en secarse, se produce la dispersión de los macroinvertebrados hacia lagunas de mayor hidroperiodo. El estrés que supone la desecación de las lagunas temporales, tanto ambiental como de depredación, se considera como una señal que dispara los procesos de dispersión y la formación de estructuras de resistencia en las comunidades de macroinvertebrados (Boix *et al.* 2004; Greig and Wissinger 2010; Jocque *et al.* 2010).

Patrones de distribución en las especies de macroinvertebrados:

Los sistemas de lagunas temporales suelen presentar una fuerte interconexión, tanto a través de la dispersión de sus organismos (principal estrategia de supervivencia de la fauna macroinvertebrada), como por la conexión física que se establece entre comunidades cuando, como en el caso de Doñana, las lagunas se conectan durante períodos de fuertes lluvias anuales (Fahd *et al.* 2007). Ambos procesos favorecen la organización de la fauna de macroinvertebrados en metacomunidades Esta estructura de metacomunidades es frecuente en los sistemas acuáticos temporales, convirtiendo estos medios en un sistema ideal para evaluar hipótesis y teorías ecológicas o evolutivas de carácter general (Urban 2004; De Meester *et al.* 2005; Van de Meutter *et al.* 2007; Joque *et al.* 2010).

Las especies de macroinvertebrados suelen distribuirse de forma diferencial según su tolerancia a determinadas condiciones ambientales, constituyendo comunidades singulares en los distintos ambientes (Waterkeyn *et al.* 2008; Porst & Irvine 2009; Bilton *et al.* 2009). Pero si estas especies se constituyen en comunidades más homogéneas pueden presentar una distribución anidada. El anidamiento es una propiedad de los sistemas que consiste en que las especies de sitios con menor riqueza específica constituyen subconjuntos de especies presentes en sitios con mayor riqueza y cuyo grado de solapamiento específico o grado de anidamiento puede llegar a medirse (McAbendroth *et al.* 2005). Sin embargo, en los sistemas anidados existen una serie de especies y comunidades que “escapan” del patrón general de anidamiento y que se denominan idiosincráticas (Atmar & Patterson 1993). Estas especies idiosincráticas suelen distribuirse bajo unas condiciones ambientales particulares, en lugares donde no se distribuyen las especies más generalistas (McAbendroth *et al.* 2005); por lo tanto, estas especies suelen formar comunidades idiosincráticas en lugares particulares que destacan por su singularidad en los sistemas anidados (Atmar & Patterson 1993). Dado que las especies idiosincráticas confieren cierto grado de heterogeneidad a los sistemas anidados, éstas son consideradas de alto interés para la conservación (McAbendroth *et al.* 2005; Heino *et al.* 2009).

La creación de patrones de distribución anidados se favorece principalmente por los procesos de colonización y extinción (Atmar & Patterson 1993), aunque la colonización como motor para generar patrones anidados suele actuar a más corto plazo (Patterson 1990; Atmar & Patterson 1993). La colonización puede variar entre especies a lo largo de un gradiente ambiental en el que las especies toleran diferentes ambientes, o espacial, según las capacidades de dispersión de las especies, o incluso temporal, según la aparición temprana ó tardía de las especies. La colonización cobra especial importancia en la creación de patrones de distribución anidados en aquellos sistemas donde el principal aporte de especies es externo al medio (Norton *et al.* 2004). Este es el caso de las lagunas temporales sometidas a ciclos anuales de inundación y desecación, en las que la gran mayoría de especies que se incorporan cada año lo hacen vía dispersión. Sin embargo, la variabilidad ambiental también puede generar estos patrones de distribución anidados (Hylander *et al.* 2005) tal y como se ha detectado para las comunidades de macroinvertebrados acuáticos (Lomolino 1996; McAbendroth *et al.* 2005; Heino *et al.* 2009). Dentro de la variabilidad ambiental de las lagunas temporales destaca el efecto del hidroperiodo, cuyo gradiente favorece que las especies se distribuyan de forma anidada entre lagunas de distinto hidroperiodo (Baber *et al.* 2004). El ciclo anual de inundación-desecación que sufren las lagunas temporales hace que las comunidades de macroinvertebrados ajusten sus ritmos de vida a esta estacionalidad. Esto puede generar una temporalidad en el grado de anidamiento de las comunidades ya que dicho anidamiento es considerado como una medida de la organización de las comunidades biológicas (Atmar & Patterson 1993).

Objetivos

El objetivo general de esta tesis es evaluar los diferentes procesos que afectan a las comunidades de macroinvertebrados de las lagunas del Parque Nacional de Doñana. Este objetivo general fue abordado a través de los siguientes objetivos parciales:

1) A la hora de determinar las comunidades, las muestras faunísticas recogidas deben ser representativas de cada laguna y de cada microhábitat muestreado, para averiguarlo se evaluaron dos técnicas de muestreo: el uso de la manga y de la nasa.

2) Determinar la presencia de especies exóticas, en particular, del coríxido exótico *Trichocorixa verticalis* que ha sido recientemente citado en las proximidades del Parque Nacional. Se analizó su distribución en las lagunas del Parque Nacional y el grado de establecimiento de poblaciones reproductoras en el área circundante.

3) Describir la estacionalidad en la composición de macroinvertebrados de 22 lagunas temporales de Doñana a lo largo de dos ciclos de inundación-desecación y su variabilidad interanual en años ampliamente diferentes en la duración de hidroperiodo de dichas lagunas.

4) Los cambios estacionales que sufre la composición de macroinvertebrados son generalmente atribuidos a cambios estacionales en las condiciones ambientales de las lagunas temporales. En esta tesis se evalúa la influencia de dichas variables ambientales en la composición de macroinvertebrados a lo largo de la estacionalidad de dos ciclos de inundación-desecación usando 22 lagunas temporales muestreadas de forma mensual.

5) Analizar la variabilidad ambiental que caracteriza el sistema de lagunas del Parque Nacional de Doñana, evaluando su efecto sobre la composición de macroinvertebrados de las lagunas. Para ello se analiza la relación de la composición de macroinvertebrados de 81 lagunas distribuidas a lo largo de todo el Parque Nacional con la composición química del agua, la estructura física de las lagunas, la proximidad a la costa y a la marisma y la estructura espacial determinada por las distancias entre lagunas.

6) Los patrones de distribución de las especies están determinados por los procesos ecológicos que tiene lugar en los ecosistemas. En esta tesis, se analiza el grado de anidamiento de las comunidades de macroinvertebrados de las lagunas del Parque Nacional de Doñana, así como las posibles causas que generan dicho patrón de distribución (colonización-dispersión, variabilidad ambiental, hidroperiodo, y variación temporal durante el ciclo de inundación-desecación).

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Área de estudio y Metodología

Área de estudio: el sistema de lagunas temporales de Doñana

El Parque Nacional de Doñana se localiza en el suroeste de la Península Ibérica, entre la desembocadura del río Guadalquivir y el Océano Atlántico.

El origen de Doñana data de la era Cuaternaria siendo la zona Sur del Parque Nacional (“Marismillas”) la que ha tenido un origen geomorfológico más reciente pues data de final del Pleistoceno, origen que se debe al continuo avance costero (Siljëstrom *et al.* 1994; Rodríguez Vidal 2006). Doñana se ha dividido tradicionalmente en dos grandes paisajes, las arenas y las marismas (García-Novo *et al.* 1991), separando al Parque Nacional en dos mitades bastante equitativas (Fig.1). El Parque Nacional ha sido cartografiado en base a su geomorfología (Siljeström *et al.* 1994) e integrando distintos procesos ecológicos y de usos del suelo en ecosecciones (Montes *et al.* 1998). En base a su geomorfología, Siljeström *et al.* (1994) clasifica tres unidades geomorfológicas en Doñana según su origen marino, marino-continental y continental generados por procesos litorales, estuáricos y eólicos respectivamente: 1) Las arenas estabilizadas del manto eólico se dividen en las de origen ancestral, que están mayorítariamente localizadas en la zona Norte del Parque, y las de origen reciente con una localización Sur, tal y como Montes *et al.* (1998) también definen en su cartografía de ecosecciones (Fig.1); 2) El sistema de dunas móviles se constituye por cordones de dunas paralelos al litoral y que alcanzan una longitud de 25 km (Fig.1). 3) Las zonas de contacto entre la marisma y las arenas, dan lugar a “La Vera” en el tramo norte del Parque que es una zona rica en lagunas temporales (Fig.1). En las zonas de contacto entre las dunas móviles y las arenas estabilizadas se encuentran las “lagunas más permanentes” (“La laguna Dulce” y “La laguna de Santa Olalla”) también llamadas lagunas “peridunares” (Fig.1) que se alimentan del drenaje del acuífero superficial del sistema de dunas móviles (García Novo *et al.* 1991). El acuífero de Doñana es conocido como la Unidad Hidrogeológica Almonte-Marismas que se extiende por un área de 3400 km^2 (Manzano & Custodio 2006). A lo largo de todo el manto eólico de Doñana, se forman anualmente un gran número de lagunas temporales. Las lagunas temporales de Doñana se sustentan por el agua de lluvia que las inunda y la descarga del acuífero al elevarse la capa freática, lo que posibilita el mantenimiento de su inundación (Manzano & Custodio 2006).

El clima de Doñana se puede caracterizar como Mediterráneo subhúmedo con inviernos suaves y veranos calurosos y secos en el que las precipitaciones se producen principalmente en otoño e invierno, periodo en el que se inundan la marisma y las lagunas temporales. El número de lagunas temporales que se forman en Doñana en años lluviosos sobrepasa los 3000 cuerpos de agua (Gómez-Rodríguez 2009).

La mayoría de las lagunas temporales se forman tras la caída de las principales precipitaciones, una vez que se han acumulado al menos 250 mm de lluvia (Díaz-Paniagua *et al.* 2010). Esto contrasta con el gradual proceso de desecación que suele comenzar al principio de la primavera, con la desecación de las lagunas de más corto hidroperiodo, mientras que las de más largo hidroperiodo suelen secarse en verano (Florencio *et al.* 2009; Díaz-Paniagua *et al.* 2010). La mayoría de las lagunas temporales se localizan en el manto eólico y, principalmente sobre las arenas estabilizadas de Doñana. Además de las lagunas temporales, en Doñana podemos encontrar otros cuerpos de aguas más permanentes y de origen antrópico, denominados "zacallones", que en verano proveen agua para la fauna salvaje y el ganado. Estos "zacallones" son particularmente frecuentes en la zona sur del Parque, "Marismillas", donde el número de lagunas temporales no es abundante. En "Marismillas", aparte de dos lagunas temporales de gran extensión ("La Laguna Larga" o "del Carrizal" y el "Lucio de Marismillas"), en años excepcionalmente húmedos se inundan también extensas praderas denominadas "navazos". En la zona norte principalmente, durante las lluvias, el agua es conducida desde las arenas hacia la marisma a través de unos arroyos intermitentes denominados "caños". Una vez que cesan las lluvias el agua se remansa en los "caños" formando pequeñas lagunitas temporales.

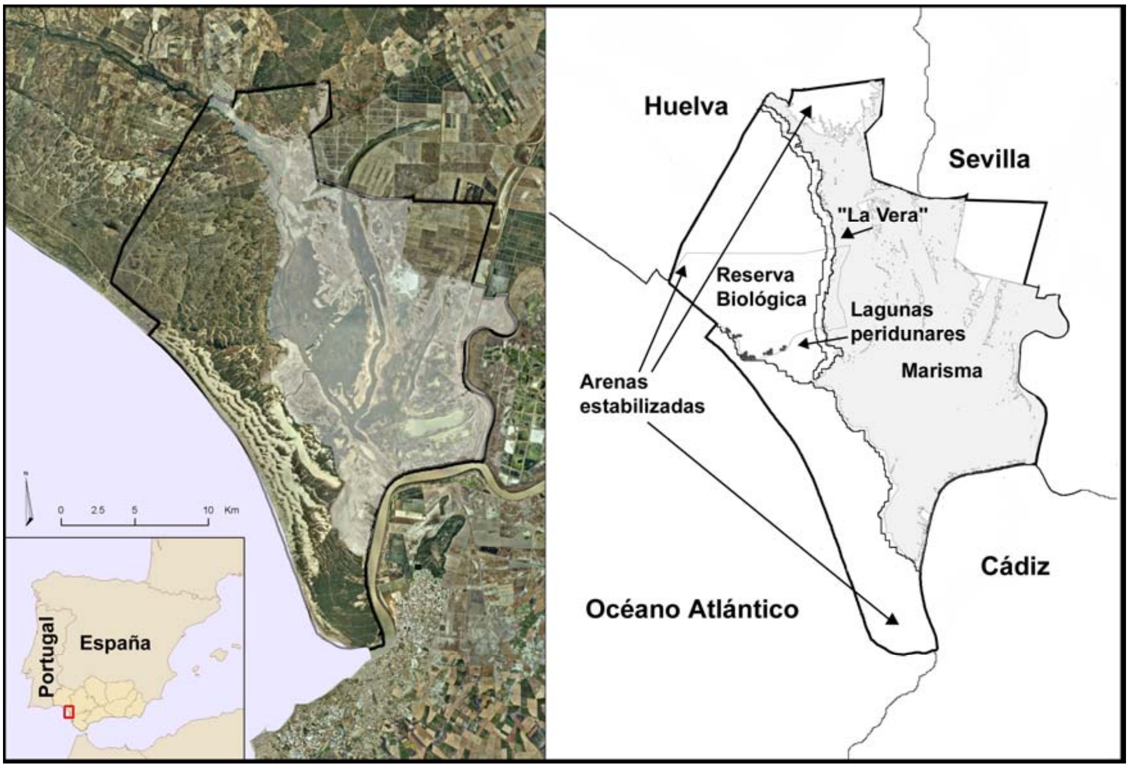

Fig.1: Localización del Parque Nacional de Doñana en el suroeste de la Península Ibérica, limitado por el Océano Atlántico y por las provincias de Huelva, Sevilla y Cádiz, como se observa en el esquema a la derecha de la foto. En la fotografía (a la izquierda), en color más claro destaca la marisma frente al sistema de arenas estabilizadas donde se localizan la mayoría de las lagunas temporales. Además en esta fotografía se puede apreciar el cordón de dunas móviles paralelo a la costa. Las zonas de “La Vera”, de las lagunas peridunares y del área delimitada por la Reserva Biológica de Doñana están destacadas de forma esquemática (a la derecha).

Periodo de estudio

El periodo de estudio abarcó dos años de muestreo cuyas precipitaciones anuales se contabilizaron desde el 1 de Septiembre al 31 de Agosto, incluyendo dos ciclos completos de inundación-desecación de las lagunas. El primer año, 2005-06, se considera un año seco, con un una precipitación anual de 468,3mm (inferior a la media de Doñana, que es de 545mm según Díaz-Paniagua *et al.* 2010). El segundo año, considerado como año lluvioso en este estudio, la precipitación fue de 716.9mm. El año previo al estudio (2004-2005) se caracterizó por una fuerte sequía (Fig.2), y por tanto no se produjo la inundación de las lagunas temporales, incluso una de las lagunas catalogadas como permanentes por Siljëstrom *et al.* (1996) se secó completamente durante aquel verano. Por ello, nosotros consideramos esta laguna, “La Laguna Dulce”, con un carácter general de semipermanente. Durante nuestro periodo de estudio, se produjo su reinundación, por lo que se puede considerar que, al menos en la fase de inundación, se comportó como una laguna temporal. En el primer año de estudio, 2005-2006, la inundación de las lagunas se produjo a final del mes de Enero (Fig.2), mientras que en el siguiente año las intensas precipitaciones otoñales favorecieron la inundación de las lagunas a final de Octubre (Fig.2). Gran cantidad de lagunas que no se habían formado en el año seco, se formaron en el área de estudio durante el año lluvioso ampliando el gradiente de hidroperiodo, por lo hubo que añadir algunas de estas ellas en el estudio para mantener el gradiente anual de hidroperiodo. Las fuertes diferencias en hidroperiodo entre los dos años de estudio se pueden observar en la Foto 1, donde una misma laguna muestreada el mes de marzo en ambos años presenta una gran diferencia en el nivel de inundación. Dicha laguna presentó una profundidad máxima de 36 cm en el año seco y de 76 cm en el año lluvioso.

Durante el periodo de estudio, las comunidades de macroinvertebrados se muestrearon de forma puntual en 91 lagunas ampliamente distribuidas a lo largo de todo el Parque Nacional, 22 de las cuales se muestrearon con periodicidad mensual dentro de la Reserva Biológica de Doñana (Fig 1).

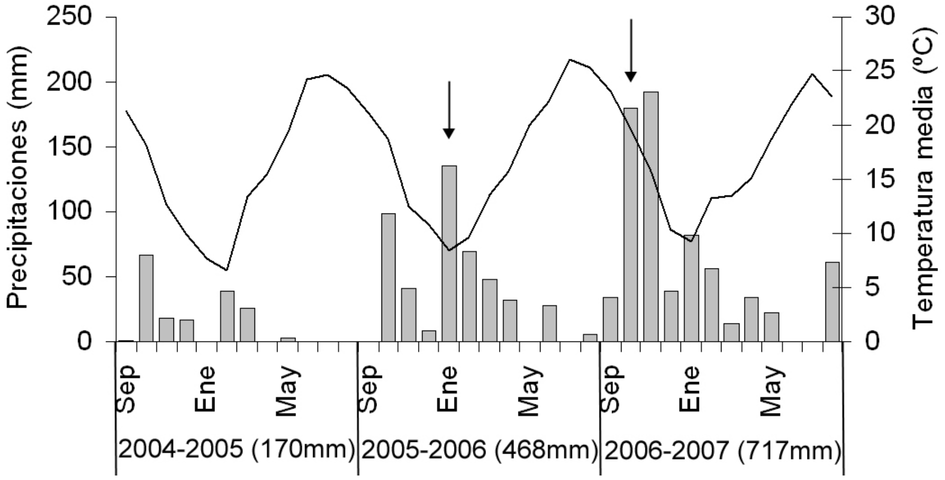

Fig. 2: Precipitaciones mensuales y temperatura mensual media de los años hidrológicos 2004-2005 (previo al estudio), 2005-2006 (el año seco del estudio) y 2006-2007 (el año lluvioso del estudio).Entre paréntesis se muestra la precipitación anual para cada año y las flechas indican el mes en el que se inundaron las lagunas temporales.

Foto 1: Laguna del Pinar de San Agustín mostrando diferencias en el grado de inundación entre Marzo de 2006 (el año seco, a la izquierda) y Marzo de 2007 (el año lluvioso, a la derecha).

Muestreo de la comunidad de macroinvertebrados de Doñana:

Nuestro muestreo se llevó a cabo mediante el uso de una manga de 39x21 cm y 1 mm de diámetro de poro de luz de malla. Cada mangueo consistió en tres pasadas de la manga por una superficie aproximada de 1.5 m, barriendo los organismos asociados al sedimento además de la fauna de la columna de agua. Los especímenes capturados se identificaron *in situ* e inmediatamente se devolvieron al medio natural salvo aquéllos de difícil identificación visual, que se preservaron en alcohol al 70% para su posterior identificación bajo lupa binocular. Hay que destacar este método de muestreo por diversas razones: 1) No es destructivo con gran parte de la fauna capturada que puede constituir decenas de individuos para cada mangueo; 2) Muchas lagunas presentan un tamaño muy reducido, como por ejemplo 4 m^2, para las cuales el investigador puede suponer una fuente importante de perturbación; 3) Reduce el tiempo de procesado de las muestras en el laboratorio mediante la toma de muestras limpias de sedimento y la identificación parcial realizada en el campo.

Para el análisis de la variación temporal de la composición específica de macroinvertebrados en las lagunas, se ha considerado importante reducir al mínimo el impacto del investigador, por lo que se evitó, en lo máximo posible, el sacrificio y la extracción de ejemplares.

La superficie que inundan las lagunas temporales de Doñana puede ser altamente variable: desde extensas praderas de escasa pendiente a pequeñas y someras lagunitas efímeras, y desde enormes a pequeñas lagunas profundas que suelen tener largo hidroperiodo. Los muestreos se realizaron a lo largo de un transecto de orilla a centro, cubriendo el gradiente de profundidad, en puntos distanciados al menos 5 m, intentando abarcar los diferentes microhábitats de cada lagunas en base a variaciones en profundidades y coberturas vegetales (ver Heyer *et al.* 1994). El número de mangueos generalmente incrementó con la superficie de las lagunas para cubrir la mayor diversidad en coberturas vegetales y/ó microhábitats que presentaron las lagunas de gran extensión. Dado que la eficiencia de cada mangueo se incrementa en lagunas temporales de pequeña superficie (Heyer *et al.* 1994), el número de mangueos aumentó con el tamaño de la laguna para aplicar un esfuerzo comparable en la detección de las especies localmente raras.

Lagunas temporales de Doñana

Las lagunas temporales se caracterizan porque sufren un periodo de desecación, que en muchos casos puede ocurrir con una periodicidad anual, como ocurre en las lagunas mediterráneas, donde el periodo seco coincide con el verano. El periodo anual durante el que las lagunas se mantienen inundadas se denomina hidroperiodo.

El hidroperiodo de las lagunas depende del momento en que se produce la inundación y de la cantidad de precipitaciones, por lo que varía en función de las condiciones meteorológicas de cada año, sufriendo en Doñana amplias variaciones. Además el momento de inundación en Doñana es bastante impredecible, produciéndose cuando se han acumulado precipitaciones por encima de 255 mm que en el 60% de las veces ocurre en otoño, y en el 40% de las veces en primavera (según Díaz-Paniagua *et al.* 2010). La inundación es sincrónica para la mayoría de las lagunas excepto para las lagunas efímeras de más corto hidroperiodo, que suelen inundarse sólo en años de fuertes precipitaciones.

Las lagunas temporales de Doñana abarcan un amplio gradiente de hidroperiodo, que unido a las variaciones interanuales, hacen difícil una clasificación en categorías discretas en función de la longitud de hidroperiodo ya que este fue contabilizado por el número de meses y días de duración. Teniendo en cuenta esto, las lagunas temporales de Doñana se han clasificado en tres grandes grupos, en los que se ha tenido en cuenta la duración del hidroperiodo de las lagunas en relación al periodo total en que se observaron las lagunas temporales inundadas cada año. A pesar de la gran variación de hidroperiodo observado entre distintos años, el orden de las lagunas dentro del gradiente anual del hidroperiodo suele mantenerse (Gómez-Rodriguez *et al.* 2009), por lo que las categorías se mantienen entre distintos años: lagunas de corto hidroperiodo, de hidroperiodo intermedio y de largo hidroperiodo:

Lagunas de corto hidroperiodo: En un año de precipitación media, estas son las lagunas de menor duración del periodo de inundación, ya que suelen comenzar a secarse a mediados de primavera, normalmente en el mes de mayo (Díaz-Paniagua *et al.* 2010). En el año seco, se formaron a final del mes de enero, con una duración de hidroperiodo menor a dos meses y medio, aunque una de las lagunas presentó un hidroperiodo de tan sólo 13 días. En el año lluvioso, las lagunas de corto hidroperiodo se dividieron entre las que se inundaron primero en octubre, con una duración de hidroperiodo menor a siete meses, y las que se inundaron a principios de Noviembre, de duración más efímera pues se inundaron más tarde y fueron las primeras en desecarse, con una duración de hidroperiodo menor de seis meses y medio. Estas lagunas de duración más efímera ni siquiera llegaron a inundarse en el año seco. Esto pone de manifiesto las fuertes diferencias en hidroperiodo que presentaron las lagunas temporales entre los dos años de estudio. En las Fotos 2 y 3 se observan lagunas de corto hidroperiodo, y en particular las de duración más efímera en el año lluvioso se observan en las Fotos 4 y 5.

Foto 2 y 3: Lagunas de corto hidroperiodo: “Laguna de la Abeja” (Abe) a la izquierda, y “Laguna adyacente al Navazo del Toro” (Ant) a la derecha.

Foto 4 y 5: Lagunas de corto hidroperiodo de duración más efímera: “Laguna de la Vaca” (Vac) a la izquierda, y “Laguna de las Armerias” (Arm) a la derecha.

Las lagunas de corto hidroperiodo son las preferidas por una fauna muy particular que aprovecha la ausencia de los depredadores (Wellborn *et al.* 1996). El principal riesgo para la fauna que habita estos medios efímeros es la desecación ocasional, que supone la adaptación de muchas de las especies de invertebrados mediante la rápida formación de huevos de resistencia (Brendonck 1996).

La vegetación acuática de estas lagunas temporales de corto hidroperiodo se caracterizó por la presencia de gramíneas como *Agrostis stolonifera*, *Paspalum paspalodes* y *Cynodon dactylon*, en ocasiones acompañadas por las especies típicas de praderas, como *Mentha pulegium*, *Baldellia ranunculoides*, *Myosotis ramosissima* e *Illecebrum verticillatum* (Díaz-Paniagua *et al.* 2010).

Lagunas de hidroperiodo intermedio: En años de precipitación media, en Doñana estas lagunas suelen permanecer inundadas hasta el final de la primavera o excepcionalmente el inicio del verano (Díaz-Paniagua *et al.* 2010). En el año seco, estas lagunas tuvieron una duración del hidroperiodo que varió entre dos meses y medio y tres meses y medio, secándose en abril-mayo. En el año lluvioso la duración del hidroperiodo fue mucho mayor para estas lagunas, entre siete meses y siete meses y medio, secándose en mayo-junio. En Doñana, estas lagunas pueden presentar una alta densidad de plantas acuáticas y macrófitos en comparación con las lagunas de corto hidroperiodo que sirven de refugio para las especies de macroinvertebrados a diferentes profundidades. Entre su vegetación características se distinguen, además de las descritas para las de corto hidroperiodo, especies propias de zonas más profundas como *Callitriche brutia* y *Callitriche obtusangula*, *Myriophyllum alterniflorum* o *Ranunculus peltatus* (Díaz-Paniagua *et al.* 2010). En las Fotos 6 y 7 pueden observarse algunos ejemplos de lagunas de hidroperiodo intermedio.

Foto 6 y 7: Lagunas de hidroperiodo intermedio: “Laguna del Camastrón” (Cam) a la izquierda, y “Laguna del Pinar Grande” (Pg) a la derecha.

Lagunas de largo hidroperiodo: Muchas de estas lagunas son las de mayor extensión, aunque puesto que la duración está principalmente asociada a la profundidad, también existen lagunas pequeñas que pueden mantenerse con largo hidroperiodo, especialmente cuando han sufrido transformaciones por el hombre. Estas lagunas suelen permanecer con agua al menos durante el principio del verano, llegando en algunos casos a prolongarse hasta el mes de agosto e incluso hasta septiembre, e incluso en años de gran inundación puede que no lleguen a desecarse completamente antes del siguiente ciclo hidrológico (como por ejemplo es el caso de “La Laguna Dulce” (Dul)). Tanto en el año seco como en el lluvioso hubo lagunas que permanecieron inundadas hasta agosto. Sin embargo, en general las primeras lagunas se secaron en mayo en el año seco y en junio en el año lluvioso. En las lagunas de largo hidroperiodo es frecuente encontrar, además de las especies de plantas acuáticas descritas para las otras categorías de hidroperiodo, especies de macrófitos de mayores requerimientos hídricos, localizadas en las zonas de mayor profundidad, como son *Potamogeton lucens* *P. natans*, o *Potamogeton pectinatus*, o incluso pueden carecer de vegetación en las zonas más profundas (Díaz-Paniagua *et al.* 2010). En la Fotos 8 puede observarse un ejemplo de laguna de largo hidroperiodo.

Foto 8: Laguna de largo hidroperiodo: “Laguna del Orfeón” (Orf).

Dentro de las lagunas de largo hidroperiodo se encuentran los **zacallones**, que son lagunas excavadas de forma artificial y que mantienen agua durante el verano para el ganado y la fauna silvestre. A pesar de su origen antrópico, la mayoría de estas lagunas, que se caracterizan por su reducido tamaño y gran profundidad, presentan un excelente estado de conservación con una alta densidad de vegetación y una alta riqueza de fauna de macroinvertebrados (Fotos 10 y 11). Suelen presentar especies de macrófitos con mayores requerimientos hídricos, tal y como hemos descrito para las zonas profundas. Estas lagunas constituyen un reservorio importante para la fauna que habita las lagunas temporales durante el verano, cuando la mayoría de lagunas temporales en el Parque Nacional se han desecado completamente.

Fotos 10 y 11: Zacallones de “Marismillas”.

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Chapter 1: Sampling macroinvertebrates in a temporary pond: Comparing the suitability of two techniques to detect richness, spatial segregation and diel activity.

Resumen:

Las lagunas temporales han despertado recientemente un gran interés como reservorios de biodiversidad. Para caracterizar de forma correcta las comunidades biológicas que albergan estos medios es necesario evaluar la eficacia de diferentes técnicas de muestreo. En este estudio se comparó la eficiencia del uso de la manga y de la nasa como técnicas de muestreo de la comunidad de macroinvertebrados de una laguna temporal localizada en el Parque Nacional de Doñana (S.O. España). Los muestreos se realizaron en cuatro momentos del día (mañana, medio día, atardecer y noche) en dos zonas de la laguna que se catalogaron como zonas someras y profundas. En total, la manga capturó mayor riqueza y abundancia de macroinvertebrados que la nasa. Sin embargo, ambas técnicas capturaron taxones de forma exclusiva que no fueron capturados por la otra técnica de muestreo. Las nasas distinguieron de forma eficaz entre especies de macroinvertebrados diurnos y nocturnos, y por tanto se considera una técnica apropiada para estudiar los ciclos de actividad de las especies de macroinvertebrados. Aquellas especies que se detectaron con actividad nocturna fueron las larvas de *Gerris thoracicus* y los adultos de *Colymbetes fuscus*, *Rhantus suturalis*, *Rhantus hispanicus* e *Hydrochara flavipes*; mientras que las especies detectadas con actividad diurna fueron las larvas de *Sympetrum fonscolombei* y *Notonecta* spp., y los adultos de *Notonecta glauca*. El ciclo diario de la comunidad de macroinvertebrados de la laguna temporal dependió del ciclo diario de las diferentes especies integrantes de dicha comunidad. La manga es considerada en este estudio como la técnica más apropiada para detectar la composición de macroinvertebrados en los diferentes microhábitats, pero la nasa fue más eficiente para capturar la fauna nocturna y con veloz capacidad natatoria. Consecuentemente, el uso de ambas técnicas, nasa y manga, sería la combinación más eficaz para obtener la composición de macroinvertebrados representativa de una laguna temporal en comparación con el uso independiente de cada una de las técnicas de muestreo.

Abstract:

Recent and increasing interest in temporary ponds as biodiversity reservoirs fosters our need to test sampling techniques for characterizing their biological communities. To compare the efficiency of dip-netting and funnel nets we sampled the macroinvertebrate assemblage of a particular pond with these two techniques. We sampled at four different times - morning, afternoon, evening and night - in deep and shallow zones of a temporary pond located in the Doñana National Park (SW Spain). Within our sampling period, dip-netting captured higher number of taxa, and higher abundances of individuals than funnel nets. However, both techniques captured exclusive taxa which were not recorded with the other device. Funnel nets distinguished between nocturnal and diurnal macroinvertebrates, and hence are more appropriate to study macroinvertebrate activity. We detected nocturnal activity in *Gerris thoracicus* larvae, adults of *Colymbetes fuscus*, *Rhantus suturalis*, *Rhantus hispanicus and Hydrochara flavipes* whereas larvae of *Sympetrum fonscolombei* and *Notonecta* spp., and adults of *Notonecta glauca* were mainly diurnal. The overall diel activity pattern of the macroinvertebrate assemblage depended on the diel activities of their integrating taxa and stages. Although dip-netting was more appropriate to sample macroinvertebrate assemblages in different microhabitats, funnel nets better captured nocturnal and fast-swimming invertebrates. Consequently, the combination of both sampling techniques would capture a better picture of the representative macrofauna of a temporary pond than either one on its own.

Key words: sampling devices, dip-net, funnel nets, night-day, macroinvertebrate assemblages, representative macrofauna

Introduction

Mediterranean temporary ponds, MTPs, which are included as priority habitats in the Habitat directive (Natura code 3170, CEE, May 21st 1992), are drawing an increasing interest for conservation due to their high vulnerability to disturbances (Grillas *et al.* 2004; Zacharias *et al.* 2007; Céréghino *et al.* 2008). These habitats are especially important for the conservation of rare or scarce taxa of aquatic macroinvertebrates (Collinson *et al.* 1995; Bilton *et al.* 2009), of which many of them may not survive or complete their life cycles in other types of aquatic habitats. From the necessity of increasing our knowledge of the biological communities in temporary ponds emerge the necessity of testing different sampling techniques for shallow and vegetated ponds (O'Connor *et al.* 2004; García-Criado and Trigal 2005; Becerra Jurado *et al.* 2008).

The characterization of macroinvertebrate assemblages in aquatic systems strongly depends on the sampling techniques used (Turner and Trexler 1997; Hyvönen and Nummi 2000; O'Connor *et al.* 2004). Macroinvertebrate surveys with different aims would require different sampling techniques. Although many studies have shown that the number and taxonomy of individuals captured may vary with time of day (Rincón and Lobón-Cerviá 1997; Céréghino and Lavandier 1998; Hampton and Duggan 2003), these differences are not considered in most studies concerning the representative species of macroinvertebrate assemblages. Dip-netting has been a technique frequently used in sampling macroinvertebrates in ponds (Maciolek 1989; Collinson *et al.* 1995; Nicolet *et al.* 2004; Bilton *et al.* 2006; Florencio *et al.* 2009), whereas specific traps have been used to sample active macroinvertebrates in different aquatic systems in order to test their diel activity, such as nets (Waters 1962), hand-made traps (Hampton and Duggan 2003) and the so-called activity traps (Murkin *et al.* 1983; Hanson *et al.* 2000). Dip-netting efficiency for monitoring macroinvertebrates of temporary ponds has been stressed (Cheal *et al.* 1993; García-Criado and Trigal 2005) but other studies found dip-netting inappropriate for sampling macroinvertebrates in shallow ponds (Muzaffar and Colbo 2002; O'Connor *et al.* 2004). The efficiency of activity traps in capturing particular groups of fast swimming invertebrates has been demonstrated (Murkin *et al.* 1983; Hanson *et al.* 2000).

Collecting techniques differ in their efficiency to capture active individuals and the whole pool of macroinvertebrates (Cellot 1989; Rincón and Lobón-Cerviá 1997). Many species present day-night changes in spatial distribution or activity that are an essential part in the behavioural dynamism of a community, for which day samples may not provide a complete picture (Elliot 2005). When sampling techniques are used to detect activity cycles of macroinvertebrates, particular species have presented high abundances and richness at night (Johnson and Covich 2000; Marklund *et al.* 2001; Hansen and Closs 2007), especially during the period just after sunset (Waters 1962; Brittain and Eikeland 1988) and before sunrise (Rincón and Lobón-Cerviá 1997). These diel activity cycles can also be associated with differential uses of the microhabitats along the day for macroinvertebrate species (Elliott 2002; 2005) and assemblages (Hampton and Duggan 2003). Macroinvertebrate diel activities also can differ among seasons, presenting peaks of activity only in particular wet phases (Waters 1962; Rincón and Lobón-Cerviá 1997; Hansen and Closs 2007).

In this study, our aim was to compare the efficiency of two sampling techniques to asses the taxa richness in an epibenthonic macroinvertebrate community. Additionally we tested the suitability of both techniques to detect spatial and diel activity differences within a temporary pond, recording information for particular taxa and macroinvertebrate assemblages.

Materials and methods:

This study is based on sampling the macroinvertebrate assemblages in a temporary pond in Doñana National Park, (37º N, 6 W, Huelva province, SW Spain) in March and April of 2007. This Park is located between the Atlantic coast and the mouth of the Guadalalquivir River, and its sandy area includes more than 3000 water bodies in a pond network that exhibit high conservation values and encompass a wide range of hydroperiods (Díaz-Paniagua *et al.* 2010). A detailed description of these temporary aquatic habitats can be found in Espinar and Serrano (2009) and Gómez-Rodríguez *et al.* (2009). The climate is Mediterranean sub-humid, with hot and dry summers, mild winters, and rainfall mainly occurring in autumn and winter. The study pond has a maximum area of approx. 4000 m^2, and during our study year it was flooded from November to May, with 54 cm maximum depth in February.

Two different sampling techniques were compared: a) Dip-netting sampling (39 x 21 cm aperture with 1 mm mesh): we swept a stretch of water of *c.a.* 1.5 m, in three successive times from one extreme to the other. b) Funnel nets (5mm mesh): These nets are commonly used in the study area to catch eels or crayfish. They have a conical structure with an inverted funnel in the aperture (aperture of 160 cm^2) which favours the entrance of animals, but prevents their exit from the trap (Fig. 1).

Fig. 1: Funnel net collecting macroinvertebrates in a pond.

Within the pond, we selected three sampling sites in each of two zones with different depth and vegetation during the study period. The shallow zone, with 11-26 cm depth, had a range of 6.7-8.6 mg l^{-1} of dissolved oxygen and of 20-22 ºC water temperature and a dense cover of meadow plants (mainly *Mentha pulegium* L., *Illecebrum verticillatum* L., and *Hypericum elodes* L.). The deep zone, with 29-39 cm depth, ranged 0.2-1.2 mg l^{-1} of dissolved oxygen, 12-15ºC of water temperature and had low-dense cover of aquatic macrophytes, (*Juncus heterophyllus* Dufour, *Myriophyllum alterniflorum* DC. in Lam and DC. and *Ranunculus peltatus* Schrank).

In order to compare the efficacy of both techniques we sampled the same sites in successive 24h period from March 16th to 18th (M1) , starting with funnel nets, that we revised every 6 hours covering the following day times: revised approx at 13 h (morning), at 18 h (afternoon), at 24 h (evening) and approx. 7 h (night). After one day without sampling, we sampled at the same six sites using dip-net, also considering similar four day-time: at approx. 9 h (morning), 15 h (afternoon), 19:30 h (evening) and 23:30 h (night). Time is shown in GMT hours. The same sampling procedure was repeated from March 24th to 26th (M2).

Most macroinvertebrates were identified and counted *in situ*, and then immediately released. The unidentified individuals were preserved in 70% alcohol for posterior identification in the laboratory. During nocturnal sampling, the inspection of funnel nets and dip-nets were made using a potent head lamp. Whenever it was possible, we identified adults, larvae or nymphs to species level, or to genus for some larvae, except for dipterans for which we mainly identified only down to the family level.

In order to assess the consistency of diel cycle variations we additionally sampled with funnel nets twice further, on April 16th (A1) and April 20th (A2).

Statistical analyses:

We carried out this study in a single pond because our purpose was to evaluate the two sampling techniques rather than study across-pond variation in macroinvertebrate assemblage composition. The sampling devices used differed in mesh size, as it is often the case as funnel-traps normally have a greater mesh size than dip-nets. Hence, we only compared abundances of trapped large bodied individuals (>5mm). Funnel nets, however, retain a certain number of small individuals (<5mm), so although their abundances are surely misrepresented, they are still suitable for detecting their presence in the pond. Therefore, we used presence-absence data for comparisons of the macroinvertebrate assemblage depicted by each sampling technique. Additionally, we tested for differences in small *vs*. large individuals recorded to evaluate the importance of mesh size in our results. Abundance and number of taxa captured at each sampling day time were compared between sampling techniques using nonparametric two-tailed Wilcoxon test. As multiple comparisons were required, we corrected the significance level using the Dunn-Sidak procedure (Sokal and Rohlf 1997).

For diel variation analyses, we considered the number of individuals recorded in funnel nets in March and April. For these analyses, we treated life-history stages separately, differentiating adults from larvae or nymphs of the same taxon (referred to as different taxa-stage), because we considered that adults and larvae differed considerably in their movements and preferred microhabitats.

To detect variations in the general composition of macroinvertebrates sampled at different day times and in different zones, we constructed a matrix including data of presence/absence of all macroinvertebrate taxa-stages per each sampling site and sampling day. Corresponding resemblance matrices were calculated through the Sorensen index (Legendre and Legendre 1998) in order to perform two-way crossed ANOSIM analyses, a routine of Primer v.6 (9999 permutations, Clarke and Warwick 2001), using sampling day hours and zones as grouping factors. We obtained a Global Spearman coefficient (Global R) to detect significant differences between the levels of these two factors, and also for every pair-wise comparison of day times. When this

Spearman coefficient (0-1) is close to 1, differences among levels of the factor are highest. In order to identify the taxa-stages which contributed the most to differences between day times and between zones, for each sampling period (M1, M2, A1 and A2), we performed a two-way SIMPER (Primer v.6; Clarke and Warwick 2001), an exploratory analysis based on the original presence data. We indicated the four taxa-stages which mainly contributed to the differences in two-way SIMPER analyses.

To asses the diel variation of particular taxa-stages, we grouped data from both monthly sampling occasions. For those taxa-stages for which we obtained n>10 individuals in a sampling month, we illustrated the number of individuals captured through different day times. Data from morning and afternoon times were grouped and considered to reveal diurnal activity, whereas evening plus night data were considered to indicate nocturnal activity. We compared if the number of individuals of each taxa-stage was captured mainly in diurnal or nocturnal times using chi-square tests.

Using the most abundant taxa-stages, we tested for differences between techniques in detecting differences in sampling day times and pond zones using repeated measures ANOVA on number of individuals captured. We used linear contrasts in planned comparison analyses to detect if taxa-stages with significant differences along day hours were more abundant during daylight hours, as diurnal taxa (morning and afternoon), or at night, as nocturnal taxa (evening and night). Abundance for each taxon was transformed as log (X+1) or 4th root transformed to meet parametric assumptions.

Results

Assessing the macroinvertebrate composition with different techniques

Comparing both sampling techniques in March, we recorded 39 taxa of which 17 were captured with both. Sixteen taxa were exclusively captured by dip-netting and five were exclusively found in funnel nets. Two of these five exclusive taxa were captured by funnel nets in spite of their larger mesh size (*Bagous* spp., *Dryops* spp.) (see Appendix 1). The total number of individuals captured through dip-nettings and funnel nets was 915 and 215 respectively. The dip-net captured significantly higher number of individuals per sampling time than nets even when we considered separately macroinvertebrates larger and smaller than 5 mm (Wilcoxon test, in all three cases Z=2.52, p=0.01, Fig. 2A, Appendix 1). The total number of taxa per sampling time recorded through dip-nettings was also larger than that of funnel nets for all body sizes (Wilcoxon test, Z= 2.52, p=0.01), the body size >5mm (Wilcoxon test, Z=2.37, p=0.02) and marginally significant for body size <5mm (Wilcoxon test, Z=1.96, p=0.05). However, both techniques captured a similar total number of taxa with small body size when we did not compare separately by each sampling day time (Fig.2B).

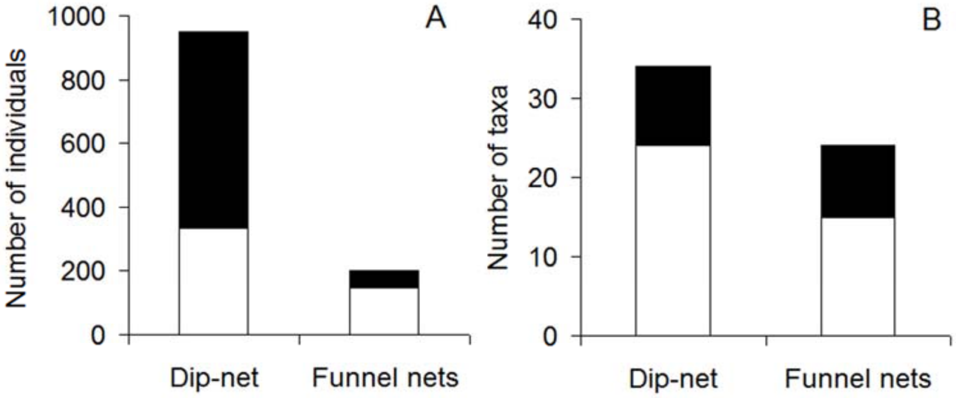

Fig. 2: Total number of macroinvertebrate individuals (A) and number of taxa (B) captured with the dip-net and funnel nets in the two sampling days of March. The number of individuals and taxa captured with body size shorter (filled fraction of bars) or larger (empty fraction) than 5mm are shown.

Variation across sampling time points: diurnal versus nocturnal activity.

Macroinvertebrate assemblages captured through dip-netting did not significantly differ among day times in March (M1 Global R=0.015, p=0.428; M2 Global R=0.053; p=0.292). On the contrary, we found significant differences among macroinvertebrate assemblages captured by funnel nets in all sampling days except for A1 (Table 1, M1 Global R=0.364, p<0.01; M2 Global R=0.350, p<0.01; A1 Global R=0.081, p=0.744; A2 Global R=0.237, p<0.05). In M1, we detected different assemblages between the following pair-wise comparisons: afternoon-morning, afternoon-evening and afternoon- night. The taxa with largest contribution to these differences were Corixidae larvae, *Cloeon dipterum* (Linnaeus, 1761) larvae, *Sympetrum fonscolombei* (Selys, 1841) larvae, *Dytiscus circumflexus* Fabricius, 1801 larvae and adults of *Colymbetes fuscus* (Linnaeus, 1758) (Table 1). In M2, we found differences between evening-morning and evening-afternoon with higher contribution of *Notonecta* spp. larvae, *S. fonscolombei* larvae, *Gerris thoracicus* Schummel, 1832 larvae, and adults of *Hydrochara flavipes* (Steven, 1808) (Table 1). In April (A2), we only detected significant differences between morning-night and afternoon-evening, with the main contribution of *C. fuscus* adults, *Notonecta glauca* Linnaeus, 1758 adults, *S. fonscolombei* larvae and *H. flavipes* adults (Table 1).

Table 1: Results of a two-way crossed ANOSIM analyses (R= Spearman coefficient) performed to detect differences between the composition of macroinvertebrate assemblages between day times through funnel nets (significant differences between day times were not detected using dip-nets). The main taxa contributing to the significant differences, resulting from a two-way crossed SIMPER test, are also shown and the day time when they were more frequent per every significant comparison. For each taxon, L indicates larvae and A indicates adults (M1= March 16th; M2= March 24th; A1= April 16th; A2= April 20th 2007).

Sampling dates	**Day hours significant comparisons**		**Spearman R**	**Main taxa**	**More frequent**	**Contribution**
M1	Afternoon	Morning	0.370*	Corixidae-L	Afternoon	16.18%
				Cloeon dipterum-L	Morning	10.15%
				Dytiscus circumflexus-L	Morning	8.24%
	Afternoon	Evening	0.767*	Corixidae-L	Afternoon	13.14%
				Sympetrum fonscolombei-L	Afternoon	10.32%
				Colymbetes fuscus-A	Evening	8.83%
	Afternoon	Night	0.630*	Corixidae-L	Afternoon	12.98%
				Sympetrum fonscolombei-L	Afternoon	10.68%
				Cloeon dipterum-L	Night	8.69%
M2	Evening	Morning	0.622*	*Sympetrum fonscolombei*-L	Morning	17.81%
				Notonecta spp.-L	Morning	17.81%
				Gerris thoracicus-L	Evening	14.44%
	Evening	Afternoon	0.694*	*Notonecta* spp.-L	Afternoon	18.21%
				Gerris thoracicus-L	Evening	14.04%
				Hydrochara flavipes-A	Evening	13.93%
A1			n.s.			
A2	Morning	Night	0.444*	*Colymbetes fuscus*-A	Night	15.05%

				Notonecta glauca-A	Morning	13.19%
				Sympetrum fonscolombei-L	Morning	11.86%
	Afternoon	Evening	0.491*	*Colymbetes fuscus*-A	Evening	15.33%
				Notonecta glauca-A	Afternoon	10.81%
				Hydrochara flavipes-A	Evening	7.07%

** = $p<0.01$
* = $p<0.05$
n.s. = non significant

In Fig. 3, we show the day time variation of the number of individuals captured by funnel nets for the most abundant taxa-stages. We detected significant higher abundances in nocturnal periods for *G. thoracicus* larvae, *Colymbetes fuscus* adults, *Rhantus suturalis* (McLeay, 1825) adults, *Rhantus hispanicus* Sharp, 1882 adults, and *H. flavipes* adults which was only captured in nocturnal samplings (Fig. 3). Other taxa reached higher diurnal than nocturnal total abundances: *S. fonscolombei* larvae and *Notonecta* spp. larvae, and adults of *N. glauca* (Fig. 3). In other taxa, we did not capture significantly different total abundances in nocturnal or diurnal samplings, e.g. adults of *G. thoracicus* despite the larvae being mainly nocturnal in March (Fig. 3). All taxa-stages showed similar tendency in March and April towards nocturnal or diurnal activity although the significant difference was only detected in the month in which they were most abundant, e.g. *S. fonscolombei* larvae was only diurnal in March and *N. glauca* only in April (Fig. 3).

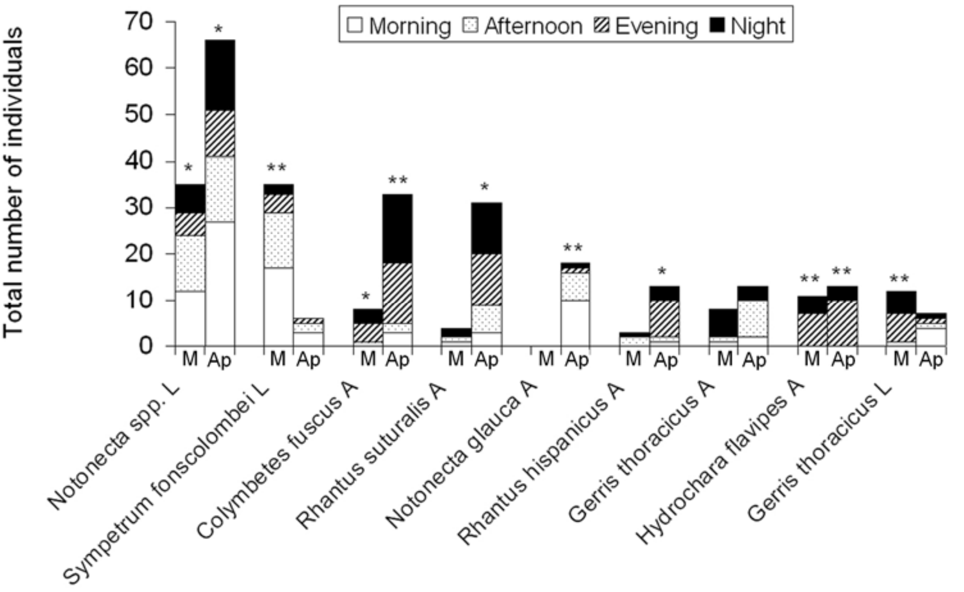

Fig. 3: Number of individuals of the most abundant taxa captured by funnel nets in the two sampling days in March, M, and in April (A1+A2), Ap, are shown. For each taxon, L indicates larvae and A indicates adults. Morning: 07:00-13:00 GMT hours, afternoon: 13:00-19:00, evening: 19:00-01:00 and night: 01:00-07:00. Significantly different abundances for diurnal (morning + afternoon) and nocturnal macroinvertebrates (evening + night) are shown (χ^2, df=1, *is $p<0.05$, **is $p<0.01$).

Spatial segregations: shallow versus deep zones.

Sampling with the dip-net, we detected significant differences in the macroinvertebrate assemblages recorded between shallow and deep zones in the two sampling occasions (M1 and M2, Table 2). *Physa* spp., larvae of *Notonecta* spp., larvae of *Laccophilus minutus*, and adults of *Berosus guttalis* Rey, 1883 were the main taxa-stages contributing to these differences (Table 2). Excluding *L. minutus* larvae, all of them were more frequent in the deep zone. Using funnel nets, we found a significant segregation only in the two sampling occasions of April (A1 and A2, Table 2), but it was not found in March (M1 R=0.078, p=0.287; M2 R=0.258, p=0.053; Table 2). In A1, the significant correlation took the lowest value with the main contribution in the pond zone differences of *Notonecta* spp. larvae and *C. fuscus* adults and *R. suturalis* adults (Table 2). In A2, with the strongest value of correlation, *Notonecta* spp. larvae, adults of *R. suturalis* and adults of *N. glauca* were the main taxa-stages contributing in the pond zone segregation (Table 2). In April, excluding *R. suturalis*, all of these taxa-stages were more frequent in the deep zone.

Table 2: Results of a two-way crossed ANOSIM analyses (R= Spearman coefficient) performed to detect differences between the composition of macroinvertebrate assemblages between deep and shallow zones of the pond, sampled through either dip-netting or funnel nets. The main taxa contributing to these differences were identified through two-way crossed SIMPER tests. The percentage contributed to the total by each taxa is also shown. Main zones indicate the zone where every taxa was abundant. For each taxon, L indicates larvae and A indicates adults (M1= March 16th-18th; M2= March 24th-26th; A1= April 16th; A2= April 20th 2007).

Funnel nets	**Sampling dates**	**Spearman R**	**Main taxa**	**Main zones**	**Contribution**
	M1	n.s.			
	M2	n.s.			
	A1	0.239*	*Notonecta* spp.-L	deep	14.72%
			Colymbetes fuscus-A	deep	13.06%
			Rhantus suturalis-A	shallow	9.05%
	A2	0.435**	*Notonecta* spp.-L	deep	14.93%
			Rhantus suturalis-A	shallow	12.95%
			Notonecta glauca-A	deep	7.45%
Dip-netting					
	M1	0.389**	*Physa* spp.	deep	9.49%
			Notonecta spp.-L	deep	7.40%
			Laccophilus minutus-L	shallow	6.92%
	M2	0.421**	*Notonecta spp.*-L	deep	11.08%
			Physa spp.	deep	10.53%
			Berosus guttalis-L	deep	8.38%

** = p<0.01
* = p<0.05
n.s. = non significant

Exploring the suitability of both techniques for particular taxa

We detected differences in the suitability of both techniques for detecting spatial and temporal variation in species abundance using the most abundant taxa-stages captured in March (see Fig. 3). In dip-netted samples of *S. fonscolombei* larvae as well as of *Notonecta* spp. larvae we did not find significant differences in the abundance captured at different day times ($F_{3,30}$=1.72, p=0.18; $F_{3,30}$=2.05, p=0.13 respectively). However, we found significant differences between the abundances captured in the two sampling zones of the pond (*S. fonscolombei* larvae: $F_{1,10}$=22.06, p<0.01; *Notonecta* spp. larvae: $F_{1,10}$=23.27, p<0.01) with higher number of individuals in the deep zone in both cases. On the contrary, in funnel net samples, we detected significant differences in the abundances at various day times for *S. fonscolombei* larvae ($F_{3,30}$=4.45, p<0.5) and *Notonecta* spp. larvae ($F_{3,30}$=5.55, p<0.01), where both taxa exhibited higher abundance over diurnal periods (planned comparisons: $F_{1,10}$=13.60, p<0.01; $F_{1,10}$=9.86, p<0.05 respectively). Funnel nets detected significant differences in species abundances between pond zones for *S. foncolombei* larvae ($F_{1,10}$=17.82, p<0.01) but not for *Notonecta* spp. larvae ($F_{1,10}$=0.463, p=0.51). No interactions between sampling day time and pond zones were detected (all p>0.319).

Discussion

Comparing the efficiency of sampling techniques

Dip-netting ensured capturing a higher number of taxa and a higher number of individuals than funnel nets. We could expect that these differences were due to the smaller mesh size of the dip-nets used, as small macroinvertebrates could escape from funnel nets with mesh size larger than their body length or width. Nevertheless, mesh size did not seem to affect these results for various reasons. Firstly, dip-netting captured higher abundances of taxa both smaller and bigger than 5mm. Moreover, the number of total taxa with reduced body size (<5mm) were equally captured by the dip-net (10 taxa) and funnel nets (9 taxa) despite the higher number of individuals captured per day time by dip-nettings. Sixteen taxa were exclusively captured by the dip-net, and only five of them had such reduced body size that individuals could escape from funnel nets [*Planorbis* spp., *Hydroporus gyllenhali* Schiödte, 1841, *Anacaena lutescens* (Stephens, 1829), *Microvelia pygmaea* (Dufour, 1833) and *Plea minutissima* Leach, 1817]. Furthermore, two of the five taxa exclusively captured by funnel nets were also smaller than 5 mm, indicating that funnel nets can also trap small species. The different suitability of both techniques for detecting macroinvertebrate assemblages' composition and its spatial and diel variation indicates that there are important differences among them, and hence complements each other. Dip-net sampling is fast and intensive, yielding a high number of individuals regardless of whether they were active or not at the moment. In contrast, funnel nets is a more passive approach that captures fewer individuals but is more likely to detect fast swimming species (e.g. the diving beetles

Colymbetes fuscus or *Dytiscus circumflexus* Fabricius, 1801) and provide information on activity patterns.

An additional important difference between our sampling techniques is that dip-netting may be more strongly affected by among-researcher variation due to idiosyncratic differences in sweeping. In contrast, funnel nets allow for an easy standardisation of the sampling procedure, an advantage already mentioned for activity traps when they were compared to dip-netting (Murkin *et al.* 1983).

In our study, the sampling technique had a great influence on species abundances. Similar conclusions were obtained in studies comparing other techniques (Brinkman and Duffy 1996; Muzaffar and Colbo 2002; O'Connor *et al.* 2004), suggesting that evaluation and comparisons of biodiversity among macroinvertebrates assemblages should take into account the sampling technique used.

Detection of diel activity cycles

Funnel nets exclusively capture active individuals, hence giving information about diel activity cycles. Indeed, although we sampled over 24-hour periods, diel activity cycles were only detected with funnel nets, not through dip-netting. This dichotomy between catching techniques has been already demonstrated in lotic aquatic systems (Cellot 1989; Rincón and Lobón-Cerviá 1997) but not in lentic macroinvertebrates of temporary ponds.

Although dip-netting was more efficient for monitoring biodiversity, five taxa were only detected by funnel nets, including nocturnal taxa such as *Colymbetes fuscus* and *Hydrochara flavipes*. The nocturnal activity of predatory beetles has been previously reported (Holomuzki 1985; Dolmen and Solem 2002) and represents an example of resource partitioning within the food chains in aquatic environments, where some prey species shift their diel rhythms to nocturnal activity in order to avoid predation risk (Gilbert and Hampton 2001).

Diel activity has been reported for particular aquatic macroinvertebrates taxa (Waters 1962; Céréghino and Lavandier 1998; Johnson and Covich 2000), but very few studies have analysed diel variations for macroinvertebrate assemblages (Rincón and Lobon-Cervia 1997; Hampton and Duggan 2003). Funnel nets detected different assemblages through day times because of the differential activity of nocturnal and diurnal species. We observed five taxa with higher abundances at night (*Gerris thoracicus* larvae, *C. fuscus* adults, *Rhantus suturalis* adults, *Rhantus hispanicus* adults and *H. flavipes* adults) whereas other three taxa were considered diurnal (*Sympetrum* *fonscolombei* larvae*, Notonecta* spp. larvae, and adults of *Notonecta glauca*). In March, the strong macroinvertebrate assemblage variation detected at sampling day times was favoured by the high contribution of diurnal taxa (*S. fonscolombei* larvae and *Notonecta* spp. larvae), although nocturnal taxa also occurred but contributed with less abundance (*C. fuscus* adults, *H. flavipes* adults, and larvae of *G. thoracicus*). In April, diurnal taxa (larvae of *Notonecta* spp. and adults of *N. glauca*) as well as nocturnal taxa (all adults of *C. fuscus*, *R. suturalis*, *R. hispanicus*, and *H. flavipes*) were abundant. In April, this high data variability caused by the strong contribution of diurnal and nocturnal taxa promoted the lack of detection of diel variation in the macroinvertebrate assemblage as a whole. This indicates that it is important to examine the variation of particular taxa to determine whether apparent lack of diel cycles in macroinvertebrate assemblages can hide opposite diel cycles of particular taxa.

Funnel nets are therefore an important supplementary technique to complement biodiversity monitoring samples obtained through dip-netting, due to a more representative fauna is obtained when different techniques are combined (Turner and Trexler 1997; Hyvönen and Nummi 2000; Becerra Jurado *et al.* 2008). As macroinvertebrate sampling is usually made during day times, the use of funnel nets during prolonged sampling periods may offer a more complete picture of the macroinvertebrate community (i.e. at least 24 h in order to collect nocturnal and diurnal species).

Spatial segregations: shallow and deep

Although some studies found dip-netting inappropriate for sampling macroinvertebrates in shallow ponds (Muzaffar and Colbo 2002; O'Connor *et al.* 2004), our results confirm this is a good method to sample a representative macrofauna of different microhabitats within a pond. In contrast, the use of funnel nets only captured differences in assemblage composition across pond zones in April and only captured different abundances per zone for *S. fonscolombei*.

Dip-netting can vary their efficiency capturing macroinvertebrates in vegetated or non vegetated zones of the pond (Becerra Jurado *et al.* 2008; O'Connor *et al.* 2004; García-Criado and Trigal 2005). In this study, the main contributing taxa to different pond zones occurred mainly in the deep zone (*Physa* spp. exclusively occurred there), but both types of zones were vegetated. Although we cannot discard the influence of increased difficulty of dip-netting in shallow vegetated areas, it is certainly not the sole cause for zone differences because some taxa showed high abundance in the shallow zone (e.g. larvae of *Agabus* spp., larvae of *G. thoracicus*, and larvae of *Laccophilus minutus*).

In conclusion, diel activity should be taken into account when sampling macroinvertebrate assemblages in temporary ponds. Dip-netting captured higher number of individuals and taxa, and better distinguished variations in assemblage composition according to microhabitat. It is hence a more appropriate technique than funnel nets if the distinction between active and inactive macroinvertebrates is irrelevant. Funnel nets were more efficient at capturing fast swimming species and provided information on diel patterns. The combination of both techniques would provide the most informative picture of the macroinvertebrate community of a pond.

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Colaborators:

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Appendix 1: Number of individuals per taxa-stage (adults, larvae and nymphs considered separately) captured by each sampling technique, dip-net and funnel nets, in two sampling events per month (the dip-net was only used in March). Taxa having adult, larvae or nymphal stages with body size <5 mm are indicated with +.

	Dip-net		**Funnel net**			
Macroinvertebrate taxa-stages	**March adults**	**March larvae**	**March adults**	**March larvae**	**April adults**	**April larvae**
Bassomatophora						
Physa spp. +	179		23		28	
Planorbis spp. +	1					
Coleoptera						
Bagous spp. +			1		5	
Dryops spp. +			1		1	
Agabus conspersus (Marsham 1802)	1				1	
Agabus nebulosus (Forster, 1771)	1		7		3	
Agabus spp. larvae +		23		1		1
Colymbetes fuscus (Linnaeus, 1758)			8		39	
Cybister lateralimarginalis (De Geer, 1774)		3	3		8	6
Dytiscus circumflexus Fabricius, 1801				2	2	2
Hydroporus gyllenhali Schiödte, 1841+	1					
Hydroporus lucasi Reiche, 1866	5					
Hydroporus spp. and *Hygrotus* spp.+				2		
Ilybius montanus (Stephens, 1828) and *Agabus bipustulatus* (Linnaeus, 1767)					6	
Laccophilus minutus (Linnaeus, 1758) +		15		1		
Rhantus hispanicus Sharp, 1882	1		3		13	
Rhantus suturalis (McLeay, 1825)	1		4		31	
Rhantus spp. +		10		1		
Helophorus spp. +	1		1		9	
Anacaena lutescens (Stephens, 1829) +	1					
Berosus affinis Brullé, 1835	1					
Berosus guttalis Rey, 1883	19		1			
Berosus signaticollis (Charpentier, 1825)	1				3	
Berosus spp. larvae +		4				10
Enochrus bicolor (Fabricius, 1792)	3					
Enochrus fuscipennis (C.G. Thomsom, 1884)	6					
Hydrobius fuscipes (Linnaeus, 1758) and *Limnoxenus niger* (Zschach, 1788) +	5	12		6	4	18
Hydrochara flavipes (Steven, 1808)			12		13	
Hygrobia hermani (Fabricius, 1775)	2					
Decapoda						
Procambarus clarkii (Girard, 1852)		3		4		2

Ephemeroptera						
Cloeon dipterum (Linnaeus, 1761) +		358		15		
Heteroptera						
Corixa affinis Leach, 1817	6		6		5	
Corixidae +		2		7		
Sigara lateralis (Leach, 1817) +					1	
Gerris thoracicus Schummel, 1832	15	22	10	12	10	7
Microvelia pygmaea (Dufour, 1833) +	1					
Anisops sardeus Herrich-Schäffer, 1849	25		5		2	
Notonecta glauca Linnaeus, 1758					19	
Notonecta maculata Fabricius, 1794					9	
Notonecta spp.		68		42		66
Plea minutissima Leach, 1817 +	1					
Saldidae	2		1			
Odonata						
Aeshna affinis Vander Linden, 1823		4				
Crocothemis erythrarea (Brullé, 1832)		1				
Sympetrum fonscolombei (Selys, 1841)		103		35		6
Sympetrum sanguineum (Müller, 1764)		4				
Sympetrum striolatum (Charpentier, 1840)		2				3
	Dip-net		**Funnel net**			
Diptera	**March larvae**	**March nymph**	**March larvae**	**March nymph**	**April larvae**	**April nymph**
Culicidae +	1	1	1			
Rhagionidae +					4	

Chapter 2: Monitoring the invasion of the aquatic bug *Trichocorixa verticalis verticalis* (Hemiptera: Corixidae) in the wetlands of Doñana National Park (SW Spain).

Resumen:

La presencia del coríxido exótico *Trichocorixa verticalis verticalis* (Fieber, 1851) que es nativo de Norteamérica ha sido detectado en los humedales de Doñana (S.O. España). Se han recolectado muestras de diferentes proyectos llevados a cabo en el área de estudio durante el periodo 2001-2007. Este coríxido exótico ha sido encontrado en 66 ocasiones de los 134 sitios muestreados. Se han detectado dos poblaciones reproductoras que podrían actuar como reservorios que fomentan la colonización hacia otros cuerpos de agua en el área. En estos reservorios donde la reproducción tuvo lugar, *T. v. verticalis* excluyó de forma competitiva la presencia de las especies de coríxidos autóctonos. Fuera de estos núcelos reproductores, la presencia de este coríxido exótico se constituyó básicamente por unos pocos individuos aislados probablemente en fase de dispersión.

Abstract:

We have detected the presence of the North American native corixid *Trichocorixa verticalis verticalis* (Fieber, 1851) in Doñana wetlands (SW Spain). We have collected data from different research projects done in the area during the period 2001-2007. We have sampled 134 different sites in Doñana and we found the exotic corixid in 66 occasions. We have found 2 reproductive populations that might act as sources for the colonization of other waterbodies in the area. When reproduction occurred *T. v. verticalis* outcompeted native corixids. Its presence out of the waterbodies where we detected reproduction was in small numbers and probably due to vagrant individuals.

Key words: Corixid, *Trichocorixa*, exotic species, invasive, Doñana.

Introduction:

The spread of exotic species occurrence worldwide is one of the major causes of global change (Vitousek et al., 1996, Ricciardi 2006). The establishment of exotic invasive species within an ecosystem usually has strong consequences, affecting ecological functions (Ricciardi et al., 1997, Maezono & Miyashita 2003) and causing a loss of indigenous biodiversity (Witte et al., 2000). Several scenarios have been described once an exotic species arrives to an ecosystem before it becomes an invasive species, and it controls ecological processes (Carlton 2006).

In this paper we consider the status and possible impact of an exotic aquatic insect recently detected in the Doñana wetlands in south-west Spain. *Trichocorixa verticalis verticalis* (Fieber, 1851) is a small predaceous corixid (< 5.5 mm) (Heteroptera) naturally distributed along the Atlantic coast of North America and on some Caribbean islands. It now occurs as an exotic species in South Africa, New Caledonia, Portugal, Morocco and Spain (Kment 2006, L'Mohdi *et al.*, in press). This species is the only aquatic alien Heteroptera recorded in Europe (Rabitsch 2008). Adult males are easily distinguished from European native corixid species by their left abdominal asymmetry and a tibial elongation over the pala (Günther 2004). This species is halobiont and usually inhabits brackish and saline water bodies, even occurring in the open sea (Hutchinson, 1931). This ability to tolerate a broad salinity range is probably a key feature of its success as an invader. *Trichocorixa verticalis verticalis* is widespread in the Portuguese Algarve which begins 80 km to the west of Doñana (Sala & Boix 2005). In Doñana itself, it has previously been cited in two locations with a hydrological connection to the Guadalquivir Estuary (Günther 2004, Millán et al., 2005), although these records are predated by some of our own observations.

Study area:

Doñana is located in the southwest of Spain in the mouth of the Guadalquivir River (Fig. 1A) and holds a great variety of waterbodies, including natural temporary ponds, natural permanent ponds, artificial permanent ponds, temporary marshes and ricefields (Garcia-Novo & Marín 2006, Serrano et al., 2006). These wetlands represent one of the most important areas for waterbirds in Europe (Rendón et al., 2008), and the core area dominated by natural, temporary wetlands is protected as a National Park, Biosphere Reserve and UNESCO World Heritage site. Surrounding fish ponds, salt ponds and ricefields are also partly protected and included within a Ramsar site and an EU Specially Protected Area.

The climate is Mediterranean with an Atlantic influence. The flooding regime of temporary ponds and marshland is highly variable among years owing to rainfall fluctuations. Mean annual precipitation is 542 mm/yr with a range of 170-1032 mm/yr. There are up to 26,000 Ha of temporary marshes mainly fed by freshwater (rainfall and runoff) and currently isolated from the tidal influence of the Guadalquivir estuary. The marshes and temporary ponds usually begin to fill by late autumn when rainfall starts (Fig.1B) and dry out completely in summer. Salinity varies from oligohaline to mesohaline according to the frequency and the duration of flooding, with a wide spatial and temporal variation depending on distance from freshwater sources, depth, etc. (Garcia-Novo & Marín 2006, Serrano et al., 2006). Recently, areas of former marshland previously drained for agriculture have been restored by removing dykes and drainage networks. The Caracoles estate (Fig. 1A) is one such area included in our study, in which 96 temporary ponds were created during restoration in 2004 (Frisch & Green 2007). Conductivity in these newly created ponds range between 7.14-51.6 mS/cm.

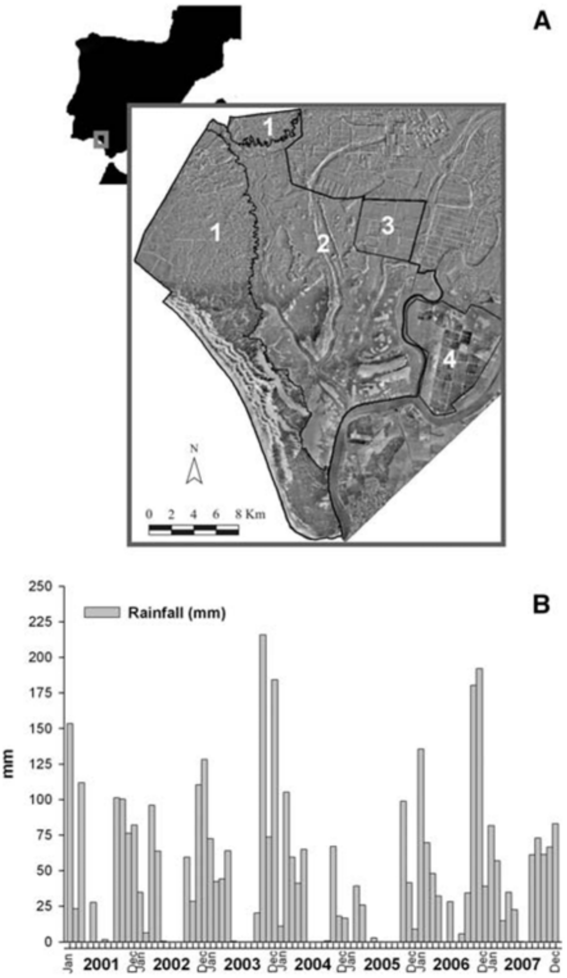

Figure 1.- **A.-** Map of the study area showing the main different areas where samples were taken: 1) Dunes and stabilized sands, 2) Natural temporary marshes, 3) Caracoles estate (restored marshland) and 4) Veta la Palma estate with fish ponds (transformed marshland). The thinest black lines are the boundaries of each area, the thickest black line is the limit of Doñana National Park.The figure was done with a digital orthophotography, source: Junta de Andalucía. 2003. Digital Orthophotografy of Andalusia. Consejería de Obras Públicas y Transportes. Instituto de Cartografía de Andalucía. Junta de Andalucía. **B.-** Rainfall record for the period of study in the area (2001-2007). Rainfall data were gathered at and provided by Doñana Biological Reserve (EBD-CSIC)

Elsewhere in Doñana National Park a large network of more than 3,000 temporary ponds occurs in an area of mobile dunes and stabilized sands (Fig. 1A, Fortuna et al., 2006, see a detailed description in Díaz-Paniagua et al, submitted, and also in Gomez-Rodriguez et al, submitted). In this area, there are also some permanent, artificial ponds made as waterholes for livestock. These *zacallones* (local name) were usually made by digging a deep hole near a natural pond or even inside the pond bed itself. Conductivity in these ponds ranged from 0.08 to 9.8 mS/cm.

Large, permanent fish ponds are located to the east of the National Park in the Veta la Palma estate (Fig. 1A), which contains 52 regular ponds. The ponds were constructed in 1992-1993 on top of what was natural marshland in the Guadalquivir estuary. All the ponds are shallow (average 30 cm, maximum depth 50 cm) and flat-bottomed with a total combined surface area of 2997 ha (see Frisch et al., 2006, Rodríguez-Pérez & Green, 2006, for more details). Each pond is dried out under rotation approximately every two years to extract fish. Ponds are interconnected via canals and a permanent flow of water taken from the Guadalquivir estuary maintains high dissolved oxygen levels. Salinity during our study varied from 10.3 mS/cm during winter months of high rainfall to 22.1 mS/cm at the end of September, after the dry summer months. pH ranged from 9.3 to 10.4.

Our study did not include salt pans in Sanlúcar de Barrameda where *Trichocorixa v. verticalis* was initially recorded (Günther 2004).

Material and methods:

We studied the distribution and abundance of *T. verticalis* in an *ad hoc* fashion from 2001 to 2007, taking advantage of several research projects designed for different purposes and using different sampling methodologies.

In 2001 and 2002 we sampled 11 ponds in Veta la Palma estate every three months (Fig. 1A.). We used a quantitative sampling methodology; a PVC pipe section of 20 cm diameter was inserted vertically down into the sediments to isolate the water within. Using a plastic jar, all the water was then scooped out and sieved through a 250 µm mesh, taking care not to extract sediments. The sieved material was then fixed with formaldehyde. Corixids were later identified and counted.

A Doñana monitoring team (Equipo de Seguimiento de Procesos Naturales de la Reserva Biológica de Doñana (http://www-rbd.ebd.csic.es/Seguimiento/mediobiologico.htm) took samples from marshes, and permanent artificial ponds in 2003, 2004 and 2005. They sampled with eel nets (5 mm mesh size) placed for 24 hours, and by dip netting (1 mm mesh size) for *ca.* 1.5 m while trampling sediments on the bed of the wetland. Samples were preserved in ethanol (70%) and later examined for the presence of *T. verticalis.*

While sampling 14 new ponds for zooplankton from April to May 2006, some corixids were incidentally included in the samples. 20 l of water was taken from a transect along the pond and filtered (see Frisch & Green 2007) before being placed in ethanol (70%). All corixids were later counted and identified. These ponds had flooded for the first time in January 2006 and dried out before July.

Finally we sampled the natural temporary ponds and zacallones located in the stabilized sands; 64 ponds in 2006 and 90 ponds in 2007. We sampled with a dip net (1 mm mesh size), sampling in the same way as the Doñana monitoring team did. All these natural ponds represented a wide hydroperiod gradient. All ponds were sampled once each year, except 19 temporary ponds, sampled monthly. In 2006, we identified species *in situ* recording only the presence or absence of each species. On the other hand, in 2007 all captured corixids (or at least 75% of them when there were too many individuals) were retrieved and fixed with ethanol (70%) and later quantified and identified with a microscope at the laboratory. When large corixid adults occurred, we recorded its presence *in situ* as *Corixa affinis* in order to its largest size and previous sampling in the area. Furthermore, we retrieved few large corixid individuals per pond in each sampling to make a correct identification under a microscope at the laboratory in order to avoid the possible confusion with *Corixa panzeri*. In any case, we have never found *C. panzeri* in these ponds in any sampling, so we can assume confidently that all large corixids found were *C. affinis*. Both species are sympatric in the Iberian Peninsula but *C. panzeri* seems to be less frequent (Niesser et al 1994). We made relative proportion of *T. v. verticalis* out of the total corixids captured in the pond (except *C. affinis*) during the entire sampling season with 2007 data.

Results:

Overall we sampled 134 different sites in the Doñana wetlands situated within a polygon of 54.000 Ha. Some of these points were sampled during several years. Sampled sites included artificial ponds (11 fish ponds, 14 shallow, new temporary ponds and 30 deep waterholes [*zacallones*]) and natural waterbodies (4 streams, 12 points in temporary marshes and 63 natural ponds). We detected the presence of *T. v.*

verticalis on 66 occasions (Fig. 2), more than half of these (53 %) being in artificial ponds. In contrast, artificial waterbodies were only 41 % of the total points sampled.

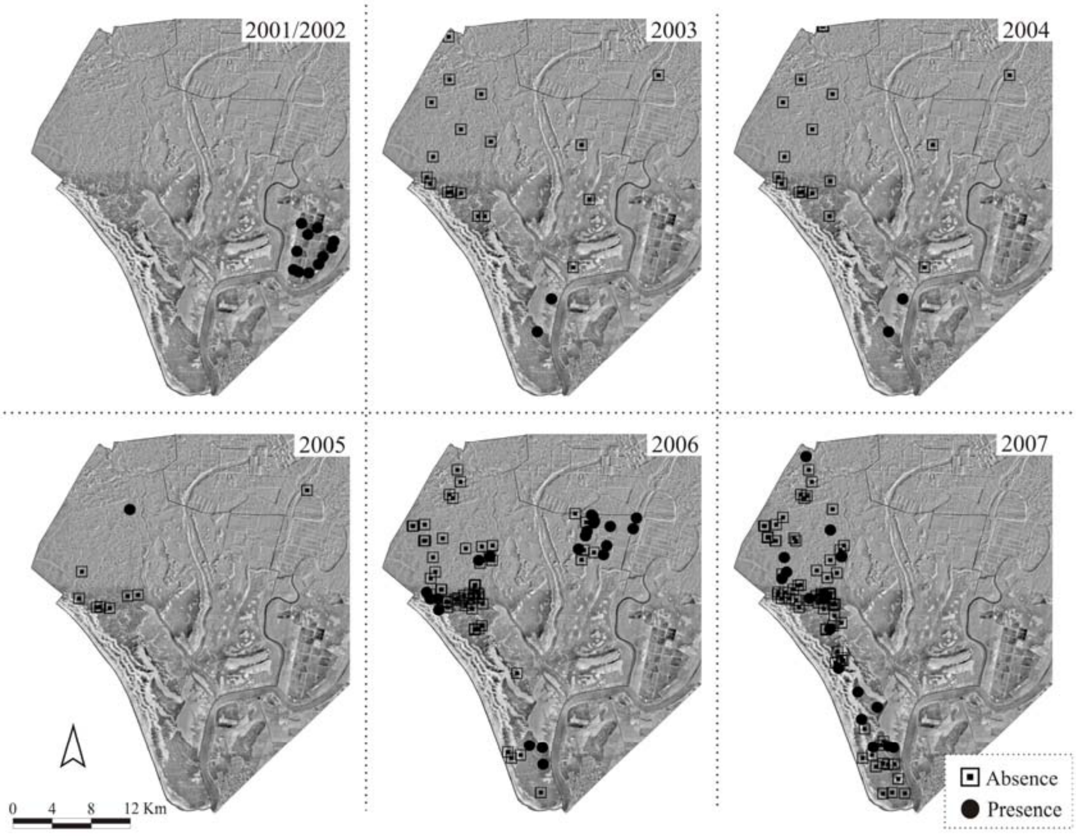

Figure 2.- Maps of the study area for each year of study with the position of the sampling sites. Black dots show the presence of *T. verticalis*, and open squares show sampled places where we did not detect *T. verticalis*. We show the results of 2001 and 2002 in the same map because there were not differences in the presences of the exotic corixid. The figure was done with a digital orthophotography, source: Junta de Andalucía. 2003. Digital Orthophotografy of Andalusia. Consejería de Obras Públicas y Transportes. Instituto de Cartografía de Andalucía. Junta de Andalucía.

Veta la Palma fish ponds and new temporary ponds in Caracoles estate:

These were the only two areas where reproductive populations of *T. v. verticalis* were recorded. In both areas *T. v. verticalis* was the dominant species, apparently outcompeting native ones. In Veta la Palma, sampled during 2001 and 2002, 179 samples were gathered with a total of 738 adult corixids, 96 % of which were *T. v. verticalis*, the remaining adults being *Sigara stagnalis* and *S. scripta*. Abundance peaked in spring and summer, with the lowest densities in autumn and winter (Fig. 3).

There was a highly significant difference in densities between seasons (Rodríguez-Pérez, 2006). The presence of juveniles suggests that reproduction continues throughout the year in this site (Fig. 3).

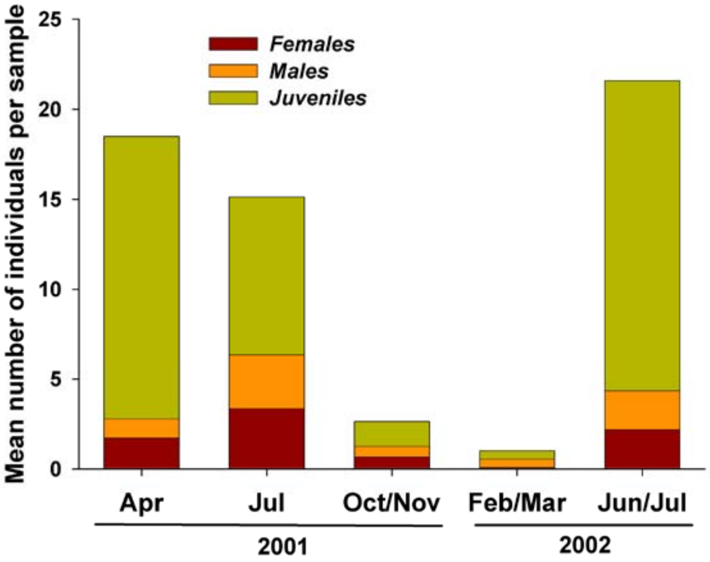

Figure 3.- The figure shows the mean number of adult males and females per sample of *T. verticalis* in each sampling campaing in Veta la Palma fish ponds during 2001 and 2002. Juveniles were mostly *T. verticalis*, but we did not identify all juveniles individuals.

In 2006 we detected a second reproductive population in the new temporary ponds in Caracoles (see Fig 1, Fig. 2), where 307 adult corixids were retrieved from 14 ponds of which 92% were *T. v. verticalis*. The other three species that occurred in this area were *Sigara lateralis* (4%), *S. stagnalis* (2%) and *S. scripta* (2%). Both here and in Veta la Palma we also identified freshly moulted adult and juvenile corixids that were surely *T. v. verticalis*. In the three new temporary ponds with the highest *T. verticalis* density (30 in our sample), conductivity was particularly high, ranging from 17.3-54.6 mS/cm.

Ponds in stabilized sands and temporary marshes:

In the other places where *T. v. verticalis* occurred, no matter the year, only adults were detected, surely these individuals were vagrant adults (we captured juvenile corixids but we identified them as other species). In these occasions *T. v. verticalis* always occurred in small numbers, and making a small proportion of the total corixids sampled. In 2007 we captured and retrived 1881 adult corixids throughout the year but only 37 of those were *T. v. verticalis*. In table 1 we show the relative proportion of *T. v. verticalis* out of the other corixid species present but only in the ponds where the exotic species occurred. *T. v. verticalis* was detected coexisting with another seven species of corixids in the area (Table 2). Only one other corixid species was present in more waterbodies than *T. v. verticalis* in 2006, and only three other species in 2007. *Paracorixa concinna* was the only species that was never observed coexisting with *T. v. verticalis* in the same pond. *T. v.verticalis* was more likely to be found in ponds than the rarer native corixids (*Sigara scripta*, *S. stagnalis*, and *S. selecta*). It is remarkable that *T. v. verticalis* has not been recorded in the main body of the temporary marsh in the samples studied as yet.

Table 1.- Percentages of presence of each corixid species recorded in the ponds where *T. verticalis* occurred in 2007. *Corixa affinis* was a frequent and abundant specie and coexisted with *Trichocorixa verticalis verticalis* in 5 ponds in 2006 and 16 ponds in 2007.

	% *Trichocorixa verticalis*	% *Sigara laterallis*	% *Sigara stagnalis*	% *Sigara scripta*	% *Sigara selecta*	% *Micronecta scholzi*	N of total corixids
Caño Arenilla	25	50	0	25	0	0	8
Laguna Estratificada	33	67	0	0	0	0	3
Zacallón Mahón	3	29	53	16	0	0	31
Zacallón de la Angostura	67	33	0	0	0	0	3
Punta de Zalabar	13	25	31	31	0	0	16
Laguna Larga o del Carrizal	1	60	39	0	0	0	277
Canal al norte sombrío	100	0	0	0	0	0	1
Zacallón pozo salinas	4	90	2	5	0	0	300
Navazo de la Higuera	3	86	8	3	0	1	76
Camino de Martinazo	20	80	0	0	0	0	5
Orfeon	100	0	0	0	0	0	1
Poli	50	25	25	0	0	0	4
Moral	100	0	0	0	0	0	1
Jiménez	25	75	0	0	0	0	4
Lagunan del Caño Martinazo	50	0	0	0	50	0	2
Adyacete al Navazo del Toro	100	0	0	0	0	0	1
Raya del Pinar	100	0	0	0	0	0	1
Leña	11	79	5	5	0	0	19

Table 2.- Number of sampling sites where each corixid species was detected out of the total number of points sampled in 2006 and 2007.

	2006 (n=76)	2007 (n=90)
Trichocorixa verticalis	21	18
Paracorixa concinna	0	4
Sigara laterallis	13	50
Sigara stagnalis	2	23
Sigara scripta	4	16
Sigara selecta	0	4
Micronecta scholzi	0	5
Corixa affinis	25	78
Without corixids	24	11

Discussion:

The dataset that we have used for this work encompasses seven years of sampling, and because we did not use the same standardized methodology in every sampling we cannot conclude conclusively that the populations of this invasive species are increasing their occurrence in the area. On the other hand the strengths of this dataset are the seven years of data itself, the high number of points that we have visited throughout the seven years in a restricted territory (54,000 Ha), and that we have sampled every kind of aquatic habitat that occurs in Doñana National Park. Despite the noted weaknesses of the dataset we show in this work evidence suggesting that an ongoing invasion is happening in the wetlands of this protected area. This fact has strong consequences for the conservation of the ponds and marshes in Doñana National Park, and it adds to other invasion events of aquatic organisms in the aquatic ecosystems of Doñana: i.e. the copepod *Acarthia tonsa* (Frisch et al., 2006), the crayfish *Procambarus clakii* (Geiger et al., 2005), the gastropod *Potamopyrgus antipodarum* (Rodríguez-Pérez, 2006), the fishes *Gambusia affinis* and *Lepomis gibbosa* (García-Berthou et al., 2007), or the fern *Azolla filiculoides* (García-Murillo et al., 2007).

Four subspecies of *Trichocorixa verticalis* occur naturally in the brackish and saline waters of North America, covering a broad geographical range from the Caribbean and Atlantic coast to the Pacific coast, and from Mexico to Central Provinces in Canada (Jansson 2002, Kment 2006). This trait of one species with highly differentiated populations distributed along its native range has been identified as an indicator of a species with high invasive potential (Lee & Gelembiuk 2008).

Within the Iberian Peninsula this species was first detected in samples collected in Algarve (South Portugal) in the mid-nineties (Sala & Boix 2005). The first evidence of its presence in Doñana is from our samples in Veta la Palma fish ponds in 2001. Given the shortage of detailed studies of corixids in Doñana and other parts of the south-west of Spain, it is impossible to know its date of arrival in Doñana whilst the limits of its current distribution beyond Doñana remain unclear. Given its abundance in Veta la Palma, it seems likely that this species colonized the fish ponds shortly after their creation in the early 1990s.

Sala & Boix (2005) suggested two different hypotheses to explain the introduction of *T. v. verticalis* in Europe. Firstly the corixid may have arrived with the introduction of *Fundulus heteroclitus* and *Gambusia hoolbroki* in the area. These two species are sympatric of *T. v. verticalis*. Secondly there may have been a natural dispersion via the marine current between the Atlantic coasts of North America and Europe, since *T. v. verticalis* has been observed in the open sea (Hutchinson 1931, Gunter & Christmas 1959). Alternatively, *T. v. verticalis* has been recently detected in Morocco (L'Mohdi *et al.*, in press). The populations in the north of Morocco and the ones in the south of Spain might be related, being the North African populations the origin of European ones or viceversa.

Over seven years we have sampled most kinds of aquatic habitat occurring in Doñana National Park and its surroundings. It is likely that the species has increased its area of distribution in Doñana over our study period, but we have not been able to demonstrate that conclusively owing to the ad hoc nature of our sampling regime. The exceptions are the new ponds in Caracoles in which *T. verticalis* immediately established itself as the dominant corixid.

The invasive character of *T. v. verticalis* in the Veta la Palma and Caracoles estates seems clear. At these sites the species has dominant reproductive populations and has overwhelmed native corixid species. Elsewhere in our study area, we did not confirm reproduction, and further work is required to establish whether the species can be considered invasive or not (see Carlton 2006). At least in the more brackish parts of Doñana, it seems likely that *T. v. verticalis* will have a significant impact on the abundance of native species and may replace *Sigara lateralis* as the most frequent and abundant corixid in the community. *T. v. verticalis* may also benefit from the increase in salinity projected for the Iberian peninsula owing to global warming (Rahel & Olden 2008).

Our results suggest that *T. v. verticalis* in Doñana is currently most abundant in areas with relatively high salinities and artificial areas that are relatively permanent. Permanent sites such as fish ponds or waterholes might act as reservoirs of *T. v. verticalis* populations during the summer, facilitating the colonization of temporary ponds and marshes when they flood in the autumn or winter. There is some evidence to suggest that, with the National Park, the temporary ponds situated closest to the fish ponds or Sanlúcar salt ponds colonized by *T. v. verticalis* are more likely to have been colonized by this species (Fig. 3), supporting the idea that these permanent, artificial sites act as a main source of exotic species colonizing surrounding areas. The Veta la Palma fish ponds similarly seem to act as a reservoir for other exotics such as the copepod *Acartia tonsa* or the gastropod *Potamopyrgus antipodarum* (Frisch et al. 2006, Rodríguez-Pérez, 2006). These ponds are widely recognized as of high value for waterbird conservation (Rendón et al. 2008). However, this benefit for biodiversity conservation in Doñana needs to be balanced against the cost of the role the ponds play in facilitating the expansion of exotic species.

Most heteropterans are extremely good dispersers and have not developed other strategies to resist the drying phase, dispersing to permanent water bodies as adults (Wiggins et al., 1980; Williams, 2006; Bilton et al., 2001). However, *Trichocorixa verticalis interiores* and *T. v. verticalis* have been reported to develop resistant resting eggs, allowing them to survive ice, hypersalinity or desiccation of pools (Tones 1977, Kelts 1979). If this is the case, it raises the possibility that *T. verticalis* will be able to withstand summer droughts in Doñana as extremely durable eggs in sediments, re-emerging during the next hydroperiod. In this scenario, the species will not be so dependent on fish ponds as a source for recolonisation of temporary waterbodies in Doñana.

Doñana contains some of the most important and diverse wetlands in Europe (García-Novo & Marín 2006, Rendón et al. 2008). Although *T. verticalis* is the first case of an alien aquatic insect, Doñana has already been invaded by a considerable number of other exotic aquatic species (Frisch et al. 2006; García-Berthou et al. 2007). Research is required into the invasion biology of *Trichocorixa verticalis*, particularly its impact on native corixids and prey species and its dispersal biology, as well as more extensive surveys to establish and monitor its distribution in south-west Spain.

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Colaborators:

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Chapter 3: Inter and intra-annual variations of macroinvertebrate assemblages are related to the hydroperiod in Mediterranean temporary ponds.

Resumen

Las comunidades de microinvertebrados de 22 lagunas temporales con diferente hidroperiodo fueron mensualmente muestreadas durante un año seco (2005-2006) y un año lluvioso (2006-2007). Los adultos de las especies de coleópteros y heterópteros fueron más abundantes hacia el final del hidroperiodo, mientras que las larvas de coleópteros, especialmente las de la familia Dytiscidae, fueron principalmente capturadas en primavera. Las comunidades de macroinvertebrados difirieron entre ambos años de estudio. La menor longitud del hidroperiodo del año seco afectó al periodo de vida acuática de los macroinvertebrados distinguiéndose tres fases en base a la composición de macroinvertebrados: la fase de inundación, una fase acuática y la fase de desecación. Debido a la mayor longitud del hidroperiodo, en el año lluvioso se diferenciaron cinco fases, en las que la fase acuática se dividió en una fase invernal, una a comienzos de primavera y otra a final de primavera. Los dispersores, como *Anisops sardeus*, *Berosus guttalis* o *Anacaena lutescens*, fueron típicos durante la fase de inundación, y *Corixa affinis* o *Enochrus fuscipennis*, durante la fase de desecación. La composición de macroinvertebrados al principio y al final de la inundación (principalmente formada por dispersores) fue similar para las lagunas de hidroperiodo intermedio, algo no observado para las lagunas que se desecaron de forma temprana y aquellas de largo hidroperiodo. Un patrón global fue descrito para ambos años de estudio en el que la variación estacional fue similar al considerar conjuntamente el gradiente anual de hidroperiodo, lo que puede estar asociado con los ciclos de vida propios de las especies de macroinvertebrados.

Abstract

Macroinvertebrate assemblages of 22 temporary ponds with different hydroperiod were sampled monthly during a dry (2005-2006) and a wet year (2006-2007). Coleopteran and Heteropteran adults were most abundant at the end of the hydroperiod whilst Coleopteran larvae, mainly Dytiscidae, were mostly recorded in spring. Macroinvertebrate assemblages differed between study years. The shorter hydroperiod of ponds in the dry year constrained the length of the aquatic period for macroinvertebrates, and three distinct wet phases of community composition could be distinguished: filling phase, aquatic phase and drying phase. In the wet year, with a longer pond hydroperiod, five phases could be identified, with the aquatic phase differentiated into winter, early spring and late spring phases. Dispersers such as *Anisops sardeus*, *Berosus guttalis* or *Anacaena lutescens* were typical during the filling phase, and *Corixa affinis* or *Enochrus fuscipennis* during the drying phase. The ponds with intermediate hydroperiod showed a similar composition (mainly dispersers) at the beginning and end of their wet period, this not being seen in early drying or long hydroperiod ponds. A general pattern was detected, with similar variation between both years, which may be associated with the life histories of the macroinvertebrate taxa recorded.

Key words: aquatic macroinvertebrates, temporal variation, wet phases, hydroperiod, community composition, life cycle

Introduction

Temporary ponds are optimal habitats for many macroinvertebrate species, being important for the conservation of their specialised fauna (Strayer, 2006). However, these ponds have been frequently neglected in conservation programs that have traditionally considered protection of extensive wetlands but not of small water bodies, despite their high biodiversity (Collinson et al. 1995; Céréghino et al., 2008). Moreover, temporary ponds are highly suitable for ecological studies due to their wide environmental gradients of salinity, temperature, vegetation, pH or hydroperiod (Herbst, 2001; Batzer et al., 2004; Waterkeyn et al., 2008; Bilton et al., 2009).

Despite the fact that permanent ponds may contain many aquatic species (Bazzanti et al., 1996; Brooks, 2000; Della Bella et al., 2005; Serrano & Fahd, 2005), temporary ponds usually harbour exclusive species or large populations of species which are scarce in, or absent from permanent waters (Collinson et al., 1995; Williams, 1997; Boix et al., 2001; Della Bella et al., 2005; Céréghino et al., 2008). Whilst the dry period may exclude many aquatic organisms from temporary ponds, the absence of large predators, such as fish, is a critical factor that determines the presence of specialist taxa (Wellborn et al., 1996).

Many macroinvertebrate species require an aquatic phase to complete their complex life cycles for which different life history strategies have been reported. Among the most important challenges for the macroinvertebrates of temporary ponds is survival during the dry period. Some adaptations for living in temporary ponds are dispersal to more permanent waters, or resistance of eggs, larvae, or adults to desiccation (Wiggins et al., 1980). Physiological and behavioural mechanisms to survive desiccation have also been described in different aquatic invertebrates (Williams, 2006). Wiggins et al. (1980) segregated groups of macroinvertebrates according to their life history strategies, justifying the presence of specific fauna in different ponds. Differences in the life history strategies of species allow the identification of functional groups which appear at different times in the ponds (Gascón et al., 2008) or to differences in optimal habitats, being able to only complete their life cycles in ponds with a long hydroperiod, but not in ephemeral ponds (Schneider & Frost, 1996).

Annual and seasonal variation of macroinvertebrate assemblages have been reported in temporary ponds (Brooks, 2000) and have been associated with seasonal changes in environmental conditions during the wet phase (Boulton & Lake, 1992). Jeffries (1994) found differences in the macroinvertebrate assemblages of the same ponds in three different years, including a low rainfall year in which ponds did not fill. Different macroinvertebrate groups have been described as characteristics of different pond phases; usually classified as filling, aquatic and drying phases, out of which the aquatic phase could be further differentiated into three additional phases (Boulton & Lake, 1992; Bazzanti et al., 1996; Boix et al., 2004).

Our study has been carried out in an area in which more than 3000 water bodies support a robust network of aquatic habitats (Fortuna et al., 2006) that exhibit high conservation values and encompass a wide range of hydroperiod and environmental conditions (Gómez-Rodríguez et al., 2009). Several studies have focused on the limnology of these ponds (García Novo et al., 1991; Serrano & Toja, 1995; Serrano et al., 2006) and their use as amphibian breeding sites (Díaz-Paniagua, 1990; Díaz-

Paniagua et al., 2005). In contrast, only preliminary data on macroinvertebrates (Agüesse, 1962; Bigot & Marazanof, 1966; Marazanof, 1967; Millán et al., 2005) and studies on abundance of Coleoptera, Heteroptera and Odonata (Montes et al., 1982) have been reported.

In this research we have studied temporal variation in macroinvertebrate abundance and composition in temporary ponds, with the following specific aims: 1) Detecting inter-annual variation; 2) Detecting seasonal variation in relation to different phases of the wet period of ponds; 3) Comparing monthly variation within ponds of different hydroperiod; and 4) Determining if there is a general pattern of temporal variation for all ponds in the study area.

Methods

The study was carried out in 22 ponds located in the Doñana Biological Reserve (Doñana National Park, Southwestern Spain, Fig.1). This area is located between the Atlantic coast and the mouth of the Guadalalquivir River. It includes a high number of temporary ponds, appearing during autumn or winter, and two permanent ponds. The type of climate is Mediterranean sub-humid, with hot and dry summers, mild winters, and rainfall mainly falling in autumn and winter (see Siljestrom et al 1994, García-Novo & Marín, 2006 for a detailed description of the area).

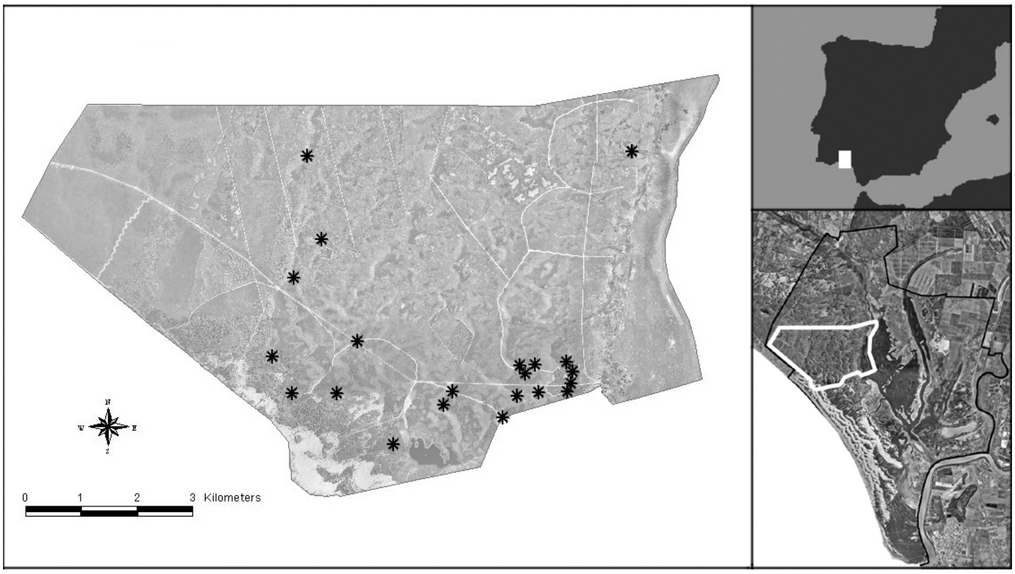

Fig.1: Location of the 22 temporary ponds in the Doñana Biological Reserve, Doñana National Park (SW Spain).

Our study period was October 2005 to July 2007. Annual rainfall was calculated as the amount of rainfall collected from 1st September to 31st August of the following year. This amounted to 468.3 mm in the first year (hereafter referred to as the dry year), when we sampled 18 temporary ponds which usually dry out every summer, and one semipermanent pond which only dries out in years of severe drought. As this pond was dry in 2005, prior to our study period, we considered it as a temporary pond. Ponds were selected to encompass the highest possible range of hydroperiods, being representative of the range of ponds found in the study area. In the second year, annual rainfall was 716.9 mm (hereafter referred to as the wet year) when a higher number of ponds with short hydroperiod were formed in the area. To assess the widest range of hydroperiod during the wet year, we sampled three of these new ponds, although the total number of ponds sampled was the same as the year before. In the dry year, most temporary ponds were wet from February to June and from October to July during the wet year, although the ponds with longest hydroperiod had water even during August in both years. A detailed description of the characteristics of Doñana temporary ponds, including most of our study ponds, is given in Gómez-Rodríguez et al. (2009). Hydroperiod and maximum depth of ponds during our study, as well as their basin areas are shown in Table 1. Pond area was extracted from a pond cartography obtained in a moment of large inundation (see Gómez-Rodríguez et al., 2008). Vegetation in the ponds was mainly composed of meadow plants as *Mentha pulegium* L., *Illecebrum verticillatum* L., or *Hypericum elodes* L., in the littoral zone, whilst aquatic macrophytes were common in deeper areas, such as *Juncus heterophyllus* Dufour, *Myriophyllum alterniflorum* DC. in Lam & DC., *Potamogeton pectinatus* L. and *Ranunculus peltatus* Schrank (García Murillo et al., 2006).

Table 1: Hydroperiod and maximum depth of every pond (named with 3 letters) are shown for the dry and wet year, and also the pond area calculated in a large inundation moment (hydroperiod is only given for the year in which each pond was sampled).

	Hydroperiod (months)		Maximum Depth (cm)		Pond area (m^2)
Pond	**Dry year**	**Wet year**	**Dry year**	**Wet year**	**Maximum inundation**
Pol	3.1	7.2	33	50	1,200
Acm	3.3	6.4	34	44	50
Rp	2.1	7.2	24	64	4,075
Pg	3.1	7.2	31	54	3,925
Jim	2.2	7.2	9.5	86	39,900
Cam	2.7	7.2	23	55	2,200
Zah	3.8	9.1	47	69	48,189
Lve	6.1	12	104	132	3,300
Dul	8.9	12	142	165	122,672
Abe	2.3	6.9	18	43	50
Bre	3.4	7.9	47	85	2,150
Pp	3.1	7.2	42	82	875
Tej	2.8	7.2	22	67	150
Orf	4.3	9	80	82	850
Ant	1.4	6.8	15	45	5,131
Wou	3.4	-	31.5	-	14,375
Mor	3.2	-	25	-	14,725
Tar	4.4	-	55	-	81,250
Arm	-	4.2	-	21	25
Vac	0.4	6.2	-	51	25
Len	-	5.5	-	24	650
Tps	-	6.1	-	39	6,375

Macroinvertebrates were sampled monthly in each pond by using a dip-net with a 1 mm mesh, netting a stretch of water of approximately 1.5 m length in each sampling unit. In the wet year, the four ponds with the shortest hydroperiod (including the three ponds only sampled during this year) were sampled every 15 days. In each pond, we sampled at different points along one or two transects from the littoral to the open water, the number of sampling points being proportional to pond size. We also took additional samples in microhabitats which were not represented in these transects. The maximum number of samples per pond ranged from 6-13 in the month of maximal inundation. As pond size decreased during the season the number of samples taken was reduced accordingly. Most macroinvertebrates captured were identified *in situ*, being counted and released. Individuals of unidentified species were preserved in 70% ethanol for identification in the laboratory. Whenever possible, individuals were identified to species level, except for Diptera, which were identified to family. For Chironomidae and Ceratopogonidae, only presence-absence data were recorded. All recorded taxa with only presence-absence data were not included in analyses.

For the analysis of the macroinvertebrate assemblage composition, we estimated the relative abundance of each taxon, as the total number of individuals captured across all samples taken in a pond, divided by the total number of samples taken in that pond. In these analyses, we differentiated adults from larvae or nymphs, and considered these as different taxa (hereafter referred to as "taxa" for simplicity) in our data matrix. Relative abundance was log transformed (X+1) to calculate the similarity matrix with the Bray-Curtis similarity index (Clarke & Warwick, 2001).

For each pond, we computed Spearman correlations between the corresponding taxa of each pair of similarity matrices of relative abundances in different months, using the RELATE program (Primer v.6, Clarke & Warwick, 2001) in order to assess **monthly variation in the macroinvertebrate assemblages within ponds.** Spearman correlation coefficient (Rho) was close to one when the monthly similarity matrices were highly corresponding. These analyses detected if the similarity among the composition of macroinvertebrates was higher in subsequent months (Serial RELATE) than in more distant months, such as the beginning and the end of each hydroperiod (Cyclic RELATE). Similarity distances among months were represented using non-metric multidimensional scaling (NMDS). Because pond hydroperiod was relatively short in the dry year, these analyses of monthly variation of macroinvertebrate assemblages were performed only for the wet year.

To assess **seasonal variation** in macroinvertebrate assemblages, we used a NMDS representation of the similarity matrices of relative abundances of all ponds and months except for the February matrix of one pond in the dry year which had been previously filled. The different groups observed in the NMDS were used as grouping factor including three or five levels depending on number of observed groups in every case. We then tested differences among observed groups using one-way ANOSIM analyses (performed with 9999 number of permutations). The ANOSIM test statistic, R, is close to one when the levels of grouping factor are different; that is to say, all dissimilarities between levels of grouping factor are larger than any dissimilarity among samples in every level of grouping factor (Clarke & Warwick, 2001). An exploratory analysis (SIMPER) was used to detect those taxa with the highest contribution to the dissimilarity of each level of grouping factor *versus* all other levels for the same factor (Primer v.6, Clarke & Warwick, 2001).

In order to explore particular questions about the temporal variation of macroinvertebrate assemblages, we averaged the relative abundances of macroinvertebrates in different ways: 1) To analyse **inter-annual variation** between the dry and wet years, we averaged the relative abundance of macroinvertebrate taxa every year by dividing by the numbers of months that every pond was sampled. These averaged matrices were represented in NMDS to observe if both years corresponded to different groups. We tested if macroinvertebrate assemblages were different in two study years through one-way ANOSIM analysis, using the year as groping factor with two levels. SIMPER analysis detected those taxa making a higher contribution to dissimilarity between the two years (Primer v.6, Clarke & Warwick, 2001). We removed the macroinvertebrate assemblages of two ponds sampled in the dry year of these analyses and NMDS representation because they only were sample once, not being comparable with the rest of ponds in both years. 2) To analyse if **a general pattern of monthly variation** occurred in both study years, we averaged the relative abundance of individual taxa across all ponds every month by dividing by the number of sampled ponds per month. Then, we used only one averaged matrix of relative abundance of macroinvertebrates per month, representing a unique similarity value per month in a NMDS. Spearman correlation between these monthly similarity values for the average matrix of relative abundance of macroinvertebrates across all ponds was calculated for each year through a Serial RELATE. Spearman correlation coefficient value (Rho) would be one in case of maximum correlation. Prior to these analyses, we tested whether the variation among months was higher than among ponds within a month, using the complete relative abundance matrix of both study years through a one-way ANOSIM analysis where months in every year were the grouping factor with a total of 18 levels.

Monthly matrices of relative abundance of macroinvertebrates were not included in the analyses when any or very scarce abundances were detected in a pond (mainly during the initial stages of annual sampling). Some taxa had to be combined to compare between years, because some species were not be identified during the first year (adults of all species of *Haliplus* were included in one taxon, as were adults of Corixidae, except for *Corixa affinis* Leach, 1817).

Results

Macroinvertebrate taxa and their monthly variation

The macroinvertebrates recorded in the Doñana ponds included 123 different taxa, including 97 species, and additionally unidentified species included in six genera, 16 families, three subfamilies and one order. The most abundant species were *Corixa* *affinis*, *Cloeon* spp. and *Anisops sardeus* Herrich-Schäffer, 1849, whilst other species such as *Coenagrion scitulum* (Rambur, 1842) appeared occasionally and with very low abundance (Table 2). Coleoptera, Heteroptera and Odonata were the orders that included the highest number of species and individuals during both years. The monthly variation of the average number of individuals caught in all the samples during both years is shown in Fig 2. Adults of Coleoptera and Heteroptera showed the highest abundance both at the end of the wet period, and at the beginning during the wet year. Dytiscidae and Hydrophilidae were the most abundant families of Coleoptera. Larvae of Coleoptera (mainly Dytiscidae) were found in the middle of the wet period, while the highest abundance of Coleoptera was reached by adults of Hydrophilidae at the end of the wet period, in July during the wet year, and in May during the dry year, when ponds had shorter hydroperiod. Adults of Heteroptera (mainly Corixidae and Notonectidae) reached their highest abundance in ponds with longer hydroperiods in summer. *Anisops sardeus* and *Corixa affinis* were the most abundant heteropterans, *Corixa affinis* being much more abundant during the wet year than the dry year. Amongst Odonata, Libellulidae (mainly *Sympetrum fonscolombei* (Selys, 1841)) were found throughout the wet period, whilst Coenagrionidae (mainly *Ischnura pumilio* (Charp., 1825)) were especially abundant at the end of this.

Table 2: Taxa of macroinvertebrates recorded in the study ponds during both years. Average and maximum number of individuals per sample is shown for adults, larvae and nymphs (*: only presence was recorded; +: new records for Doñana National Park).

		Average		Maximum	
Taxa	**Family**	**Adult**	**Larva**	**Adult**	**Larva**
Acari					
Hydrachnellae	-	0.029		11	
Bassomatophora					
Physa spp.	Physidae	1.849		446	
Planorbidae	Planorbidae	1.486		259	
Coleoptera					
Donacia spp.	Chrysomelidae	*		*	
Bagous spp.	Curculionidae	0.017		3	
Bagous revelieri Tournier, 1884 +	Curculionidae	*		*	
Bagous subcarinatus Gyllenhal, 1836 +	Curculionidae	*		*	
Bagous vivesi González, 1967 +	Curculionidae	*		*	
Dryops luridus (Erichson, 1847)	Dryopidae	*		*	
Dryops spp.	Dryopidae	0.602	0.01	54	2
Agabus bipustulatus (Linnaeus, 1767)	Dytiscidae	*		*	
Agabus conspersus (Marsham 1802)	Dytiscidae	0.026		5	
Agabus didymus (Olivier, 1795)	Dytiscidae	0.001		1	

Agabus nebulosus (Forster, 1771)	Dytiscidae	0.012		3	
Agabus spp.	Dytiscidae		0.397		19
Cybister (Scaphinectes) lateralimarginalis (De Geer, 1774)	Dytiscidae	0.014	0.025	3	2
Dytiscus circumflexus Fabricius, 1801	Dytiscidae	0.001	0.02	1	2
Eretes griseus (Fabricius, 1781)	Dytiscidae	0.001		1	
Graptodytes flavipes (Olivier, 1795)	Dytiscidae	0.004		1	
Hydaticus (Guignotites) leander (Rossi, 1790)	Dytiscidae	0.001	0.001	1	1
Hydroglyphus geminus (Fabricius, 1792)	Dytiscidae	0.516		92	
Hydroporus gyllenhali Schiödte, 1841	Dytiscidae	0.023		5	
Hydroporus lucasi Reiche, 1866	Dytiscidae	0.079		22	
Hygrotus confluens (Fabricius, 1787)	Dytiscidae	0.012		3	
Hygrotus lagari (Fery, 1992)	Dytiscidae	0.478		47	
Hydroporus spp. or *Hygrotus spp.*	Dytiscidae		0.151		12
Hyphydrus aubei Ganglbauer, 1892	Dytiscidae	0.009	0.033	2	5
Ilybius montanus (Stephens, 1828)	Dytiscidae	0.003		1	
Laccophiluis minutus (Linnaeus, 1758)	Dytiscidae	0.171	0.358	65	33
Liopterus atriceps (Sharp, 1882)	Dytiscidae	0.044		15	
Rhantus (Rhantus) hispanicus Sharp, 1882	Dytiscidae	0.063		5	
Rhantus (Rhantus) suturalis (McLeay, 1825)	Dytiscidae	0.02		6	
Colymbetes fuscus (Linnaeus, 1758)	Dytiscidae	0.063		19	
Rhantus spp. or *Colymbetes fuscus*	Dytiscidae		0.442		18
Gyrinus (Gyrinus) dejeani Brullé, 1832	Gyrinidae	0.007	0.007	1	1
Haliplus (Liaphlus) andalusicus Wehncke, 1874	Haliplidae	0.018		4	
Haliplus (Liaphlus) guttatus Aubé, 1836	Haliplidae	0.01		2	
Haliplus (Neohaliplus) lineatocollis (Marsham, 1802)	Haliplidae	0.008		2	
Haliplus spp.	Haliplidae		0.020		3
Helophorus spp.	Helophoridae	0.367	0.001	93	1
Helophorus (Trichohelophorus) alternans Gené, 1836	Helophoridae	*		*	
Helophorus (Rhopalohelophorus) longitarsis Wollaston, 1864	Helophoridae	*		*	
Hydraena (Hydraena) rugosa Mulsant, 1844	Hydraenidae	0.012		2	
Limnebius furcatus Baudi, 1872	Hydraenidae	0.001		1	
Ochthebius (Asiobates) dilatatus Stephens, 1829	Hydraenidae	0.018		9	
		Average		**Maximum**	
Taxa	**Family**	**Adult**	**Larva**	**Adult**	**Larva**
Ochthebius (Ochthebius) punctatus Stephens, 1829	Hydraenidae	0.004		1	
Ochthebius (Ochthebius) auropallens Fairmaire, 1879	Hydraenidae	0.060		17	
Hydrochus flavipennis Küster, 1852	Hydrochidae	0.029		12	
Anacaena (Anacaena) lutescens (Stephens, 1829)	Hydrophilidae	1.165		421	
Berosus (Berosus) affinis Brullé, 1835	Hydrophilidae	0.455		136	
Berosus (Enoplurus) guttalis Rey, 1883	Hydrophilidae	0.165		13	
Berosus (Berosus) signaticollis (Charpentier, 1825)	Hydrophilidae	0.201		12	
Berosus spp.	Hydrophilidae		0.084		5
Enochrus (Lumetus) bicolor (Fabricius, 1792)	Hydrophilidae	0.059		6	
Enochrus (Lumetus) fuscipennis (C.G. Thomsom, 1884)	Hydrophilidae	1.192		242	
Enochrus spp.	Hydrophilidae		0.007		1
Helochares (Helochares) lividus (Forster, 1771)	Hydrophilidae	0.029		14	
Hydrobius convexus Brullé, 1835	Hydrophilidae	*		*	
Hydrobius fuscipes (Linnaeus, 1758) & *Limnoxenus niger* (Zschach, 1788)	Hydrophilidae	0.369		40	
Hydrobius spp. or *Limnoxenus niger*	Hydrophilidae		0.084		21

Hydrochara flavipes (Steven, 1808)	Hydrophilidae	0.023	0.007	6	2
Hydrophilus (Hydrophilus) pistaceus (Laporte, 1840)	Hydrophilidae	0.001	0.007	1	2
Laccobius (Hydroxenus) revelierei Perris, 1864	Hydrophilidae	0.003		2	
Paracymus scutellaris (Rosenhauer, 1856)	Hydrophilidae	0.222		110	
Hygrobia hermanni (Fabricius, 1775)	Paelobiidae	0.029	0.107	12	8
Noterus laevis Sturm, 1834	Noteridae	0.019		11	
Hydrocyphon spp.	Scirtidae		0.019		4
Decapoda					
Procambarus clarkii (Girard, 1852)	Cambaridae		0.027		7
Ephemeroptera					
Cloeon spp.	Baetidae		6.179		394
Haplotaxida					
Lumbricidae & Sparganophilidae	Lumbricidae & Sparganophilidae		*		*
Tubificidae	Tubificidae		*		*
Heteroptera					
Corixa affinis Leach, 1817	Corixidae	8.879		2209	
Micronecta scholzi (Fieber, 1860)	Corixidae	0.001		1	
Paracorixa concinna (Fieber, 1848)	Corixidae	0.006		3	
Sigara (Vermicorixa) lateralis (Leach, 1817)	Corixidae	0.391		59	
Sigara (Vermicorixa) scripta (Rambur, 1840)	Corixidae	0.04		14	
Sigara (Halicorixa) selecta (Fieber, 1848)	Corixidae	0.003		1	
Sigara (Halicorixa) stagnalis (Leach, 1817)	Corixidae	0.037		6	
Trichocorixa verticalis (Fieber, 1851)	Corixidae	0.009		2	
Corixidae spp.	Corixidae		1.258		99
Gerris (Gerris) cf maculatus Tamanini, 1946	Gerridae	0.002		1	
Gerris (Gerris) thoracicus Schummel, 1832	Gerridae	0.229		2	
Gerris spp.	Gerridae		0.282		12
Microvelia pygmaea (Dufour, 1833)	Microveliidae	0.011		2	
Naucoris maculatus Fabricius, 1798	Naucoridae	0.01	0.03	5	12
Nepa cinerea Linnaeus, 1798	Nepidae	0.008	0.009	5	6
Anisops sardeus Herrich-Schäffer, 1849	Notonectidae	3.704		272	
Notonecta glauca Linnaeus, 1758 ssp. *glauca*	Notonectidae	0.025		4	
Notonecta glauca Linnaeus, 1758 ssp. *meridionalis* Poisson, 1926	Notonectidae	0.039		4	
Notonecta maculata Fabricius, 1794	Notonectidae	0.011		3	
Notonecta viridis Delcourt, 1909	Notonectidae	0.029		6	
Notonectidae spp.	Notonectidae		1.219		91
Plea minutissima Leach, 1817	Pleidae	0.677	0.208	130	38
Saldidae	Saldidae	0.018		12	
Isopoda					
Asellus aquaticus (Linnaeus, 1758)	Asellidae	0.014		11	
Lumbricula					
Lumbriculidae	Lumbriculidae	*		*	
Notostraca					
Triops mauritanicus (Ghigi, 1921)	Triopsidae	0.055		6	
Spinicaudata					
Cyzicus grubei Simon, 1886	Cyzicidae	*		*	
Maghrebestheria maroccana Thiéry, 1988	Leptestheriidae	*		*	
Anostraca					
Branchipus cortesi Alonso y Jaume, 1991	Branchipodidae	*		*	
Branchipus schafferi Fischer de Waldheim, 1834	Branchipodidae	*		*	
Tanymastix stagnalis (Linnaeus, 1758)	Tanymastigiidae	*		*	
Chirocephalus diaphanus Desmarest, 1823	Chirocephalidae	*		*	
Odonata					

Aeshna affinis Vander Linden, 1823	Aeshnidae		0.005		1
Aeshna mixta Latreille, 1805	Aeshnidae		0.012		2
Anax imperator Leach, 1815	Aeshnidae		*		*
Hemianax (Anax) ephippiger (Burmeister, 1839)	Aeshnidae		0.003		1
Coenagrion scitulum (Rambur, 1842)	Coenagrionidae		0.001		1
Ischnura elegans (Vander Linden, 1820)	Coenagrionidae		0.052		9
Ischnura pumilio (Charp., 1825)	Coenagrionidae		0.525		50
Lestes barbarus (Fabr., 1798)	Lestidae		0.028		16
Lestes dryas Kirby, 1890	Lestidae		0.008		2
Lestes macrostigma (Eversm., 1836)	Lestidae		0.001		1
Lestes virens (Charpentier, 1825)	Lestidae		0.002		1
Crocothemis erythraea (Brullé, 1832)	Libellulidae		0.033		5
Sympetrum fonscolombei (Selys, 1841)	Libellulidae		0.252		9
Sympetrum meridionale (Selys, 1841)	Libellulidae		0.024		3
Sympetrum sanguineum (Müller, 1764)	Libellulidae		0.038		5
Sympetrum striolatum (Charpentier, 1840)	Libellulidae		0.048		4
		Average	**Maximum**		
Taxa	**Family**	**Larva**	**Nymph**	**Larva**	**Nymph**
Diptera					
Ceratopogoninae	Ceratopogonidae	*		*	
Chaoborus spp.	Chaoboridae	*		*	
Chironomidae sp.	Chironomidae	*	0.001	*	1
Chironomus plumosus (Linneo, 1758)	Chironomidae	*	0.001	*	1
Culicidae	Culicidae	0.567	0.132	253	8
Dixa spp.	Dixidae	0.01	0.004	2	2
Dolichopodidae	Dolichopodidae	0.016		11	
Ephydridae	Ephydridae	0.023	0.013	12	4
Orthocladiinae	Chironomidae	*		*	
Rhagionidae	Rhagionidae	0.009		2	
Scatophagidae	Scatophagidae	0.001		1	
Sciomyzidae	Sciomyzidae	0.001		1	
Syrphidae	Syrphidae	0.005	0.004	3	2
Tabanidae	Tabanidae	0.005		1	
Tanypodinae	Chironomidae	*	0.001	*	1
Thaumelidae	Thaumelidae		0.001		1
Tipulidae	Tipulidae	0.011	0.004	2	2

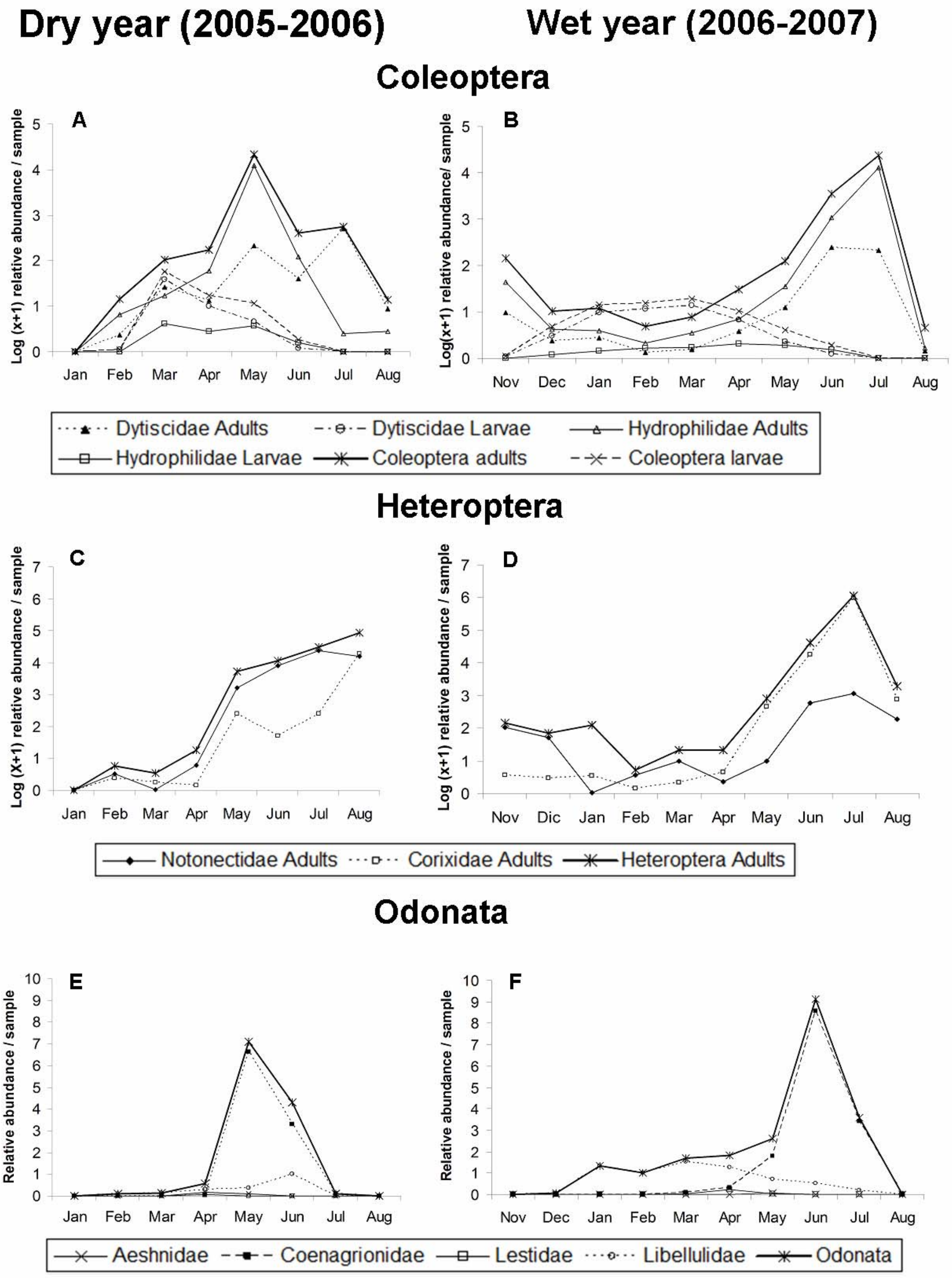

Fig.2: Monthly variation in the relative abundance of individuals of different taxa of macroinvertebrates averaging data of all ponds in a dry and a wet year: Coleoptera (A,B), Heteroptera (C,D) and Odonata (E,F).

Inter-annual variation

Macroinvertebrate compositions of every pond were segregated in two groups corresponding with both study years, although the dissimilarity between the dry and the wet year was not very strong (ANOSIM: R=0.235; p=0.02) (Fig.3). SIMPER analyses showed that adults of *Hydroglyphus geminus* (Fabricius, 1792) (13.67%), *Anacaena lutescens* (Stephens, 1829) (13.64%) and Notonectidae larvae (10.91%) mainly contributed to these differences in the dry year, whilst *Cloeon* spp. (11%) and adults of *Anisops sardeus* (10.81%) had a larger contribution in the wet year.

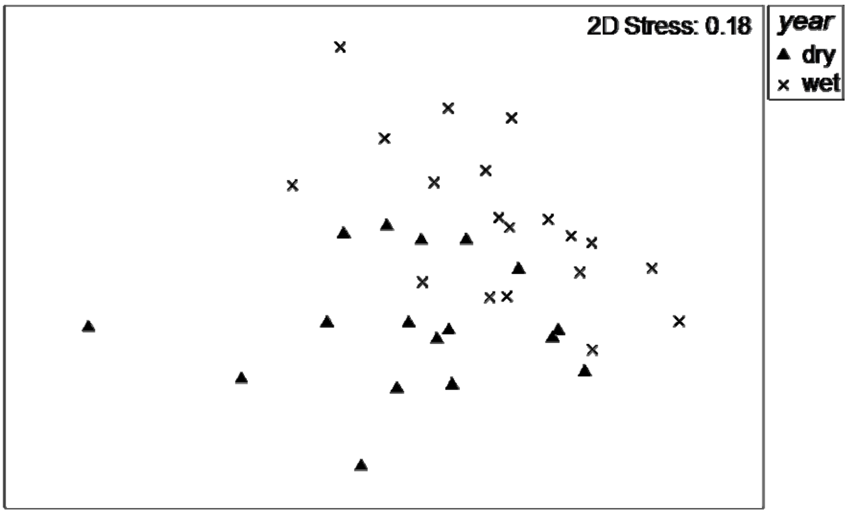

Fig.3: NMDS ordination of the relative abundance of macroinvertebrates in every pond, showing their inter-annual variation. It is averaging the number of individuals per month in the dry and the wet year.

Seasonal variation

During the dry year we observed three groups in the NMDS composed by different macroinvertebrate compositions detected in every pond and month, which corresponded to different wet phases of the ponds (Fig.4): filling phase (February), aquatic phase (March and April) and drying phase (May, June, July, August and September) (ANOSIM, global R=0.615, p=0.01). In the aquatic phase we also distinguished a weak segregation in two subgroups: early spring (March) and late spring (April) phases (ANOSIM, R=0.297, p=0.02). We identified the main taxa that contributed to the dissimilarity of the three phases with a SIMPER analysis: Adults of *Berosus affinis* Brullé, 1835 (17.32%), *Helophorus* spp. (17.24%), *Anacaena lutescens*

(15.74%), Corixidae (without *Corixa affinis*) (12.52%) and *Dryops* spp. (11.97%) in the filling phase; Notonectidae larvae (14.14%) and adults of *Hydroglyphus geminus* (13.69%) in the aquatic phase; adults of Corixidae (without *Corixa affinis*) (25.83%) and *Anisops sardeus* (19.18%) in the drying phase. During the wet year, we observed five consecutive groups of macroinvertebrates compositions of every pond and month in the NMDS that corresponded to different wet phases (Fig.4): filling phase (November), winter (December and January), early spring (February and March), late spring (April) and drying phase (May, June, July and August). The wet phases presented different similarities according to an ANOSIM analysis (global R=0.538, p=0.01). The highest R value in the pairwise comparison of wet phases during the wet year were for filling phase *versus* early spring (R=0.826; p=0.01), and filling phase *versus* late spring (R=0.912; p=0.01). SIMPER analysis revealed that the taxa with highest contribution to global dissimilarity were: adults of *Anisops sardeus* (23.90%) and *Berosus guttalis* Rey, 1883 (12.30%) in the filling phase; *Anisops sardeus* (adults) (31.41%) and *Cloeon* spp. (19.34%) in the winter phase; *Cloeon* spp. (39.79%) in the early spring phase; *Gerris* spp. larvae (14.04%), Notonectidae larvae (11.94%) and *Cloeon* spp. (11.93%) in the late spring phase; and adults of *Corixa affinis* (25.15%) and *Enochrus fuscipennis* (C.G. Thomsom, 1884) (10.44%) in the drying phase.

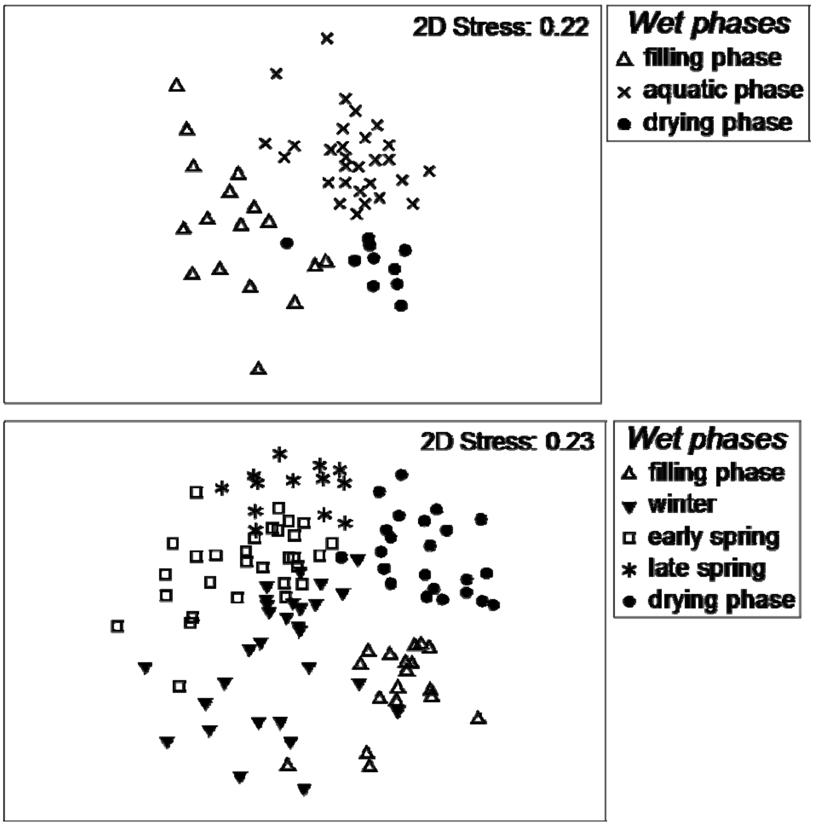

Fig.4: NMDS ordination of the relative abundance of macroinvertebrates in different ponds and months during the dry (top) and wet year (bottom). Different phases identified are indicated on the plot.

Monthly variation of macroinvertebrate assemblages within ponds

In the wet year, the Spearman correlations comparing the similarity matrices of monthly macroinvertebrate assemblages in each pond tended to present higher Rho values in serial than in cyclic correlations in 13 ponds (Table 3, Fig. 5A). In contrast, in five ponds they tended to present higher cyclic correlations (Table 3, Fig. 5B). In one pond, the Rho value for these similarity matrices was not significant in the case of serial correlation and was very low in the case of cyclic correlation (Table 3). The correlations were higher in three of the four ponds sampled monthly compared to every 15-day-sampled-ponds. In these three ponds, monthly macroinvertebrate assemblages presented higher serial than cyclic correlations, whilst in one pond they only presented a significant cyclic correlation for 15-days samples (Table 3). These ponds exhibited a high variability among 15-day-samples in the NMDS representation, pointing out their fluctuating trajectory which was not detected among monthly samples (Fig. 5D and 5C).

Table 3: Spearman correlation coefficients (Rho) calculated amongst monthly macroinvertebrate assemblages in each pond (Serial and Cyclic RELATE analyses) during the wet year. For ponds sampled every 15 days, both monthly and 15-day analyses are shown. Number of samples in each correlation analysis is given in brackets. ** = p<0.01; * = p<0.05. The highest significant Rho value (cyclic or serial correlations) for each pond is marked in bold.

	Spearman correlation (Rho)			
	Monthly		**Every 15 days**	
Pond	**Serial**	**Cyclic**	**Serial**	**Cyclic**
Pol	**0.732** (7)**	0.491** (7)		
Acm	**0.627** (6)**	0.466* (6)		
Rp	**0.618** (7)**	0.472** (7)		
Pg	**0.736** (7)**	0.616** (7)		
Jim	**0.757** (7)**	0.478** (7)		
Cam	**0.641** (7)**	0.287** (7)		
Zah	**0.881** (9)**	0.658** (9)		
Lve	**0.653** (10)**	0.614** (10)		
Dul	**0.724** (12)**	0.665** (12)		
Abe	**0.593* (7)**	0.549** (7)		
Bre	0.241 (9)	**0.474** (9)**		
Pp	0.511* (7)	**0.636** (7)**		
Tej	0.555* (7)	**0.742** (7)**		
Orf	0.391* (9)	**0.471** (9)**		
Ant	0.35 (6)	0.086* (6)		
Arm	0.156 (6)	0.362 (6)	0.309 (8)	**0.428* (8)**
Vac	**0.724** (7)**	0.684** (7)	**0.553** (11)**	0.42** (11)
Len	0.736* (7)	0.736* (7)	**0.509** (10)**	0.358** (10)
Tps	**0.583** (6)**	0.265* (6)	**0.506** (10)**	0.291* (10)

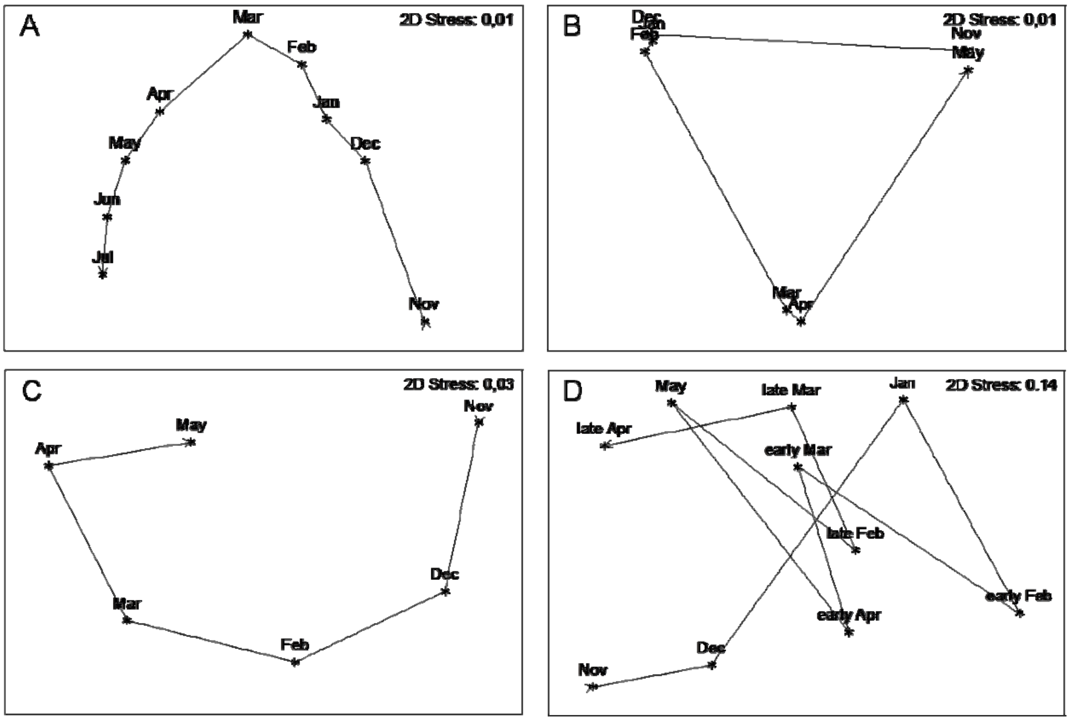

Fig.5: Monthly variation of the macroinvertebrate assemblage in three different temporary ponds (A, B and C) represented in a NMDS during the wet year. Pond A (Zah) presented a higher serial correlation whilst B (Pp) presented a higher cyclic correlation. C and D show the same pond (Len) with monthly samples (C) and 15-day-samples (D). Pond shown in C and D was occasionally dried in January when we could not record data for the monthly sample (C), but only for the 15-day sample (D).

General pattern of monthly variation in the macroinvertebrate community

We detected differences in the macroinvertebrate assemblages of all ponds and months using the sampling month in both study years as grouping factor, with an ANOSIM analysis (global R=0.475, p=0.01). It showed that the variation amongst months was higher than amongst ponds for each month. The monthly variation of the community showed a similar pattern in both years, observed in the NMDS representation. The highest similarity was found between the months of April of both years (Fig. 6). The monthly macroinvertebrate community presented a strong serial correlation (Serial RELATE) in both the dry (Rho=0.916; p=0.01) and the wet year (Rho=0.788; p=0.01).

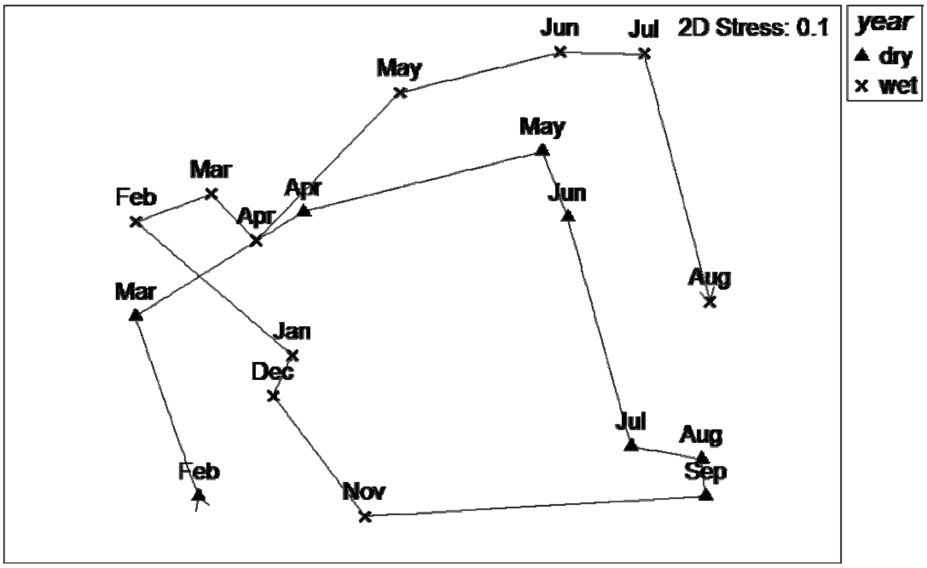

Fig.6: NMDS ordination of the relative abundance of macroinvertebrates showing monthly variation after averaging across all the study ponds per month in the consecutive dry and wet years.

Discussion

Macroinvertebrate fauna of temporary ponds

Our temporary ponds had a rich macroinvertebrate fauna with similar or even higher richness than other temporary (Bazzanti et al., 1996; Schneider & Frost, 1996; Brooks, 2000; Boix et al., 2001) or permanent ponds (Heino, 2000; Della Bella, 2005). The high richness found in our study does not correspond to a single pond, but to a system of temporary ponds which allow movement and dispersal of individuals among ponds (Fortuna et al., 2006). In this kind of systems, the high connectivity and non-fragmentation area are very important factors to conserve their invertebrate biodiversity (Van de Meutter et al., 2006; Briers & Biggs, 2005). In the past, temporary ponds were usually excluded from conservation plans for wetlands, neglecting the diversity of their associated fauna due to their small size and temporal behaviour (Williams et al., 2001; Grillas et al., 2004; Williams, 2006; Zacharias et al., 2007). The high richness of macroinvertebrates in temporary ponds justifies the necessity of their conservation, this also being important since they include different fauna from permanent aquatic habitats, including many rare species (Collinson et al., 1995). These temporary habitats also allow the occurrence of many species which are vulnerable to predation, and adapted to survive their characteristic dry phase (Wellborn et al., 1996; Williams, 2006)

Inter-annual variation

Temporary ponds are fluctuating habitats, and in this study we have detected significant changes in their macroinvertebrate composition. Many physical characteristics of temporary ponds are widely dependent on rainfall, with important variation from dry to wet years. Consequently, macroinvertebrate assemblages may differ among wet and dry years (Jeffries, 1994). Historical events, such as very dry years, may affect the macroinvertebrate community composition as much as site-specific abiotic differences amongst ponds (Boulton & Lake, 1992). Between our dry and wet study years, the same ponds differed in their hydroperiod, as well as in water depth and area, and accordingly we also found significant differences in the macroinvertebrate composition between years, despite these being consecutive. The shorter hydroperiod of the ponds in the dry year constrained the length of the aquatic period for macroinvertebrates. Thus, the occurrence of larvae of Coleoptera and Odonata was more concentrated, and we detected differences in the peak of abundance of Coleoptera and Odonata that occurred in May in the dry year, one or two months earlier than in the wet year (June-July).

Seasonal variation

From filling to desiccation, temporary ponds experience large physicochemical variations (García Novo et al., 1991; Serrano & Toja, 1995; Gómez-Rodríguez et al., 2009), characterizing different phases according to the wet period (Bazzanti et al., 1996). Particular macroinvertebrate compositions have been described as characteristic of different wet phases of such ponds. They are explained as a consequence of the changes experienced in these aquatic habitats, which present optimal environmental conditions for different macroinvertebrates (Boulton & Lake, 1992; Boix et al., 2004; Culioli et al., 2006). In fact, different taxa of macroinvertebrates show wide differences in their life strategies, such as in reproduction, feeding, development or dispersal, and other particularities of their life cycle (Bilton et al., 2001a; Williams, 2006; Verberk et al., 2008). The macroinvertebrate groups obtained in the NMDS representation of ponds and months also revealed this variation in macroinvertebrate assemblages (including adults, larvae and nymphs), changing through the different wet phases of the ponds. However, the shorter pond hydroperiod of the dry year also reduced the number of phases observed in this year relative to the wet year. During the dry year, only three distinct phases were identified: filling phase, aquatic phase and drying phase, whilst during the wet year five phases were detected: filling phase, winter, early spring and late spring phases (aquatic phase) and drying phase. The reduction of the number of phases in years of low rainfall causes macroinvertebrates to synchronize their life histories (Wiggins et al., 1980; Nilsson, 2005b) concentrating biological processes into the short hydroperiod available. In ponds with very short hydroperiods the number of phases may be even lower than three (Boix et al., 2004). In dry years, organisms with long life cycles may be the taxa most affected by short hydroperiods, as they cannot successfully complete their aquatic development (Schneider & Frost, 1996; Taylor et al., 1999).

The filling phase, just after pond formation, is characterized by the arrival of coleopterans and heteropterans through dispersal from other (more permanent) ponds (Wiggins et al., 1980). The taxa most characteristic of this phase did not coincide in our two study years, probably because the date of filling occurred in different seasons in both years, affecting the activity cycles of the species. We also found other macroinvertebrates taxa that usually spend the dry period in the mud, such as adults of *Berosus signaticollis* (Charpentier, 1825) (Boix et al., 2001) or adults of some species of Hydrophilidae which have a period of flight to colonize new habitats in newly filled ponds (Wiggins et al., 1980; Hansen; 2005; Williams, 2006).

The aquatic phase was longer in the wet year, and also the species characteristic of this or these phases were different amongst years, except for Notonectidae larvae which mostly appeared in the late spring phase of the wet year, having their peak abundance in the same month of the dry year. The environmental conditions of the wet year appear to have favoured particular species, such as *Cloeon* spp. which was very abundant in the wet year, being the only taxon characteristic of the three phases, winter, early and late spring that constituted the aquatic phase of this year. In contrast, its abundance was not high during the dry year.

The taxa most characteristics of the drying phases of both years did not coincide either in both study years, although adult corixids were characteristic of this phase in both the dry and wet year. In the drying phase, adult heteropterans and coleopterans were the most common taxa in our study ponds, as described in other studies (Boulton & Lake, 1992; Culioli et al., 2006; Garrido & Munilla, 2008). Some beetles and almost all hemipterans possess excellent dispersal capabilities (Wiggins et al., 1980; Bilton et al., 2001). The high abundance of these taxa may be explained by the arrival of dispersers, moving from dry ponds to other ponds whilst dispersing to more permanent habitats to survive during dry periods (Wiggins et al., 1980; Higgins & Merrit, 1999; Bilton et al., 2001, Williams, 2006). We observed some dispersing individuals landing in some of our study ponds during the drying phase, such as *Colymbetes fuscus* (Linnaeus, 1758), *Gerris thoracicus* Schummel, 1832 and *Corixa affinis*. In the dry phase, as well as in the filling phase, the increase in number of species recorded in particular ponds were mainly due to dispersers, as reported for summer and autumn seasons in Verberk et al. (2005).

Monthly variation of macroinvertebrate assemblages within ponds

The variation of the macroinvertebrate composition in the ponds was not only attributable to differences between a wet and a dry year, or to the wet phases. Our study ponds had been chosen within a wide hydroperiod gradient, and whilst most of them filled in approximately the same month of each year, they clearly differed in the timing of desiccation, with some ponds drying earlier than others. As a consequence of the different desiccation times of the ponds, we observed different monthly variation in macroinvertebrate assemblages: some ponds showing cyclic correlation with similar assemblage composition at the beginning and the end of the hydroperiod, whilst others differed at these two phases, showing serial correlations instead. These differences may be explained in relation to the capability of many species to move between ponds via dispersal (Bilton et al., 2001; Rundle et al., 2002; Williams, 2006). At the end of hydroperiod, many Dytiscidae and Hydrophilidae suddenly leave the water, dispersing to more permanent waters (Nilsson, 2005a). Some adults and larvae can also leave the water and bury into the mud for pupation or as resistance stages (Hansen 2005, Nilsson, 2005b; 2005c; 2005d) waiting for the next filling phase. Ponds with serial correlations would correspond to: a) early drying ponds in which coleopterans and heteropterans were forced to move as desiccation progressed, b) ponds with very long hydroperiod with a high abundance of heteropterans (mainly corixids) in summer. In contrast, ponds with cyclic correlations would correspond to intermediate hydroperiod ponds which still have water when the other ponds are drying and could act as intermediate sites during dispersal of organisms towards more permanent aquatic habitats, with similar taxa occurring in drying and filling phases. All the ponds with non-significant or weak correlation values had short hydroperiods, indicating that they were much more fluctuating than the other ponds. It was detected mainly in 15-day-sampled pond compositions when compared with monthly samples of the same ponds. Richness and biodiversity has been related to the stability along of time (White, 2004), being invertebrate assemblages more stable in ponds with more permanence of water and highly fluctuating in ephemeral ponds (Shurin, 2007). We recommend increasing the frequency of samples along the time in ephemeral ponds with respect more permanent ponds to record all the variability of their macroinvertebrate assemblages, and maybe of other groups like macrophytes, amphibians and other invertebrates.

General pattern of monthly variation in the macroinvertebrate community

Despite differences in macroinvertebrate composition amongst ponds, and in the same ponds at seasonal and inter-annual scales, a general pattern was detected, with similar variation between both years. This may be associated with the general development of the life cycle of many macroinvertebrates within the hydrological cycle of temporary ponds. From flooding to desiccation we detected the successive appearance of adults, larvae and nymphs at different phases of the ponds. In long hydroperiod years this general pattern may be extended from autumn to summer whilst in short hydroperiod years it is concentrated. In our two study years, we detected a similar variation in macroinvertebrate composition through both wet phases. We also detected a similar composition between filling months although it occurred in February in the dry and November in the wet year, as well as between the last month of the drying phases (August). This consistent general pattern revealed a high monthly correlation during both years, which was apparently repeated in the two years of the study.

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Chapter 4: Do seasonal patterns of macroinvertebrate assemblages follow environmental fluctuations in temporary ponds?

Resumen:

Las variaciones estacionales asociadas a las comunidades de microinvertebrados son normalmente atribuidas a diferencias estacionales en los parámetros ambientales. Para evaluar la fortaleza de estas relaciones a lo largo de los ciclos de inundación-desecación, 22 lagunas temporales fueron mensualmente muestreadas durante su periodo de inundación en dos años sucesivos con diferentes precipitaciones. Las lagunas presentaron fuertes diferencias en sus características ambientales, lo que supone una gran variabilidad de habitats que favorecen una alta diversidad de macroinvertebrados en el área. Aunque las lagunas difirieron en sus características ambientales entre los dos años de estudio, apenas se encontraron diferencias entre las tres fases estacionales descritas para las comunidades de macroinvertebrados en el área de estudio (las fases de inundación, la fase acuática y la fase de desecación). A pesar de ello, las variables ambientales contribuyeron de forma diferente a la estructura de las comunidades de macroinvertebrados a lo largo de dichas fases. La mayor contribución se produjo durante la fase de inundación mediante la conductividd, el pH y la profundidad máxima de la columna de agua. Sin embargo, las características ambientales de las laguas apenas influenciaron la estructura de las comunidades de macroinvertebrados durante la las fase acuática y la fase de desecación. Ambas fases estuvieron principamente asociadas a diferencias en la longitud del hidroperiodo de las lagunas a través del pH, la profundidad máxima de la columna de agua y las concentraciones de fósfotro y hierro del sedimento. Cuando las lagunas se aproximaron al momento de desecación sufrieron un cambio drástico en todas sus características ambientales. Esto puede suponer un estrés ambiental para las especies de macroinvertebrados que pone en marcha el desarrollo de las estrategias de supervivencia frente a la desecación, tales como la dispersión hacia cuerpos de agua más permanentes, y la formación de estructuras de resistencia. El hidroperiodo es considerado en este estudio como el factor principal que afecta a las comunidades de macroinvertebrados a lo largo de las fases estacionales en las lagunas temporales de Doñana. En estas lagunas la fase de inundación y la desecación de las lagunas parecen ser procesos clave que afectan al establecimiento de estas comunidades.

Abstract:

Seasonal variations of macroinvertebrate assemblages are usually attributed to seasonal differences in environmental variables. In order to assess the strength of these relationships throughout the inundation-desiccation cycle, twenty-two temporary ponds were monthly sampled during their inundation period in two successive years which widely differed in rainfall. Environmental characteristics strongly differed among ponds, suggesting a high distinctiveness in the pond habitat which provides excellent conditions to support high macroinvertebrate diversity in the area. Although ponds differed in their environmental characteristics between the two study years, they hardly differed among the three phases described for macroinvertebrate assemblages in this area (filling, aquatic and drying phases). However, environmental conditions varied in their contribution to the structure of macroinvertebrate assemblages throughout the different phases. The highest contribution occurred during the filling phase and was that of water conductivity, pH and maximum depth. In contrast, pond environmental characteristics poorly influenced macroinvertebrate assemblage structure during the aquatic and the drying phases; these phases were mainly associated to differences in pond hydroperiod with the only contribution of pH, maximum depth and total sediment P and Fe concentrations. Ponds experienced a strong change in all their environmental conditions when they were close to desiccation which is suggested as stressful conditions triggering macroinvertebrate survival strategies such as adult dispersal towards more permanent ponds and the development of drought-resistant forms. Throughout seasonal wet phases, we identified the hydroperiod as the factor with a highest effect in the structure of macroinvertebrate assemblages of Doñana temporary ponds, in which pond filling and desiccation seem to be key processes for the establishment of these assemblages.

Key words: Mediterranean ponds, environmental characteristics, invertebrate composition, hydroperiod, pond filling, pond desiccation

Introduction:

Mediterranean temporary ponds are considered priority habitats in the EU and are included in the Habitat Directive with a high status of conservation (Natura code 3170, CEE, May 21st 1992, see Ruíz 2008). Temporary ponds are fluctuating aquatic systems which usually form at the onset of the rainy season and present a recurrent dry phase which duration is variable and quite unpredictable (Williams 1997). Unpredictability is a general characteristic of temporary pond systems; they present high inter- and intra-annual variability in their environmental characteristics (Serrano and Toja 1995; Bazzanti *et al.* 1996; Gómez-Rodríguez *et al.* 2009), as well as in their macroinvertebrate assemblages (Serrano and Toja 1998; Jeffries 1994; Florencio *et al.* 2009a).

Different environmental characteristics of ponds can structure particular macroinvertebrate assemblages in different temporary ponds (Waterkeyn *et al.* 2008; Porst and Irvine 2009; Bilton *et al.* 2009). Assessing the environmental variability among ponds is essential to preserve macroinvertebrate diversity in pond networks (Urban 2004).

Doñana temporary ponds present a natural pond network with a high status of conservation, included in the RAMSAR convention since 1982, and designated as a World Heritage Site by UNESCO in 1995. In years of heavy rainfalls, more than 3,000 waterbodies can form in the Doñana landscape (Gómez-Rodríguez 2009). Although seasonal and annual variations in pond environmental features (Serrano and Toja 1995; Serrano *et al.* 2006; Gómez-Rodríguez *et al.* 2009) and macroinvertebrate assemblages (Florencio *et al.* 2009a) are characteristic of Doñana temporary ponds, seasonal effects of these environmental factors on the structure of macroinvertebrate assemblages has not been assessed in Doñana temporary ponds.

During the annual period in which temporary ponds are inundated, seasonal variation in macroinvertebrate assemblages is characterized by successive wet phases, from the filling to the desiccation of all ponds in an area (Bazzanti *et al.* 1996; Boix *et al.* 2004; Culioli *et al.* 2006; Florencio *et al.* 2009a): 1) the filling phase is a short period which takes places just after pond formation at the beginning of the rainy season; 2) the intermediate or aquatic phase is a long period in which macroinvertebrate assemblages become well established and species develop their life cycles; 3) the drying phase is a period in which pond desiccation occurs, forcing macroinvertebrate species to display survival strategies facing desiccation. In Doñana, most temporary ponds fill in approximately the same time, but their desiccation is gradual according to the water permanence (i.e. hydroperiod) (Díaz-Paniagua *et al.* 2010). In the drying phase, most adult macroinvertebrates leave the drying ponds and disperse towards more permanent ponds which still hold water. Whilst most species leave drying ponds, more permanent ponds received those dispersers, resulting in different macroinvertebrate assemblages between both types of ponds (Florencio et al. 2009a). Environmental seasonal variation is suggested as the main force influencing the structure of macroinvertebrate assemblages throughout these wet phases (Boulton and Lake 1992; Bazzanti *et al.* 1996; Angélibert *et al.* 2004; Culioli *et al.* 2006) in addition to the species life cycles themselves (Florencio *et al.* 2009a).

In temporary ponds, the length of the hydroperiod, particularly its desiccation, is a key factor for organisms completing their life cycles (Williams 2006). When ponds are drying, a drastic reduction of the pond surface takes place (Higgins and Merritt 1999) accompanied by a degradation of environmental conditions such as a temperature increase, deoxygenation (Bazzanti *et al.* 1996), and acidic waters (Culioli *et al.* 2006). By this time, most insects have completed their larval development and emerge as adults, while most crustacean species produce drought-resistant eggs (Higgins and Merritt 1999). The environmental stress and high predator densities during pond desiccation have been suggested as a trigger in macroinvertebrate strategies to survive the dry season (Boix *et al.* 2004; Greig and Wissinger 2010; Jocque *et al.* 2010).

We assessed the seasonal variation in environmental characteristics of 22 temporary ponds with a wide hydroperiod range in two consecutive years differing in rainfall. We aimed to determine whether environmental variables differed among temporary ponds and between years with different pond hydroperiods. We hypothesised that seasonal variation in environmental variables can drive the seasonal structure of macroinvertebrate assemblages across the wet phases. In the drying phase, we also aimed to describe the effect of environmental variables that trigger the drastic changes in macroinvertebrate abundance observed in drying ponds.

Methods

Study area

We analysed the environmental characteristic of 22 temporary ponds located in the Doñana Biological Reserve (Doñana National Park, Southwestern Spain). This area is located between the Atlantic coast and the mouth of the Guadalalquivir River. The climate is Mediterranean sub-humid, with mild winters and hot and dry summers; rainfall is highly variable and heavy floods tend to occur in autumn-winter (see Siljestrom *et al* 1994, García-Novo and Marín 2006 for a detailed description of the area). Most ponds in Doñana are fed by rainfall and a shallow water table that rises above the surface after heavy rainfall, thus creating a pond network of more than 3000 waterbodies (Gómez-Rodríguez 2009). Most of them are temporary ponds that hold water during autumn and winter (Díaz-Paniagua *et al.* 2010). A detailed description of the characteristics of Doñana temporary ponds, including most of our study ponds, is given in Gómez-Rodríguez *et al.* (2009) and Florencio *et al.* (2009a). Vegetation in the ponds was mainly composed of meadow plants such as *Mentha pulegium* L., *Illecebrum verticillatum* L., or *Hypericum elodes* L., in the littoral zone, whilst aquatic macrophytes such as *Juncus heterophyllus* Dufour, *Myriophyllum alterniflorum* DC. in Lam & DC., *Potamogeton pectinatus* L. and *Ranunculus peltatus* Schrank were common in deeper areas (Díaz-Paniagua *et al.* 2010).

Our study period was October 2005 to August 2007. Annual rainfall was calculated as the amount of rainfall collected from 1st September to 31st August of the following year. This amounted to 468.3 mm in the first year (hereafter referred to as the dry year), and to 716.9 mm in the second year (hereafter referred to as the wet year). Study ponds were selected within the widest range of hydroperiod in each study year, being a good representation of pond heterogeneity in the study area. In the dry year we sampled 19 temporary ponds. In the wet year, a higher number of ponds with short hydroperiod were formed in the area. To assess the widest range of hydroperiod during the wet year, we sampled three of these new ponds, although the total number of ponds sampled was the same as in the previous year. In the dry year, most temporary ponds were inundated from February to June; in the wet year, from October to July; however, one pond with the longest hydroperiod held water even during August in both years. Most ponds filled in at similar time, after the first heavy rainfall each year, but the number of ponds which held water decreased successively from March in the dry year and from April in the wet year. Then, temporary ponds were dry until the first rainfalls which usually occur in autumn or winter. We calculated hydroperiod as the number of months in which ponds were flooded within a given year, and classified it in relation to the longest hydroperiod seen in each year. In the dry year, hydroperiod categories were short (< 2.5 months), intermediate (2.5-3.5 months) and long (> 3.5 months). In the wet year, we considered an additional category of ephemeral ponds, which were formed in the following 13 days after the start of the filling phase and were also the first ponds that dried up. Therefore they had the shortest hydroperiod that year (< 6.4 months) in comparison of short (6.4-7 months), intermediate (7-7.5 months) and long (> 7.5 months) hydroperiod ponds (for further details on hydroperiod of these ponds see Florencio *et al.* 2009a).

To analyse the seasonal variation of pond environmental characteristics and macroinvertebrate assemblages, we grouped the sampling period in successive wet phases: the filling phase (February in the dry year and November in the wet year); two sub-phases of the aquatic phase in the dry year (early spring phase in March and late spring phase in April); and three sub-phases in the wet year (winter phase in December and January, early spring phase in February and March, and late spring phase in April); and the drying phase (May-August in both years). These wet phases were grouped according to the seasonal variation described for macroinvertebrate assemblages of these ponds in Florencio *et al.* (2009a).

Sampling of macroinvertebrate assemblages

We sampled the macroinvertebrates of the study ponds monthly during their wet period. Using a 1-mm mesh size dip net, we covered a stretch of water of approximately 1.5 m length in each sampling unit. In each pond, we sampled at points separated by a minimum distance of 5 m along one or two transects from the littoral to the open water, the number of sampling points being proportional to pond size. We also took additional samples in microhabitats which were not represented in these transects. The maximum number of samples per pond ranged from 6 to 13 in the month of maximal inundation. As pond size decreased during the season the number of samples taken was reduced accordingly. Most macroinvertebrates captured were identified *in situ*, being counted and then released. Individuals of unidentified species were preserved in 70% ethanol for identification in the laboratory. We identified separately adults and larvae (hereafter referred to as "taxa" for simplicity). In general, taxa were identified to species (most adults) or genus (most larvae) level, although most members of the orders Bassomatophora, Diptera, Haplotaxida, Lumbricula and saldid bugs were identified only to family level. Each year, we analysed the monthly variation in the number of macroinvertebrate taxa. For analyses, we estimated the relative abundance of each macroinvertebrate taxon as the total number of individuals captured in a pond divided by the total number of samples taken in that pond.

Pond characteristics

In each pond and sampling month, we measured the maximum depth (with a graduated pole at the deepest point of the pond), electrical conductivity at 20ºC (on bed using HI 9033), pH (on bed using HANNA 991000), dissolved oxygen concentration and temperature (ºC) (both on bed using YSI 550A) *in situ*. Surface water (500 ml) was also collected to measure, after acidic digestion in the laboratory (Golterman 2004), the concentration of total P in the water. Surface sediment samples (5 cm depth) were collected and the following variables were measured in the laboratory: organic matter, in three replicates (lost on ignition, 450 ºC, 5 h); sediment total P, in two replicates, as dissolved inorganic phosphate following the method of Murphy & Riley (1962) after acid digestion of the ignited sediment with 0.5 M H_2SO_4 and $K_2S_2O_8$ (0.5–1 g) at 120 ºC for 4h (Golterman 2004); and total Fe concentrations, determined colorimetrically in two replicates after digestion, by means of o-phenantroline using ascorbic acid as reducing agent (Golterman 2004). For statistical analyses, we did not consider water temperature because of its dependence on seasonal climate variations. Monthly data for ponds in which we could not record all environmental variables were also excluded from the analyses.

Macroinvertebrate assemblage and environmental characteristic data

We constructed a macroinvertebrate matrix and an environmental matrix with the data obtained per pond and month (including the two study years), using the relative abundance of taxa and the values of the environmental variables, respectively. Environmental variables (except sediment total Fe and organic matter) were $\log_e (X+1)$ transformed to approximate normality. Additionally, we extracted from these matrices: 1) two independent annual matrices including only data for each study year; 2) independent matrices including only data per each wet phase; and 3) a matrix which only included May and April data of the wet year, using only those ponds which were dried in June.

Data analyses

We performed ANOSIM analyses (Primer v.6, 9999 permutations, Clarke & Warwick 2001) on the environmental matrices, using Euclidean distances, to evaluate environmental differences among the study years, ponds, months and wet phases. The ANOSIM test statistic, R, is close to one when levels of the grouping factor are different; i.e. all dissimilarities between levels of grouping factor are larger than any dissimilarity among samples in every level of grouping factor (Clarke & Warwick, 2001). For assessing the environmental differences between the dry and wet years and among the study ponds, we performed one-way ANOSIM analyses (Clarke & Warwick 2001). To control for differences in pond environmental characteristics in each year, we used pond as one of the grouping factors in two-way crossed ANOSIM analyses of two types: 1) with no replication (ρ coefficients, see Clarke & Warwick 2001), to explore environmental differences among the sampling months; and 2) with replication (Global

R, see Clarke & Warwick 2001), to explore environmental differences among the wet phases. The environmental matrix and the macroinvertebrate matrix which only included May and April data of the wet year (after applying Euclidean distances and Bray-Curtis index respectively) were used to perform one-way ANOSIM analyses in order to select those ponds without significant differences and analyse, for these ponds, the differences between the two months previous to desiccation. A non-metrical multidimensional scaling (NMDS) representation was used to depict pond dissimilarity in environmental characteristics (Clarke & Warwick 2001). To explore the main environmental variables contributing to the environmental difference (>10% of contribution), we performed one-way SIMPER analyses (Clarke & Warwick, 2001).

We performed canonical correspondence analyses (CCA) with the annual macroinvertebrate matrix and the annual environmental matrix per each wet phase (CANOCO software v.4.5) to detect relationships between environmental variables and macroinvertebrate assemblages in each wet phase. For each year, we performed non-parametric Spearman correlations (Spearman r) between each pair of variables in order to exclude co-variables in CCA analyses. We used a stepwise forward procedure to select the environmental variables and Monte Carlo test to assess their significance (999 permutations, Leps and Smilauer 2003).

Results:

Monthly environmental variation in ponds

Doñana temporary pond waters generally presented high variability in their environmental characteristics; e.g. pH ranged between 4.5 and 9.5, although it was typically circumneutral (Fig.1, Fig.2). When ponds filled in, waters were well-oxygenated and presented very low values of electrical conductivity (<200 µS cm^{-1} for most ponds) except in long hydroperiod ponds, which generally presented high conductivity values (Fig.1, Fig.2). From filling onwards, dissolved oxygen concentration was drastically reduced while conductivity values were gradually increased until the complete desiccation of ponds. Organic matter and total sediment P and Fe concentrations generally presented moderate values throughout the inundation period, although total P concentrations in the water were more variable in ephemeral hydroperiod ponds (Fig.1). However, long hydroperiod ponds presented the maximum total water P concentration (1,000 µg l^{-1}), organic matter (21%) and total sediment P and Fe concentrations (700 µg g^{-1} dw and 20 mg g^{-1} dw respectively) during the latest months in which long hydroperiod ponds held water. Temperature was clearly dependent on the intra-annual climate variations, with low values during winter. Organic matter and total sediment P and Fe concentrations were highly inter-correlated variables: organic matter and sediment total P were significant co-variables in the dry year (Spearman $r=0.9$, $p<0.0001$), and total sediment P and Fe concentrations were significant co-variables in the wet year (Spearman $r=0.83$, $p<0.0001$; we excluded all rest of significant co-variables with $r<0.6$).

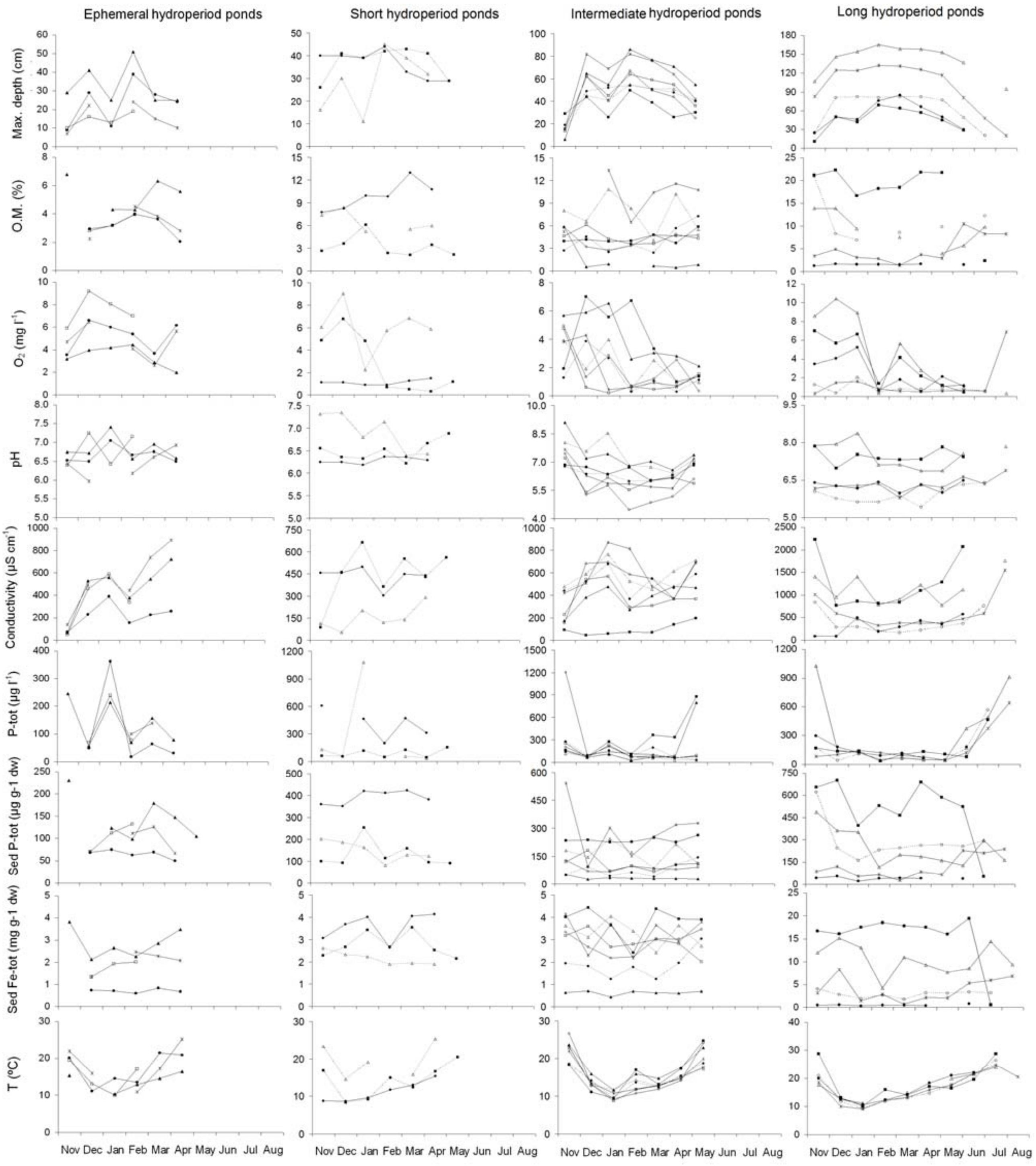

Fig. 1: Monthly variation of environmental variables in the wet year during the inundation period (Max depth is the maximum depth of water column, O.M. is the organic matter, O_2 is the dissolved oxygen concentration, P-tot is the total P concentration in the water, Sed P-tot is the sediment total P concentration, Sed Fe-tot is the sediment total Fe concentration, T is the temperature). Ponds were grouped according to their relative hydroperiod in ephemeral, short, intermediate and long hydroperiod ponds.

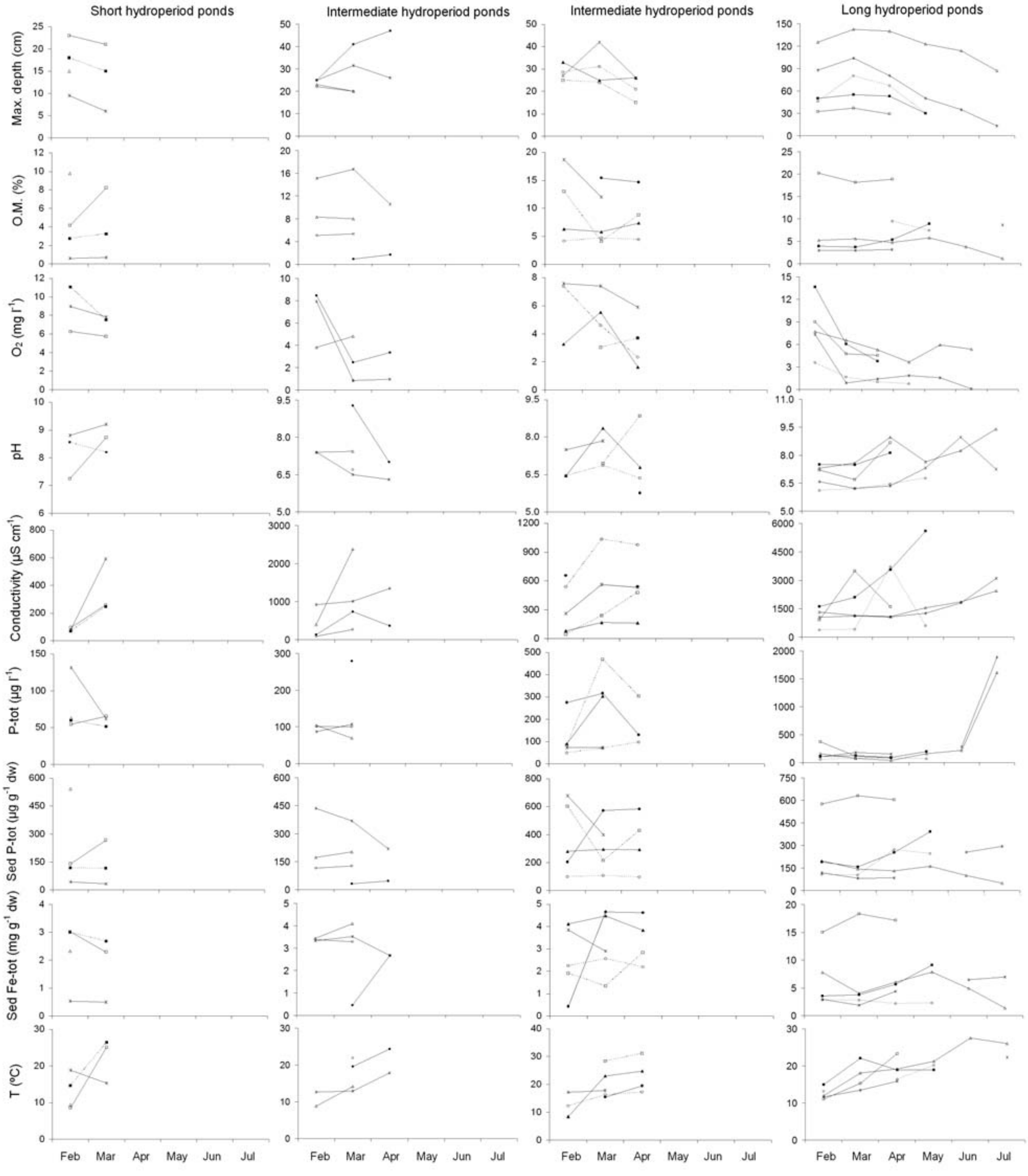

Fig.2: Monthly variation of environmental variables in the dry year during the inundation period (Max depth, the maximum depth of the water column; O.M., organic matter; O_2, dissolved oxygen concentration; P-tot, total P concentration in the water; Sed P-tot, sediment total P concentration; Sed Fe-tot, sediment total Fe concentration; T, water temperature). Variables were grouped according to the relative hydroperiod of ponds in short, intermediate and long hydroperiod ponds.

Analysing the differences in pond environmental characteristics between both years, we detected a significant segregation between the environmental variables of ponds in the dry and the wet year (one-way ANOSIM analysis, R=0.335, p<0.0001, Fig.5A). The main contributing variable to these differences was organic matter (one-way SIMPER analysis, 22.62% contribution), which reached higher values in the dry year (Fig.5B).

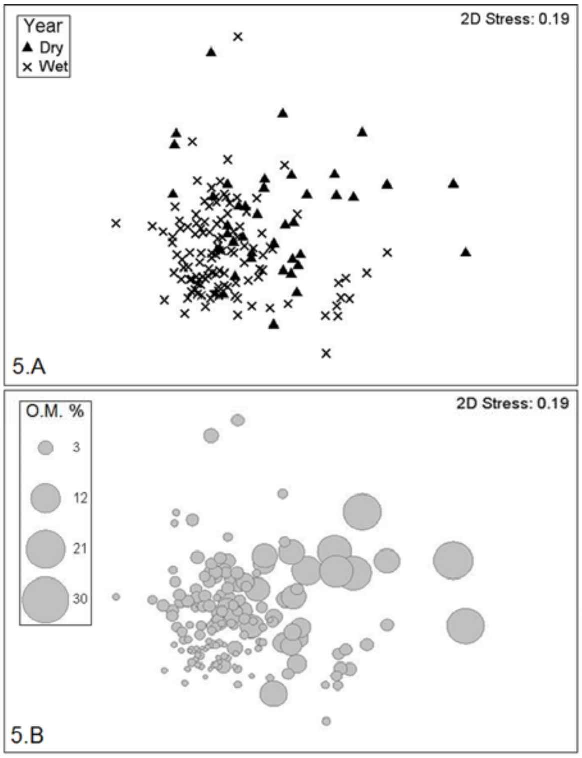

Fig.5: NMDS ordination of ponds according to Euclidean distances of the environmental variables per pond and month. The dissimilarity between the dry and the wet year is shown (5.A). A bubble plot representation on the NMDS ordination shows the proportion of organic matter (O.M. %) per pond (5.B). Bubble size range 3-30 % is corresponding with the minimum and maximum values of organic matter, which is observed higher in the dry year (5.B).

Monthly macroinvertebrate taxon richness variation in ponds

Despite the longer hydroperiod of the ponds in the wet year, the monthly pattern of the macroinvertebrate number of taxa was similar in the dry and the wet year (Fig.4). In short and intermediate hydroperiod ponds in both study years, and also in ephemeral hydroperiod ponds in the wet year, the number of taxa gradually increased from pond filling until the month in which ponds dried up, although some ponds reduced their number of taxa in the last month (Fig.4). In long hydroperiod ponds, the increase in taxon richness continued after the desiccation of the other ponds, reaching the maximum number of taxa the same or the following month in which intermediate hydroperiod ponds dried up.

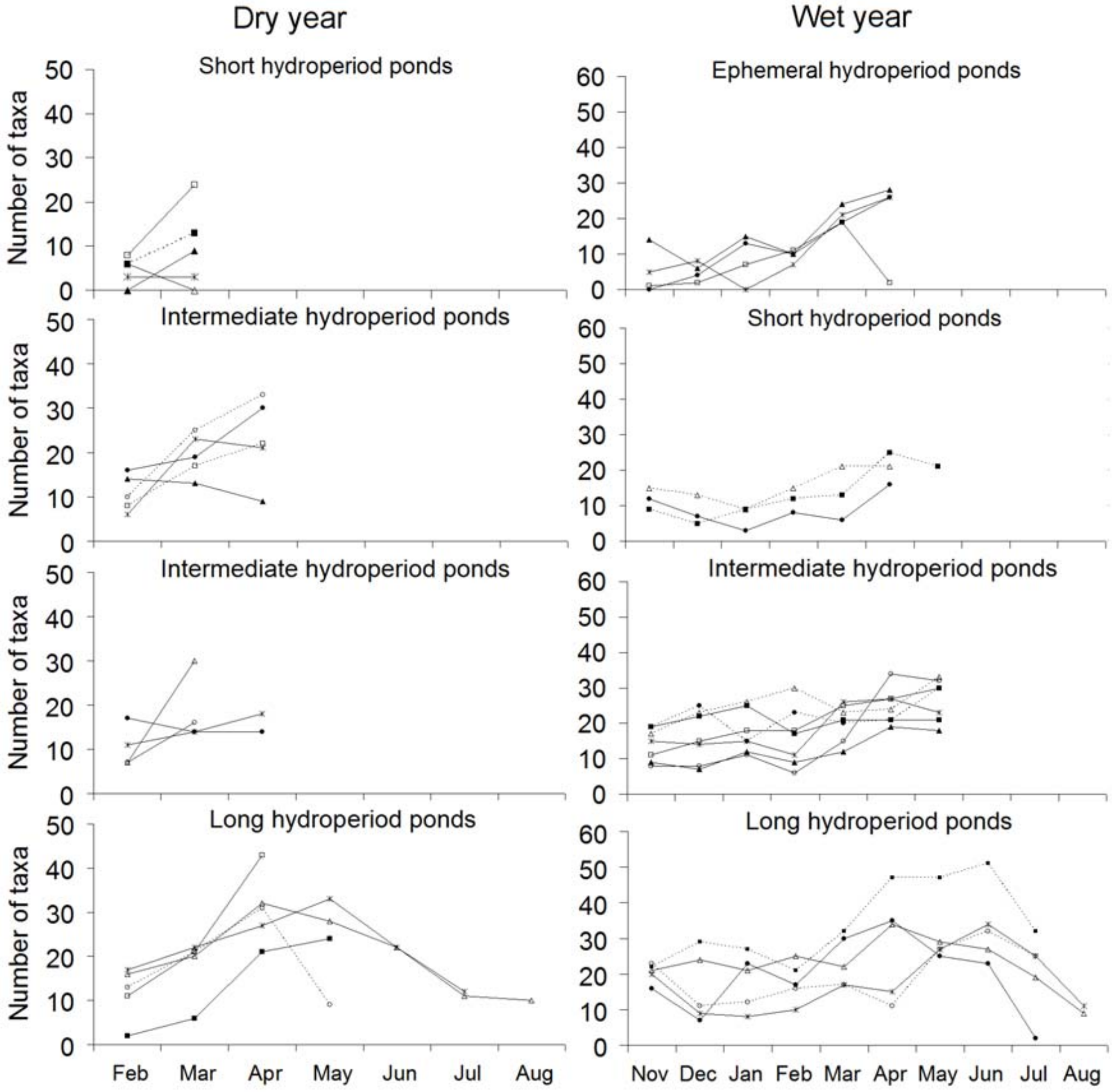

Fig.4: Monthly variation in the number of taxa per pond in the dry and wet years. Ponds were grouped in short, intermediate and long hydroperiod ponds in the dry year and in also in ephemeral hydroperiod ponds in the wet year.

Environmental differences in ponds and wet phases

Analysing the environmental characteristics per pond and month each year, we detected an important segregation among the environmental characteristics of ponds in both study years (one-way ANOSIM analyses, dry year: Global R= 0.598, p<0.0001;

wet year: Global R=0.531, p<0.0001). However, pond environmental variables hardly differed among sampling months, with non-significant differences in the dry year (two-way crossed ANOSIM analysis, ρ=0.443, p=0.067) and significant but weak differences in the wet year (two-way crossed ANOSIM analysis, ρ=0.252, p<0.0001).

Analysing the pond environmental characteristics of the wet phases (filling, early spring, late spring and drying phases in the dry year; and additionally the winter phase in the wet year), we did not detect significant differences in the environmental variables of the wet phases in the dry year (two-way crossed ANOSIM, Global R= -0.056, p=0.55) and either in the pair-wise comparisons between the wet phases (all p>0.5). In the wet year, we detected a low but significant segregation between the pond environmental characteristics of the wet phases (two-way crossed ANOSIM analysis, Global R=0.084, p<0.01), only detecting the filling phase as significantly different in the pair-wise comparisons between the wet phases (filling phase and late spring phase: R=0.288, p<0.001; filling phase and early spring phase: R=0.277, p<0.001; filling phase and drying phase: R=0.251, p<0.001).

Macroinvertebrate and environmental relationships throughout the wet phases

Analysing the particular relationships between environmental variables and macroinvertebrate assemblages in each wet phase, we found that pond environmental variables influenced the structure of macroinvertebrate assemblages, with the highest explained variation occurring in the filling phase of both study years (explained variation: dry year=29.3%, wet year= 22.9%). In the filling phase, electrical conductivity and pH were the significant explanatory variables affecting the structure of macroinvertebrate assemblages in the dry year (N=13, F-ratio=1.92, p<0.01) and maximum depth and pH in the wet year (N=18, F-ratio=2.03, p<0.01). In the dry year, the significant explanatory variables of macroinvertebrate assemblage structure were maximum depth in the early spring phase (N=15, F-ratio=2.02, p<0.001; explained variation=13.5%) and pH in the late spring phase (N=7, F-ratio=1.51, p<0.05; explained variation=23.3%). In the wet year, there were no significant explanatory variables in the winter phase (N=34, all p>0.075); maximum depth and total sediment P concentration were significant explanatory variables in the early spring phase (N=33, F-ratio=1.7, p<0.05; explained variance=10.2%); and total sediment Fe concentration in the late spring phase (N=15, F-ratio=2.51, p<0.001; explained variance=16.2%). In the drying phase, we did not obtain significant explanatory variables affecting the structure the macroinvertebrate assemblages in the dry year (N=5, all p>0.116) and only the sediment total Fe concentration in the wet year (N=17, F-ratio=3.74, $p<0.001$; explained variation= 20%).

Environmental and macroinvertebrate characteristics of drying ponds

In order to analyse environmental characteristics of ponds which were close to desiccation, we selected six ponds which were not significantly different in both their environmental characteristics (one-way ANOSIM analysis, Global R=0.322, p=0.077) and macroinvertebrate assemblages (one-way ANOSIM analysis, Global R=0.333, p=0.072).

We detected significant differences in pond environmental characteristics between the two months previous to desiccation, April and May (one-way ANOSIM test, Global R=0.293, $p<0.05$). All the environmental variables (maximum depth, organic matter, dissolved oxygen concentration, pH, electrical conductivity, total water P concentration, total sediment P and total sediment Fe concentrations) had an important contribution (one-way SIMPER analysis, each variable contributed >10%) to the environmental differences between May and April. Drying ponds in May generally exhibited higher dissolved oxygen, and total water and sediment P concentrations than in April. Particularly, total water P presented a peak of concentration in some ponds in May (see intermediate hydroperiod ponds in Fig.1). Electrical conductivity and pH values increased and water depth decreased in May (see intermediate hydroperiod in Fig.1). In May, organic matter and total sediment Fe concentration values experienced an important change respect to the April values, although the directionality of these changes differed among ponds (see intermediate hydroperiod ponds in Fig.1). Regarding macroinvertebrate assemblages, there was a strong significant segregation between the assemblages of April and May (one-way ANOSIM analysis, Global R=0.585, $p<0.01$). The water boatman *Corixa affinis* Leach, 1817 was the main contributing taxon (11.94%) to these differences. In April, the main contributing taxa to the macroinvertebrate assemblages were the larvae of *Gerris* spp. (19.33%), *Sympetrum fonscolombei* (Selys, 1841) (12.48%), *Cloeon* spp. (12.30%) and *Notonecta* spp. (12.09%); in May, adults of *C. affinis* (23.72%) were the main contributing taxon to macroinvertebrate assemblages.

Discussion

Seasonal environmental and macroinvertebrate variations

Doñana pond waters generally exhibited moderate nutrient concentrations in comparison to other Mediterranean temporary ponds (Della Bella *et al.* 2008; Boix *et al.* 2008; Waterkeyn *et al.* 2008). In the inundation period, low nutrient concentrations occurred in months of maximum flooding, while peaks in total water P concentrations occurred in months close to pond desiccation. Maximum flooding dilution can keep low nutrient and solute concentrations, which increase with the dehydration of ponds until their desiccation (Serrano and Toja 1995). Hence, in ephemeral ponds, high variations in water depth were followed by drastic variations in total water P concentrations. Flooding dilution affecting ion concentration was also responsible for the gradual increase in electrical conductivity during the inundation period, something often observed in temporary ponds (Serrano and Toja 1995; Hancock and Timms 2002; Culioli *et al.* 2006). However, the high electrical conductivity values observed in long hydroperiod ponds are supported more by the contribution of the regional ground-water discharges than by evaporation, as Sacks *et al.* (1992) suggested for two natural ponds of the Doñana National Park which usually held water during the summer.

Differences in environmental pond characteristics between our two study years were expected because of the much longer hydroperiod of ponds in the wet year. The high organic matter characteristic of the dry year is explained by inter-annual differences in water level; i.e. lower concentration of organisms occurred in the wet year, in which more extensive areas were inundated compared to the dry year. This water dilution in the wet year could cause the differences in organic matter between years. Despite the important difference in hydroperiod length between years, monthly variation of macroinvertebrate taxa richness of each year was similar, suggesting that most species succeeded to complete their life cycles in both study years.

Macroinvertebrate and environmental relationships throughout the wet phases

We hardly detected seasonal differences in environmental pond characteristics among the wet phases, as well as among the sampling months in the two study years, with the exception of the filling phase. In this phase, which experienced high environmental variation (e.g. initial high values of dissolved oxygen concentration and low values of electrical conductivity), environmental pond characteristics (electrical conductivity and pH in the dry year, and pH and maximum depth in the wet year) had an important effect on the structure of macroinvertebrate assemblages. Electrical conductivity and pH are strongly dependent on each other because of the high acid neutralizing capacity of alkalinity (Brönmark and Hansson 2005). Initial low pH could be due to heavy rains, which fill in ponds and produce slightly low initial pH (Jarret *et al.* 1993; Brönmark and Hansson 2005). Humic substances, which are washed from the upland vegetation into Doñana ponds during the filling phase (Serrano 1992; Serrano 1994), could have also reduced initial water pH. During the filling phase, low pH and electrical conductivity are usually important variables triggering the hatching of resting eggs for passive dispersers (Brendonck 1996; Brendonck *et al.* 2008). Hence, electrical conductivity, pH and maximum depth are considered variables which influence the establishment of pioneer colonists in temporary ponds, affecting dispersers and conditioning the emergence of resting forms (Boulton and Lake 1992). In our study, hydroperiod was a main influence for the establishment of macroinvertebrate assemblages during the filling phase, due to the strong differences in electrical conductivity and maximum depth exhibited by the ponds with different hydroperiods.

During the aquatic phase, environmental variables were less important in structuring macroinvertebrate assemblages in comparison with the filling phase. Biotic interactions such as competition and predation become stronger with the increase in hydroperiod (Schneider and Frost 1996; Wellborn *et al.* 1996; Spencer *et al.* 1999; Bilton *et al.* 2001). Thus, the structure of well-established populations after pond filling could be more affected by biotic interactions than by environmental variables during the aquatic phase. In this phase, pH, maximum depth and total sediment P and Fe concentrations appeared to influence the structure of macroinvertebrate assemblages. Except for pH, which is related to photosynthetic and respiratory activities, these variables reached high values in long hydroperiod ponds. Hence, total sediment P and Fe concentrations are co-variables of hydroperiod length (Florencio *et al.* 2009b).

During the drying phase, no variable was detected affecting the structure of pond macroinvertebrate assemblages in the dry year, when only long hydroperiod ponds held water. In contrast, in the wet year, when also ponds with intermediate hydroperiod held water, total sediment Fe concentration strongly influenced macroinvertebrate assemblage structure. As total sediment Fe concentration is indicative of hydroperiod length (see Florencio *et al.* 2009b), this variable may be simply indicating differences in the macroinvertebrate assemblages of intermediate and long hydroperiod ponds.

After the desiccation of intermediate hydroperiod ponds, we observed an increase in macroinvertebrate taxon richness in long hydroperiod ponds, which is attributed to flying adults dispersing towards more permanent ponds. We also noticed that larvae were characteristic of ponds sampled in April, while adults of *C. affinis* dominated the ponds when desiccation had started in May. Both situations indicate that most species were able to leave the pond prior to desiccation, through dispersal and drought-resistant forms, both typical macroinvertebrate strategies to survive the dry period of temporary ponds (Williams 2006). In our study, a general impoverishment of pond environmental characteristics such as water depth reduction and high concentration of nutrients and solutes took place during the drying phase. This could have determined stressful conditions for macroinvertebrate species, triggering their survival strategies to face desiccation.

Environmental variability in temporary pond characteristics regarding conservation

Temporary ponds are threatened and labile ecosystems, and the conservation of these aquatic ecosystems has received great interest in the last years (Oertli *et al.* 2005; Zacharias *et al.* 2007; Céréghino *et al.*, 2008). We showed that Doñana temporary ponds are highly heterogeneous in terms of environmental characteristics throughout the inundation period of our two study years. Given that environmental heterogeneity is known to promote the presence of diverse macroinvertebrate assemblages (Urban 2004; Jeffries 2005), our results demonstrate the high conservation value of our study temporary ponds. Environmental variables explaining macroinvertebrate assemblage structure of the study ponds throughout two inundation-desiccation cycles suggest pond hydroperiod as the main factor influencing species occurrences. Consequently, the wide hydroperiod variability in the Doñana temporary ponds importantly contributes to sustain the environmental variability of the system as a key factor to preserve the high macroinvertebrate diversity of the whole pond network.

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Colaborators:

Carmen Díaz-Paniagua, Carola Gómez-Rodríguez and Laura Serrano have colaborated in the elaboration of this manuscript which is *in preparation* for being submitted in a scientific journal.

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Cahpter 5: Relationships between macroinvertebrate assemblages and environmental characteristics in Mediterranean ponds

Resumen

Las especies de macroinvertebrados son consideradas unos de los indicadores de la calidad del agua por excelencia en diversos ecosistemas acuáticos. En este estudio se ha evaluado la variabilidad ambiental que caracteriza el sistema de lagunas de Doñana, S.O. España, y la influencia de esta variabilidad ambiental en la estructura de las comunidades de macroinvertebrados que albergan. El sistema de lagunas de Doñana se caracterizó por una fuerte variabilidad ambiental, principalmente entre las lagunas localizadas en el norte y en el sur del Parque Nacional y entre las lagunas de origen antrópico y las lagunas temporales de Doñana. Sin embargo, las comunidades de macroinvertebrados de estas lagunas sólo presentaron leves diferencias. La conductividad, y sus covariables (pH, alcalinidad y la concentración de iones) fueron las principales variables que influenciaron estas escasas diferencias en las comunidades de macroinvertebrados según su localización Norte-Sur. Las especies de coleópteros se revelaron como excelentes indicadores de la calidad del agua, sobre todo en perturbaciones que fomentan su turbidez y las altas concentraciones de nutrientes. Las variables químicas (las concentraciones de fósforo total del sedimento y fosfato inorgánico disuelto, la materia orgánica, la turbidez y la conductividad), físicas de las lagunas (el grado de aislamiento, el area y la riqueza de especies de plantas acuáticas), de distancias (a la marisma y a la costa más cercana) y las variables espaciales constribuyeron también a la estructura de las comunidades de macroinvertebrados. Los análisis de partición de varianza detectaron el efecto independiente de las siguientes variables: 1) las variables químicas, especialmente la conductividad, tuvieron un efecto significativo en las comunidades de macroinvertebrados; 2) Las variables físicas sólo afectaron a las comunidades de coleópteros; 3) las variables espaciales no tuvieron un efecto independiente en las comunidades de macroinvertebrados, lo que sugiere que no hubo limitaciones de dispersión en el área de estudio. El hecho de que especies singulares aparezcan bajo condiciones ambientales particulares en un sistema tan similar en sus comunidades de macroinvertebrados demuestra la importancia de la variabilidad ambiental para preservar una alta diversidad de macroinvertebrados en las lagunas de Doñana.

Abstract

Macroinvertebrate species have traditionally been used as excellent indicators of water quality in diverse aquatic ecosystems. We analysed environmental variability in the Doñana natural pond network, SW Spain, in order to assess its influence on structuring pond macroinvertebrate assemblages. A wide environmental variability among Doñana waterbodies was observed, mainly between ponds from the northern and southern areas of the park and between human-made and temporary ponds. However, these ponds only slightly differed in their macroinvertebrate assemblages. Conductivity and its co-variables (pH, alkalinity and ion concentrations) were the main variables influencing the small differences of macroinvertebrate assemblages between the North-South pond locations. Coleoptera species were considered the best indicator of water perturbations in terms of turbidity and high nutrient concentrations. Chemical (sediment total phosphorus and dissolved inorganic phosphate concentrations, organic matter, turbidity, conductivity), physical (pond isolation, pond area, water depth, aquatic plant species), distance (marsh and coast proximity) and spatial variables contributed to structure the pond macroinvertebrate assemblages. Variation partitioning detected the independent effects of these variables: 1) chemical variables, mainly conductivity, had a significant effect in macroinvertebrate assemblages; 2) physical variables were only significant for Coleoptera assemblages; and 3) spatial variables had non-independent effects on macroinvertebrate assemblages which suggests no dispersal limitations in the study area. As particular pond environmental characteristics promoted the occurrence of some particular species within an otherwise similar macroinvertebrate community, the high environmental variability of ponds is considered essential to preserve high macroinvertebrate diversity in Doñana ponds.

Key-words: aquatic insects, temporary ponds, invertebrate composition, spatial descriptors, conductivity, Doñana

Introduction

Diversity and abundance of macroinvertebrates can be used as indicators of environmental quality of wetlands (Trigal *et al.*, 2007; Sharma & Rawat, 2009). Because those macroinvertebrate species which have specific ecological requirements usually occur under particular environments (Tarr *et al.*, 2005; Garrido & Munila, 2008;

Gascón *et al.*, 2008), pond networks with high variability in environmental conditions support high macroinvertebrate diversity (Urban, 2004; Jeffries, 2005).

Water chemistry and physical pond characteristics have an important influence on macroinvertebrate composition and abundance in wetlands (Wissinger, 1999; Brönmark & Hansson, 2005; Williams, 2006). Salinity is one of the most frequent chemical constrictors of macroinvertebrate (Waterkeyn *et al.*, 2008), zooplankton (Frisch *et al.*, 2006) and macrophyte diversity in ponds (Della Bella *et al.*, 2008). Particularly, acidic water has negative effects on macroinvertebrate species diversity by means of changes in the resistance of species to tolerate saline conditions (Zalizniak *et al.*, 2009), in natural decomposition processes (Kok & Van der Velde, 1994) and in acid-basic balance of organisms (Radke *et al.*, 2003). Controversial results have been published about nutrient concentrations, though in general it appears that nutrients can promote macroinvertebrate species occurrences at moderate levels of concentrations (Oertli *et al.*, 2008); but when high levels of concentration are reached, they can have a negative effect in species occurrences (Declerck *et al.*, 2005). Applying the theory of island biogeography (MacArthur & Wilson, 1967) to lakes and ponds, high macroinvertebrate species richness is harboured in large ponds (Friday, 1987; Nicolet *et al.*, 2004; Studinski & Grubbs, 2007), which usually contain a high diversity of aquatic vegetation. Inter-pond distances can also affect the incidence of species in particular pond assemblages in relation to the dispersal limitations of species (Briers & Biggs, 2005; Sanderson *et al.*, 2005).

Ponds are diverse and vulnerable habitats which have been largely degraded and fragmented during recent decades with the consequent disappearance of endemic flora and fauna species (Oertli *et al.*, 2005). Temporary ponds are particularly labile and contain high species diversity; a factor that has attracted great interest in their conservation in recent years (Grillas *et al.*, 2004; Oertli *et al.*, 2005; Zacharias *et al.*, 2007; Céréghino *et al.*, 2008). Many temporary ponds spanning a wide range of hydroperiods are found in one of the most pristine wetlands in Europe, the Doñana National Park. The area of Doñana has been included in the RAMSAR convention since 1982 and was later designated as a World Heritage Site by UNESCO in 1995. In this area, environmental characteristics have previously been assessed for some of the ponds (Serrano & Toja, 1995; García-Novo *et al.*, 1991; Serrano *et al.*, 2003; Serrano *et al.*, 2006; Gómez-Rodríguez *et al.*, 2009; Espinar & Serrano, 2009) and invertebrate taxa have been listed for zooplankton (Galindo *et al.*, 1994; Fahd *et al.*, 2000; Serrano &

Fahd, 2005; Fahd *et al.*, 2009) and macroinvertebrates (Agüesse, 1962; Bigot & Marazanof, 1966; Marazanof, 1967; Montes *et al.*, 1982; Millán *et al.*, 2005; Florencio *et al.*, 2009a). Nevertheless, there are no studies describing the particular environmental characteristics which favour the presence of macroinvertebrate species in Doñana ponds. This kind of study has been carried out in other systems, providing valuable information on the ecology and conservation status of species in temporary and fluctuating ponds (Boix *et al.*, 2004; Studinski & Grubbs, 2007; Bilton *et al.*, 2009; Porst & Irvine, 2009).

Doñana National Park constitutes an excellent system to analyse the influence of environmental variables on the presence and abundance of macroinvertebrate species. In this study, we aimed to asses the environmental variability in this natural pond network and to identify any relationships with macroinvertebrate assemblages in order to detect the contribution of pond environmental variability to macroinvertebrate diversity.

Methods

Study area

We carried out a survey of 80 ponds (late March-middle June of 2007) which widely differed in pond area and water depth and were widespread across the whole area of the Doñana National Park (SW Spain). This area is located between the mouth of Guadalquivir River and The Atlantic Ocean (SW Spain). Doñana has been dated from the Quaternary age with a clear geomorphological distinction between the ancient northern area and the southern area of the park, locally named "Marismillas" that has a more recent marine origin (see Siljeström *et al.,* 1994 and García-Novo & Marín, 2006 for a detailed description of the area). There, three main types of landscape are described: A sandy area with stabilized dunes, a mobile dune system and an extensive marsh area (see Siljeström *et al.,* 1994 for a detailed geomorphological description of the area). The climate is Mediterranean sub-humid, with mild winters and hot and dry summers and heavy rains mainly falling in autumn or winter (mean annual rainfall= 544.6±211.3 mm with a wide inter-annual variability, see Díaz-Paniagua *et al.,* 2010).

This area contains a pond network with more than 3000 waterbodies in wet years (Gómez-Rodriguez, 2009) mainly composed of temporary ponds. These ponds are annually fed by rainfall and a shallow water-table that rises above the surface after heavy rainfall, in autumn or winter, and generally dry out during summer (Serrano *et al.*, 2006; Díaz-Paniagua *et al.,* 2010). Ponds are heterogeneous in area, depth and hydroperiod and are very abundant in the area of stabilized dunes and frequent in the contact areas between the three types of landscapes (Díaz-Paniagua *et al.*, 2010). In Doñana, there are only two wide permanent (or semi-permanent) ponds, which only occasionally dry out after successive years of severe drought. There are also human made ponds, (locally named as zacallones, and so hereafter referred to) which have been deepened to supply water for cattle and wild fauna during summer. They are present through the whole Park, but are the main waterbodies present in the southern areas. In the contact area between stabilized dunes and marsh there are some intermittent streams (locally named and hereafter referred to as "caños") in which water flows towards the marsh mainly during the wet season and generally form ponds after rainfall. In this study we have included 46 temporary ponds, one of the two semi-permanent ponds, 27 zacallones and 6 caños.

Sampling macroinvertebrates

We sampled the macroinvertebrate assemblages of the 80 study ponds using a dip-net (1 mm mesh size). In each sampling point of the ponds we made three successive sweeps in a stretch of water of approximately 1.5 m length. Ponds were sampled according to the number of microhabitats, in terms of depth and vegetation cover, which usually increase in extensive ponds (Heyer *et al.*, 1994). As dip-netting efficiency usually increases in small ponds (Heyer *et al.*, 1994), we typically carried out a higher number of samples in more extensive ponds with diverse microhabitats in order to achieve comparable effort in detecting rare species. Macroinvertebrates were identified *in situ* and then released again into the pond. Only unidentified individuals were preserved in 70% ethanol for later identification in the laboratory. We identified individuals to species or genus level, except for Basomatophora, Diptera, Oligochaeta and saldid bugs, which were identified to family level. For those species in which we identified larvae and adults, we considered both stages separately, and then our data were referred to as taxa-stages.

Environmental variables

In order to characterise the environmental gradients in the Doñana ponds, we measured different groups of variables.

A) Physical variables: We visually estimated in the field the proportion of water surface of each pond free of surface vegetation (Open water), terrestrial grassland plants in the vegetated margins of pond (Grassland), pond surface divided into separate pools (Pools) and reed cover grouped in root ball (Reed) and identified the taxa for the aquatic plant richness (Aq plant R). Maximum water depth was measured at the deepest point of the pond with a graduated pole (Max depth). Pond surface area (Pond area), the total number of ponds with extension > 150 m^2 (Pond number) in a 200m buffer area around each pond and the total flooded surface area (Flooded area) in a 200m buffer area around each pond were extracted from a GIS-based map of ponds obtained from a hyperspectral image taken at a moment of high inundation of the area (see Gómez-Rodríguez *et al.* 2008 for details).

b) Chemical variables: We recorded *in situ* pH (on bed using HANNA 991000), dissolved oxygen concentration ([O_2]) (on bed using YSI 550A), electrical conductivity at 20ºC (EC) (on bed using HI 9033) and turbidity (in water column using HANNA HI93703). Surface water (500 ml) was collected to determine nutrient concentrations (dissolved inorganic phosphate (i-P), nitrate, nitrite and ammonium), alkalinity and main cation and anion concentrations (Cl^-, Na^+, Ca^{2+}, K^+, Mg^{2+} and SO_4^{2-}). Ion concentrations were analysed using an Inductively Coupled Plasma Mass Spectrophotometer (ICP), while nutrient concentrations were measured colorimetrically using an Auto Analyzer (Bran + Luebbe). Alkalinity was analysed according to the titration method described in APHA (1998). Surface sediment samples (5 cm depth) were collected and the following variables measured in the laboratory: organic matter (O.M.) in three replicates (lost on ignition, 450 ºC, 5 h), sediment total P (TP-s) in two replicates as dissolved inorganic phosphate following the method of Murphy & Riley (1962) after acid digestion of the ignited sediment with 0.5 M H_2SO_4 and $K_2S_2O_8$ (0.5–1 g) at 120 ºC for 4h (Golterman, 2004). Total Fe concentration in the sediment (TFe-s) was determined colorimetrically, in two replicates after digestion, by means of o-phenantroline using ascorbic acid as reducing agent (Golterman, 2004). The Na^+/Ca^{2+} ratio was considered for its biological implications in regulating processes associated with the acid-base balance of the organisms (Radke *et al.*, 2003). We did not use nitrite and nitrate concentration in the analyses because most values were negligible.

C) Distance variables: Minimum linear distances from each pond to the border of the marsh and to the coast were also estimated using the GIS pond map.

D) Spatial variables: spatial descriptors based on inter-pond distances were generated through Moran's eigenvector maps (MEMs) (spacemakeR package, R software, Dray *et al.*, 2006), a general framework of principal coordinates of neighbour matrices (PCNM, see Borcard & Legendre, 2002). Selection procedure of spatial descriptors for controlling for Type I error in the analyses was carried out according to

Peres-Neto & Legendre (2010). Spatial descriptors were used only in two analyses described below (RDA analyses and variation partitioning) in order to evaluate the effect of inter-pond distances on the structure of macroinvertebrate assemblages.

Statistical analyses

We constructed an **environmental matrix** with the values of the physical, chemical, and distance variables. Additionally, each group of variables (physical, chemical, distance and spatial) was considered in individual environmental matrices. Each variable was previously independently transformed to approximate normality (see Table 1 for transformations). The environmental matrix was standardized (see Clarke & Warwick, 2001) and Euclidean distance was applied in order to obtain the environmental resemblance matrix. We constructed an **aquatic plant matrix** (a resemblance matrix) by applying the Sorensen index (Legendre & Legendre, 1998) on the presence-absence data of the aquatic plant taxa per pond. Finally, we also constructed a macroinvertebrate matrix including the number of samples with presence of every taxa-stage divided by the total number of samples taken in a pond. Bray-Curtis index (Clarke & Warwick, 2001) was applied on the macroinvertebrate matrix in order to obtain the macroinvertebrate resemblance matrix. Subsets of the macroinvertebrate matrix were extracted for the main taxonomical orders Coleoptera, Heteroptera and Odonata.

We analysed the differences in pond environmental characteristics (using the environmental resemblance matrix) and macroinvertebrate assemblage compositions (using the macroinvertebrate resemblance matrix) between temporary ponds and zacallones and, independently, between ponds located in southern areas (in “Marismillas”) and northern areas of the park with one-way ANOSIM analyses on the environmental and macroinvertebrate matrices (Primer v.6, 9999 permutations, Clarke & Warwick, 2001). In ANOSIM analyses R is close to one when the levels of grouping factor are completely different. Due to the limited sample size, the caños (n=6) and the semi-permanent pond were not included in the ANOSIM analyses. Non-metric multidimensional scaling (NMDS) representations (Clarke & Warwick, 2001) were performed in order to illustrate the differences among ponds in environmental characteristics and also in macroinvertebrate assemblage compositions.

We analysed how closely the patterns among ponds in terms of aquatic plant composition were mirrored by the patterns in macroinvertebrate composition (using the aquatic plant matrix and the macroinvertebrate resemblance matrix) through a RELATE analysis (Primer v.6, 9999 permutations, Clarke & Warwick, 2001). In RELATE analysis, Spearman correlation coefficient (ρ) measures the degree of agreement between two resemblance matrices ranking 0-1 which only became 1 in a perfect matching pattern.

In order to detect which environmental variables influenced the macroinvertebrate assemblage structure of ponds, we performed **a constrain redundancy analysis** (RDA) using each of the physical, chemical, distance and spatial variables matrices and the macroinvertebrate matrix and, independently, the Coleoptera, Odonata and Heteroptera matrices. Only taxa-stages which occurred in more than five ponds were included in RDA analyses in order to avoid the disrupting effect of rare species (Leps & Smilauer, 2003). In order to exclude those co-variables with poor explanatory power in RDA analyses, we performed Spearman rank correlations between each pair of environmental variables. We used a stepwise forward procedure to select the environmental variables and Monte Carlo test to assess their significance (999 permutations) in CANOCO software v. 4.5. **Variation partitioning** was performed in order to measure the independent effect of chemical, physical, distance and spatial variables (see Borcard *et al.*, 1992); only including those significant explanatory variables extracted after RDA analyses. In variation partitioning, we used an adjustment of the multiple coefficient of determination (Adj.R^2) required in matrices with different number of variables (Peres-Neto *et al.*, 2006). Variation partitioning was performed using ‘varpart’ command and significance was tested after Monte Carlo permutation tests (R software, ‘vegan’ package, Oksanen *et al.*, 2008).

We analysed if significant explanatory variables after RDA analyses constrained particular groups of macroinvertebrate assemblages. LINKTREE analysis (Primer v.6, Clarke & Warwick, 2001), a framework of multivariate regression tree (De’ath, 2002), was applied on the macroinvertebrate pseudo-quantitative matrix. SIMPROF analyses extracted only significant divisions at 0.05 levels constructing a dendrogram named linkage tree (Clarke *et al.*, 2008). Pair-wise differences between each group of macroinvertebrate assemblages detected in the linkage tree were assessed through one-way ANOSIM analysis. We explored the main contributing taxa-stages to these pair-wise differences (>10% of contribution) through one-way SIMPER analysis (Primer v.6, Clarke & Warwick, 2001).

Results

Variability in pond environmental characteristics and macroinvertebrate assemblages

The ordination of ponds according to environmental variables is shown in a NMDS representation (Fig.1). A significant but weak segregation between temporary ponds and zacallones was detected (one-way ANOSIM analysis, R= 0.309, p<0.001). The high dissimilarity observed among temporary ponds (Fig.1) evidences their high environmental variability in comparison with zacallones.

Fig.1: NMDS ordination of the 80 study ponds according to Euclidean distance of the chemical, physical and distance variables. Temporary ponds (temp), zacallones (z), caños (caño) and the semi-permanent pond (semip) are highlighted as well as the location of ponds in southern (South) and northern (North) areas of the park.

Temporary ponds presented a wide variability in physical variables showing high proportion of Grassland and general shallow waters (mean of maximum depth=60 cm, Table 1). Water chemistry was characterized by a circumneutral pH, general low EC values (<1 mS cm^{-1}) and low alkalinity values and ion concentrations (Table 1). Zacallones were small ponds with deep waters (mean of maximum depth=100 cm) and high proportion of Open water (Table 1). They presented the highest values of EC, alkalinity, ion concentration and pH but low values of nutrients in the water column (NH_4^+ and i-P) and in sediment (O.M. and TP-s, Table 1). Although caños presented high turbidity, i-P and TP-s concentrations, they exhibited similar variability in the physical and chemical variables to temporary ponds (Table 1). The semi-permanent pond was the biggest and deepest waterbody in the study and exhibited the highest TFe-s concentration and Aq plant R (Table 1). This pond presented similar water and sediment nutrient concentrations and O.M. to zacallones whilst physical variables, alkalinity and ion concentrations were more similar to temporary ponds (Table 1).

Table 1: Mean and standard deviation (SD) is shown for the environmental characteristics of sampled ponds: temporary ponds (Temporary), human-made ponds (Zacallones), caños and the semi-permanent ponds (Semip) (see methods for a detailed description of study ponds; N= number of ponds). Chemical variables with negligible values are indicated as negligible variables. Those correlated variables with positive (+) and negative (-) significant Spearman coefficient higher than 0.6 (all $p<0.01$) are shown. Independent transformations to approximate normality are also shown (Sqr is square root transformation; Log is Log(X+1) transformation; _ is no transformation required; Alk is alkalinity; Marsh and Coast are distances of ponds to the marsh and to the coast).

	Temporary (N=46)		**Zacallones (N=27)**		**Semip**	**Caños (N=6)**		**Transf.**	**Correlations >0.6**
Physical variables	**Mean**	**SD**	**Mean**	**SD**	**(N=1)**	**Mean**	**SD**		
Open water (%)	43.6	31.9	92.8	14.8	65	54.2	29.4	_	Grassland (-), Reed (-), Pond area (-)
Grassland (%)	33.4	33.1	4.8	11.4	15	29.2	36	_	Pond area (+), Open water (-)
Pools (%)	3.7	10	0.9	3.4	10	0.8	2	Sqr	
Reed (%)	19.3	15.5	1.9	5	10	15.8	15.3	_	Open water (-)
Aq plant R	7.4	3.7	3.9	3	14	6.2	3.5	_	Pond area (+)
Max depth (cm)	59.8	31.8	103.8	13.2	118	52.2	34.2	_	
Pond area (m^2)	3,390	5,586	119	329	122,672	538	408	Log	Aq plant R (+), Open water (-), Grassland (+)
Pond number	5.1	4	3.1	3.4	11	2.8	2.2	_	Flooded area (+)
Flooded area (m^2)	3,814	6,268	1,649	3,397	63,282	1,227	1,993	Log	Pond number (+)
Chemical variables	**Mean**	**SD**	**Mean**	**SD**	**(N=1)**	**Mean**	**SD**		
NH_4^+(mg l^{-1})	0.17	0.54	0.12	0.15	0.05	0.14	0.12	Log	
i-P (mg l^{-1})	0.11	0.13	0.08	0.1	0.01	0.21	0.28	Log	
TP-s (ug g^{-1} d.w.)	258.36	314.59	105.29	121.8	162.56	328.4	333.52	_	TFe-s (+), O.M. (+)
TFe-s (mg g^{-1} d.w.)	3.3	3.18	2.36	2.61	7.61	4.57	4.41	_	TP-s (+), O.M. (+)
[O_2] (mg l^{-1})	2	1.8	2.5	3.6	2.8	3.6	3.1	Log	
pH	6.5	0.7	7.7	1.1	6.9	6.7	0.6	Log	EC (+), Alk (+), Na^+ (+), Ca^{2+} (+), K^+ (+), Mg^{2+} (+)

EC (µS cm^{-1})	670.8	1,270.60	1,304.70	1,226.40	1,224	479.2	356.3	Log	pH (+), Alk (+), Cl^{-1} (+), Na^+ (+), Ca^{2+} (+), K^+ (+), Mg^{2+} (+), coast (-)
Alk (meq l^{-1})	1.99	2.72	4.96	4.22	1.47	1.57	0.82	Log	pH (+), EC (+), Cl^{-1} (+), Na^+ (+), Ca^{2+} (+), K^+ (+), Mg^{2+} (+)
Turbidity (NTU)	27	51	17	18	3	192	385	Log	
O.M. (%)	7.02	6.6	2.63	3.49	3.93	6.08	6	_	TP-s (+) and TFe-s (+)
Cl^{-1} (meq l^{-1})	4.2	7.81	8.65	11.83	4.24	3.79	2.91	Log	EC (+), Alk (+), Na^+ (+), Ca^{2+} (+), K^+ (+), Mg^{2+} (+)
Na^+ (meq l^{-1})	3.88	6.47	7.28	7.87	4.3	3.19	2.41	Log	EC (+), Alk (+), Cl^{-1} (+), Ca^{2+} (+), K^+ (+), Mg^{2+} (+)
Ca^{2+} (meq l^{-1})	0.85	1.15	2.19	2.41	0.59	0.67	0.47	Log	EC (+), Alk (+), pH (+), Cl^{-1} (+), Na^+ (+), K^+ (+), Mg^{2+} (+)
K^+ (meq l^{-1})	0.16	0.32	0.33	0.38	0.03	0.1	0.06	Log	EC (+), Alk (+), pH (+), Cl^{-1} (+), Na^+ (+), Ca^{2+} (+), Mg^{2+} (+)
Mg^{2+} (meq l^{-1})	0.92	1.34	3.34	3.86	1.05	0.71	0.63	Log	EC (+), Alk (+), pH (+), Cl^{-1} (+), Na^+ (+), Ca^{2+} (+), K^+ (+), Mg^{2+} (+)
SO_4^{2-} (meq l^{-1})	0.5	0.67	1.44	4.41	0.29	0.7	0.25	Log	
Na^+/Ca^{2+} ratio	14.49	58.08	4.92	4.8	7.24	4.93	1.24	Log	
Distance variables	**Mean**	**SD**	**Mean**	**SD**	**(N=1)**	**Mean**	**SD**		
Marsh (m)	2,293	1,798	2,180	1,450	3,519	234	410	_	
Coast (m)	5,097	2,529	3,085	2,099	2,168	9,523	2,806		EC (-)
Negligible variables	**Range**		**Range**		**(N=1)**	**Range**		_	
NO_3^- (mg l^{-1})	<0.15-0.38		<0.15-0.38		0.18	<0.15-0.60		_	_
NO_2^- (mg l^{-1})	<0.15		<0.15		< 0.15	<0.15		_	_

Considering distance variables, temporary ponds were sparsely distributed in different areas of the park except in southern areas; caños were close to the nearby marsh and far from the coast; and zacallones in our study were on average located close to the coast, in southern areas of the park (Table 1). We detected a significant segregation between the southern and the northern ponds in the National Park based on their environmental characteristics (Fig.1; one-way ANOSIM analysis, $R=0.458$, $p<0.0001$).

In contrast to the ordination based on environmental characteristics, the NMDS representation of macroinvertebrates assemblages evidenced high similarities among ponds (Fig.2). Macroinvertebrate assemblages hardly differed between temporary ponds and zacallones (one-way ANOSIM analysis, $R=0.106$, $p<0.01$). In contrast to the marked differences in environmental variables detected between southern and northern ponds, macroinvertebrate assemblages presented a significant but weak segregation between those pond locations (Fig.2; one-way ANOSIM analysis, $R=0.148$, $p<0.05$).

Fig.2: NMDS ordination of the 80 study ponds according to Bray-Curtis similarities of the macroinvertebrate assemblages. Temporary ponds (temp), zacallones (z), caños (caño) and the semi-permanent pond (semip) are highlighted as well as the location of ponds in southern (South) and northern (North) areas of the park.

We detected a significant agreement between the pond macroinvertebrate assemblages and the aquatic plant communities (RELATE analysis, $\rho=0.261$, $p<0.0001$).

Pond environmental variables influencing macroinvertebrate assemblages

The study ponds presented an average of 23.5 taxa-stages per pond (SD=8.5). The significant explanatory variables influencing pond macroinvertebrate compositions detected in RDA analyses were: EC among the chemical variables (F-ratio=4.91, $p<0.001$; Table 2); Aq plant R among physical variables (F-ratio=2.31, $p<0.05$; Table

2); both the distance of ponds to the marsh and to the coast (F-ratio=2.56, $p<0.05$; Table 2); and two spatial descriptors related to inter-pond distances (F-ratio=2.3, $p<0.05$, Table 2). EC presented the highest effect (lambda, Table 2) on the macroinvertebrate community, although the distance variables and the spatial variables were also important (Table 2).

Table 2: Significant explanatory variables obtained after forward selection in redundancy analyses (RDA) which were performed separately for chemical, physical, distance variables and spatial descriptors. RDA analyses were performed using the macroinvertebrate matrix (community), and also considering the Coleoptera, Odonata and Heteroptera matrices independently. Lambda shows the magnitude of the effects of each significant explanatory variable on the macroinvertebrate assemblages (V1 and V2 are eigenvectors extracted from the inter-pond distance based on the Moran's eigenvector map; *=$p<0.05$; **=$p<0.01$; n.s. non significant variables).

Variables/ Lambda	**Community**	**Coleoptera**	**Odonata**	**Heteroptera**
Chemical variables				
EC	0.06**	0.06**	n.s.	0.09**
Turbidity	n.s.	0.02*	n.s.	n.s.
i-P	n.s.	0.03*	n.s.	n.s.
O.M.	n.s.	n.s.	0.06*	n.s.
Physical variables				
Aq plant R	0.03*	n.s.	n.s.	0.04*
Pond area	n.s.	0.05**	n.s.	n.s.
Max depth	n.s.	0.03**	n.s.	n.s.
Pond number	n.s.	n.s.	n.s.	0.03*
Distance variables				
Marsh	0.04**	0.04**	0.06*	n.s.
Coast	0.03*	0.03**	n.s.	n.s.
Spatial descriptors				
V1	0.03*	0.02*	n.s.	n.s.
V2	0.03*	n.s.	n.s.	n.s.

Coleopterans averaged 10.8 taxa-stages per pond (SD=5.2). For this order, we detected that the four groups of environmental variables had important effects on the structure of Coleoptera assemblages. EC, turbidity and i-P were the significant explanatory chemical variables (F-ratio=2.16, p<0.05, Table 2); among the physical variables, pond area and Max depth were significant (F-ratio=2.46, p<0.01, Table 2); both distance variables, the distance of ponds to the marsh and to the coast, were significant (F-ratio=2.69, p<0.01, Table 2); and one spatial descriptor had a significant effect among the spatial variables (F-ratio=1.89, p<0.05, Table 2). On the contrary, the structure of Odonata assemblages (average= 2.4 taxa per pond, SD=2.1) was only significantly explained by the chemical variable O.M. (F-ratio= 3.6, p<0.05, Table2) and distance of ponds to the marsh (F-ratio=3.64, p<0.05, Table 2; rest of variables all p> 0.14). For Heteroptera assemblages (average= 7.1 taxa-stages per pond, SD=2.8), EC was the only significant chemical explanatory variable (F-ratio=7.2, p<0.01, Table 2) whilst Aq plant R and Pond number were the significant explanatory variables in the physical matrix (F-ratio=2.88, p<0.05, Table 2; rest of variables all p>0.10).

Variation partitioning

Variation partitioning analyses revealed that only chemical variables (EC) presented a significant independent effect on the structure of macroinvertebrate assemblages (Table 3). Chemical (EC, turbidity and i-P) and physical (Aq plant R) variables presented significant independent effects on the structure of Coleoptera assemblages; only chemical variables (EC) had a significant independent effect on the structure of Heteroptera assemblages; and there were not any explanatory variables with a significant independent effect on the structure of Odonata assemblages (Table 3).

Table 3: Variation partitioning performed among the significant explanatory physical, chemical, distance and spatial variables. Independent effects of these variables on the structure of macroinvertebrate assemblages (community) and independently on the Coleoptera, Odonata and Heteroptera assemblages are indicated with the adjusted square R (Adj.R^2; 0-1). Significance was calculated after Monte Carlo permutations (199 permutations, *=p<0.05, **=p<0.01).

Adj.R^2	**Community**	**Coleoptera**	**Odonata**	**Heteroptera**
Chemical variables	0.017*	0.030**	0.023	0.039**
Physical variables	-0.002	0.021**	_	0.011
Distance variables	-0.006	0.001	0.023	_
Spatial descriptors	0.011	0.007	_	_

Pond macroinvertebrate assemblages under specialized environments

Despite the high similarity detected among the macroinvertebrate assemblages of ponds throughout the park, some environmental variables favoured the occurrence of particular species in particular groups of ponds (Fig.3, Table 4). These groups had a differential contribution to the global dissimilarity of the whole macroinvertebrate community (Fig.3). Fourteen taxa-stages mainly contributed to the pond assemblage differences according to particular environmental thresholds (Table 4): four generalist taxa-stages occurred under multiple environments (adults of *Corixa affinis* Leach, 1817, adults of *Anisops sardeus* Herrich-Schäffer, 1849, larvae of *Cloeon* Leach, 1815 spp. and larvae of Notonectidae), *Sympetrum fonscolombei* (Selys, 1841) occurred in three different environments whilst the other ten taxa-stages mainly occurred under particular environmental conditions (Table 4). Adults of *Anacaena lutescens* (Stephens, 1829), larvae of *Chironomus plumosus* (Linneo, 1758) and adults of *Hydrobius fuscipes* (Linnaeus, 1758) and *Limnoxenus niger* (Zschach, 1788) were important in ponds with EC lower than 222 µS cm^{-1} and closer than 6220m to the coast (A-C splits in Fig.3, Table 4). Adults of *Gerris thoracicus* Schummel, 1832 and larvae of Corixidae mainly occurred in ponds located less than 1600m from the marsh, less than 6220m from the coast and in ponds with more than three Aq plant R (A-E splits in Fig.3, Table 4). Adults of *Helophorus* Fabricius, 1775 spp., adults of *Hygrotus lagari* (Fery, 1992) and *Physa* spp. occurred in ponds located adjacent the marsh and more than 6250m from the coast (A-K splits in Fig.3, Table 4). The first split in the linkage tree (A split in

Fig.3) contributed to 65% of the global dissimilarity of the whole macroinvertebrate community and segregated macroinvertebrate assemblages of ponds with high EC (>3915 µS cm^{-1}, Table 4). On the contrary, ponds with the lowest contribution to the global dissimilarity (A-F splits in Fig.3, global dissimilarity=29%) presented values of EC lower than 830 µS cm^{-1} and were located more than 1610m from the marsh and less than 6220m from the coast; adults of *Sigara lateralis* (Leach, 1817) was the main contributing taxa (Table 4).

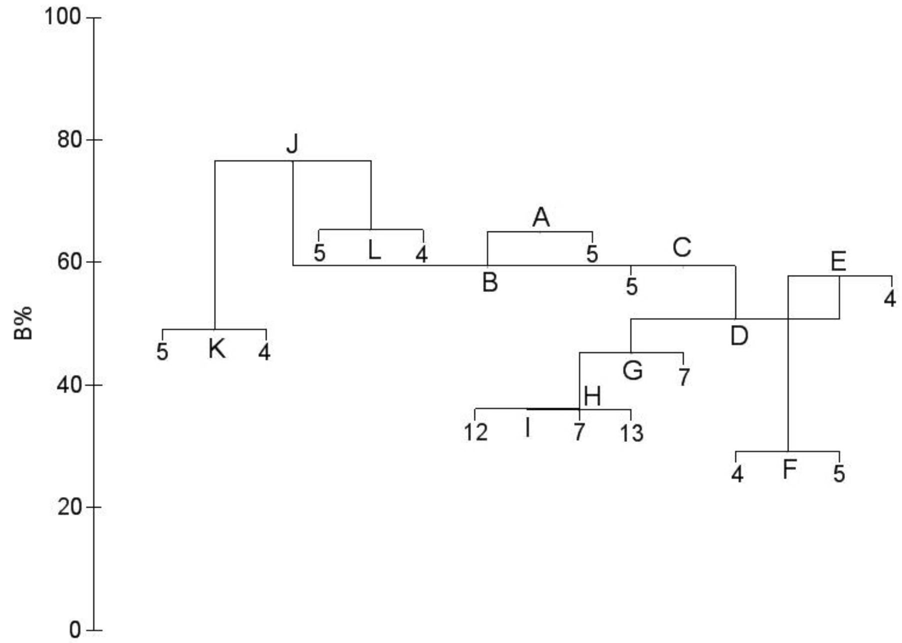

Fig. 3: Linkage tree representation showing significant divisive clustering of macroinvertebrate assemblages (ponds) constrained by particular environmental variables (see Table 4). Each successive split is conditioned by the environmental thresholds of previous splits. In the dendrogram, capital letters indicate each split and numbers indicate the number of ponds included in each split. B% is the contribution of each binary partition to the global dissimilarity (ranged 0-100%).

Table 4: Main taxa-stages contributions (>10% of contribution) and environmental thresholds which determined the successive splits observed in Fig.3 (each letter is corresponded with each split in Fig.3). The first inequality defines a group to the left in the linkage tree, second inequality groups to the right (see Fig.3). R is the Spearman coefficient giving the dissimilarity value in every split (-A: adults; -L: larvae; _: taxa-stages contribution <10%; d coast: distance of the ponds to the coast; d marsh: distance of the ponds to the marsh).

Groups	**Spearman R**	**Variable**	**Groups to the left**	**Groups to the right**
A:	R=0.25	EC (µS cm^{-1})	<2000	>3915
			Corixa affinis-A (13.9%)	*Corixa affinis*-A (20.9%)
			Notonectidae-L (10.9%)	*Anisops sardeus*-A (11.2%)
			_	*Cloeon* spp.-L (10.7%)
B:	R=0.24	d coast (m)	>6250	<6220
			Corixa affinis-A (14.4%)	*Corixa affinis*-A (13.5%)
			Anisops sardeus-A (11.5%)	Notonectidae-L (11.4%)
			Cloeon spp.-L (11.1%)	_
C:	R=0.34	EC (µS cm^{-1})	<222	>226
			Anacaena lutescens-A (16.4%)	Corixa affinis-A (13.6%)
			Anisops sardeus-A (13.3%)	Notonectidae-L (11.9%)
			Chironomus plumosus-L (12.1%)	_
			Hydrobius fuscipes & Limnoxenus niger-A (10.7%)	_
D:	R=0.26	R Aq plants	<2	>3
			Corixa affinis-A (22.7%)	*Corixa affinis*-A (13.6%)
			Anisops sardeus-A (13.9%)	Notonectidae-L (11.9%)
E:	R=0.44	d marsh (m)	>1610	<1600
			Corixa affinis-A (24.3%)	*Gerris thoracicus*-A (21.6%)
			Anisops sardeus-A (14.4%)	Corixidae-L (17.6%)
			_	*Corixa affinis*-A (12.4%)
F:	R=0.51	EC	<830	>880
			Corixa affinis-A (24.0%)	*Corixa affinis*-A (19.4%)
			Anisops sardeus-A (22.9%)	Notonectidae-L (11.1%)
			Sigara lateralis-A (13.9%)	_
G:	R=0.3	EC	>1163	<1144
			Corixa affinis-A (14.6%)	Notonectidae-L (13.5%)
			Notonectidae-L (13.0%)	
H:	R=0.25	d marsh (m)	>1460	<1340
			Notonectidae-L (12.0%)	Notonectidae-L (12.6%)
			Cloeon spp.-L (12.6%)	*Corixa affinis*-A (10.0%)
I:	R=0.36	d marsh (m)	<3760	>4080
			Corixa affinis-A (13.8%)	*Cloeon* spp.-L (15.6%)
			Notonectidae-L (12.8%)	Notonectidae-L (12.2%)
			Cloeon spp.-L (12.2%)	_
J:	R=0.47	d marsh (m)	<788	>1020
			Corixa affinis-A (27.3%)	*Cloeon* spp.-L (26.2%)
			_	*Sympetrum fonscolombei*-L (18.4%)
			_	Notonectidae-L (12.7%)

K:	R=0.33	d marsh (m)	=0	>0
			Corixa affinis-A (18.0%)	*Corixa affinis*-A (30.9%)
			Helophorus spp.-A (12.9%)	*Anisops sardeus*-A (13.3%)
			Hygrotus lagari-A (12.5%)	_
			Physa spp. (11.1%)	_
L:	R=0.26	EC (µS cm^{-1})	<208	>213
			Anisops sardeus-A (18.8%)	*Cloeon* spp.-L (40.7%)
			Cloeon spp-L (17.1%)	*Sympetrum fonscolombei*-L (27.4%)
			Notonectidae-L (13.7%)	_
			Sympetrum fonscolombei-L (12.0%)	_

Discussion:

Environmental variability

The dissimilarity detected among the environmental variables of temporary ponds, zacallones, caños and the semi-permanent pond shows Doñana with a heterogeneous pond network. Temporary ponds showed the highest environmental heterogeneity whilst the other waterbodies additionally increased the total heterogeneity of aquatic habitats of the area. Particularly, human made ponds (zacallones) extended the availability of aquatic habitats in summer, without which the area would contain only a few semi-permanent ponds as available habitat during these months. However, the environmental characteristics assessed in this study are not representative of the general conditions of ponds due to the high inter-annual and seasonal variability in the area, as described in previous studies which included a smaller fraction of the Doñana ponds (Serrano & Toja, 1995; Serrano *et al.*, 2006; Gómez-Rodríguez *et al.*, 2009; Gómez-Rodríguez *et al.* 2010).

In this study area, we observed important differences in the environmental characteristics of temporary ponds and zacallones. Zacallones are ponds with long hydroperiods and small surface areas; this is in contrast to the general pattern described for natural ponds in which their hydroperiod increases with increasing surface area (see Tarr *et al.*, 2005; Waterkeyn *et al.,* 2008; Vanschoenwinkel *et al.*, 2009). As pond area and aquatic plant richness were co-variables in our study, higher numbers of plant species occur in extensive and shallow waters but not in more permanent ponds.

Differences in the environmental characteristics of southern and northern ponds were even greater than those differences detected between zacallones and temporary ponds. The main difference between the northern and southern areas of the park is the sand richness in $CaCO_3$. The southern area is higher in $CaCO_3$ as a consequence of its more recent marine origin (Siljeström *et al.*, 1994). This distinct mineralogical composition of the southern ponds due to the larger contribution of Ca^{2+} to the sand composition explains the lower Na^+/Ca^{2+} ratios. In addition, all southern study ponds were zacallones except two temporary ponds and consequently exhibited typical environmental characteristics of zacallones: low proportion of vegetation in the margins of ponds and on the water surfaces, small surface areas, high electrical conductivities, alkalinities and ion concentrations. As electrical conductivity and distance of the ponds to the coast were co-variables, electrical conductivity values increased from north to south, with the southern pond, located closest to the coast, reaching the highest value.

Macroinvertebrate assemblage variability

Despite the high heterogeneity in pond environmental characteristics, the differences between the macroinvertebrate assemblages of zacallones and temporary ponds and either of southern and northern areas of the park were not strong. In comparison to the high number of taxa-stages collected per pond, only four species were exclusively observed in southern ponds: *Hygrotus inaequalis* Fabricius, 1777, *Yola bicarinata* (Latreille, 1804), *Limnebius furcatus* Baudi, 1872 and *Potamopyrgus antipodarum* (Smith, 1889); and two species were more abundant in southern than in northern ponds: *Micronecta scholzi* (Fieber, 1860) and the exotic species *Trichocorixa verticalis* (Fieber, 1851) (see Rodríguez-Pérez *et al.*, 2009). The generally good dispersal ability of the typical species associated with temporary waters such as Heteroptera, Coleoptera, Odonata, Ephemeroptera and Diptera (Wiggins *et al.*, 1980; Bilton *et al.*, 2001; Williams, 2006) favours their wide distribution throughout the study area and explains the homogeneity detected among pond macroinvertebrate compositions.

Relationships between macroinvertebrate assemblages and pond characteristics

Macroinvertebrate and aquatic plant assemblages presented a matching pattern with a certain degree of association between their pond characteristics; this is concordant with other studies carried out in temporary and fluctuating ponds (Bilton *et al.*, 2009). Moreover, aquatic plant richness and the correlated variable pond area were important explanatory variables of the structure of the macroinvertebrate community and strongest for the Coleoptera assemblages. This close relationship between aquatic plant species and macroinvertebrate taxa is not surprising because local plant species composition is considered the best predictor for arthropod assemblages (Schaffers *et al.*, 2008). Environmental conditions influencing local plant compositions can also influence macroinvertebrate compositions, but also each plant species' architecture affects biological processes such as predator-prey interactions, presence of oviposition sites and microclimatic conditions (Schaffers *et al.* 2008). Hence, aquatic vegetation through its quality and quantity of vegetation cover, species richness and biomass has frequently been detected as an important explanatory variable for macroinvertebrate assemblages of temporary ponds (Della Bella *et al.*, 2005; Nicolet *et al.*, 2004; Bilton *et al.*, 2009).

Electrical conductivity was revealed as the main environmental variable determining macroinvertebrate (Coleoptera and Heteroptera) assemblages; although Doñana ponds did not exhibit extremely saline waters (82-8,800 $\mu S\ cm^{-1}$). Aquatic invertebrate species are well-adapted to low salinity concentrations through simple osmoregulation whilst only a few species can survive in hypersaline waters (Herbst, 2001). Thereby, salinity has been detected in several studies to have an important effect on macroinvertebrate diversity (e.g. Velasco *et al.*, 2006; Garrido & Munilla, 2008; Waterkeyn *et al.*, 2008). In particular, salinity has already been proved to be an important variable influencing the presence and abundance of corixid species (Van De Meutter *et al.*, 2010). Co-variables of electrical conductivity (i.e. alkalinity, pH and ion concentrations) may also have important influences on the structure of macroinvertebrate assemblages, although their particular effects have not been analysed in this study.

In Doñana National Park, distance variables and spatial descriptors are important influences on the macroinvertebrate assemblage structures; however, the north to south electrical conductivity gradient is suggested as the factor with the greatest effect on macroinvertebrate compositions. When we considered electrical conductivity and spatial descriptors independently in variation partitioning analyses only electrical conductivity significantly affected the assemblage structures. The importance of the distance of ponds to the marsh reveals that Doñana marshes can act as a source of invasive predator species such as the red-swamp crayfish *Procambarus clarkii* (Girard, 1852) and *Gambusia holbrooki* (Girard, 1859) as well as other non-native fish species that can influence the structure of the native aquatic communities (Fahd *et al.*, 2007).

To explain the structure of Coleoptera assemblages, we needed a larger number of environmental variables than for the whole of the macroinvertebrate assemblages. Adult Coleoptera are frequently used as surrogates for macroinvertebrate assemblages in different aquatic systems (Sánchez-Fernández *et al.*, 2004; 2006; Picazo *et al.*, 2010).

In our study adults, but also larvae, of Coleoptera taxa were distributed under more special conditions than the whole macroinvertebrate assemblages. This suggests Coleoptera species are a better indicator of pond water perturbations than the whole macroinvertebrate community, which included other more tolerant species. As turbidity and dissolved inorganic phosphate concentration were important variables influencing the structure of Coleoptera assemblages, Coleoptera species of the Doñana ponds are suggested as good indicators of turbid waters and eutrophication. These variables could negatively affect the coleopteran species occurrences because 1) turbidity has been assessed as an important constrictor of macroinvertebrate density (Stewart & Downing, 2008; Sharma & Rawat, 2009) resulting in differences between macroinvertebrate assemblages in turbid and clear waters (Van De Meutter *et al.*, 2005; Waterkeyn *et al.*, 2009); and 2) high nutrient concentrations, particularly phosphorus and nitrogen, typically constrain macroinvertebrate species richness (Jeffries, 1991; Batzer & Wissinger, 1996; Biggs *et al.*, 2005; Menetrey *et al.*, 2005). Heteroptera and Odonata assemblages of the Doñana ponds were not constrained by environmental variables. The lack of spatial effects on these assemblages reveals that most Heteroptera and Odonata species are not dispersal limited, and may cope with adverse environmental conditions through their good dispersal ability. In this study, electrical conductivity was an important influence on the Heteroptera assemblage structures. The high electrical conductivity values of zacallones seems to be related to the presence of some Heteroptera species, mainly Notonectidae and Corixidae species, which are abundant in Doñana ponds in summer (Florencio *et al.*, 2009a). Given that aquatic bug species can develop various generations per year in warm latitudes (Jansson, 2005), the widespread location of zacallones can favour the reproduction of these species in the study area. Pond Odonata assemblages were only constrained by organic matter and the distance of ponds to the marsh, both with a weak effect. Organic matter is directly related to water permanence (Rubio *et al.*, 1995; Florencio *et al.*, 2009b) which is an important factor determining larval development for Odonata species (De Block *et al.*, 2005). However, its weak effect on Odonata assemblages may be because damselfly and dragonfly species are well adapted to temporary habitats e.g. *Lestes* and *Ischnura* species can survive desiccation of the ponds as drought-resistant eggs and larvae (Grillas *et al.*, 2004; Williams, 2006; Hassall & Thompson, 2008).

Despite the high similarity of the pond macroinvertebrate assemblages in the Doñana National Park, we detected that some environmental variables conditioned the occurrence of particular species, thus contributing to the diversity of the system. Among the 14 taxa and life history stages which particularly occurred in these assemblages, four taxa-stages occurred in multiple environments (*C. affinis*; *A. sardeus,* larvae of Notonectidae and larvae of *Cloeon* spp.) whilst the other 10 taxa-stages mainly occurred under particular pond characteristics (*A. lutescens* adults, *H. fuscipes* and *L. niger* adults, *G. thoracicus* adults, *S. lateralis* adults, larvae of Corixidae, *H. lagari* adults, *C. plumosus* larvae, *Helophorus* spp. adults, *Physa* spp, and larvae of *S. fonscolombei*). These ten taxa-stages were listed among the 59 taxa-stages that departed from the general nested pattern described for the Doñana pond network (after Florencio *et al.*, *submitted*). Although *S. fonscolombei* occurred in different environments this species was strongly constrained by the pond location (i.e. only occurred in the North of the park) and by EC (i.e. only occurred in waters with a value lower than 2 mS cm^{-1}). All these species, except the water boatman *S. lateralis*, significantly contributed to the global dissimilarity of the macroinvertebrate community and confirmed that species which depart from the general nested pattern are distributed under particular conditions throughout the Doñana pond network. As the conservation of these species receives greater interest (McAbendroth *et al.*, 2005; Heino *et al.*, 2009), the characterization of their particular environmental requirements provide good guidelines for conservation in a highly nested system. Consequently, the environmental variability described for Doñana ponds is a key factor in preserving high macroinvertebrate diversity as the occurrence of species with specific requirements is favoured in heterogeneous pond networks with diverse availability of environments.

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Chapter 6: Spatio-temporal nested patterns in macroinvertebrate assemblages across a pond network with a wide hydroperiod range

Resumen

El anidamiento ha sido ampliamente utilizado como una medida de la estructura de las comunidades biológicas que se produce cuando los sitios pobres en especies constituyen subconjuntos de los sitios con mayor riqueza específica. En este estudio se ha examinado el patrón de anidamiento de las comunidades de macroinvertebrados de 91 lagunas en el Parque Nacional de Doñana, España, y se ha explorado la variabilidad temporal de dicho patrón de anidamiento y de la riqueza específica en 19 lagunas temporales durante dos años de estudio con diferentes precipitaciones. Las comunidades de macroinvertebrados de las lagunas de Doñana presentaron un patrón significativamente anidado, siendo, tanto la distribución espacial de las lagunas, como la variabilidad ambiental del sistema, responsables de dicho patrón anidado. Una serie de taxones y de lagunas se desviarón de este patrón anidado (denominados idiosincráticos) de manera que su presencia fue más frecuente de lo esperable en los sitios pobres en especies o/y constituyeron comunidades dominadas por especies que estuvieron prácticamente ausentes en los sitios de mayor riqueza. Los estados adultos con capacidad de vuelo, considerados como los dispersores, presentaron una distribución más anidada que los taxones y los estadios de desarrollo sin capacidad de dispersión. Los taxones idiosincráticos se localizaron en lagunas de diferente hidroperiodo, aunque el grado de anidamiento fue mayor en las lagunas de hidroperiodo más largo. Los muestreos mensuales demostraron un incremento gradual de riqueza específica y del grado de anidamiento desde la inundación hasta abril-mayo, cuando las lagunas de más corto hidroperiodo empezaron a secarse. Las laguna variaron su grado de anidamiento a lo largo de los meses de estudio, pero sin embargo, el grado de anidamiento anual fue prácticamente idéntico para los dos años de estudio a pesar de sus fuertes diferencias en hidroperiodo. Nuestros resultados sugieren que tanto la colonización diferencial como la variabilidad ambiental son procesos clave que determinan el alto grado de anidamiento en las lagunas de Doñana, y, además, que las comunidades de macroinvertebrados cambian de una manera predecible cada año en respuesta a los ciclos de inundación-desecación de las lagunas.

Abstract

Nestedness has been widely used to measure the structure of biological communities and occurs when species-poor sites contain subsets of species-rich ones. Here we examine nested patterns across the macroinvertebrate assemblages of 91 ponds in Doñana National Park, Spain, and explore temporal variation of nestedness and species richness in 19 temporary ponds over two years with differing rainfall. Macroinvertebrate assemblages were significantly nested, both pond spatial arrangement and environmental variation being important in driving nested patterns. Despite the nested structure observed, we detected a number of taxa and ponds which deviate from this pattern (termed idiosyncratic), by occurring more frequently than expected in species poor sites, or having assemblages dominated by species largely absent from species rich sites. Aquatic adults of winged insects, capable of dispersal, were more highly nested than non-dispersing taxa and life-history stages. Idiosyncratic taxa were found in ponds spanning a wide range of hydroperiods, although nestedness was higher in more permanent waterbodies. Monthly sampling demonstrated a gradual increase of species richness and nestedness from pond filling to April-May, when the most temporary ponds examined started to dry out. Although the degree of nestedness of individual pond assemblages varied from month to month, the overall degree of nestedness in the two study years was practically identical despite marked differences in hydroperiod. Our results suggest that both differential colonization and environmental variation are key processes driving the nested structure of Doñana ponds, and that macroinvertebrate assemblages change in a predictable manner each year in response to cycles of pond wetting and drying.

Key-words: colonization, community composition, dispersal, nestedness, temporary ponds

Introduction

Nested systems occur when species-poor sites contain subsets of the assemblages found in species-rich sites, and the degree of nestedness thus quantifies the overlap in species composition between high and low diversity areas (McAbendroth *et al.* 2005). Nestedness has been widely used to measure the structure of biological communities (Fleishman and Murphy 1999; Hylander *et al.* 2005; Meyer and Kalko

2008; Elmendorf and Harrison 2009), including lentic freshwaters, (Baber *et al.* 2004; McAbenthroth *et al.* 2005; Angeler *et al.* 2008; Wissinger *et al.* 2009), although few studies have explored the factors which may drive this pattern. Factors that lead to consistent differences amongst species in immigration or extinction rates cause strong patterns of nestedness across species assemblages (Wright and Reeves 1992). Although originally extinction was assumed to be the main cause of nestedness (Atmar and Patterson 1993), immigration can also be very important in generating nestedness under particular conditions (Cook and Quinn 1995). Whilst extinction is important in structuring biological communities over long timescales (Patterson 1990; Hausdorf and Henning 2003), differential colonization can be important over shorter term scales (Patterson 1990; Atmar and Patterson 1993), particularly when colonization drives community assembly in originally empty sites such as new and ephemeral habitat patches (Norton *et al.* 2004). In addition to selective immigration and extinction, variation in environmental conditions, including habitable area, across sites can also generate nested patterns (Lomolino 1996; Baber *et al.* 2004; McAbendroth *et al.* 2005; Heino *et al.* 2009), although studies of nestedness which assess the importance of immigration-extinction dynamics and environmental heterogeneity are rare in any habitat.

Temporary ponds are fluctuating waterbodies with recurrent seasonal phases of flooding and desiccation in most years (Grillas *et al.* 2004; Brönmark and Hansson 2005; Williams 2006). Although there are exceptions (see Batzer and Wissinger 1996), dispersal to more permanent waterbodies is the main strategy through which macroinvertebrates survive dry phases in these temporary aquatic habitats (Wiggins *et al.* 1980; Bilton *et al.* 2001; Garrido and Munilla 2008). As a consequence, much of the invertebrate biota of temporary ponds is assembled through colonization on each re-wetting cycle, making these an ideal system in which to explore the role of colonization in generating nestedness. Such a dispersal-driven system can have important implications for nestedness patterns, and isolated ponds would show lower nestedness if the regional species pool is dominated by poor dispersers (Cook and Quinn 1995; McAbendroth *et al.* 2005). In lentic freshwaters, hydroperiod duration is well established as key factor driving assemblage structure (e.g. Baber *et al.* 2004, Urban 2004; Waterkeyn *et al.* 2008). As nestedness can be related to assemblage stability (Atmar and Patterson 1993), this could be expected to increase with hydroperiod length due to the presence of species which require more time to complete their life cycles which are not able to survive even occasional dry phases. The temporal variability of nested patterns in biological communities can shed light on the role of colonization and extinction in generating nestedness (Patterson 1990; Loo *et al.* 2002; Bloch *et al.* 2007), and the temporal cycle of wetting and drying in ephemeral waters makes them an ideal system in which to explore such processes. Despite this, no studies to date have explored temporal variation in nestedness in these systems over their wetting/drying cycles.

Idiosyncratic species and sites are those that depart from the general nested pattern, reducing the value of matrix nestedness (Atmar and Patterson 1993). Idiosyncratic species are those which occur more frequently than one would expect in species-poor sites, and/or less frequently in species rich ones, something which could result through habitat specialisation or competition avoidance, as well as being a locally infrequent habitat generalist (see McAbendroth *et al.* 2005). Individual sites are considered idiosyncratic when they contain a higher proportion of idiosyncratic species than one would predict from their species richness – in other words, their assemblages contain a number of species which are largely absent from the most species rich sites. The detection of idiosyncratic species and sites is of potential interest for conservation (McAbendroth *et al.* 2005; Heino *et al.* 2009) as idiosyncratic sites may harbour specialist taxa not present in species rich localities, and idiosyncratic species may require specialized conditions, present in relatively few sites in a region (Atmar and Patterson 1993).

Here we examine nestedness and its temporal variation, across a natural pond network in southern Spain, spanning a wide range of hydroperiods. We determine the degree of nestedness across the pond network as a whole by analysing data from close to 100 ponds, collected over a single season. We use this dataset to determine whether nestedness increases with: 1) the dispersal ability of macroinvertebrates; 2) the degree of isolation of ponds, and; 3) the length of hydroperiod. We go on to examine whether variation in environmental conditions across sites may contribute to nestedness, and evaluate the relative importance of colonization and environmental variation in generating observed patterns. Additionally, we explore whether species richness and nestedness vary in a predictable manner over the hydrological cycle in a subset of ponds sampled monthly across two years with differing rainfall regimes. Finally we consider the possible implications of our findings for the conservation of lentic biodiversity in the region.

Materials and Methods

Study area

Our study was performed in Doñana National Park, located between the mouth of the Guadalquivir River and the Atlantic Ocean in south-western Spain (see Siljeström *et al.* 1994). The climate of this area is Mediterranean sub-humid with most rainfall in autumn and winter, hot and dry summers, and mild winters. In this area, two main regions can be differentiated: an extensive marsh and an adjacent sandy area where numerous temporary waters form in natural depressions; more than 3,000 occurring in wet years. Ponds in the park differ widely in surface area and hydroperiod, and constitute a heterogeneous network of aquatic habitats which has been listed under the RAMSAR convention since 1982. Temporary ponds in Doñana are seasonally flooded after heavy rains, usually filling in autumn or winter, and persisting until late spring or early summer. There are also two large semi-permanent ponds which only occasionally dry in years of severe drought, and some natural ponds have been artificially deepened to supply water for cattle and wild mammals, making them permanent in most years. A detailed description of the environmental characteristic of these ponds is given in Gómez-Rodriguez *et al.* (2009), Espinar and Serrano (2009) and Díaz-Paniagua *et al.* (2010).

Sampling surveys

Two linked sampling surveys were carried out, and these provide the data which form the basis of this study:

1) Extensive macroinvertebrate survey: In order to assess the degree of nestedness across the pond network as a whole, we sampled a total of 91 ponds which ranged from 24-122,672 m^2 in area between end March and mid June 2007. Pond assemblages in Doñana were relatively stable over this time period (no consistent change in species richness between March and June; linear regression: R^2=0.017, p=0.22), which was the minimum window over which all sites could be visited. Ponds were chosen to span the full range of hydroperiod variation present within the study area, and cover all areas of the park where discrete lentic waterbodies occur (Fig 1). Based on their depth, area, and previous observations (see Florencio *et al.* 2009), ponds were assigned to one of three categories based on their degree of permanence: short hydroperiod (N=28), intermediate hydroperiod (N=22), and long hydroperiod (including permanent ponds; N=41). Pond surface area varied considerably within each hydroperiod category, and did not increase with pond duration. Although short and

intermediate hydroperiod categories did not differ in area, long hydroperiod ponds were actually significantly smaller (one-way ANOVA, $F_{(2,88)}=10.919$, $p<0.0001$; Tukey HSD, $p<0.0001$, see Table 1), often being relatively small pools which had been artificially deepened (see above).

Table 1: Environmental characteristics of ponds in the extensive survey, showing the mean, minimum (min) and maximum (max) values ([b] indicates a legend at the bottom of the Table)

Environmental variables	**Mean (min-max)**
Pools (%)	3.8 (0-80)
Maximum depth (cm)	74.2 (9-126)
Pond area (m^2)	4,103 (24-122,672)
-Short hydroperiod	3,427 (25-23,097)
-Intermediate hydroperiod	4,517 (25-37,544)
-Long hydroperiod	4,370 (24-122,672)
Pond number [b]	4.4 (0-16)
NH_4^+ (mg l^{-1})	1.34 (0.04-3.59)
i-P [b] (mg l^{-1})	0.11 (0.01-0.75)
$[O_2]$ [b] (mg l^{-1})	2.2 (0.2-16.8)
EC [b] (µS cm^{-1})	962.8 (82-8,800)
Turbidity (NTU)	35.0 (1.1-975.5)
Organic matter (%)	5.43 (0.17-27.74)
SO_4^{2-} (meq l^{-1})	0.82 (0.04-23.24)

[b]: Pond number is the number of ponds >150m^2 within 200m of each pond; i-P is dissolved inorganic phosphate; $[O_2]$ is dissolved oxygen concentration; EC is electrical conductivity.

2) Monthly macroinvertebrate survey: To analyse temporal variation in nestedness over wet-dry cycles, we sampled aquatic macroinvertebrates in 19 temporary ponds during their wet phases, from February 2006-August 2007. All these ponds were distributed within the central area of the Park (Fig 1). The first study year was relatively dry (468 mm rainfall; hereafter referred to as the dry year), and most ponds were only wet from February to May. The second study year was wetter (716.9 mm rainfall; hereafter referred to as the wet year), and most ponds were flooded from November to July. Due to the increase in the number of ponds formed in the second year (as a result

of higher rainfall), we sampled three additional ponds replacing three ponds only sampled in the dry year in order to include ponds spanning the highest range of hydroperiod observed in the area. For the monthly survey we calculated hydroperiod as the number of months ponds were flooded in a given year, and classified this in relation to the longest hydroperiod seen in each year. In the dry year, hydroperiod categories were short (< 2.5 months), intermediate (2.5-3.5 months) and long (> 3.5 months). In the wet year, short hydroperiod was < 7 months, intermediate 7-8 months and long > 8 months (for further details on hydroperiod of these ponds see Florencio *et al.* 2009).

Macroinvertebrate sampling and taxon identification:

We sampled macroinvertebrates with a dip-net (39 x 21 cm, 1 mm mesh), netting a stretch of water of approximately 1.5 m length in each sampling unit. In each pond, we sampled all different available microhabitats, based largely on differences in aquatic plant cover and depth (Heyer *et al.* 1994). As the efficiency of dip-netting increases in small ponds (Heyer *et al.* 1994), we took more samples in larger ponds, which also typically contained a higher number of microhabitats, in order achieve comparable effort in detecting rare species. In all ponds, sampling was concentrated amongst vegetation, where most macroinvertebrate species are located. In most cases a minimum of three samples were taken (maximum 13), with the exception of five small ponds sampled at the end of their dry phase, which were so small that three discrete 1.5 m sampling areas could not be identified. Most macroinvertebrates were identified *in situ* and then returned to the pond, with those taxa which could not be determined in the field preserved in 70% ethanol for examination in the laboratory. Identifications were performed to the lowest taxonomic level possible, in general to species (most adults) or genus (most larvae), although Basomatophora, Diptera, Oligochaeta and saldid bugs were identified only to family. We kept the different taxonomic levels reached for adults and larvae, using these stages of taxa separately (hereafter referred to as taxa/stages), to assess the role of each stage in generating nested patterns.

Environmental variables:

In order to determine whether pond characteristics influence macroinvertebrate nestedness, we measured a range of environmental variables across the ponds (see Table 1). In the field, we measured the proportion of the pond surface divided into separate pools, maximum water depth (with a graduated pole at the deepest point of the pond), conductivity (on bed using HI 9033), dissolved oxygen concentration (on bed using YSI 550A) and turbidity (in water column using HANNA HI93703). Surface water (500 ml) was also collected to determine nutrient (dissolved inorganic phosphate and ammonium - using an Auto Analyzer (Bran + Luebbe)) and SO_4^{2-} anion concentrations (using Inductively Coupled Plasma Mass Spectrophotometer (ICP)). Surface sediment samples (5 cm depth) were collected and organic matter measured in the laboratory (mean of 3 replicates via loss on ignition, 450 ºC, 5 h). Pond area and the number of ponds > 150 m^2 within 200m of each pond were extracted from a GIS-based map constructed at time of maximum inundation (see Gómez-Rodríguez *et al.* 2008).

Statistical analyses:

We constructed several matrices using presence-absence data of macroinvertebrate occurrence by pond:

For the extensive survey we built: i) the **taxon matrix,** pooling adults and larvae of individual taxa, to the highest taxonomic resolution possible; ii) the **stage matrix**, with adults and larvae separated; iii) the **disperser matrix**, including only adults of taxa capable of flight; iv) the **non-disperser matrix,** including only larvae and taxa with non-flying adults. To evaluate whether longer hydroperiod increased nestedness, we built three sub-matrices of the stage matrix which included the ponds catalogued as short, intermediate or long hydroperiod for each matrix. A χ^2 test was used to evaluate whether idiosyncratic taxa or stages were associated with ponds of any of the hydroperiod categories. A χ^2 test was also used to evaluate whether idiosyncratic ponds were more common in some hydroperiod categories than others.

In order to evaluate how the degree of nestedness observed across ponds changed through their wetting-drying cycle, we built a stage matrix as described above for each month when > 2 ponds were wet (4 months in the dry year; 9 months in the wet year). To compare nestedness between the two study years, we also built two annual presence-absence matrices with the occurrence of taxa/stages pooled across months (one matrix per year). The size of monthly matrices varied between sampling months with the gradual desiccation of ponds, as not all ponds were flooded in all months. Also, different numbers of taxa/stages were recorded each month.

We used the program ANINHADO, a package which has advantages over the more widely used Nestedness Temperature Calculator (Atmar and Patterson 1995), to analyse nestedness using both *T/N* and *NODF* (- see http://www.guimaraes.bio.br - Guimaraes and Guimaraes 2006). ANINHADO orders matrices according the number of presences per row (ponds), and a diagonal between presences and absences is drawn to calculate *T*, based on unexpected absences (Atmar and Patterson 1993). Although *T* reaches low values in ordered systems (Atmar and Patterson 1993), here we use N, which indicates the degree of nestedness, or order, with values between 0 and 1 (maximum nestedness). N is calculated as $N= (100 - T) / 100$ (Bascompte *et al.* 2003). As we compared nestedness between matrices with different sizes, and N is dependent on matrix size (Atmar and Patterson 1993; Almeida-Neto *et al.* 2008), we calculated the relative nestedness ($N^{*}= [N - N_{R}]/ N_{R}$) where N is the degree of nestedness of the original matrix and N_{R} is the average nestedness of the 1,000 null model matrices (Bascompte *et al.* 2003). *NODF* is a more recently developed metric to calculate nestedness which varies from 0 to 100 (maximum nestedness). *NODF* is obtained from the paired degree of nestedness for each pair of columns and for each pair of rows in an ordered matrix and has the advantage of being independent of both matrix size and shape (Almeida-Neto *et al.* 2008). We include both *NODF* and N here to allow comparison with previous studies, most of which have employed variations of the latter metric.

ANINHADO allows the degree of nestedness present within a system to be assessed against four separate null models, all of which were tested here: 1) presences assigned at random across the matrix; 2) a fixed-fixed null model, with both column sums and row sums fixed; 3) only column sums fixed; 4) only row sums fixed (see http://www.guimaraes.bio.br). The significance of observed values of nestedness was assessed using 1,000 permutations of each null model. Using N and *NODF*, whenever significant nestedness was obtained with the fixed-fixed model, it was also obtained using the other three, and we therefore used the fixed-fixed null model (the most restrictive; incurring fewer Type I errors (Gotelli 2000; Ulrich and Gotelli 2007a, 2007b; Almeida-Neto *et al.* 2008)) to assess the degree of nestedness. To detect idiosyncratic ponds and taxa we calculated N for each pond and taxon/stage, and considered as idiosyncratic those taxa/stages whose N values were lower than the value of the original matrix (Atmar and Patterson 1993). Since the degree of nestedness can be affected by sample size, and the number of samples taken differed between ponds, we explored the effect of sample number on nestednesss via sample-based rarefaction (Gotelli and Colwell 2001) with the minimum number of samples per pond and month using EcoSim Version 7 (Gotelli and Entsminger 2004). In all cases we obtained similar nested patterns to those produced with raw data, and consequently present only the latter here.

Pond spatial isolation was used to assess the contribution of differential colonization to nestedness patterns, following Lomolino (1996). We evaluated whether the degree to which a pond departed from nestedness (*N*) was affected by the spatial arrangement of sampled ponds (i.e. whether the level of nestedness observed depended on relative isolation), using distance-based Moran's eigenvector maps (MEM- see Dray *et al.* (2006)), a general framework of principal coordinates of neighbour matrices (PCNM, see Borcard and Legendre 2002). We also compared the results for disperser and for non-disperser matrices in order to evaluate the role of dispersal in generating the nested pattern. We used the Delaunay triangulation criterion (see Legendre and Legendre 1998) which was appropriated to connect large distances, but did not consider edge effects since the Park boundaries are not the limit of the aquatic systems in the region. The number of eigenvectors was reduced using the 'ortho.AIC' command in R software ('spacemakeR' package, Dray *et al.* 2006). Only significant eigenvectors were used directly as explanatory variables in a multiple forward stepwise regression with the nestedness of ponds (in STATISTICA 6.0). This complete procedure is an effective way of controlling for Type I error (Peres-Neto and Legendre 2010).

To explore the role of inter-pond environmental variation in driving nestedness in the macroinvertebrate community we used the Monte Carlo approach of Lomolino (1996). Analyses here were restricted to a subset of 80 ponds for which full environmental variables were available. The ponds of the stage matrix were ranked by each environmental variable, and the number of unexpected absences followed by a presence quantified as the departure (*D*) from perfect nestedness (Honney *et al.* 1999). Statistical significance was estimated as the number of randomizations of the fixed-fixed null model giving $D<$ observed (see Lomolino and Davis 1997). Environmental variables with identical values in two or more ponds (proportion of pools, maximum depth, nutrient concentrations and the number of ponds > 150 m^2 within 200m) were not analysed, as the approach requires unique values for each site (Lomolino 1996).

To assess the relative role of pond spatial arrangement and local environmental variation in generating nested patterns, we used a variance partitioning approach. As a measure of the spatial arrangement of ponds we used spatial descriptors extracted from MEMs; the effect of environmental variation being summarised using the first axis scores from a principal components analysis (PCA) of all environmental variables (Primer v.6, Clarke and Warwick 2001). Variance partitioning was performed using the 'varpart' command in R software ('vegan' package, Oksanen *et al.* 2008), which obtains an adjusted multiple coefficient of determination (Adj.R^2, 0-1) in order to compare explanatory variables (Peres-Neto *et al.* 2006). Significance was tested using a multiple forward stepwise regression for the spatial descriptors, and a linear regression for the PC1 scores.

Results

Nestedness in the Doñana ponds network

The macroinvertebrate assemblages of Doñana ponds were highly nested overall (stage matrix: *N*=0.81, 16.43% fill; taxon matrix: *N*=0.83 17.65% fill, Table 2). The taxon matrix was more highly nested than the stage matrix (including adults and larvae separately), in which we detected a higher number of idiosyncratic taxa and stages (Table 2). The disperser matrix exhibited higher nestedness than the non-disperser matrix (Table 2).

Table 2: Degree of nestedness (*N, N** and *NODF*) detected in the extensive macroinvertebrate survey for different matrices: the taxon matrix, the stage matrix (which included adult and larval stage separately), matrix of dispersers (adults with capability of flight) and non-dispersers (larvae and non-flying adults). Columns are the number of taxa/stages; idio-taxa the number of idiosyncratic taxa/stages; idio-ponds the number of idiosyncratic ponds. All nested values were significant at p<0.001.

	N	***N****	***NODF***	**Columns (taxa)**	**Idio-ponds**	**Idio-taxa**
Stage matrix	0.81	0.43	34.52	135	34	59
Taxon matrix	0.83	0.46	45.20	102	37	32
Disperser matrix	0.82	0.48	42.32	66	40	24
Non-disperser matrix	0.88	0.34	41.55	68	30	24

Out of the 135 taxa/stages recorded, 59 were idiosyncratic (Table 3), occurring in 1 to 53 ponds, across all hydroperiod classes. Some idiosyncratic beetles (*Yola bicarinata* (Latreille, 1804), *Hygrobia hermanni* (Fabricius, 1775), *Hygrotus confluens* (Fabricius, 1787)) and damselflies (*Lestes virens* (Charpentier, 1825)) were significantly more abundant in long hydroperiod ponds, wheras some idiosyncratic flies (Culicidae and *Chaoborus* spp.) were significantly more frequent in short hydroperiod ponds.

Sympetrid dragonflies (*Sympetrum fonscolombei* (Selys, 1841), *S. meridionale* (Selys, 1841), *S. striolatum* (Charpentier, 1840) and *S. sanguineum* (Müller, 1764)) were significantly more frequent in short and intermediate hydroperiod ponds rather than those with long hydroperiods (Table 3). Out of the 91 studied ponds, 34 were idiosyncratic at the assemblage level (Table 4). These idiosyncratic ponds tended to cluster in both northern and southern areas of the park, suggesting an effect of isolation, but did not correspond to a particular hydroperiod category (Fig. 1). Four significant spatial descriptors extracted from MEMs analyses were strongly correlated with the degree of nestedness of the ponds (*N*) of the stage matrix (R^2=0.52, $F_{(24,66)}$=2.987, $p<0.001$); i.e. the spatial arrangement of ponds, based on their inter-pond distances (pond isolation), can partially drive the macroinvertebrate nested structure. This effect was much higher in the disperser matrix with six significant spatial descriptors (R^2=0.54, $F_{(21,68)}$=3.849, $p<0.0001$) than in the non-disperser matrix with only a single significant spatial descriptor (R^2=0.23, $F_{(10,79)}$=2.354, $p<0.05$). This means dispersers were much more affected by the spatial structure and isolation of ponds than non-dispersers. We detected significant differences in the proportion of idiosyncratic ponds in each hydroperiod category (χ^2 = 11.99, df=2, $p<0.01$). Fifty four percent of short hydroperiod ponds were idiosyncratic, 23 % of intermediate and 34 % of long hydroperiod ponds (Table 4). Although the macroinvertebrate assemblages of ponds with different hydroperiod categories were similar nested using *NODF*, with *N** we observed the highest nestedness in the macroinvertebrate assemblages of long hydroperiod ponds (*N**=0.41, *NODF*=33.66, both indices $p<0.001$) whilst short and intermediate hydroperiod ponds showed similar degrees of nestedness (*N**=0.38, *NODF*=32.81, both indices $p<0.001$; and *N**=0.34, *NODF*=35.17, both indices $p<0.001$ respectively).

Table 3: Idiosyncratic taxa/stages (adults or larvae) with their degree of nestedness (*N*), the number of ponds in which they occurred (pond occurrence) and hydroperiod category of these ponds: short, intermediate (Interm) and long (*= significant over-representation in one pond hydroperiod category at level $p<0.05$, χ^2 test).

Order	**Adults**	***N***	**Pond occurrence**	**Short**	**Interm**	**Long**
Bassomatophora	*Physa* spp.	0.42	33	10	6	17
Coleoptera	*Gyrinus dejeani* Brullé, 1832	0.47	15	4	3	8
Heteroptera	*Trichocorixa verticalis* (Fieber, 1851)	0.50	13	5	4	4
Coleoptera	*Hydrobius fuscipes* (Linnaeus, 1758)/*Limnoxenus niger* (Zschach, 1788)	0.54	36	12	13	11
Coleoptera	*Hydroglyphus geminus* (Fabricius, 1792)	0.54	28	5	5	18
Coleoptera	*Rhantus hispanicus* Sharp, 1882	0.62	31	15	6	10
Heteroptera	*Notonecta meridionalis* Poisson, 1926	0.63	25	8	6	11
Coleoptera	*Ochthebius dilatatus* Stephens, 1829	0.64	5	3	0	2
Coleoptera	*Helochares lividus* (Forster, 1771)	0.66	10	4	1	5
Heteroptera	*Notonecta viridis* Delcourt, 1909	0.67	23	4	5	14
Coleoptera	*Agabus nebulosus* (Forster, 1771)	0.67	19	8	3	8
Coleoptera	*Hydroporus lucasi* Reiche, 1866	0.67	41	12	13	16
Coleoptera	*Yola bicarinata* (Latreille, 1804)	0.67	6 *	0	0	6
Coleoptera	*Hygrotus confluens* (Fabricius, 1787)	0.70	21*	3	3	15
Coleoptera	*Hygrobia hermanni* (Fabricius, 1775)	0.71	29*	5	4	20
Heteroptera	*Gerris thoracicus* Schummel, 1832	0.71	55	18	16	21
Coleoptera	*Berosus signaticollis* (Charpentier, 1825)	0.71	15	4	6	5
Heteroptera	*Notonecta glauca* Linnaeus, 1758	0.72	18	6	6	6
Coleoptera	*Enochrus fuscipennis* (Thomson, 1884)	0.73	32	7	11	14
Coleoptera	*Hygrotus lagari* (Fery, 1992)	0.73	34	5	9	20
Bassomatophora	Planorbidae	0.74	13	2	3	8
Heteroptera	*Sigara scripta* (Rambur, 1840)	0.74	12	3	2	7
Heteroptera	*Plea minutissima* Leach, 1817	0.75	26	4	6	16
Heteroptera	*Nepa cinerea* Linnaeus, 1798	0.75	8	2	2	4
Coleoptera	*Anacaena lutescens* (Stephens, 1829)	0.75	49	16	12	21
Heteroptera	*Micronecta scholzi* (Fieber, 1860)	0.77	6	1	0	5
Coleoptera	*Helophorus* spp.	0.78	36	10	9	17
Coleoptera	*Noterus laevis* Sturm, 1834	0.78	6	1	1	4
Coleoptera	*Ilybius montanus* (Stephens, 1828)	0.78	11	1	4	6
Coleoptera	*Laccobius revelierei* Perris, 1864	0.79	1	1	0	0
Heteroptera	*Sigara lateralis* (Leach, 1817)	0.79	39	11	8	20
Coleoptera	*Berosus affinis* Brullé, 1835	0.79	17	4	6	7
Heteroptera	*Notonecta maculata* Fabricius, 1794	0.80	14	3	4	7
Coleoptera	*Laccophilus minutus* (Linnaeus, 1758)	0.80	29	6	9	14

Heteroptera	*Sigara stagnallis* (Leach, 1817)	0.80	15	4	2	9
Coleoptera	*Colymbetes fuscus* (Linnaeus, 1758)	0.80	35	10	10	15
Order	**Larvae**					
Coleoptera	*Hydrobius fuscipes* /*Limnoxenus niger*	0.44	23	5	10	8
Diptera	Culicidae	0.49	27 *	15	6	6
Coleoptera	*Hyphydrus aubei* Ganglbauer, 1892	0.55	22	4	4	14
Diptera	*Chironomus plumosus* (Linnaeus, 1758)	0.61	53	11	12	30
Odonata	*Sympetrum fonscolombei* (Selys, 1841)	0.61	46*	17	16	13
Odonata	*Ishnura pumilio* (Charp., 1825)	0.62	33	9	12	12
Diptera	*Chaoborus* spp.	0.64	5*	2	3	0
Heteroptera	Corixidae	0.72	52	16	13	23
Coleoptera	*Cybister lateralimarginalis* (De Geer, 1774)	0.73	21	7	6	8
Coleoptera	*Haliplus* spp.	0.73	7	2	4	1
Coleoptera	*Laccophilus minutus*	0.73	47	15	10	22
Coleoptera	*Colymbetes fuscus/Rhantus* spp.	0.75	17	7	6	4
Odonata	*Sympetrum meridionale* (Selys, 1841)	0.77	16*	8	6	2
Odonata	*Sympetrum striotalum* (Charpentier, 1840)	0.78	21*	10	7	4
Odonata	*Aeshna mixta* Latreille, 1805	0.78	7	2	2	3
Diptera	Tanypodinae	0.78	8	2	2	4
Odonata	*Lestes virens* (Charpentier, 1825)	0.78	8*	0	0	8
Odonata	*Lestes barbarus* (Fabricius, 1798)	0.79	13	3	5	4
Coleoptera	*Agabus* spp.	0.79	9	1	3	5
Heteroptera	*Nepa cinerea*	0.79	8	1	1	6
Odonata	*Sympetrum sanguineum* (Müller, 1764)	0.79	20*	7	9	4
Coleoptera	*Berosus* spp.	0.80	22	8	7	7
Odonata	*Ischnura elegans* (Vander Linden, 1820)	0.80	24	4	10	10

Table 4: Idiosyncratic ponds in which macroinvertebrate assemblages departed from the nested pattern obtained with the stage matrix. Degree of nestedness (*N*), taxon/stage richness and hydroperiod category of the ponds are also shown.

Pond	***N***	**Taxon/stage richness**	**Hydroperiod**
3011	0.34	5	Short
Cry	0.36	8	Long
429	0.43	9	Short
1502	0.47	12	Short
3425	0.49	11	Long
Trp	0.50	32	Short
3411	0.57	7	Long
313	0.61	7	Long
308	0.62	12	Short
3431	0.64	12	Long
140	0.64	13	Intermediate
1300	0.65	13	Short
389	0.66	15	Short
3339	0.67	11	Short
Fre	0.69	16	Long
3362	0.70	14	Short
630	0.70	16	Short
3286	0.71	26	Long
3413	0.71	15	Short
3345	0.74	9	Intermediate
3432	0.74	18	Long
3315	0.74	26	Short
3410	0.75	7	Long
Trj	0.76	15	Long
Arm	0.76	2	Short
310	0.77	18	Intermediate
Acm	0.77	18	Intermediate
604	0.78	22	Intermediate
321	0.79	18	Short
311	0.79	7	Long
Orf	0.79	12	Long
3371	0.80	25	Long
299	0.80	16	Short
3398	0.80	19	Long

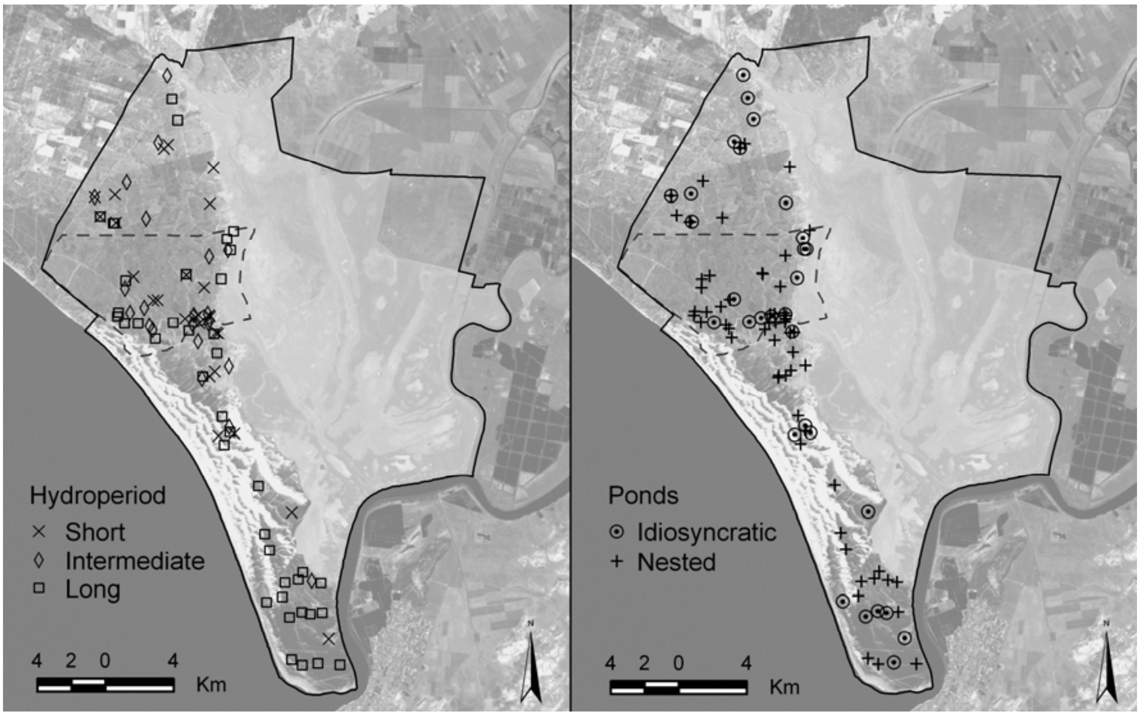

Fig1. Location of the ponds sampled in Doñana National Park, with A) hydroperiod categories and B) idiosyncratic or nested character of their macroinvertebrate assemblages. The discontinuous line demarcates the area including the 19 ponds sampled for the monthly survey. The lighter coloured area to the right indicates the marsh, where discrete ponds are largely absent during the flooding period

All measured environmental variables made a significant contribution to the nested pattern according to the number of Lomolino's departures (*D*) from perfect nestedness (Table 5). Pond area produced the lowest *D*, making the greatest contribution to the macroinvertebrate nestedness (Table 5). Despite the contribution of environmental variables to the nested pattern, variance partitioning showed that spatial descriptors were more strongly associated with nestedness (pure explained variation Adj.R^2=0.117, $p<0.001$) than environmental variables (pure explained variation Adj.R^2=0.041, $p<0.01$).

Table 5: Number of departures (*D*) by Lomolino's approach calculated with a subset of 80 ponds of the stage matrix. Ponds were ranked by the value of each environmental variable. We did not find any randomization of the fixed-fixed null model giving D> observed (1000 randomizations, all p=0.001).

Ponds ranked by:	***D***
Pond area	1083
Richness	1091
SO_4^{2-}	1145
O_2	1146
EC	1149
O.M.	1155
Turbidity	1165

EC is electrical conductivity; O_2 is the dissolved oxygen; O.M. is the organic matter.

Monthly variation in species richness and nestedness

Most of the ponds which were sampled monthly differed in hydroperiod between dry and wet years (Table 6). In the dry year, the monthly variation in taxon/stage richness per pond in each of the three hydroperiod categories reached a maximum in April, except for short hydroperiod ponds which had already dried up (Fig. 2). In the wet year, maximum richness occurred in March-April for short hydroperiod ponds, but in May for those with intermediate and long hydroperiods (Fig. 2). The maximum taxon/stage number per pond occurred in long hydroperiod ponds with up to 30 taxa and/or stages (Fig. 2). In ponds of all three hydroperiod categories, we observed an initial increase in the proportion of adult dispersers with respect to larvae just after ponds filled. From then on, adult dispersers and larvae remained in similar proportions until April, when the relative proportion of dispersers increased again (Fig. 2). A higher total number of taxa and/or stages were recorded in the wet year (n=132) than in the dry year(n=108).

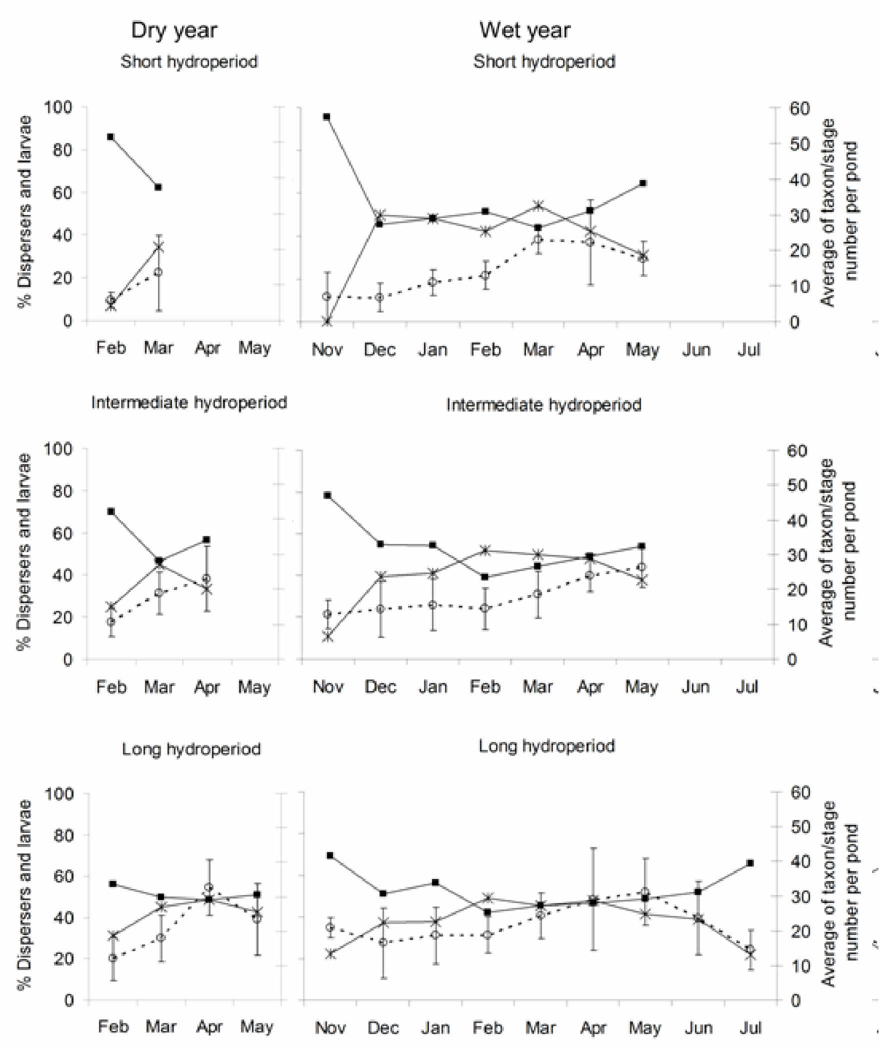

Fig2. Monthly variation (mean ± SD) in the number of taxa/stages per pond (open circles). The percentage of adult dispersers (full squares) and the percentage of larvae (asterisks) are also shown for the temporary ponds of each hydroperiod category for both dry and the wet years. Ponds with shorter hydroperiod dried out before intermediate and long hydroperiod ponds

In both years we observed a similar seasonal shift in the degree of nestedness, with a gradual increase from the time at which ponds first became wet (November and February in the wet and dry years respectively) to April-May in both years when nestedness decreased or disappeared (Fig. 3). Significantly nested assemblages were found in more months using *N** than *NODF* (Fig. 3). The nested pattern observed was similar using *N** and *NODF*, these differing only when *NODF* was non-significant (Fig. 3). We obtained a highly similar degree of nestedness for the pool of macroinvertebrate taxa and stages recorded every year (dry year: *N**=0.65, *NODF*=61.62, both indices p<0.001; wet year: *N**=0.64, *NODF*=60.15, both indices p<0.001) With one exception, the idiosyncratic character of ponds varied between months as well as between study years (Table 6). While a lower number of ponds were detected as idiosyncratic in the wet year (Table 6), a higher number of taxa/stages were detected as idiosyncratic in the wet year (n=53) than in the dry year (n=38).

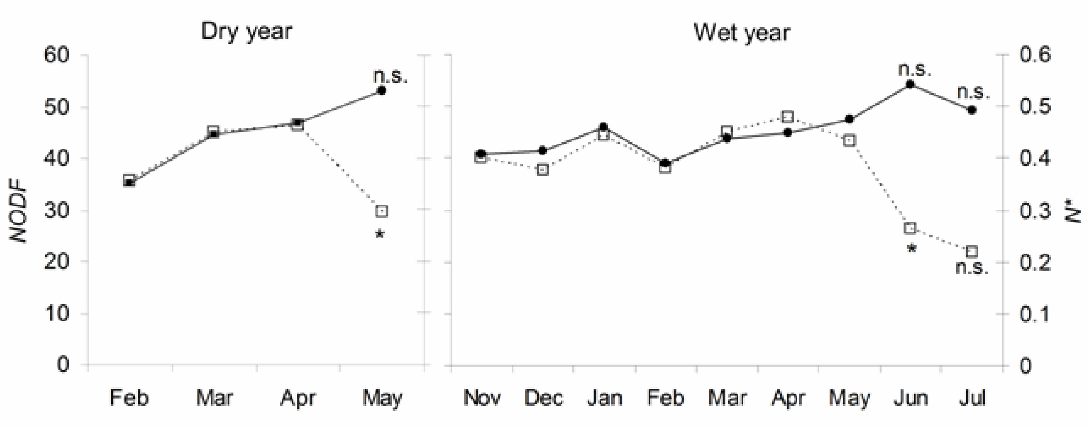

Fig3. Monthly variation in the nestedness of the macroinvertebrate assemblages of temporary ponds in the wet and dry years, calculated with *N** (open squares) and *NODF* (full circles). Nestedness was significant at p<0.01 level in all months, except as indicated: * = p<0.05 and n.s. = non significant

Table 6: Ponds showing idiosyncratic macroinvertebrate assemblages which departed of the general nested pattern by month through the wet and dry years are marked with X (D indicates pond dry; _ indicate lack of sample).

	Dry year: Idiosyncratic ponds						Wet year: Idiosyncratic ponds										
Pond	**Hydroperiod**	**Feb**	**Mar**	**Apr**	**May**	**Total**	**Hydroperiod**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Total**
Pol	interm	X	X	X	D		interm								D	D	
Acm	interm	X			D	X	interm	X		X	X	X		D	D	D	
Rp	short			D	D		interm								D	D	
Pg	interm		X		D		interm		X		X		X		D	D	
Jim	short	X	X	D	D	X	interm	X	X		X	X		X	D	D	
Cam	interm		X	D	D		interm		X						D	D	
Abe	short	X	X	D	D		interm	X	X			X			D	D	
Pp	interm	X			D		interm	X	X			X			D	D	
Tej	interm			D	D	X	interm	X			X	X			D	D	
Zah	long	X	X	X	D	X	long	X		X	X	X	X	X	X	X	X
Lve	long	X			X	X	long	X		X		X	X				
Dul	long					X	long	X	X							X	X
Bre	interm		X	X	D	X	long		X	X		X			X	D	
Orf	long				X	X	long						X				
Ant	short	X	D	D	D	X	short			X				X	D	D	
Wou	interm		X	X	D		-	-	-	-	-	-	-	-	-	-	-
Mor	interm				D		-	-	-	-	-	-	-	-	-	-	-
Tar	long	X	X				-	-	-	-	-	-	-	-	-	-	-
Arm	-	-	-	-	-	-	short		X					D	D	D	X
Vac	short	D	X	D	D		short							X	D	D	
Len	-	-	-	-	-	-	short	X	X	X	X			X	D	D	X
Tps	-	-	-	-	-	-	short		X				X		D	D	X

Discussion

Nestedness in the macroinvertebrate assemblages of Doñana ponds

We detected a high degree of nestedness in the macroinvertebrate assemblages of Doñana ponds overall, when surveyed in a single season, despite this being a highly dynamic aquatic system (Gómez-Rodríguez *et al.* 2009; Florencio *et al.* 2009; Díaz-Paniagua *et al.* 2010). Such finding is in line with some previous studies of lentic macroinvertebrate assemblages (e.g. McAbendroth *et al.*, 2005; Baber *et al.*, 2004), but such a pattern is not universal. Urban (2004), for example did not detect nestedness in the macroinvertebrates of the temporary ponds of a 200-ha region. Nestedness of Doñana macroinvertebrate assemblages implies that a high proportion of taxa (including adult and larval stages) may be found in most of the ponds in the park, with the exception of those species and ponds which are idiosyncratic. The high degree of nestedness found contrasts with the high proportion of idiosyncratic taxa/stages (43%) and the presence of idiosyncratic assemblages in 37% of the sampled ponds. Soininen (2008) detected a similar proportion of idiosyncratic taxa amongst nested diatom assemblages, suggesting good dispersal amongst sites to be the major driver of nestedness. In our highly nested pond network, we also suggest that nestedness is partly driven by differential colonization amongst these temporary aquatic systems; whilst the high number of idiosyncratic taxa/stages and assemblages may be partly due to the presence of specialized environments in some sites.

Nestedness can be used as a measure of stable conditions in biological communities (Atmar and Patterson 1993), and in our study, such stability can be related to the degree of unpredictability in the composition of macroinvertebrate assemblages which is related to hydroperiod (Florencio *et al.* 2009). Hence, short duration ponds would be expected to generally exhibit lower nestedness, since only species adapted sudden desiccation events could persist, than long hydroperiod ponds, where more stable environmental conditions allow more sensitive species to complete their life cycles (Baber *et al.* 2004). In our study, however, we detected only slightly higher nestedness in long hydroperiod ponds compared to those of short and intermediate hydroperiod. Similarly, Urban (2004) did not detect differences in nestedness related to hydroperiod, because although long hydroperiod ponds harboured higher numbers of species, temporary ponds in their study contained species which did not occur in long hydroperiod ponds, an observation which is also likely to apply in our system.

The Doñana pond network is mainly comprised of temporary ponds, which are usually filled with autumn or winter rainfall and dry out in summer, when only a few permanent ponds retain water. The number of ponds and the filling date each year depend on the quantity of rainfall, which shows wide inter-annual variation, which plays an important role in driving unpredictability in the system (Serrano and Zunzunegui 2008; Díaz-Paniagua *et al.* 2010). Macroinvertebrates which are well adapted to temporary ponds adopt different physiological and behavioural mechanisms to survive desiccation (Williams 2006), with dispersal into permanent ponds being the most common strategy followed by dormant life-history stages such as resistant eggs, larvae and adults burying into the mud (Wiggins *et al.* 1980; Higgins and Merrit 1999; Bilton *et al.* 2001). Dispersal also is favoured by physical links between sites which can occur following heavy rainfall (Fahd *et al.* 2007). As high connectivity is one of the main drivers of nestedness in biological communities (Boecklen 1997; Cook and Quinn 1995; Wright *et al.* 1998; Higgins *et al.* 2006) and dispersal has been suggested as being important in driving nested patterns (Patterson 1990; Atmar and Patterson 1993; Loo *et al.* 2002; Hausdorf and Hennig 2003), we expected, and found, good dispersers to be more highly nested than poor and non-dispersing taxa (Cook & Quinn 1995). Isolation can affect the degree of nestedness observed across systems (Wright *et al.* 1998; McAbendroth *et al.* 2005; Monaghan *et al.* 2005), something that we have observed in distant ponds located in northern and southern areas of the park, which had more idiosyncratic assemblages. The fact that the spatial arrangement of ponds affected their degree of nestedness was much more evident for adult dispersers than for non-dispersing life history stages or taxa. As nested patterns may arise through inter-pond colonisation processes operating on a more local scale within the pond network, (McAbendroth *et al.* 2005; Soininen 2008), our results suggest that isolation-driven differential colonization contributed to the observed nested pattern.

All environmental variables measured contributed significantly to the nested pattern, despite the wide physicochemical variation observed across ponds, with area being identified as the most important factor. Pond area has been identified as an important cause of nestedness in macroinvertebrate assemblages of pond networks in other regions (Baber *et al.* 2004; McAbendroth *et al.* 2005), and patch-area-dependent extinction is believed to be an important mechanism driving nested patterns (Lomolino 1996; Honney *et al.* 1999). In our study, however, local extinction risk is not higher in small ponds, since the long hydroperiod ponds, supporting higher numbers of taxa, were mainly artificially deepened small ponds. Instead, in our systems, the effect of pond area on macroinvertebrate nestedness may result from the fact that larger ponds have higher habitat heterogeneity.

As colonization of more permanent sites is the main strategy many macroinvertebrates use to survive desiccation in temporary ponds (Wiggins *et al.* 1980; Bilton *et al.* 2001; Williams 2006), we expected colonization to be one of the main drivers of nestedness in our system. Indeed, our results suggest that colonization is important in driving nestedness across the Doñana pond network. Although dispersal events may be concentrated during the filling and desiccation of ponds, they are also likely to be occurring continuously to avoid sub-optimal conditions e.g. adverse conditions of temperature and food or in association with vital processes such as feeding and reproduction (Bilton *et al.* 2001; Williams 2006). Such inter-pond movements would help maintain the nested structure of the macroinvertebrate assemblages across the pond network.

Some of the idiosyncratic taxa were largely restricted to ponds with particular hydroperiods, although many had distributions which were not apparently driven by water regime. Taxa whose occurrence was influenced by hydroperiod included the diving beetles *Yola* bicarinata (Latreille, 1804) and *Hygrotus confluens* (Fabricius, 1775), the squeak beetle *Hygrobia hermanni* (Fabricius, 1775) and larvae of the damselfly *Lestes virens* (Charpentier, 1825), largely restricted to long hydroperiod sites, and some *Sympetrum* dragonfly larvae, which preferentially occurred in ponds with short and intermediate hydroperiods. Such taxa were apparently idiosyncratic through habitat specialization, something which may also apply to a number of taxa not restricted to ponds with a particular hydroperiod. Such taxa may have niches unrelated to the overall drivers of species diversity, or be distributed genuinely at random, reflecting stochastic colonisation processes. Either way, this would result in them occurring in species poor sites more often than expected, given their occupancy (McAbendroth *et al*., 2005). Snails such as *Physa* spp, as well as the whirligig beetle *Gyrinus dejeani* Brullé, 1832 and the invasive water boatman *Trichocorixa verticalis* (Fieber, 1851) apparently fall into this category. The number of idiosyncratic ponds in the three hydroperiod categories differed significantly. Amongst ponds detected as idiosyncratic, a similar proportion had short and long hydroperiods, whilst only five intermediate hydroperiod ponds were detected as idiosyncratic probably due to their intermediate position in the hydroperiod gradient allowing them to support eurytopic taxa which also occur in ponds with long and short hydroperiods.

Temporal variation in species richness and nestedness

Since temporary ponds are characterized by an annual process of filling and desiccation, every year much of their fauna undergoes a colonization-establishment and dispersal-emigration cycle. In the two study years, monthly nestedness increased from the month when ponds were first recorded as wet up until April-May, showing a similar pattern despite the longer inundation period during the wet year. Ponds began to dry up gradually from March onwards in the dry year and from May onwards in the wet year, short hydroperiod ponds drying first. We observed a high proportion of adult dispersers during the drying phase of ponds in all hydroperiod categories. At the end of this drying phase, only permanent ponds retained water, these supporting all aquatic macroinvertebrates except those with resistant stages such as some beetle larvae, which we have observed burying into the mud of drying ponds. When ponds are filled again the next year, macroinvertebrates start to recolonize (Wiggins *et al.* 1980; Taylor *et al.* 1999; Grillas *et al.* 2004; Williams 2006), reflected by the high proportion of adult dispersers at the beginning of the hydroperiod. The temporary ponds in our study receive the majority of macroinvertebrates via dispersal after the dry phase, making colonization potentially important in generating nestedness. In contrast, resident fauna with resistant stages may reduce the degree of nestedness, and such taxa (e.g. large branchipods - Brendonck 1996; Brendonck *et al.* 2008) represent a small proportion of the macroinvertebrates present in our sites (Florencio *et al.*, 2009).

The monthly change in the number of taxa per pond, following initial colonization, mirrored the monthly nestedness pattern; both increasing and decreasing together. Shurin (2007) suggested that higher invertebrate richness could be associated with more stable environmental conditions, and the increase of nestedness detected here along the annual hydroperiod may result from the tendency of macroinvertebrate assemblages to stabilize in composition, as a result of interspecific interactions, following initial, more stochastic assembly – a process which continues until ponds begin to dry out. As ponds dry, the degree of nestedness of the macroinvertebrate assemblages decreased or became insignificant. Whilst drying ponds may present more stressful environmental conditions for some taxa (e.g. lower oxygen content and increased temperature -Bazzanti *et al.* 1996), longer hydroperiod ponds retain optimal conditions for macroinvertebrates for longer. As a consequence, two distinct macroinvertebrate assemblages can be found in the study area: those with non-restrictive ecological traits in drying ponds, and those more specific to optimal conditions, explaining the reduction of nestedness during the drying phase. The variability in the monthly nested pattern along the annual hydroperiod coincided with their changeable idiosyncratic character between months and study years. This variability in the idiosyncratic character of temporary ponds could be due to unpredictability in environmental conditions in different seasons and years (García Novo *et al.* 1991; Serrano and Toja 1995; Gómez-Rodríguez *et al.* 2009) or because ponds supported different macroinvertebrate stages through their hydroperiods, depending on the life history traits of individual species (Florencio *et al.* 2009).

Implications for conservation

In nested systems the best conservation strategy may be to preserve a network of habitat patches within an area (Wright and Reeves 1992; Boecklen 1997). In systems where colonization plays an important role in creating nestedness, effective conservation of the regional biota must allow colonization and dispersal processes to take place (Cook and Quinn 1995; Monaghan *et al.* 2005). To allow the annual restructuring of the macroinvertebrate community in Doñana, it is therefore essential that the network of sites with differing hydroperiods are retained within the region. Nestedness, as detected in our study, is also an indicator of high quality habitats, with wide environmental gradients (Hylander *et al.* 2005), something which favours the conservation of natural temporary aquatic habitats, which have declined catastrophically elsewhere in Europe (Williams *et al.* 1997; Zacharias *et al.* 2007). Alongside the general nested pattern observed across the ponds studied here, a relatively high number of idiosyncratic taxa and sites were detected. From a conservation perspective, this finding also emphasises the need to maintain a diverse network of ponds to maintain regional biodiversity (Bilton *et al.* 2009), since even species-poor sites may contain specialist taxa, not found elsewhere within the region.

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Colaborators:

Carmen Díaz-Paniagua, Laura Serrano and David T. Bilton have colaborated in the elaboration of this manuscript which has been submitted to the journal “Oecologia” and is “*under review*”.

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Discusión

1) El estado de conservación de las especies de macroinvertebrados que habitan el sistema de lagunas temporales de Doñana

El sistema de lagunas temporales de Doñana se caracteriza por una alta variabilidad ambiental. Ésta se favorece, tanto por los cambios temporales que sufren las lagunas temporales, desde la inundación a la desecación y en años con diferente cantidad de precipitaciones, como por el amplio gradiente de hidroperiodo que las caracteriza a lo largo de todo el Parque Nacional. Esto se encuentra en consonancia con otros estudios realizados en estas lagunas (Serrano & Toja 1995; García-Novo *et al.* 1996; Serrano *et al.* 2006; Gómez-Rodríguez *et al.* 2009).

Por otra parte, unido a la variabilidad ambiental, la gran abundancia de lagunas y la alta conectividad que caracteriza al sistema de charcas temporales favorecen el intercambio de especies de macroinvertebrados con excelentes habilidades dispersivas, por lo que las lagunas temporales de Doñana constituyen un sistema altamente anidado en cuanto a la composición de macroinvertebrados. El anidamiento es una propiedad de los sistemas que presentan un patrón de distribución de especies con un alto grado de solapamiento entre medios con alta y baja riqueza específica (Atmar & Patterson 1993). En un sistema anidado como Doñana, donde la composición de macroinvertebrados de los distintos medios es bastante homogénea, se observa que determinadas especies presentan una distribución exclusiva, en lugares con características ambientales particulares. Estas especies singulares, así como las características ambientales que las condicionan son de especial interés para la conservación en los sistemas anidados ya que confieren cierta heterogeneidad y favorecen una alta diversidad en estos sistemas. Muchas de estas especies (denominadas idiosincráticas) se encuentran en lugares en los que no sería esperable encontrarlas dentro de una distribución anidada, constituyendo una composición específica que difiere de la composición general del resto de lagunas del sistema (McAbendroth *et al.* 2005).

La distribución anidada de las especies se ve favorecida en sistemas que incluyen excelentes dispersores (Cook & Quinn 1995) así como en los que presentan una alta variabilidad ambiental (Hylander *et al.* 2005). En el caso del sistema de lagunas de Doñana existen ambas características: es un sistema altamente conectado que se coloniza por dispersores que difieren en capacidades de dispersión, sobre todo entre los dispersores activos y pasivos. Por tanto, la alta variabilidad ambiental del sistema, el alto grado de anidamiento de las especies, sus excelentes capacidades de dispersión y la alta riqueza específica de los medios determinan que la comunidad de macroinvertebrados del sistema natural de lagunas temporales de Doñana se pueda considerar en excelentes condiciones de conservación, donde se favorece una alta diversidad específica a pesar de las características fluctuantes propias de los medios acuáticos temporales.

Estatus de conservación de las especies de macroinvertebrados de Doñana

La riqueza de macroinvertebrados detectada en las lagunas del Parque Nacional de Doñana resultó elevada en comparación con otros estudios que han registrado niveles taxonómicos similares a nuestro estudio, en Doñana se registraron 23.5 taxa/laguna mientras que en las lagunas mediterráneas del noreste de la Península Ibérica, Boix *et al.* (2008) sólo registraron un promedio de 2.7 especies/laguna, aunque hay que tener en cuenta que en Doñana se han considerado como taxones independientes las larvas y adultos de las especies. Por otra parte, considerando la riqueza total detectada en el área, en Doñana se superaron los 123 taxa, de los cuales el 80 % estuvieron identificados a nivel de especie. Esta riqueza se puede considerar alta comparada con otros sistemas de lagunas temporales mediterráneas (como se observa en Bazzanti *et al.* 1996; Boix *et al.* 2001; Waterkeyn *et al.* 2008). En nuestro estudio la mayoría de los dípteros se identificaron a nivel de familia, por lo que podrían incrementar aún más la alta riqueza específica de las laguna de Doñana ya que este grupo puede llegar a constituir la fauna dominante en determinadas lagunas, como ocurre en determinadas lagunas Mediterráneas del Norte de la Península (Trigal *et al.* 2007).

En las lagunas de Doñana, al igual que en otros sistemas de lagunas temporales, los principales grupos de macroinvertebrados fueron los coleópteros, los heterópteros y los odonatos (Taylor *et al.* 1999; Williams 2006). Las especies más frecuentes en Doñana pertenecieron a las familias de coleópteros Dytiscidae e Hydrophilidae, a las familias de heterópteros Corixidae y Notonectidae y en particular a los géneros de odonatos *Sympetrum*, *Lestes*, *Aeshna* e *Ischnura*.

La fauna de macroinvertebrados de las lagunas de Doñana se caracteriza por una mayor similitud con la fauna de ambientes lénticos del Sur Peninsular que con la del Norte Peninsular. Mientras que el 76% de las especies de coleópteros de Doñana (en base a la lista taxonómica de Picazo *et al.* 2010) y el 75% de las especies de heterópteros de Doñana (en base a la lista taxonómica de Millán *et al.* 2001) han sido registradas en lagunas del Sureste Peninsular, tan sólo el 40% de la fauna de coleópteros así como el 40% de la fauna de heterópteros han sido registrados en lagunas costeras del Norte de la Península (en base a la lista taxonómica de Garrido & Munilla 2008). Las especies de coleópteros y heterópteros del Norte Peninsular que fueron comunes a las que se citan en este estudio en Doñana, además de constituir un bajo número de especies difirieron en sus frecuencias de aparición y de abundancia entre ambas regiones: por ejemplo *Notonecta glauca* Linnaeus, 1758 ssp. *glauca* que es una especie rara en el Norte Peninsular (según Garrido & Munilla 2008), es una especie común que aparece en un gran número de lagunas en el área de Doñana. Además, las especies más habituales de coleópteros y heterópteros difirieron ampliamente entre el Norte y el Sur Peninsular: por ejemplo *Gerris gibbifer* Schummel, 1832, es el único gérrido citado en el Norte Peninsular (según Garrido & Munilla 2008), una especie no citada en las lagunas de Doñana que, sin embargo, presentaron de forma abundante la especie de gérrido *Gerris throacicus* Schummel, 1832 en este estudio. O por ejemplo, la especie más abundante detectada en las lagunas costeras del Norte de la Península según Garrido & Munilla (2008) fue *Hesperocorixa linnei* (Fieber, 1848), mientras que el género *Hesperocorixa* está completamente ausente en las lagunas de Doñana. En cuanto a las especies que se han detectado como localmente raras en Doñana, por ejemplo *Limnebius furcatus* Baudi, 1872 e *Hygrotus inaequalis* (Fabricius, 1777), éstas resultaron contrariamente abundantes en el Norte Peninsular (según Garrido & Munilla 2008).

A diferencia de la fauna del Norte Peninsular, la fauna de coleópteros de Doñana fue mucho más afín a la detectada en los ambientes lénticos del Sureste Peninsular donde las lagunas naturales presentaron como especies típicas *Agabus bipustulatus* (Olivier, 1795) y *Ochthebius dilatatus* (Stephens, 1829) (según Picazo *et al.* 2010), ambas especies también detectadas en las lagunas de Doñana en este estudio. En las lagunas naturales del Sureste Peninsular cabe destacar la ausencia de *Eretes griseus* (Fabricius, 1781) (según Picazo *et al.* 2010), un coleóptero de la familia Dytiscidae que destaca por su rareza en las lagunas de Doñana. Aunque en Doñana hay citas antiguas de *Eretes sticticus* (Linnaeus, 1767), Castro *et al.* (2003) sugiere que estas citas podrían corresponder a *E. griseus* y no a *E. sticticus*, especie que no ha sido citada en las lagunas de Doñana en este estudio, ni en estudios recientes sobre la fauna de coleópteros acuáticos en el área de Doñana (según Castro *et al.* 2003; Millán *et al.* 2005) ni entre las especies de coleópteros de ambientes lénticos citadas en el Sureste Peninsular (según Picazo *et al.* 2010). El coleóptero perteneciente a la familia Hydrophilidae *Enochrus fuscipennis* (C.G. Thomson, 1884) ha sido citado en el Sureste Peninsular como una especie de interés faunístico por su rareza en otros medios acuáticos (según Millán *et al.* 2001), y cuya identificación taxonómica ha sido recomendada por Millán *et al.* (2005) para el área de Doñana. En este estudio podemos confirmar que se trata de una especie frecuente y abundante en las lagunas temporales de Doñana, principalmente asociada a los márgenes someros y vegetados de las lagunas. En el Sureste Peninsular, dentro de las especies que se han detectado con más frecuencia completando sus ciclos de vida destacan *Gerris thoracicus* y *Naucoris maculatus* Fabricius, 1798. Ambas especies han sido detectadas en esta tesis como especies frecuentes en las lagunas temporales de Doñana, destacando la alta abundancia y el gran número de lagunas en las que apareció *G. thoracicus* y la abundancia de *N. maculatus,* aunque su aparición estuvo restringida a un menor número de lagunas. Algunas de las especies de coleópteros acuáticos citadas por primera vez en la región de Albacete (S.E. Peninsular) según Millán *et al.* (2001): *Agabus conspersus* (Marsham 1802), *Ilybius montanus* (Stephens, 1828), *Rhantus hispanicus* Sharp, 1882, *Hydaticus leander* (Rossi, 1790), y *Ditiscus circumflexus* Fabricius, 1801 aparecieron de forma común en las lagunas de Doñana incluidas en este estudio, a excepción de *H. leander* que puede considerarse una especie localmente rara en el área de estudio. Sin embargo, algunas de las especies encontradas en Doñana son catalogadas como localmente raras en el Sureste Peninsular (según Sánchez-Fernández *et al.* 2004), tales como *Cybister tripunctatus africanus*, *Berosus guttalis* Rey, 1883, *Hydrochus flavipennis* Küster, 1852, *Limnebius furcatus* Baudi, 1872 e *Hydrochara flavipes* (Stephens, 1808), esta última, además, estuvo catalogada como vulnerable para dicha región. A excepción de *Berosus guttalis*, que fue una de las especies más frecuentes en las lagunas de Doñana, en general estas especies se registraron de forma ocasional en el área de Doñana, especialmente *L. furcatus* que fue una especie rara en las lagunas temporales de Doñana. *Hydrochus flavipennis* fue principalmente detectada en el margen de aquellas lagunas próximas a su desecación a final de primavera o comienzos del verano. *Hydrochara flavipes*, a pesar de su rareza y vulnerabilidad en el Sureste Peninsular, fue una especie fácil de localizar en las lagunas de Doñana aunque no de forma abundante.

Dentro de las especies que han sido poco estudiadas en la Península Ibérica entre las que se encuentran las pertenecientes a la familia Scirtidae, cuyos adultos son de vida terrestre, y a la tribu Bagoini (según Millán *et al.* 2005), se han detectado especies pertenecientes a ambos grupos en las lagunas temporales de Doñana. Dentro de la familia Scirtidae, se han detectado larvas del género *Hydrocyphon,* que aunque apareció de forma frecuente en una de las lagunas temporales estudiadas, la laguna del Orfeón (Orf), no se trata de una especie frecuente en el resto de las lagunas del Parque

Nacional. Dentro de la tribu Bagoini, destacan las tres nuevas citas para el género *Bagous* en el área de Doñana que completarían la lista taxonómica de coleópteros acuáticos aportada por Millán *et al.* (2005): *B. revelieri* Tournier, 1884, *B. subcarinatus* Gyllenhal, 1836 y *B. vivesi* González, 1967.

Podemos concluir que las especies de macroinvertebrados de Doñana son más similares a la mitad Sur Peninsular y Norte de África que al Norte de la Península, tal y como sugieren los trabajos de Ribera *et al.* (1996) y Ribera (2000). En el área de Doñana se encuentran especies de coleópteros que presentan una distribución restringida iberoafricana, entre las que destacan (según Ribera *et al.* 1998): *Hygrotus lagari* (Fery, 1992), *Hydroporus lucasi* Reiche, 1866, dos especies de aparición frecuentes en las lagunas temporales de Doñana, *Laccobius revelierei* Perris, 1864, *Cybister tripunctatus africanus* (Castelnau, 1834) y *Ochthebius auropallens* Fairmaire, 1879, especies localmente raras en el área de estudio, y *Bagous revelieri* y *B. vivesi*, ambas nuevas citas para Doñana. Entre las especies de Heterópteros, sólo dos especies se han detectado en las lagunas de Doñana con dicha distribución iberoafricana (según Millán *et al.* 2001): *Microvelia pygmaea* (Dufour, 1833) y *Sigara scripta* (Rambur, 1840). Igualmente, la especie de notostráceo *Triops mauritanicus* (Ghigi, 1921) presenta una distribución restringida al Norte de África y Sur Peninsular, a diferencia de la especie *Triops cancriformis* (Lamarck, 1801) que es común a toda Europa (Korn *et al.* 2006).

También cabe destacar la presencia de dos especies en las lagunas de Doñana por su rareza en el territorio nacional: *Haliplus andalusicus* Wehncke, 1874 en Doñana, que constituye una de las especies más amenazada del Norte de Marruecos (Bennas *et al.* 2009), y *Rhantus hispanicus* Sharp, 1882, que es destacada por su interés biogeográfico y faunístico en la Península Ibérica (Millán *et al.* 2001; Millán *et al.* 2005). Sin embargo, ambas especies *H. andalusicus* y *R. hispanicus* fueron frecuente en las lagunas de Doñana, con la particularidad de que *R. hispanicus* fue una especie abundante en las lagunas en las que apareció.

Para algunas especies, Doñana representó su límite de distribución más meridional, como ocurre con *Hydroporus gyllenhalii* Schiödte, 1841, *Limnoxenus niger* (Zschach, 1788) e *Hydraena rugosa* Mulsant, 1844, que son especies típicas del Norte de España (según Ribera *et al.* 1998), destacando *L. niger* que además es característica del Norte de Europa (según Millán *et al*. 2001).

La singularidad de la fauna de Doñana se debe principalmente debida a la similitud que presenta con la fauna del Norte de África, así como por la mayor rareza o escasez de especies características de la Península Ibérica o de la fauna europea. Sin embargo, no son frecuentes especies incluidas en catálogos de conservación, como las especies catalogadas como amenazadas en las Listas Rojas de la IUCN (IUCN 2010). De hecho, tan sólo 19 especies de macroinvertebrados de Doñana se encuentran catalogados de alguna manera en las Listas Rojas de invertebrados de España (Verdú & Galante 2006), de Andalucía (Barea-Azcón *et al.* 2008) o de la Región Mediterránea (Riservato *et al.* 2009). La mayoría de los invertebrados y macroinvertebrados acuáticos constituyen grandes ausencias en las Listas Rojas de la IUCN a excepción de las especies de Odonatos (IUCN 2010), probablemente debido a la carencia de información respecto a su estado de conservación. De hecho, 18 especies de los Odonatos citados en Doñana aparecieron catalogados en las Listas Rojas, lo que contrasta con el hecho de que haya una sola especie de coleóptero acuático, *Acilius duvergeri* Gobert, 1874, y ninguna de heterópteros, notostráceos, conscostráceos ni anostráceos. En particular, la presencia de *A. duvergeri* no ha sido citada en estudios faunísticos recientes de coleópteros acuáticos en la región de Doñana (Millán *et al.* 2005), ni durante los dos años de muestreos intensivos realizados en este estudio, lo que sugiere la posible extinción de dicha especie a nivel local. En general, la ampliación del conocimiento sobre la distribución y abundancia de las especies de macroinvertebrados acuáticos, y en particular de las lagunas temporales, debe considerarse esencial para asignar una correcta clasificación en las categorías correspondientes de conservación.

En los sistemas de lagunas altamente conservados, pueden aparecer especies que aún siendo abundantes, presentan unos requerimientos ecológicos especiales que permiten su aparición. Tanto estas especies como los parámetros ecológicos que rigen su presencia son de consideración importante para la conservación de los sistemas de lagunas temporales ya que, además de incrementar la riqueza de un área, hacen singular a la comunidad y pueden denotar problemas de conservación que afectan a su abundancia. En este estudio, consideramos de gran interés la conservación de las especies de macroinvertebrados que presentaron una distribución idiosincrática. Entre estas especies idiosincráticas, se identificaron aquellas que aparecieron bajo condiciones ambientales particulares y que contribuyeron a la disimilaridad global del sistema: *Sympetrum fonscolombei* (Selys, 1841), *Anacaena lutescens* (Stephens, 1829), *Chironomus plumosus* (Linneo, 1758), *Hydrobius fuscipes* (Linnaeus, 1758) &

Limnoxenus niger (Zschach, 1788), *Gerris thoracicus* Schummel, 1832, *Sigara lateralis* (Leach, 1817), *Helophorus* spp., *Physa* spp, *Hygrotus lagari* (Fery, 1992) y las larvas de Corixidae. A pesar de presentar distribuciones particulares, estas especies singulares fueron frecuentes y abundantes en las lagunas temporales de Doñana. En total se han clasificado 59 especies de macroinvertebrados con una distribución idiosincrática en el área de Doñana. La simple presencia de estas especies en el área de Doñana, lo configura como un lugar de importancia para la conservación de macroinvertebrados acuáticos.

Las especies de macroinvertebrados en las lagunas de distinto hidroperiodo:

La longitud del hidroperiodo de las lagunas temporales representa el periodo en que las especies acuáticas pueden habitarlas, por lo que condiciona el desarrollo de la fase acuática en muchas especies. En función de éste, se pueden encontrar distintas especies en las lagunas temporales. En las láminas 1 y 2 se observan lagunas catalogadas con distinto hidroperiodo desde su inundación a comienzos de noviembre hasta el mes de su desecación, Mayo para las lagunas de corto hidroperiodo e hidroperiodo intermedio, y Julio para la de largo hidroperiodo.

Las lagunas de corto hidroperiodo: En estas charcas la fauna más singular se caracteriza por los denominados "grandes branquiópodos". Estas especies son habituales principalmente en las lagunas de duración más efímera. En Doñana se han detectado anostráceos como *Branchipus cortesi* Alonso y Jaume, 1991, *B. schafferi* Fischer de Waldheim, 1834 o *Tanymastix stagnalis* (Linnaeus, 1758); concostráceos como *Cyzicus grubei* Simon, 1886 y *Maghrebestheria maroccana* Thiéry, 1988; y el notostráceo *T. mauritanicus*. A pesar de que estás especies son típicas de las lagunas de corto hidroperiodo en Doñana, éstas son de aparición temprana y son sustituidas por otras especies conforme avanza el hidroperiodo. Entre estas especies de aparición más tardía destacan las larvas depredadoras de gran tamaño de la familia Dytiscidae (*Dytiscus circumflexus* Fabricius, 1801, *Cybister lateralimarginalis* (De Geer, 1774) *y C. tripunctatus africanus*), que pueden contribuir a la desaparición de las especies de branquiópodos por depredación. En estas lagunas de corto hidroperiodo de Doñana se observan también preferentemente otras especies, como *Agabus conspersus* (Marsham 1802), *A. nebulosus* (Forster, 1771) y *A. didymus* (Olivier, 1795), así como las larvas de la familia Culicidae, excelentes presas para *T. mauritanicus* y para las especies de coleópteros depredadores. Hay que señalar, que la aparición de larvas de coleópteros en estas lagunas no asegura que las especies completen sus ciclos de vida, ya que no todas las especies de larvas han sido observadas en su estado adulto hacia el final del hidroperiodo. A pesar de la mayor riqueza de macroinvertebrados de las lagunas de hidroperiodo más largo, las especies típicas de las de corto hidroperiodo son exclusivas o poco frecuentes en el resto de lagunas constituyendo una pieza clave para mantener la biodiversidad en los sistemas de lagunas temporales (Collinson *et al.* 1995; Williams 1997; Céréghino *et al.* 2008).

Las lagunas de hidroperiodo intermedio: Estas lagunas en Doñana pueden presentar una fauna de macroinvertebrados común a las de corto hidroperiodo, como *T. mauritanicus* y otros anostráceos. También presentan especies comunes con lagunas de más largo hidroperiodo, dada su posición intermedia en el gradiente de hidroperiodo. De hecho, una especie de anostráceo, *Streptocephalus torvicornis* (Waga, 1842), que además constituye la primera cita para Doñana (según Fahd *et al.* 2009), fue encontrada en dos lagunas de hidroperiodo intermedio en todo el Parque Nacional. Estas lagunas presentan orillas con abundnte vegetación ricas en coleópteros de la familia Hydrophilidae como las especies *Hydrobius convexus* Brullé, 1835, *H. fuscipes*, *Hydrochara flavipes*, *Enochrus fuscipennis*, *E. bicolor* (Fabricius, 1792), o los pequeños coleópteros *A. lutescens* y *Paracymus scutellaris* (Rosenhauer, 1856), que además son especies que suelen coexistir en zonas someras embarradas. Además, estas zonas de orilla son las preferidas por el escorpión de agua *Nepa cinerea* Linnaeus, 1798, que suele habitar bajo la vegetación, y el ditíscido *Hydroglyphus geminus* (Fabricius, 1792), que suele hacer pequeños agujeritos en el sedimento libre de vegetación. En las zonas más profundas donde crecen los macrófitos sumergidos y semiflotantes, se encuentran coleópteros que son buenos nadadores, como por ejemplo los ditíscidos *Colymbetes fuscus* (Linnaeus, 1758), *Rhantus hispanicus* o *R. suturalis* (McLeay, 1825); así como algunas especies de heterópteros como *Naucoris maculatus* Fabricius, 1798; o las larvas de especies de odonatos como las de los géneros *Sympetrum* (*S. fonscolombei*, *S. striolatum* (Charpentier, 1840), *S. meridionale* (Selys, 1841) y *S. sanguineum* (Müller, 1764)), *Aeshna* (*A. affinis* Vander Linden, 1823 y *A. mixta* Latreille, 1805) y *Lestes* (*L. dryas* Kirby, 1890, *L. barbarus* (Fabr., 1798), *L. macrostigma* (Eversm., 1836) y *L. virens* (Charpentier, 1825)). Aunque la mayoría de los odonatos requieren más de un año para completar sus ciclos de vida, las especies adaptadas a los medios acuáticos temporales presentan desarrollos larvarios rápidos (Wiggins *et al.* 1980). Este es el caso de las especies presentes en Doñana, tanto del género *Lestes* como de las especies de la familia Coenagrionidae, como por ejemplo *Ischnura pumilio*, que además, forman estructuras de resistencia frente a la desecación (Askew 1988; Williams 2006; Hassall & Thompson 2008; Samraoui 2009).

Lagunas de largo hidroperiodo: Suelen ser las que presentan mayor riqueza de especies de macroinvertebrados (Schneider & Frost 1996; Bilton *et al.* 2001a; Waterkeyn *et al.* 2008), ya que las especies disponen de más tiempo para completar sus ciclos de vida. El incremento de riqueza en estas laguna es especialmente notable hacia el final del hidroperiodo anual, cuando reciben gran cantidad de especies dispersantes que provienen de las lagunas de desecación más temprana. Además, muchas especies son de aparición tardía en las lagunas de Doñana con lo que requieren lagunas con hidroperiodo más largo, como es el caso de las especies de la familia Coenagrionidae *Ischnura pumilio* (Charp., 1825) e *Ischnura elegans* (Vander Linden, 1820). Muchas especies de las lagunas de hidroperiodo largo de Doñana son comunes a las que hemos citado para las de hidroperiodo intermedio. Hay que subrayar la presencia de dos especies de odonatos catalogados como vulnerables, *Coenagrion scitulum* (Rambur, 1842) y *Lestes macrostigma* (Eversm., 1836), que exclusivamente fueron encontrados en cinco y en dos lagunas de hidroperiodo largo, respectivamente. Es muy característico que estas lagunas presenten una gran abundancia de heterópteros hacia el final del hidroperiodo, entre ellos, las especies más frecuentes son *Sigara lateralis* y *Anisops sardeus* Herrich-Schäffer, 1849, además de *Corixa affinis* Leach, 1817, *Notonecta glauca* Linnaeus, 1758, *N. maculata* Fabricius, 1794 y *N. viridis* Delcourt, 1909. Las lagunas temporales naturales más permanentes de Doñana, que se alimentan de la descarga directa del freático (Sacks *et al.* 1992), presentan en las orillas abundantes zonas encharcadas que se mantienen a lo largo de gran parte del periodo de inundación albergando especies que persisten en estos hábitats como por ejemplo *Dryops luridus* (Erichson, 1847), *Noterus laevis* Sturm, 1834, *E. fuscipennis* y *E. bicolor*. Además, otras especies que se observan frecuentemente en las lagunas de largo hidroperiodo de Doñana son los coleópteros *Hygrobia hermanni* (Fabricius, 1775) y *Berosus affinis* Brullé, 1835. En algunas de estas lagunas, puede encontrarse temporalmente el pez exótico *Gambusia holbrooki* (Girard, 1859), que las coloniza en momentos de gran inundación en los que se produce la conexión con otros medios acuáticos del área (Fahd *et al.* 2007).

Los **zacallones** son un tipo de lagunas de largo hidroperiodo de carácter antrópico, ya que han sido excavadas por el hombre. Presentan un reducido tamaño con menor heterogeneidad de microhábitats que las demás lagunas y generalmente tienen alta densidad pero no gran diversidad de vegetación acuática. Sin embargo, los zacallones son importantes porque sustentan una gran riqueza de macroinvertebrados, especialmente los del sur del Parque Nacional en la zona más árida denominada "Marismillas", donde constituyen el único reservorio acuático para las especies de macroinvertebrados durante la desecación de las lagunas temporales.

2) Las comunidades de macroinvertebrados en el sistema de lagunas de Doñana

La composición faunística detectada en un estudio está relacionada con la intensidad de muestreo y con la efectividad de las técnicas utilizadas. En nuestro estudio, la manga permitió una caracterización eficiente de las comunidades de macroinvertebrados que habitan las lagunas temporales de Doñana. La alta riqueza de taxones detectados podría ser aún superior si se hubiera combinado el uso de la manga con otras técnicas de menor tamaño de poro que favorecería la captura de especies de dípteros (por ejemplo especímenes de la subfamilia Tanypodinae), nematodos y otros invertebrados, tales como pequeños crustáceos de cladóceros, copépodos y rotíferos. El uso combinado de estas técnicas con la nasa, que resultó una técnica eficiente para capturar especies nadadoras activas y que presentan ciclos diarios de actividad, sería la combinación perfecta para representar de forma más completa la comunidad de macroinvertebrados acuáticos de las lagunas temporales de Doñana. Dado que la manga es una técnica efectiva en la captura de la fauna más representativa de las lagunas de Doñana, su uso permitió detectar la presencia y distribución del coríxido exótico *Trichocorixa verticalis verticalis* (Fieber, 1851) en las lagunas del Parque Nacional. Además, se ha detectado una población reproductora de esta especie en Veta la Palma, localizada en los alrededores del Parque Nacional, y otra en Caracoles, dentro de un área recientemente regenerada e incluída como Parque Nacional. Ambas poblaciones podrían constituir un foco de dispersión para esta especie que sólo presentó individuos dispersores de forma aislada en las lagunas de Doñana. Sin embargo, la frecuencia de ocurrencia de esta especie exótica fue muy superior a algunas de las especies autóctonas de coríxidos del Parque Nacional tales como *Paracorixa concinna* (Fieber, 1848), *Sigara selecta* (Fieber, 1848) y *Micronecta scholzi* (Fieber, 1860). En particular, los zacallones de la zona sur de "Marismillas" que se caracterizaron por una mayor frecuencia de especies de coríxidos, y en particular, de esta especie exótica, podrían llegar a constituir un reservorio para esta especie en Doñana. Consideramos la detección temprana de esta especie exótica en Doñana como el primer paso para prevenir el establecimiento de las poblaciones exóticas (según Genovesi 2007), y en particular en las lagunas de Doñana.

La alta variabilidad que caracteriza las lagunas temporales de Doñana en sus condiciones ambientales (Serrano & Toja 1995; Serrano *et al.* 2006; Gómez-Rodríguez *et al.* 2009; Gómez-Rodríguez *et al.* 2010) así como en la composición de su fauna asociada por ejemplo anfibios y macroinvertebrados (Florencio *et al.* 2009a, Gómez-Rodríguez *et al. in press*), dificulta, e incluso, imposibilita el establecimiento de especies no adaptadas a medios temporales (Williams 2006). La variación estacional de la composición de macroinvertebrados en el sistema de lagunas temporales de Doñana se clasificó en las fases que caracterizan a este sistema y que se denominaron: fase de inundación, fase acuática y fase de desecación (siguiendo la terminología de Bazzanti *et al.* 1996 y Culioli *et al.* 2006). Aunque la variación de la composición de la comunidad de macroinvertebrados a lo largo de estas fases estacionales suele estar atribuída a variaciones ambientales (Boulton & Lake 1992; Boix *et al.* 2004; Culioli *et al.* 2006), en Doñana se debe principalmente a las diferentes estrategias de vida que presentan las especies a través de sus ciclos de desarrollo. En la fase de inundación, en la que se produce la formación de la mayoría de las lagunas temporales de Doñana, fueron abundantes los coleópteros y heterópteros dispersores. La dispersión junto con la emergencia de las formas de resistencia son las principales estrategias con las que los macroinvertebrados colonizan las lagunas temporales tras la inundación (Wiggins *et al.* 1980; Bilton *et al.* 2001b; Williams 2006). De hecho, la fase de inundación determina el éxito de ambos procesos, constituyendo la fase estacional en la que las condiciones ambientales a través del pH, la conductividad y la profundidad máxima, son más influyentes en la estructura de las comunidades de macroinvertebrados. Estas variables ambientales son determinantes para la emergencia de la fauna residente (Brendonck 1996; Brendonck *et al.* 2008) así como para el establecimiento de la fauna dispersante (Boulton & Lake 1992). Durante la fase acuática, la más estable en composición faunística y en variaciones ambientales de las lagunas, las formas larvarias fueron abundantes y las especies desarrollaron sus ciclos de vida. En esta fase, las variables ambientales apenas tuvieron influencia sobre la estructura de las comunidades de macroinvertebrados que probablemente se vieron más afectadas por las interacciones bióticas, como la depredación, ya que el número de depredadores tiende a incrementarse a lo largo del hidroperiodo (Schneider & Frost 1996; Wellborn *et al.* 1996; Spencer *et* *al.* 1999; Bilton *et al.* 2001a). Algunas variables están relacionadas con la longitud de hidroperiodo, aunque con poco poder explicativo, destacando la profundidad máxima, que fue mayor en las lagunas de más largo hidroperiodo, y el fósforo y el hierro total del sedimento cuyas concentraciones son mayores en lagunas de más largo hidroperiodo (Florencio *et al.* 2009b). La fase de desecación es un proceso gradual que comienza con la desecación de las lagunas de duración más efímera y termina con la desecación de las lagunas de más largo hidroperiodo. Esta fase se caracteriza por la variabilidad que se observa en las características ambientales y en la composición de macroinvertebrados de las lagunas, sobre todo entre las lagunas que se aproximan a su desecación y las de más largo hidroperiodo. Tanto los coleópteros como los heterópteros se suelen dispersar durante esta fase desde las lagunas que comienzan a desecarse hacia las lagunas de más largo hidroperiodo (Wiggins *et al.* 1980; Higgins & Merrit 1999; Bilton *et al.* 2001b, Williams 2006), lo que supone un brusco incremento en la riqueza específica de las lagunas que reciben a los dispersantes. Los cambios ambientales que se observaron durante el proceso de desecación particular de las lagunas fueron drásticos para todas las variables analizadas (profundidad máxima, materia orgánica, concentración de oxígeno disuelto, pH, conductividad, y las concentraciones totales de fósforo inorgánico disuelto y de hierro y fósforo del sedimento). Estos cambios pueden ser considerados como condiciones estresantes para las especies de macroinvertebrados que favorecen el inicio de las diferentes estrategias de supervivencia frente a la desecación, principalmente la formación de estructuras de resistencia y la dispersión (Wiggins *et al.* 1980; Hansen 2005; Williams 2006).

El grado de anidamiento se utiliza como una medida de la estructura de las comunidades en diferentes grupos taxonómicos (por ejemplo: Fleishman and Murphy 1999; Hylander *et al.* 2005; Meyer and Kalko 2008; Elmendorf & Harrison 2009). De la misma manera, el análisis del grado de anidamiento permite medir la variación estructural de las comunidades de macroinvertebrados a lo largo del ciclo de inundación-desecación de las charcas temporales. En nuestro estudio, el grado de anidamiento incrementó desde la inundación hasta el comienzo de la desecación junto con la riqueza de macroinvertebrados. La mayor estabilidad de la composición de especies parece determinar la mayor riqueza de invertebrados a lo largo del hidroperiodo (Shurin 2007), la cual podría favorecer el incremento del grado de anidamiento a lo largo de dicho hidroperiodo. Las diferencias ambientales que se observaron entre lagunas que se encontraban próximas a la desecación y las de más largo hidroperiodo, probablemente determinaron que la composición de macroinvertebrados no presentara una distribución anidada durante la fase de desecación en el sistema.

Las composición de macroinvertebrados en las lagunas fue bastante homogénea a lo largo de todo el Parque Nacional durante la primavera de 2007, aunque se observaron ciertas diferencias entre la zona Norte y la zona Sur ("Marismillas) del Parque. La escasa variabilidad en la composición de macroinvertebrados a lo largo de todo el Parque Nacional estuvo influenciada por un elevado número de variables relacionadas con la composición química del agua (el fosfato inorgánico disuelto, la materia orgánica, la turbidez y la conductividad), la estructura física de las lagunas (el número de lagunas circundantes, el área, la profundidad máxima y la riqueza de especies de plantas acuáticas), la proximidad a la costa y a la marisma, y la estructura espacial determinada por las distancias entre lagunas. Entre todas estas variables, la conductividad asociada espacialmente al gradiente Norte-Sur en el Parque Nacional, fue la variable que presentó mayor influencia en la composición de macroinvertebrados. Las distancias entre lagunas no afectaron a la composición de macroinvertebrados cuando se consideraron de forma independiente al resto de variables, demostrando las excelentes capacidades dispersivas que presentan la mayoría de especies en el área de estudio. Esto no es sorprendente dada la gran capacidad de dispersión que caracteriza a las especies de coleópteros y heterópteros acuáticos (Wiggins *et al.* 1980; Bilton *et al.* 2001b). La homogénea composición de macroinvertebrados observada en las lagunas de Doñana coincide con el alto grado de anidamiento detectado en estas comunidades, un patrón de distribución que se considera generado por la colonización diferencial de las especies (Patterson 1990; Atmar & Patterson 1993; Loo *et al.* 2002; Hausdorf & Hennig 2003) y sus excelentes capacidades dispersivas (Cook & Quinn 1995), la alta conectividad entre lagunas (Boecklen 1997; Cook & Quinn 1995; Wright *et al.* 1998; Higgins *et al.* 2006), y la variabilidad ambiental del sistema (Hylander *et al.* 2005).

Las especies catalogadas como idiosincráticas en este estudio son de interés potencial para la conservación, tal y como han sido consideradas en otros estudios (McAbendroth *et al.* 2005; Heino *et al.* 2009), ya que sus distribuciones, bajo condiciones ambientales particulares, incrementan la heterogeneidad y diversidad del sistema.

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Láminas

Laguna de corto hidroperiodo: Laguna “Adyacente al Navazo del Toro” (Ant)

Noviembre 2006 Diciembre 2006 Enero 2007 Febrero 2007 Marzo 2007

Abril 2007 Mayo 2007

Laguna de hidroperiodo intermedio: Laguna del “Camastrón” (Cam)

Noviembre 2006 Diciembre 2006 Enero 2007 Febrero 2007 Marzo 2007

Abril 2007 Mayo 2007

Lámina 1

Laguna de hidroperiodo intermedio: Laguna "Pinar Pequeño" (Pp)

Noviembre 2006 Diciembre 2006 Enero 2007 Febrero 2007 Marzo 2007

Abril 2007 Mayo 2007

Laguna de largo hidroperiodo: Laguna del "Zahillo" (Zah)

Noviembre 2006 Diciembre 2006 Enero 2007 Febrero 2007 Marzo 2007

Abril 2007 Mayo 2007 Junio 2007 Julio 2007

Lámina 2

Conclusions / Conclusiones

Conclusions:

1. Sampling techniques can have an important influence in the analysis of macroinvertebrate communities. Dip-netting is an efficient technique for estimating richness and abundance of macroinvertebrates, and hence obtaining a representative picture of these communities in temporary ponds. However, this technique does not detect diel activity rhythms of the species, which are better analysed with other sampling techniques, such as funnel nets. The combination of both sampling techniques would draw the best picture of the macroinvertebrate community.
2. The exotic bug *Trichocorixa verticalis* is expanding over the ponds of the Doñana National Park where vagrant-disperser individuals were detected. There are two important reproductive populations of this exotic species, in the surrounding area of the Park (Veta la Palma) and in an area recently included into the National Park (Caracoles), which constitute a source for its dispersal.
3. The seasonal variation of the macroinvertebrate assemblages of Doñana temporary ponds can be grouped at least in three wet phases: an initial phase in which most ponds across the whole network are filled, characterised by the macroinvertebrate pond colonization through dispersal and emergence of resident fauna; the intermediate or aquatic phase, in which macroinvertebrates species develop their life-cycles; and the drying phase consisting in the desiccation of most ephemeral water bodies, onset of dispersal toward more permanent ponds, and the production of drought-resistant forms.
4. Macroinvertebrate assemblage compositions of Doñana temporary ponds differed between years with different pond hydroperiod. Nonetheless, the assemblages showed a similar overall seasonal pattern of composition between years.
5. In Doñana temporary ponds, environmental characteristics differed among ponds as well as between years with different hydroperiods. However, pond environmental characteristics within years did not vary across the seasonal phases described for macroinvertebrate assemblages of these ponds.
6. Electrical conductivity, maximum depth and pH significantly affected the structure of macroinvertebrate assemblages in temporary ponds throughout the inundation-desiccation cycle. Hydroperiod was the variable of greater influence

in macroinvertebrate assemblage structures, followed by conductivity, maximum depth and total sediment phosphorus and iron concentrations.

7. During pond desiccation, there was a drastic change in environmental variables (maximum depth, organic matter, dissolved oxygen concentration, pH, conductivity, total dissolved phosphorus concentration and total sediment phosphorus and iron concentrations). This environmental stress triggers the onset of survival mechanisms for typical species of temporary waters.
8. The main environmental difference among ponds of the Doñana National Park was observed in spring 2007 between temporary ponds and human-made ponds (zacallones). This pond differentiation occurred across a North-South axis mainly differing in electrical conductivity and their co-variables pH, alkalinity and ion concentrations.
9. Environmental variables that significantly influenced the macroinvertebrate assemblages across the Doñana National Park ponds in spring 2007 were: dissolved inorganic phosphate, organic matter, turbidity and electrical conductivity as water chemical composition; the number of surrounding ponds, area, maximum depth and aquatic plant species richness as physical variables; distance from the ponds to the marsh and to the coast; and the spatial effects determined by inter-pond distances. Among these variables, water chemistry, mainly electrical conductivity, was the most important variable affecting the structure of these assemblages.
10. Coleopterans are excellent indicators of the pond environmental characteristics in the Doñana National Park.
11. The high environmental variability of the pond network in the Doñana National Park is essential to preserve a high macroinvertebrate diversity and favours the occurrence of singular species in particular environments.
12. The macroinvertebrate assemblages of Doñana were constituted as a highly nested system, sustained by the annual colonization of ponds, through the species differential dispersal capabilities, and the environmental variability of ponds.
13. Macroinvertebrate assemblages of Doñana temporary ponds showed a similar seasonal nested pattern in the two study years. This pattern showed a gradual increase in the degree of nestedness until the start of the drying phase.

14. Idiosyncratic species were mainly detected in ponds with particular environmental characteristics. These species and habitats should be considered with high interest for conservation in Doñana National Park.

Conclusiones:

1. Las técnicas de muestreo pueden tener una gran influencia en el análisis de la composición de macroinvertebrados de un medio. La manga es una técnica de muestreo efectiva para la captura de una alta riqueza y abundancia, y muestra una composición representativa de los macroinvertebrados de una laguna temporal. Sin embargo, no refleja la influencia de los ritmos de actividad diaria de las principales especies que sí puede ser detectada con otras técnicas, como las nasas. El uso combinado de ambas técnicas ofrece una imagen más completa de la comunidad.
2. La invasión del coríxido exótico *Trichocorixa verticalis* se encuentra en expansión dentro de las lagunas del Parque Nacional de Doñana, con individuos dispersantes aislados. Existen dos importantes poblaciones reproductoras, en el área circundante del Parque Nacional (Veta la Palma) y en un área de reciente inclusión en el Parque nacional (Caracoles) que constituyen un foco para dicha expansión.
3. La variación estacional de la comunidad de macroinvertebrados de las lagunas temporales de Doñana se divide al menos en tres fases: la fase inicial de inundación del sistema de lagunas, en la que los macroinvertebrados colonizan las lagunas a través de la dispersión y con la emergencia de la fauna residente; la fase intermedia o acuática en la que se produce el desarrollo de los ciclos de vida de las especies de macroinvertebrados; y la fase de desecación del sistema de lagunas que comienza con la desecación de las lagunas más efímeras en las que las especies activan sus estrategias de supervivencia, como la dispersión o formación de fases de resistencia. Las comunidades de macroinvertebrados que oscilan más fuertemente a lo largo de este patrón estacional son las que pertenecen a las lagunas de corto hidroperiodo.
4. Las comunidades de macroinvertebrados de las lagunas temporales de Doñana difirieron entre ambos años de estudio acorde con las diferencias interanuales en hidroperiodo. A pesar de estas diferencias, las comunidades mostraron un patrón

global de variación estacional general para ambos años al considerar conjuntamente el gradiente anual de hidroperiodo.

5. Las lagunas temporales de Doñana mostraron características ambientales singulares entre lagunas y en los dos años de estudio. Sin embargo, no se produjo un patrón estacional en estas características ambientales concordante con las variaciones estacionales de las comunidades de macroinvertebrados.
6. Las comunidades de macroinvertebrados de las lagunas temporales estuvieron influenciadas por la variabilidad ambiental a lo largo del periodo de inundación. El pH, la conductividad y la profundidad máxima influyeron significativamente en el establecimiento de las especies dispersantes y la emergencia de las formas residentes. Podemos considerar la longitud del hidroperiodo, a través de sus co-variables (conductividad, profundidad máxima y las concentraciones de P y Fe del sedimento), como la variable que tiene mayor influencia a lo largo de la inundación-desecación en la estructura de las comunidades de macroinvertebrados de las lagunas temporales de Doñana.
7. La desecación de las lagunas temporales se caracterizó por un cambio drástico en los valores de todas las variables ambientales (profundidad máxima, materia orgánica, concentración de oxígeno disuelto, pH, conductividad, y las concentraciones totales de fósforo inorgánico disuelto y de hierro y fósforo del sedimento). Esto supone un estrés ambiental que dispara el desarrollo de las estrategias de supervivencia para las especies de macroinvertebrados adaptadas a estos medios temporales.
8. La principal diferencia ambiental entre las lagunas del Parque Nacional de Doñana se observó en la primavera de 2007 entre las lagunas temporales y las lagunas de origen humano denominadas zacallones a lo largo del eje Norte-Sur, mostrando las principales diferencias en conductividad y en sus co-variables pH, alcalinidad y concentración de iones.
9. Las variables ambientales que significativamente influenciaron las comunidades de macroinvertebrados de las lagunas distribuidas por todo el Parque Nacional de Doñana fueron el fosfato inorgánico disuelto, la materia orgánica, la turbidez y la conductividad como componentes de la química de agua; el número de lagunas circundantes, el área, la profundidad máxima y la riqueza de especies de plantas acuáticas como variables físicas del medio ; la distancia a la marisma y a la costa; y el efecto espacial determinado por las distancias entre lagunas. Entre

estas variables, la composición química del agua, y sobre todo la conductividad, fue la variable con mayor influencia en la estructura de estas comunidades.

10. Los coleópteros acuáticos resultaron excelentes indicadores de las condiciones ambientales de las lagunas del Parque Nacional de Doñana.

11. La alta variabilidad ambiental del sistema de lagunas del Parque Nacional de Doñana es esencial para preservar una alta diversidad de macroinvertebrados, dando lugar a comunidades singulares.

12. La colonización, a través de la dispersión diferencial de las especies, y la variabilidad ambiental de las lagunas determinaron que las comunidades de macroinvertebrados del Parque Nacional de Doñana se constituyeran como un sistema altamente anidado.

13. Se detectó un patrón estacional en el grado de anidamiento de las comunidades de macroinvertebrados de las lagunas temporales de Doñana que fue similar durante los dos años de estudio. El anidamiento mostró un incremento gradual desde el la fase de de llenado hasta el comienzo de la fase de desecación.

14. Se detectaron especies idiosincráticas principalmente en lagunas con características ambientales particulares. Estas especies y sus hábitats deben considerarse de alto interés para la conservación en el sistema de lagunas de Doñana.

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