Universitat de Girona

LIMNOLOGICAL CHARACTERISTICS AND ZOOPLANKTON COMMUNITY STRUCTURE OF MEDITERRANEAN COASTAL LAGOONS UNDERGOING RESTORATION

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Memòria presentada per Anna Badosa i Salvador per optar al grau de Doctor per la Universitat de Girona

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A la Quimeta, en Pere i en Carles,
els de casa.

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ABSTRACT

The Baix Ter Wetlands (NE Iberian Peninsula) are illustrative of wetlands located in a western Mediterranean coastal plain, where the large population, and intense industrial, agricultural and tourist activities exert strong impacts on them. Between 1999 and 2003, a restoration EU LIFE project was developed in order to recover and conserve the ecological value of the Baix Ter Wetlands. In these wetlands two different kinds of coastal ecosystems are found: La Pletera salt marsh, a confined brackish ecosystem, and the Ter Vell lagoon, a coastal freshwater ecosystem. Restoration and management actions were specific for each ecosystem since the environmental problem was different in each case. The present study was carried out in the framework of the scientific research behind this project in order to establish the natural functioning of both ecosystems, analyze their environmental problem and also to evaluate the effects of the restoration measures.

La Pletera salt marshes were affected by an urbanisation plan, which was only partially developed in 1987. As a consequence, the natural water regime changed, several lagoons and wetlands disappeared and, since then, salt marshes have remained almost totally isolated from the river and sea. The creation and stocking of new refuges (permanent brackish lagoons) for an endangered fish, the Iberian toothcarp (*Aphanius iberus*), were the main restoration actions carried out in La Pletera, where the distribution of this species had been restricted to only one lagoon. The role of the hydrological regime in the nutrient and zooplankton composition and dynamics was analysed in five permanent brackish lagoons, including the new ones (*A. iberus* refuges). In all of these waterbodies, the hydrology was determined by a prolonged period of confinement without water inputs, irregularly interrupted by sudden flooding events (sea storms or intense rainfalls). While the dynamics of oxidized nitrogen compounds depends on the water inputs variability, the internal load of phosphorus, total nitrogen and organic matter is related more to the cumulative mechanisms during the confinement periods. Accumulation processes may be easily related to lagoon age since old lagoons have a higher content of

nutrients and organic matter. This fact suggests a progressive accumulation of nutrients and organic matter during successive confinement events over the years.

The zooplankton community integrates the effects of both factors, the hydrological regime and the lagoon age since the former determines the temporal pattern and the latter explains differences in composition and structure between old and new lagoons. The zooplankton community structure in all the salt marsh lagoons was also analysed by means of the combination of taxon- and size-based approaches using a taxonomic and a size diversity measure, respectively. Both diversities showed different responses to several ecological factors. Whereas size diversity was mainly related to trophic interactions, such as fish predation or inter/intraspecific competition for food, taxonomic diversity appeared to be more sensitive to abiotic factors, such as nutrient concentrations.

As hydrology and nutrient dynamics, invertebrate community in the new lagoons was also similar to that of one old lagoon, which was the natural habitat of *A. iberus* in the marsh. Dense submerged *Ruppia* prairies, which provide refuge and decrease the predation risk for this species, developed rapidly in one of the new lagoons. The fast growth in size of the new *A. iberus* population recorded during the first two years after the stocking of new lagoons suggested that the new habitat provided a favourable refuge for this species, at least in the short-term.

The Ter Vell lagoon is a eutrophic coastal lagoon which has been flooded mainly by the excess irrigation water during the last decades. In this lagoon the restoration actions were mainly focused on the improvement of the water quality. To achieve this aim, constructed wetlands were built to reduce nutrient inputs, and sediment dredging was performed to reduce the organic content of the lagoon. Simultaneously, but independent of the restoration activities, water management in agriculture changed the irrigation system. As a consequence, there was a drastic reduction in the freshwater inflow and, therefore, a change in the hydrological regime. The short-term effects of this hydrological change on the limnological characteristics of the lagoon were analysed.

Before the hydrological change, the hydrology of the lagoon was artificial since the freshwater flooding period was prolonged due to the high agricultural demand. As a result, the lagoon had an exorheic character with a high water turnover rate. After the change, the lagoon showed a more climate-dependent hydrology and it had a more endorheic character, with scarce water inputs and prolonged confinement periods. Then, the nutrient composition and dynamics tended to be more similar to those observed in the confined lagoons in La Pletera salt marshes. Nutrient inputs diminished, and the internal organic load and salinity increased because the internal recycling and the accumulation processes became more relevant. However, zooplankton did not tend to resemble to that of the salt marsh lagoons, at least in the short-term. The observed effects on nutrients were, in fact, caused by the reduction in the freshwater inflow which impeded, in turn, the correct functioning of the constructed wetlands. Since the Ter Vell lagoon is in its origin a riverine wetland ecosystem, water managers should maintain a regular and continuous freshwater inflow to the constructed wetlands, at least during the winter-spring seasons.

In order to comply with the Water Framework Directive, all the management actions in an ecosystem should be focused on protecting or even on improving its ecological status. Thus, the short-term effects of water management actions, and the subsequent hydrological change, on the ecological status of the Ter Vell lagoon were analysed. Several commonly used physicochemical (Trophic State Indexes) and biological indicators (rotifers indicative of eutrophy, the *QAELS* index, community decriptors, for example) were applied to assess the ecological status before and after the management actions. Some specific results raised doubts about the suitability of using in the Ter Vell lagoon those indicators developed for ecosystems with very different hydrological functioning. However, if the most suitable indicators are taken into account, it can be concluded that a general, but small improvement of the ecological status of the Ter Vell lagoon took place after water management actions.

RESUMEN (in Spanish)

Los humedales del Baix Ter (NE Península Ibérica) son un ejemplo de humedales localizados en una llanura costera mediterránea sobre los que la elevada densidad de población y la intensa actividad agrícola, industrial y turística ejercen fuertes impactos. Entre 1999 y 2003 se desarrolló un proyecto europeo LIFE de restauración para recuperar y conservar los valores ecológicos de los humedales del Baix Ter. En estos humedales se encuentran dos tipos distintos de ecosistemas: la marisma de La Pletera, un ecosistema salobre confinado, y la laguna del Ter Vell, un ecosistema costero de agua dulce. Las acciones de restauración y gestión fueron específicas para cada ecosistema ya que su problemática ambiental era muy distinta. El presente estudio forma parte del seguimiento científico que acompañó este proyecto y tiene como objetivo establecer el funcionamiento de ambos ecosistemas, analizar su problemática ambiental, y a la vez evaluar los efectos de las medidas de restauración.

La marisma de La Pletera se vio afectada por un plan de urbanización que se desarrolló parcialmente in 1987. Como consecuencia, el régimen hidrológico cambió, varias lagunas y humedales desaparecieron y desde entonces la marisma ha permanecido casi totalmente aislada del río y del mar. La creación de nuevos refugios (lagunas salobres permanentes) para un pez amenazado, el fartet (*Aphanius iberus*) y la liberación de individuos de esta especie en estos refugios fueron las principales actuaciones de restauración en La Pletera, dónde la presencia de esta especie se había restringido en una única laguna. Se analizó el papel del régimen hidrológico en la composición y dinámica de los nutrientes y del zooplancton de cinco lagunas salobres permanentes, incluyendo las nuevas lagunas o refugios. En todas ellas la hidrología fue determinada por un largo período de confinamiento sin entradas de agua, interrumpido irregularmente por súbitas inundaciones (temporales de mar y precipitaciones intensas). Mientras que la dinámica de los compuestos oxidados del nitrógeno dependió de la variabilidad de las entradas de agua, la carga interna de fósforo, el nitrógeno total y la materia orgánica se relacionaron con

procesos de acumulación durante los períodos de confinamiento. Estos procesos de acumulación se pueden relacionar fácilmente con la edad de la laguna ya que las antiguas lagunas presentan un mayor contenido de nutrientes y de materia orgánica. Este hecho sugiere una progresiva acumulación de los nutrientes y la materia orgánica durante los sucesivos períodos de confinamiento a lo largo de los años.

La comunidad de zooplancton integra los efectos de ambos factores, el régimen hidrológico y la edad de la laguna, ya que el primero determina su patrón temporal y el segundo explica las diferencias en la composición y estructura entre las antiguas y las nuevas lagunas. La estructura de la comunidad del zooplancton en las lagunas de la marisma se analizó a través de la combinación de aproximaciones taxonómicas y de tamaños utilizando una medida de diversidad taxonómica y una de diversidad de tamaños, respectivamente. Ambas diversidades presentaron respuestas diferentes frente a distintos factores ecológicos. La diversidad de tamaños se relacionó principalmente con interacciones tróficas como la depredación por peces o la competencia intra e interespecífica por el alimento, mientras que la diversidad taxonómica fue más sensible a factores abióticos como la concentración de nutrientes.

Al igual que la hidrología y la dinámica de nutrientes, la comunidad de invertebrados en las nuevas lagunas fue similar a la de una de las antiguas lagunas, el hábitat natural de *A. iberus* en la marisma. Densas praderas sumergidas de *Ruppia*, que proporcionan refugio y disminuyen el riesgo de depredación, se desarrollaron rápidamente en una de las nuevas lagunas. El rápido crecimiento de la nueva población de *A. iberus*, detectado dos años después de la primera liberación de individuos, sugirió que el nuevo hábitat ha proporcionado un refugio favorable para esta especie, al menos a corto plazo.

La laguna de Ter Vell es una laguna eutrófica que durante las últimas décadas ha estado alimentada principalmente por el agua excedente de regadío. Las acciones de restauración en esta laguna se centraron principalmente en la mejora de la calidad del agua. Las principales acciones fueron la reducción de las entradas de nutrientes por medio de unos humedales construidos, y el dragado de sedimento para reducir el contenido orgánico de la laguna. Simultáneamente a estas acciones, pero de forma independiente, la gestión del agua en la agricultura cambio el sistema de irrigación. Como consecuencia, tuvo lugar una drástica reducción del caudal de entrada de agua dulce y, por tanto, un cambio en el régimen hídrico. Se analizaron los efectos a corto plazo que este cambio en la hidrología tuvo sobre las características limnológicas de la laguna.

Antes del cambio en el régimen hídrico, la laguna presentaba una hidrología artificial ya que el período de inundación de agua dulce se había prolongado debido a la elevada demanda agrícola. Consecuentemente, la laguna presentó un carácter exorreico con una elevada tasa de renovación del agua. Después del cambio, la hidrología fue más dependiente del clima y la laguna presento un carácter más endorreico, con escasas entradas de agua y largos períodos de confinamiento. Como consecuencia, la composición y la dinámica de los nutrientes tendieron a semejarse a las observadas en las lagunas confinadas de la marisma de La Pletera. Las entradas de nutrientes disminuyeron, y la carga orgánica interna y la salinidad aumentaron debido a que el reciclaje interno y los procesos de acumulación se hicieron más relevantes. No obstante, el zooplancton no tendió a semejarse al observado en las lagunas de la marisma, al menos a corto plazo. Todos los efectos observados en los nutrientes en realidad fueron causados por la reducción en el caudal de entrada de agua dulce el cual impidió a su vez el correcto funcionamiento de los humedales construidos. El hecho que la laguna de Ter Vell es un ecosistema de origen fluvial, la gestión del agua debería asegurar un flujo de entrada de agua hacia los humedales construidos continuo y regular, al menos durante las estaciones de invierno y primavera.

Para cumplir con la Directiva Marco del Agua, todas las actuaciones de gestión en un ecosistema deberían enfocarse en la protección o incluso en la mejora de su estado ecológico. Así, se analizaron los efectos a corto plazo de la gestión del agua, y del subsecuente cambio hidrológico, sobre el estado ecológico de la laguna de Ter Vell. Distintos indicadores físicoquímicos (Trophic State Indexes) y biológicos (rotíferos indicadores de eutrofia, el índice *QAELS*, parámetros descriptores de la comunidad, etc.), comúnmente utilizados, fueron aplicados en Ter

Vell para determinar su estado ecológico antes y después de las actuaciones de gestión. Algunos resultados obtenidos ofrecieron dudas sobre la conveniencia de utilizar en la laguna de Ter Vell aquellos indicadores desarrollados para ecosistemas con un funcionamiento hidrológico muy distinto. No obstante, si se tienen en cuenta los indicadores más adecuados, se puede concluir que después del cambio hidrológico hubo una ligera mejora del estado ecológico de la laguna.

RESUM (in Catalan)

Els aiguamolls del Baix Ter (NE Península Ibèrica) són un exemple d'aiguamolls situats en una plana costanera mediterrània on l'elevada població i les intenses activitats agrícoles, indústrials i turístiques exerceixen una forta pressió sobre ells. Entre el 1999 i el 2003 es va desenvolupar un projecte europeu LIFE de restauració per tal de recuperar i conservar els valors ecológics dels aiguamolls del Baix Ter. En aquests aiguamolls s'hi troben dos tipus diferents d'ecosistemes: la maresma de La Pletera, un ecosistema salobre confinat, i la llacuna del Ter Vell, un ecosistema costaner d'aigua dolça. Les accions de restauració i gestió foren específiques per cada ecosistema ja que la seva problemàtica ambiental era molt diferent. El present estudi forma part del seguiment científic que va acompanyar el projecte i té com a objectiu establir el funcionament d'ambdós ecosistemas, analitzar la seva problemàtica ambiental, i alhora evaluar els efectes de les accions de restauració.

La maresma de La Pletera es va veure afectada per un pla d'urbanització el qual es portà a terme parcialment en el 1987. Com a conseqüència el règim hídric va canviar, diverses llacunes i aiguamolls van desaparèixer, i des de llavors la maresma ha restat quasi totalment aïllada del riu i del mar. La creació de nous refugis (llacunes salobres permanents) per a un peix amenaçat, el fartet (*Aphanius iberus*), i l'alliberament d'individus d'aquesta espècie en els refugis han estat les principals actuacions de restauració a La Pletera, on la presència d'aquesta espècie estava restringida a una única llacuna. Es va analitzar el paper del règim hídric en la composició i dinàmica dels nutrients i del zooplancton de cinc llacunes permanents, incloent les noves llacunes o refugis. En totes elles, la hidrologia va ser determinada per un llarg període de confinament sense entrades d'aigua, interromput irregularment per sobtades inundacions (temporals de mar i precipitacions intenses). Mentre que la dinàmica de les formes oxidades del nitrogen va dependre de la variabilitat de les entrades d'aigua, la càrrega interna de fòsfor, el nitrogen total i la matèria orgànica es van relacionar amb els mecanismes d'acumulació durant els

períodes de confinament. Aquests processos d'acumulació es poden relacionar fàcilment amb l'edat de la llacuna ja que les llacunes antigues van presentar un major contingut de nutrients i matèria orgànica. Aquest fet suggereix una acumulació progresiva de nutrients i matèria orgànica durant successius períodes de confinament al llarg dels anys.

La comunitat zooplanctònica integra els efectes d'ambdós factors, el règim hídric i l'edat de la llacuna, ja que el primer en determina el patró temporal i el segon n'explica les diferències entre les llacunes antigues i les noves pel que fa la composició i estructura. L'estructura de la comunitat zooplanctònica també va ser analitzada per mitjà de la combinació d'aproximacions basades en la taxonomia i en la mida. Es van utilitzar una mesura de diversitat taxonòmica i una mesura de diversitat de mides, respectivament. Ambdues mesures van presentar respostes diferents davant diversos factors ecològics. La diversitat de mides es va relacionar principalment amb interaccions tròfiques com la predació per peixos o la competència intra- o interespecífica pel menjar, mentre que la diversitat taxonòmica es va mostrar més sensible a factors abiòtics com la variació de nutrients.

Així com la hidrologia i la dinàmica de nutrients, la comunitat d'invertebrats de les noves llacunes també va ser similar a la de una de les llacunes antigues, l'hàbitat natural d' *A. iberus* a la maresma. Densos prats submergits de *Ruppia*, que proporcionen refugi per a l'espècie i disminueixen el risc de predació, es van desenvolupar ràpidament en una de les noves llacunes. El ràpid creixement de la nova població d'*A. Iberus*, detectat durant els primers dos anys després de l'alliberament d'individus, va suggerir que el nou hàbitat havia esdevingut el refugi adequat per a l'espècie, almenys a curt termini.

La llacuna del Ter Vell és una llacuna eutròfica que durant les últimes dècades ha estat alimentada per l'aigua excedent de regadiu. Les accions de restauració en aquesta llacuna es van centrar principalment en la millora de la qualitat de l'aigua. Les principals actuacions foren la reducció de l'entrada de nutrients per mitjà d'uns aiguamolls construïts, i el dragatge de sediment per reduir el contingut orgànic de la llacuna. Simultàniament a aquestes actuacions, però de manera independent, la

gestió de l'aigua en l'agricultura va canviar el sistema de regadiu. Com a conseqüència va haver-hi una dràstica davallada del cabal d'entrada d'aigua dolça i, per tant, un canvi en el règim hídric. Es van analitzar els efectes a curt termini d'aquest canvi sobre les característiques hidrològiques de la llacuna.

Abans del canvi en el règim hídric, la llacuna presentava una hidrologia artificial ja que el període d'inundació d'aigua dolça s'havia allargat degut a l'elevada demanda agrícola. Com a conseqüència la llaguna presentava un caràcter exorreic amb una elevada taxa de renovació de l'aigua. Després del canvi, la hidrologia va dependre més de la climatologia i la llacuna va presentar un caràcter més endorreic, amb poques entrades d'aigua i llargs períodes de confinament. Com a conseqüència, la composició i la dinàmica dels nutrients van tendir a semblar-se més a les observades a les llacunes confinades de la maresma. Les entrades de nutrients van disminuir i la càrrega orgànica interna i la salinitat van augmentar degut a que el reciclatge intern i els processos d'acumulació van esdevenir més rellevants. No obstant, el zooplàncton no va semblar-se a l'observat a les llacunes de la maresma, almenys a curt termini. Tots els efectes observats en els nutrients foren en realitat causats per la reducció del cabal d'entrada d'aigua dolça que va impedir el correcte funcionament dels aiguamolls construïts. Ja que Ter Vell és una llacuna d'origen fluvial, la gestió de l'aigua hauria de garantitzar l'arribada d'un cabal d'aigua dolça regular i continu als aiguamolls construïts, com a mínim durant les estacions d'hivern i primavera.

Per tal de complir amb la Directiva Marc de l'Aigua, totes les actuacions de gestió en un ecosistema haurien d'enfocar-se en protegir i fins i tot millorar el seu estat ecològic. Així, es van analitzar els efectes a curt termini de la gestió de l'aigua, i el subseqüent canvi hidrològic, sobre l'estat ecològic de la llacuna del Ter Vell. Diversos indicadors físicoquímics (Trophic State Indexes) i biològics (rotífers indicadors d'eutròfia, l'índex *QAELS*, paràmetres descriptors de la comunitat, etc.), àmpliament utilitzats, van ser aplicats per determinar l'estat ecològic abans i després de les actuacions de gestió. Alguns dels resultats obtinguts van qüestionar la conveniència d'utilitzar a la llacuna del Ter Vell aquells indicadors que havien estat desenvolupats per ecosistemas que presentaven un funcionament hidrològic molt diferent. No obstant, si es tenen en compte els indicadors adequats es pot conclure que després de la gestió hi va haver una lleugera millora de l'estat ecològic de la llacuna.

SECTION I

General Introduction

CHAPTER 1
INTRODUCTION

1.1. Coastal wetlands

Coastal wetlands, as transition zones between the land and the sea, are fluctuating ecosystems which have been considered one of the most diverse and productive ecosystems of the world (Mitsh and Gosselink, 1993; Gopal et al., 2000). These highly dynamic environments exhibit strong temporal and spatial variability in their physical and chemical variables and, in turn, in their biotic communities (Chapman, 1977; Pearce and Crivelli, 1994; Wolanski et al., 2004). This is basically due to energetic forcings such as tides, waves, river floods and storms, which occur over different spatial and temporal scales and determine its functioning, morphology and evolution. Coastal wetlands may operate as sources or as sinks of a wide range of compounds (nutrients, organic matter, heavy metals, etc.), either from endogenous (internal cycling) or exogenous (external input) origin (Boorman, 1999; Day et al., 2000; de Jonge et al., 2002).

In the Mediterranean basin, the largest coastal wetlands are located in the main deltaic areas, such as those of the rivers Po (Italy), Nile (Egypt), Danube (Romania), Rhône (France) and Ebro (Spain). Formation of extensive deltaic areas protruding into the sea is favoured by the small amplitude of marine tides. Thus, such deltaic ecosystems are more river-dominated than estuarine ecosystems in macrotidal seas, where periodical and predictable tidal inundations play a crucial role in their functioning and structure, especially as homogenizing agents (Mitsh and Gosselink, 1993; Ibáñez et al., 1997). In the Mediterranean, the lack of tides and the low currents velocities favour the shallowness of the waters, water stagnation and the isolation of waterbodies from the sea, making coastal wetlands more lagunal or isolated in nature (Britton and Crivelli, 1993; Ibáñez et al., 2000; De Stefano, 2004). In reference to this, Guelorget and Perthuisot (1983) introduced the term “confinement” (related to a low water turnover rate) as a characteristic feature of the Mediterranean coastal wetlands where exchanges with the sea are restricted to some degree.

1.2. Types of Mediterranean coastal wetlands

Mediterranean coastal wetlands exhibit a great diversity of aquatic environments which occur on a water permanence gradient, from ephemeral to permanent waterbodies, depending on the soil or sediment type and the balance between surface and groundwater inputs, rainfalls and droughts (Skinner and Zalewski, 1995; Álvarez-Cobelas et al., 2005). With regard to the hydrological dynamics, two main types of Mediterranean coastal ecosystems could be differentiated on a flooding-confinement gradient: those receiving continuous freshwater inputs (mostly of riverine origin) with a high water turnover rate (e.g. Serra et al., 1984, Comín and Valiela, 1993) and those which are semi-confined or confined with restricted water inputs, a low flushing rate and high marine influence (Trobajo et al., 2002; Quintana et al., 1998a).

In the ecosystems where freshwater flux is very active due to continuous surface inputs and outputs, the salinity values are low (although occasionally marine intrusion can occur) and nutrient supplies, mostly as nitrates, are high and usually related to fertilizer application in agriculture (e.g. Kormas et al., 2001; Lucena et al., 2002). In such environments that sustain high nitrogen inputs, phosphorus usually becomes the limiting nutrient (Anderson et al., 2002). In those ecosystems which do not receive continuous freshwater inputs, the scarce and irregular water and nutrient inputs (meteorological disturbances) remain confined during prolonged periods when these waterbodies tend to desiccation (Quintana et al., 1998a; Brucet et al., 2005a). Salinity is higher and variable, exceeding that of the seawater in the most confined waterbodies. A differential confinement of nutrients takes place and, whereas phosphorus remains cumulated in the sediment (Quintana et al., 1998a) nitrogen tends to disappear mainly by denitrification, usually becoming the limiting nutrient.

1.3. Zooplankton community in Mediterranean coastal wetlands

The degree of confinement plays an important role in determining biological communities (Basset et al., 2006b and references therein) and several factors closely related with it such as salinity, water turnover, water permanence, and productivity generally determine the composition and structure of zooplankton communities (e.g. Quintana et al, 1998b; Brucet et al., 2005a), and also the predominance of different types of primary producers (e.g. Trobajo et al., 2002; López-Flores et al., 2006) or the spatial distribution of benthic communities (e.g. Gifre et al., 2002; Gascón et al., 2005). Intra/interannual variability of these environmental factors is very high (Álvarez-Cobelas et al., 2005; Britton and Crivelli, 1993) and seasonal patterns are usually not observed in aquatic invertebrate dynamics in Mediterranean coastal wetlands (Quintana et al., 2006 and references therein). Nevertheless, several regular situations have been identified in the zooplankton temporal pattern of Mediterranean confined salt marshes in relation to characteristic hydrological events such as, for example, flooding disturbances, winter hydrological stability and summer confinement (Quintana et al., 1998b; Brucet et al., 2005a).

Dominance of a single zooplankton species is characteristic of each situation leading to in general low diverse communities. Thus, zooplankton studies dealing with taxon-based approaches, such as those that have been traditionally carried out in Mediterranean coastal wetlands (e.g. Menéndez and Comín, 1986; Pretus, 1989; Oltra and Miracle, 1992), would provide insufficient information about the community organization and functioning, which is probably based on ecological interactions where the organism's body size acquires great relevance (competition, predation, etc.; Quintana et al., 2006). Taking into account the size distribution would provide a more functional perspective of the community structure (Sprules and Holtby, 1979; Stein et al., 1988), and the use of size-based approaches would supplement the information provided by the taxonomic ones (Rodríguez and Magnan, 1993; Jennings, 2005). In Mediterranean salt marsh ecosystems the combination of both approaches has led to a better understanding of the functioning and dynamics of the zooplankton (Brucet et al., 2005a, 2006).

Widespread knowledge of the zooplankton taxonomic composition in Mediterranean coastal wetlands and its relation to trophic state variables allows its use as an indicator of ecological status. Although zooplankters are very important components of shallow ecosystems, sometimes acting as keystone species, they have not been considered as a biological element for the classification of ecological status in the EU Water Framework Directive. This fact can be considered mystifying (Moss et al., 2003) and several attempts have recently been made in Mediterranean shallow waters to assess the potential of several groups of zooplankters as indicators of ecological status (e.g. Bianchi et al., 2003; Boix et al., 2005). The suitability of zooplankton as an indicator of water quality has been broadly demonstrated in a great variety of aquatic environments (e.g. Gannon and Stemberger, 1978; Gulati, 1983; Mäemets, 1983; Sládeček, 1983; Pontin and Langley, 1993; Attayde and Bozelli, 1998; Duggan et al., 2001).

1.4. Main anthropic impacts in Mediterranean coastal wetlands

In the Mediterranean region, coastal wetlands are nowadays receiving high anthropic impact due to the resident population (> 50% of the coastline is heavily urbanised), tourism (it is the world's leading holiday destination) and the anthropic activities within their catchment areas (EEA, 1999; De Stefano, 2004; Álvarez-Cobelas et al., 2005). Traditionally, agriculture has been the main human activity affecting coastal wetlands but, since the second half of the 20th century, urban, industrial and tourism activities have become more relevant. Land reclamation, alterations of the hydrological pattern and degradation of water quality are the main anthropic impacts and the main causes of wetlands degradation and disappearance (Pearce and Crivelli, 1994; Day et al., 2000; de Jonge, 2002; Green et al., 2002). In countries such as France, Greece, Italy and Spain, coastal wetland losses exceeding 50% of their original area have been recorded throughout the last century and many of the remaining Mediterranean wetlands are still threatened today by pollution, water extraction, eutrophication and sedimentation (Skinner and Zalewski, 1995; Flower, 2001; De Stefano, 2004).

Freshwater ecosystems of riverine origin whose functioning and dynamics are determined by inflowing watercourses have been hydrologically altered mainly due to agricultural activities, leading to a prolonged freshwater flooding period (e.g. Etang du Vaccarès, in France; l'Encanyissada lagoon, in Spain). On the other hand, those ecosystems with high marine influence and, therefore, low agricultural potential have been primarly drained and/or physically modified by coastal works (channelisation, impoundments, housing, etc.) and aquaculture activities (e.g. Mar Menor lagoon, in Spain; Venice lagoon, in Italy). In all these ecosystems, changes in the salinity balance and in the biological communities in addition to eutrophication processes are the most widespread effects (Pérez-Ruzafa et al., 1991; Heurteaux, 1992; Comín and Valiela, 1993; Marchini et al., 2004). Eutrophication is usually due to an increased loading of nutrients through agriculture, aquaculture, industrial and domestic wastewaters discharges (Becklioglu et al., in press). Nutrient stimulation usually leads to an imbalance between the processes of algal production and consumption (e.g. Albufera de Valencia, in Spain; Venice lagoon; Oltra and Miracle, 1992; Libralato et al. 2004), followed by dystrophic crises due to oxygen depletion (e.g. Étang de Thau, France and Sacca di Goro, Italy; Mazouni et al., 1998; Bartoli et al., 2001) and the subsequent benthic animal mortalities and fish and/or shellfish kills (e.g. Stagno di Santa Giusta, in Italy; Sechi et al., 2001).

Wetland disappearance and degradation contributes to the decline of species that depend on them to survive but, after the loss of habitat, invasive species are the second leading cause of biodiversity loss (Vitousek, 1997; Mack et al., 2000). Mediterranean countries such as Spain, Cyprus, Portugal and Greece have been considered as "recipient" countries with regard to the most frequently introduced aquatic species in Europe (García-Berthou et al., 2005). The introduction and establishment of invasive species in Mediterranean wetlands have been particularly evident in the past few years as a result of human activities (Cognetti and Maltagliati, 2000; Alcaraz et al., 2005), causing negative impacts on native species (e.g. American mosquitofish *Gambusia* spp.; Rincón et al., 2002; Pleguezuelos 2002) but also water quality degradation and food web alterations (e.g. Lousiana crayfish *Procambarus clarckii*; Geiger et al., 2005; Rodríguez et al., 2005).

1.5. Conservation, restoration and management of coastal wetlands

In the Mediterranean region, with strong developmental pressures and a still-expanding population, restoration and management programmes are currently required to protect and conserve coastal wetlands, but also to recover old wetlands areas that are currently highly degraded. It is well known that ecological restoration requires an integration of scientific research and management strategies in order to develop the appropriate goals, monitoring programmes and criteria to evaluate the success of restoration efforts (Elliott et al., 1999; Frid and Clark, 1999). In fact, the relative absence of research into that management has occasionally been a serious obstacle to the achievement of conservation goals (Saunders et al., 2002 and references therein).

The main goal of ecological restoration should be to assure a complete ecological function of the restored ecosystem and for this reason previous deep knowledge about the ecosystem functioning and structure should be taken into account when restoration goals are set (Hobbs and Harris, 2001). In fact, the EU Water Framework Directive (WFD 2000/60/EC) intends that the quality of the structure and functioning of an ecosystem will express its ecological status (Pollard and Huxham, 1998). The WFD, which aims to protect and enhance the ecological status of European aquatic ecosystems by means of their restoration if necessary (Irvine, 2004), presents a number of scientific challenges such as developing ecotype classification systems, establishing the ecological status and the reference conditions ("very minor" anthropogenic alterations) for each of these types, and designing statistically robust monitoring regimes (Elliott et al., 1999). The assessment of the ecological status includes hydromorphological, physical, chemical and biological "quality elements" or descriptors, but focuses more on the condition of the biological ones (Pollard and Huxham, 1998; Elliott et al., 1999). The identification and selection of proper descriptors, which requires deep knowledge of ecosystem structure, organization and functioning, is a major challenge for the scientific community, especially with regard to the transitional waters in the Mediterranean region (i.e. river mouth ecosystems, coastal ponds and lagoons, etc.), where there is still a poor understanding of the organization and functioning of these ecosystems (Basset et al., 2006a). The identification of a typology of water bodies or ecotypes is indispensable, and the ecological status should be defined within ecosystem type since the optimal biological descriptors or indicators utilized are likely to be ecosystem type specific (Basset et al., 2006b). This fact suggests that descriptors or indicators developed for other kinds of ecosystems with different functioning, in other regions or in the same region, will not offer a correct assessment of the ecological status.

CHAPTER 2
STUDY SITE

2.1. The Baix Ter Wetlands

The Baix Ter Wetlands are located in the northeastern Iberian Peninsula (Catalonia, Spain) in the deltaic plain of the Ter and Daró rivers (Fig. 2.1). Since the first wetland desiccation activities in the 18th century and the deviation of the last tract of the course of the Ter River in the 19th century, the deltaic plain has been subjected to intensive agricultural use. Furthermore, since the 1960s the seacoast has been especially vulnerable to strong anthropogenic pressures due to mass tourism, often poorly conceived urbanization, the construction of marinas etc. Nowadays, this area is an example of a western Mediterranean coastal plain, densely populated, industrialized and with intensive agricultural land use. The Baix Ter Wetlands appear as a fragmented and poorly interconnected wetlands area formed by two river mouths (Ter and Daró rivers), several freshwater (Ter Vell and Basses d'en Coll) and confined brackish-hyperhaline coastal lagoons (Fra Ramon and Bassa del Pi) and some temporary flooded areas. Since 1992, the Baix Ter Wetlands have been legally protected under the government of Catalonia's PEIN (Plan for Spaces of Interest for Nature, Law 12/1985), and have been proposed as a SCI (Site of Community Importance) included in the NATURA 2000 Network (EU Habitats Directive 92/43/EEC).

During the 1990s, several projects for the recovery and conservation of these coastal wetlands were proposed but only some restoration actions were carried out (Giró et al., 1988, 1990; Martinoy and Quintana, 1991; Quintana et al., 1988). An EU LIFE project was developed between 1999 and 2003 in order to recover and conserve the ecological value of two coastal ecosystems located at the north of the Ter River mouth: a confined brackish ecosystem, La Pletera salt marshes, and a fluctuating freshwater ecosystem, the Ter Vell lagoon (Fig. 2.1)

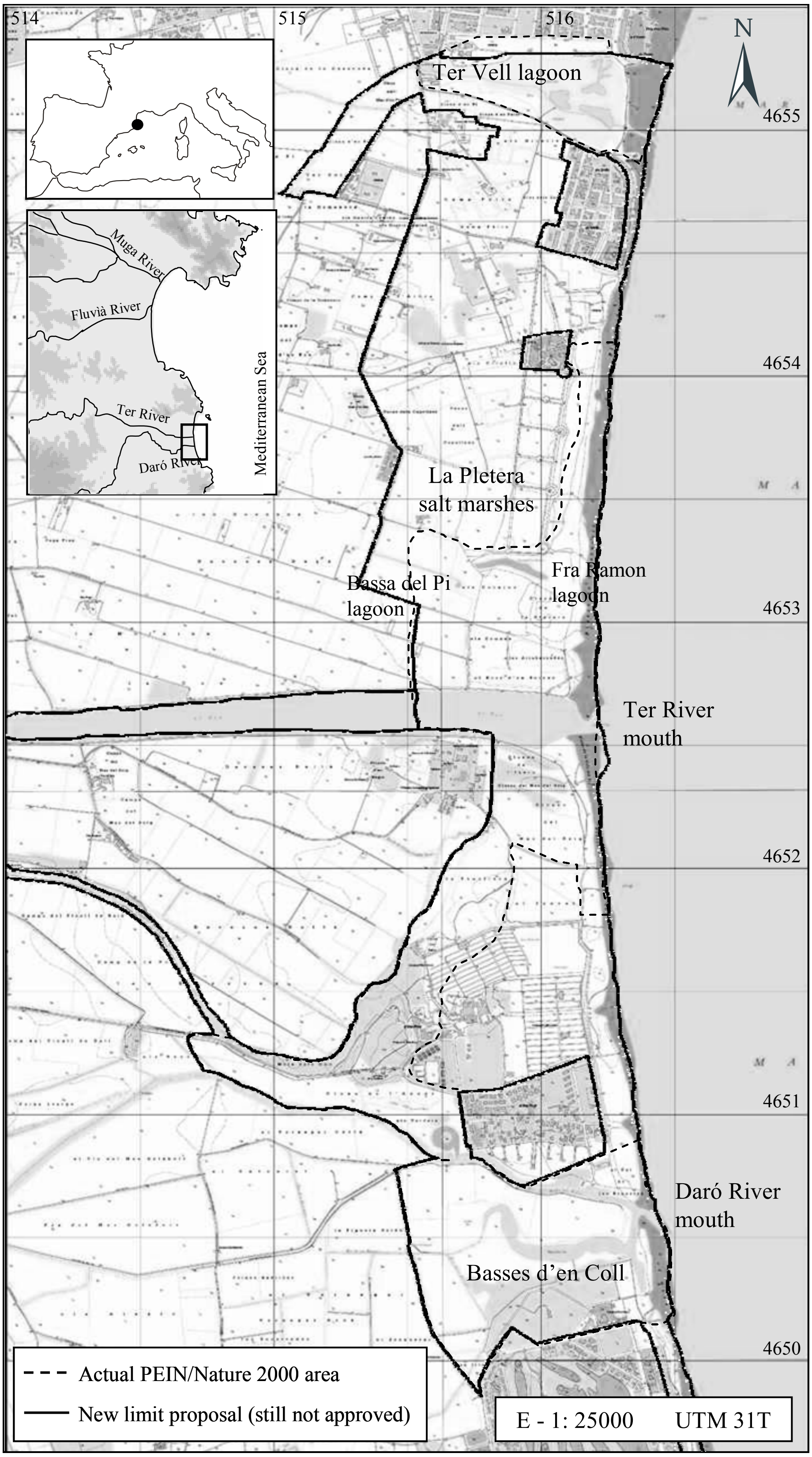

Figure 2.1. Map of the Baix Ter Wetlands.

2.1.1. La Pletera salt marshes

La Pletera is a *Salicornia*-dominated salt marsh which covers 37.5 ha of the coastal area located on the north side of the Ter River mouth, immediately behind the sand dune ridge. It is a flat area with a topographic level below 2 m a.s.l. (above sea level) for 96% of its surface. Since the 1970s, the hydrology has been modified due the channelisation of the Ter River and the construction of levees at several points of the marsh. In 1987, partial urbanisation of the marshland modified the landscape since some areas of the marsh were filled in and several roads and a 1 km long promenade were constructed (Photo 1A). As a consequence, the natural water regime changed, several lagoons and wetlands disappeared and salt marshes remained almost totally isolated from the river and sea (Photo 2). Since the urban reclassification carried out in 1999 by the Torroella de Montgrí Council, these salt marshes have remained as a non-urbanizable area due to their ecological value.

Until the beginning of the LIFE project, there were only two shallow permanent lagoons, Bassa del Pi and Fra Ramon, in the marsh (Photo 3). Both lagoons have been classified as confined coastal brackish-hyperhaline ponds (Trobajo et al. 2002) and have their origin in an old river distributary. Bassa del Pi has a maximum depth of about 1 m b.s.l. (below sea level) and is surrounded at the western south side by an artificial 2-3 m high levee. Fra Ramon, with a maximum depth of 1.75 m b.s.l., is almost entirely surrounded by an artificial 4 m high levee. Bassa del Pi is situated further from the seashore than Fra Ramon and is the most confined lagoon in the marsh. In both lagoons, *Chaetomorpha* and *Enteromorpha* are the dominant genera in the algal mats. There are dense perennial and annual populations of the macrophyte *Ruppia cirrhosa* (Gesti et al., 2005) only in the Fra Ramon lagoon, where the colonial polychaete *Ficopomatus enigmaticus* also reaches high densities. Until 1999, Fra Ramon was the only lagoon that supported a population of the endangered cyprinodont Iberian toothcarp *Aphanius iberus* (Moreno-Amich et al. 1999a). Occasionally, when the hydrology is very stable and water levels are very low in the Bassa del Pi lagoon the water colour becomes pink due to the presence of *Chromatium*-like phototrophic bacteria (Photo 3B).

2.1.2. The Ter Vell lagoon

The Ter Vell lagoon is a freshwater coastal lagoon which covers a total area of about 23 ha and is situated behind the coastal dunes. Its topographic level ranges from 1.25 m b.s.l. in the deepest basins of the lagoon to 4 m a.s.l. on the artificial levees which surround part of the lagoon perimeter. The 95% of the total surface of this lagoon has a topographic level below 2 m a.s.l. The lagoon was the old mouth of the Ter River until the middle of the 19th century, when the river was diverted to its current location. Now the Ter Vell is a freshwater coastal lagoon fed by a channel which diverts water from the river for agricultural irrigation and, in turn, collects runoff from the cultivated plain and the adjacent urban areas. The lagoon empties through a drainage channel connecting it to the sea. Trobajo et al. (2002) have classified this lagoon as a fluctuating freshwater system.

The Ter Vell is a typical example of a eutrophic freshwater coastal lagoon which has experienced the effects of high agricultural and tourism pressures, especially during the second half the 20th century (Photo 1B). Since the 1960s the lagoon has been supplied by the excess irrigation water, rainfall runoff and urban wastewater. Consequently, fluctuations of water level and the degree of eutrophy have increased and the free-water surface has drastically diminished due to the high proliferation of the common reed *Phragmites australis* subsp. *australis* (Quintana and Comín, 1989), which is especially adapted to high water level fluctuations and anoxic sediments (Ailstock et al., 2001; Deegan et al., 2006). In the last 50 years the free-water surface has decreased from 6 to 1.7 ha and nowadays, only 5 permanent basins can be distinguished (Photo 2). The common reed surface has increased from 2 to more than 10 ha to the detriment of the halophytic communities, the potential vegetation of these coastal wetlands (Fig. 2.2). Fortunately, the reduction of the urban wastewater inputs in the 1980s and the excavation of new basins in the first restoration projects in the 1990s stopped the common reed proliferation, but the degree of eutrophy still remained very high.

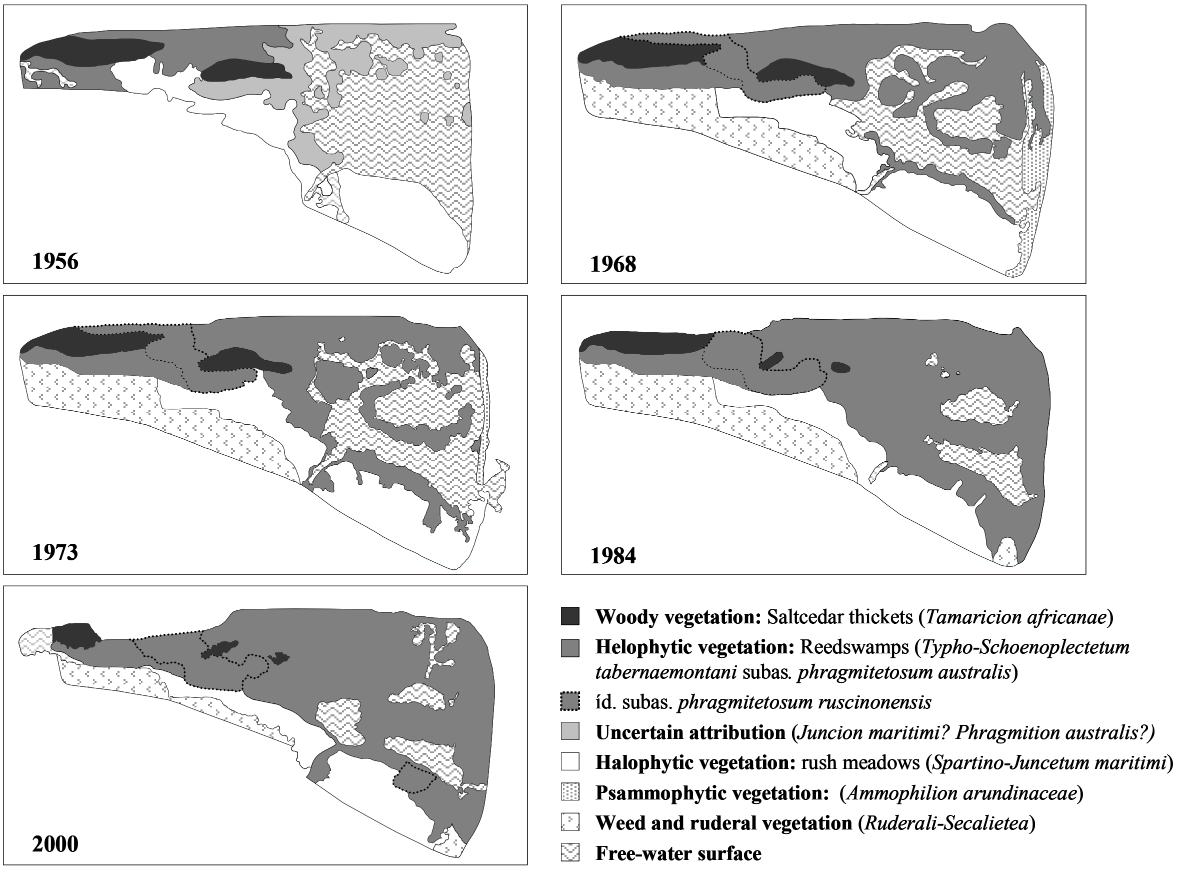

Figure 2.2. Historical evolution of the vegetation communities in the Ter Vell lagoon during the last 50 years (modified from Gesti, 2003). Note that between 1984 and 2000 the free-water surface increased with the excavation of new basins in the western end, in the central part and in the northeastern vertex of the lagoon surface. Such basins were created during the first restoration projects carried out in the 1990s.

2.1.3. Climate of the Baix Ter Wetlands

The Baix Ter Wetlands are free from tidal influence and have Mediterranean littoral-type climate characterized by a very mild summer and the lack of a true winter. Temperatures are generally moderate with an average annual temperature of 16 ºC. Mean monthly temperatures range from 7-9 ºC in the winter low to 22-26 ºC in the summer high, so the annual thermal amplitude is usually lower than 15 ºC. (Fig. 2.3 A). The wind pattern plays an important role in modulating the local climate conditions and is determined by the dominance of the north wind (called 'tramuntana') in the winter months and the southwest wind (called 'garbí') in the summer. East-southeast winds (mainly 'llevant') are responsible for the sea storm events, which occur mainly between autumn and spring. Total annual precipitation ranges from 500 to 700 mm. There is a great interannual variation, particularly in the timing and quantity of rainfall, but generally the highest rainfalls are recorded in autumn and spring. In autumn, rainfall is usually more intense and often appears accompanied by sea storms (Fig. 2.3 B).

Sea level changes are a fundamental factor influencing Mediterranean coastal wetlands and are mainly caused by wind surges and changes in barometric pressure. These meteorological tides are higher than astronomical tides (Britton and Crivelli, 1993; Ibáñez et al., 2000) and they strongly affect the phreatic level of coastal aquatic ecosystems. The monthly average sea level remains especially low during the winter anticyclones and the highest level occurs in autumn, with a secondary maximum in spring, usually coinciding with sea storms or wind surges (Fig. 2.3 B). In the Baix Ter Wetlands, sea storms are punctual events which do not usually last for more than two days and the wave height always exceeds 3 m (according to J.Pascual, Meteorological Station of l'Estartit).

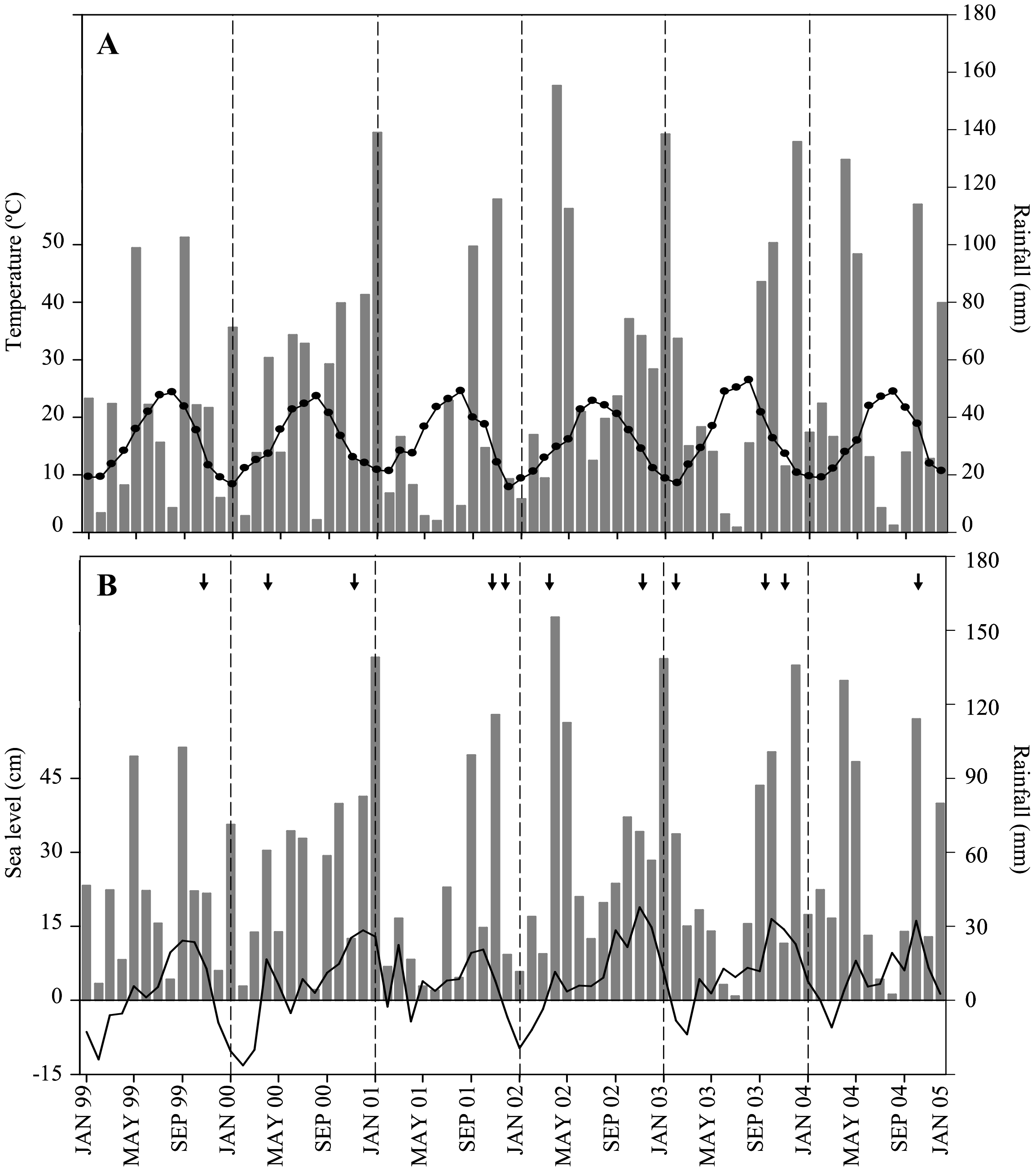

Figure 2.3. A) Ombrothermic diagram for the study area from 1999 to 2004. Average monthly temperature (ºC) and monthly total rainfall (mm) are shown.
B) Variation in the monthly average sea level (cm above or below the average sea level) in the study area from 1999 to 2004. Total monthly rainfall (mm) is also represented. Black arrows indicate sea storms events (waves higher than 3 m). Data come from the Meteorological Station of L'Estartit (J. Pascual, pers. comm.) located about a half kilometre to the north of the study area (station no. 385 J of the Spanish National Institute of Meteorology).

CHAPTER 3

The EU LIFE project : “Arrangement and management of the Baix Ter coastal lagoons and marshes”

3.1. Project goals

The EU LIFE project “Arrangement and management of the Baix Ter coastal lagoons and marshes” (LIFE 99 NAT/E/006386) was developed in the Baix Ter Wetlands between 1999 and 2003 for the restoration and management of confined brackish ecosystems (La Pletera salt marshes) and a fluctuating freshwater ecosystem (Ter Vell lagoon). Although these ecosystems are located less than 1 km from each other (Fig. 3.1), their environmental problem was significantly different and therefore the actions proposed in the framework of this project were specific for each one. In La Pletera salt marshes actions were mainly based on the creation and stocking of new refuges for the Iberian toothcarp (*Aphanius iberus*) since habitat loss was the main cause of the fragmented distribution of this species (Planelles-Gomis, 1999; Doadrio, 2001). In the Ter Vell lagoon the actions were mainly focused on the improvement of the water quality through nutrient reduction in the inflowing waters. High freshwater and nutrient inputs and the irregularity of freshwater inflow were the main causes of the environmental degradation in this lagoon.

The project proposals also included the recovery and the conservation of the sand dunes and marshland vegetation by reducing vehicular traffic and establishing itineraries for visitors.

3.2. Creation and stocking of new refuges for the Iberian toothcarp in La Pletera salt marshes

In La Pletera salt marshes, the main goal of the LIFE project was the improvement of the conservation status of the endangered cyprinodont Iberian toothcarp (*Aphanius iberus*; Photo 7). In the entire Baix Ter Wetlands, this endemic fish of the Iberian Peninsula, threatened with extinction (Doadrio, 2001), was only present in the Fra Ramon lagoon (Moreno-Amich et al. 1999a). Consequently, this population is particularly vulnerable to any disturbance that takes place in the lagoon, especially during summer when tourist visitor numbers are highest. Thus, the LIFE project was developed in order to increase the number of toothcarp populations. Two main strategies were followed: (1) the creation of new refuges (permanent brackish lagoons) and (2) their subsequent stocking.

3.2.1. Creation of new refuges

The creation of refuges in La Pletera salt marshes was carried out with the goals of (1) recreating the most suitable habitat for *A. iberus* in the marshland, where the surface covered by permanent brackish lagoons had reduced from 2.3 ha to 1.2 ha in the last 50 years and, (2) establishing a new self-sustaining population of *A. iberus*.

The criteria followed in the creation of the new refuges were:

- New lagoons should be situated on public property and/or at an authorized place and located in flat areas where vegetation is degraded or nonexistent. Destruction of the mature and well-conserved vegetation should be avoided in the process of construction.
- Their morphology and orientation should be similar to those of the characteristic coastal lagoons in the area: elongated and narrow, either parallel or perpendicular to the coastline, following the direction of the interdunal depressions or the ancient river runoffs, respectively (Bach, 1990).
- Their hydrological regime should be that typical of confined Mediterranean coastal lagoons: no freshwater courses should supply them, but rather they should be fed only by marine groundwater inputs and, occasionally, by surface marine and/or freshwater inputs (e.g. sea storms, rainfalls runoff). Lagoons should remained confined during a large part of the year with no water inputs, leading to large salinity oscillations (Quintana et al., 1998a; Gascón et al., 2005).

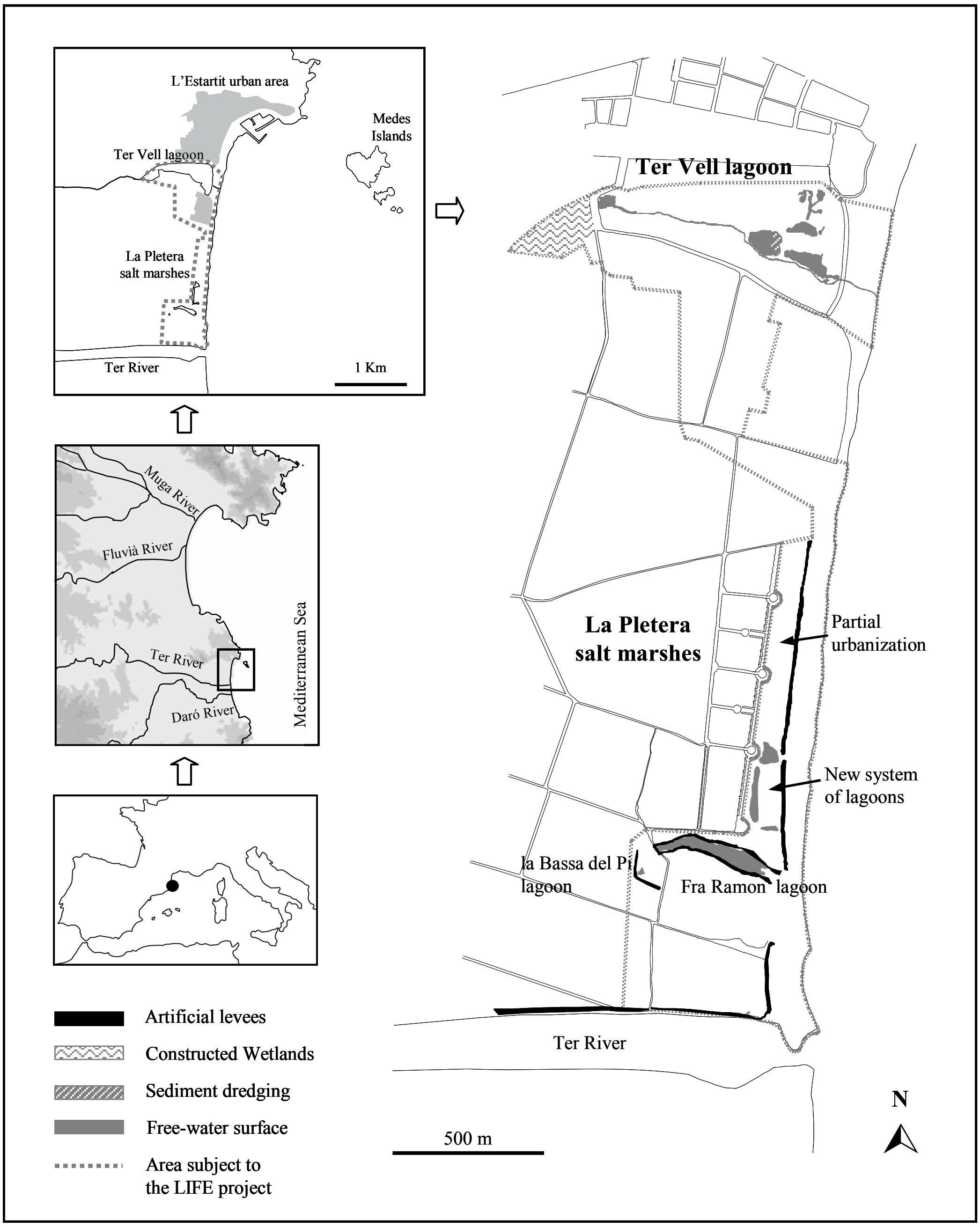

Figure 3.1. Sketch map of the area of the Baix Ter Wetlands subject to the LIFE project.

- Their hydrology should ensure the exclusion of introduced freshwater species such as *G. holbrooki*, as well as, marine competitors and predators of *A. iberus* (Planelles-Gomis, 1999) because the high salinity fluctuations limit the development and establishment of these species (Crivelli, 1995; Doadrio, 2001)
- The summer water permanency in the new lagoons, guaranteed by the lowest topographic level (below the average sea level), would favour the development of *Ruppia cirrhosa*, the most abundant macrophyte in this habitat, which develops dense prairies in those areas where the water level remains between -20 and +20 cm (relative to the average sea level; Gesti et al., 2005). These submerged prairies are commonly used by *A. iberus* as a refuge (García-Berthou and Moreno-Amich, 1992; Fernández-Delgado et al., 1999).

3.2.2. Fish stocking of the new refuges

The stocking was carried out through captive breeding and release, as well as through translocation of wild individuals (Photo 8). Captive breeding is often used as a fish conservation measure for stocking newly created habitats or restored ecosystems to obtain of a self-sustaining population (Philippart, 1995). Since species restoration efforts should strive to maintain local genetic diversity (García-Marin et al., 1990), which usually is related to variable local conditions (Maltagliatti, 2002), individuals for captive breeding were collected from the Fra Ramon lagoon. The extraction of individuals was carried out at the end of the spring 2002 to ensure large individuals with bigger reproductive potential.

Additionally, wild individuals from the local population of the Fra Ramon lagoon were translocated in order to ensure a successful stocking of the new habitats. The incorporation of wild individuals would help to minimize the loss of genetic diversity in the initial founding of the captive bred population and to reduce the effects of genetic drift. The maintenance of high genetic diversity is important for adaptation to changes in environmental conditions and assures the establishment of the new population (Philippart, 1995; Schönhuth et al., 2003).

3.3. Improvement of the water quality in the Ter Vell lagoon

In the Ter Vell lagoon the LIFE project was developed to improve the water quality. Two main strategies were followed to achieve this aim: (1) reducing nutrient loads in the inflowing waters and (2) reducing the organic load in the sediment. The depuration of the inflowing waters with constructed wetlands and the dredging of sediment in several points of the lagoon were performed to achieve the two proposals, respectively.

3.3.1. Constructed wetlands

The main objectives were to reduce the nutrient loads and retain the suspended solids of the freshwater inputs. In turn, these artificial wetlands will provide flood control to mitigate water level fluctuations in the lagoon and recover lost wetland functions. At the same time, the establishment of vegetation communities characteristic of permanent and temporary freshwater wetlands will be favoured.

The constructed wetlands were located in an old flooded area highly degraded, situated close to the Ter Vell, and next to the irrigation channel that supplies the lagoon (Photo 9). They became operative in summer 2002. Freshwater inflow is routed through a total area of 2 ha, which can absorb an average inflow of 750 m^3 day^{-1} and has been designed to reduce 95% of the suspended solids and 65% of the total nitrogen and phosphorus. A residence time of 8 days is expected. A shallow sheet flow of a depth of around 20 cm depth is distributed by gravity through a system of successive terraces or treatment cells which show a progressive gradient toward the lagoon (Photo 9A). This ensures a unidirectional flow along the cells, separated by 20 cm high berms. Lateral flow is avoided by the construction of 50 cm high berms. Two sluice gates in the main distribution channel regulate the level of water supplying the wetlands and deviate the excess of inflow toward the lagoon during intense flooding periods. The topographic level in the constructed wetlands ranges from 0.10 m a.s.l. in the permanent flooded area to 1 m a.s.l. in the preserved “islands” of terrestrial vegetation. In the outlet of the last treatment cell, a terminal deep zone has been designed to provide water level and flow control. This permanent flooded area collects and routes, at a uniform rate, the water flow from the other cells to the Ter Vell lagoon. The preserved "islands" of terrestrial vegetation and the highest berms have been useful in the creation of walks for the maintenance activities and visits to the constructed wetlands.

3.3.2. Sediment dredging

The main objective was to reduce the organic load of the sediment at the points of the lagoon where organic matter had accumulated in excess. Another aim was an increase in the free-water surface to enhance the presence of aquatic birds.

The criteria followed in the sediment dredging were:

- Sediment removal should be carried out in those areas with the highest organic content.
- Deep sediment dredging in basins of natural origin should be avoided.
- Fragmentation of the surface of helophytic vegetation should be avoided in order to maintain a sufficiently broad area to be used as a refuge and nidification site for the fauna associated with this habitat.
- Sediment dredging should be performed where vegetation communities had low botanical interest or a lower conservation status. Damage to the original, undegraded vegetation should be avoided as much as possible.

A total of 0.2 ha of common reed were dredged in summer 2002 in the area which showed the highest organic content, located at the eastern part of the central basin of artificial origin which had a bird observatory (Fig. 3.2; Photo 10). The dredged area showed a progressive gradient with the deepest part located next to the basin. This will prevent common reed expansion, ensuring an increase of the free-water surface which would, in turn, enhance the presence of aquatic birds. This is very positive for this lagoon since a bird observatory was constructed during the first restoration actions in the 1990s. The area to be dredged was located through a previous analysis of the sediment organic content carried out at 20 points, distributed throughout the Ter Vell lagoon (Fig. 3.2). In other basins of the lagoon where the organic content was also high, but sediment dredging was not feasible or involved too much habitat perturbation, the surface layer of mud sediment was removed by aspiration (Photo 11).

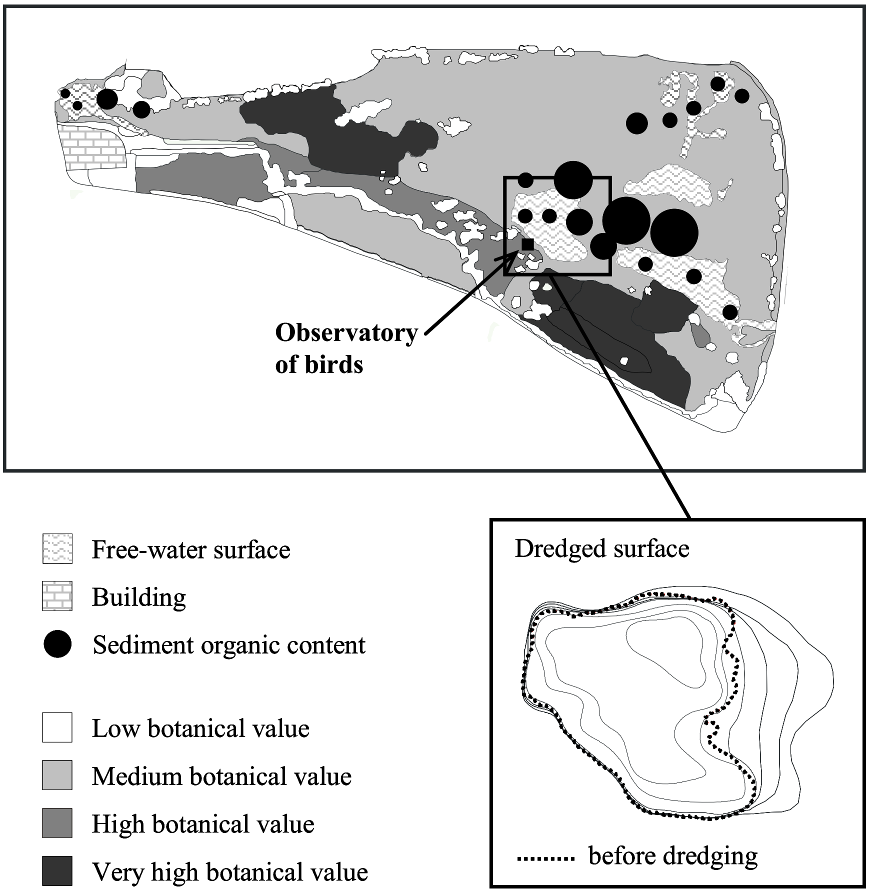

Figure 3.2. Sketch map of the vegetation communities in the Ter Vell lagoon with botanical information. The sediment organic content is also indicated: the bigger the black circles, the greater organic content. There is also a sketch map of the dredged surface in the central basin (modified from Badosa et al., 2001 and Gesti, 2003).

3.4. Other restoration activities

The LIFE project also aimed to recover and conserve the sand dune and marshland vegetation. In those littoral areas where sand dunes were highly degraded, artificial mechanisms to retain sand were installed. They were formed by a successive square cells (5 m side x 1 m high) fixed in the substratum and grouped in modules which were parallelly oriented to the seashore (Photo 13A). Once sand had been retained due to the wind action, vegetation recovery took place mainly by spontaneous colonization (Photo 12). When difficulty with spontaneous colonization was foreseen, psammophytic species, cultivated from seeds collected in the area, were planted to accelerate the sand dune fixation.

To ensure the conservation and recovery of these managed wetlands, a network of itineraries was established to regulate accessibility to the area, either by car, bicycle or foot. In the areas of more natural interest motor vehicle access has been restricted. In order to guide the visits and sensitize the visitors, several informative posters explaining the main restoration activities have been incorporated along the planned routes (Photo 13B).

3.5. Agriculture water management

In November 2001 and independently of the restoration actions, agricultural water managers changed the irrigation system of the coastal plain and water diversion for irrigation became subterranean. This meant a drastic reduction in the surface freshwater inflow to the Ter Vell lagoon leading to a change in the hydrological regime. Since then, the irrigation channel has supplied water to the lagoon only during intense rainfall events, except in August and September 2002 when agricultural surface irrigation took place again.

CHAPTER 4
STUDY APPROACH

4.1. Study approach

Scientific research in ecosystems that are subject to restoration and management is necessary, not only for the application of the restoration measures and management programmes, but also to assure their success and improve future management plans. The present study collects the scientific research developed during the LIFE project with the goal of determining the natural functioning of La Pletera salt marshes and the Ter Vell lagoon and their environmental problem. Limnological studies during monitoring campaigns before and after restoration allowed us to guide the development of such actions and to evaluate results obtained afterwards. Environmental problematic in La Pletera salt marshes and in the Ter Vell lagoon are very different, as are the restoration measures required and the research objectives for each one. Thus, results of the present study have been divided into those obtained in La Pletera salt marshes (Section II) and those obtained in the Ter Vell lagoon (Section III):

In **La Pletera salt marshes (Section II)**, the main objectives of the present study were (1) to describe the limnological functioning of the old lagoons (see section 2.1.1, Chapter 2) and the new created ones (see section 3.2.1, Chapter 3) and (2) to asses the suitability of the latter as new refuges for *A. iberus*. Additionally, this limnological study gave us the opportunity to expand our knowledge of the trophic interactions established within the planktonic community in this kind of coastal lagoons and, especially, of the role that *A. iberus* plays as a target species in the zooplankton structure, in addition to other ecological factors.

In Chapter 5, the role of the hydrological regime in the nutrient and zooplankton composition and dynamics was analysed in the five permanent lagoons of the marshland, the old ones (Bassa del Pi and Fra Ramon) and the three new ones. Differences between the old and the new lagoons allowed us to also analyse the effect of ‘lagoon age’ on nutrient and zooplankton composition and dynamics.

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In Chapter 6, the influence of several ecological factors such as hydrology, nutrient composition, food resource availability and predation on zooplankton structure has been analysed in both the old and the new lagoons. Taxon- and size-based approaches have been used in the study of the zooplankton community structure since it has previously been demonstrated that their combined use provides complementary information. This chapter has been published as a research article:
Badosa et al. / Estuarine, Coastal and Shelf Science 71 (2007), 335-346.

In Chapter 7, the feasibility of the newly created lagoons as suitable refuges for *A. iberus* has been analysed through the comparison of the limnological characteristics (hydrology, physical and chemical parameters, submerged vegetation, invertebrate community) of the new refuges with those of the current habitat of this species in the marshland (the Fra Ramon lagoon). The short-term evolution of the new *A. iberus* population in the new habitats was also analysed. This chapter has been submitted as a research article:
Badosa et al. / Aquatic Conservation: Marine and Freshwater Ecosystems
(It is currently being revised following favourable reviews)

In the **Ter Vell lagoon (Section III)**, the main objectives of the present study were (1) to describe the limnological functioning and to relate it to the environmental quality degradation and (2) to analyse whether the restoration actions and changes in agricultural water management had affected the limnological functioning and ecological status. In turn, this study allowed us to analyse whether the limnological functioning of an ecosystem would condition the indicators that should be used when determining its ecological status. This meants if the use of indicators developed for a specific type of ecosystem could lead to erroneous conclusions when applying them in ecosystems with different functioning.

In Chapter 8, the short-term effects of both the restoration actions and the agricultural water management on the limnological characteristics and the zooplankton community of the lagoon were analysed through the comparison of two hydrological cycles, one before and one after the actions. Since agricultural water management drastically reduces the freshwater flooding period, the limnological characteristics of the Ter Vell after the management actions were compared with those of the confined lagoons of La Pletera in order to analyse if the degree of confinement of the Ter Vell had increased. This chapter has been published as a research article:

Badosa et al. / Marine Pollution Bulletin (in press).

In Chapter 9, the short-term effects of both the restoration actions and the agricultural water management on the ecological status of the Ter Vell lagoon were analysed. Ecological status was determined using commonly used indicators of trophic state and water quality, based on physical and chemical characteristics and on the invertebrate community. The suitability of such indicators for use in this kind of coastal wetlands was also discussed. This chapter has been submitted as a research article:

Badosa et al. / Aquatic Conservation: Marine and Freshwater Ecosystems

(It is currently being revised following favourable reviews)

4.2. Objectives

The main objective of this study was to determine the natural functioning of the waterbodies in La Pletera salt marshes and the Ter Vell lagoon, in terms of hydrology, physical and chemical parameters and zooplankton composition and structure and, in turn, to evaluate the results of the restoration actions.

Within this main objective, the following specific objectives can be identified:

- To assess how the variability in the hydrological pattern determines the nutrient and zooplankton composition and dynamics in old and new lagoons of La Pletera salt marshes.

- To analyse if “lagoon age” can explain, at least partially, the differences in the nutrient composition and the zooplankton community between old and newly created lagoons.

- To assess the usefulness of the combined use of taxon- and size-based approaches to studying zooplankton structure in La Pletera salt marshes.

- To establish if the community structuring factors: predation, food resource availability, nutrient composition and hydrology affect the taxonomic and the size structure of the zooplankton community in the lagoons of La Pletera salt marshes.

- To validate the suitability of the new lagoons of La Pletera as refuges for *Aphanius iberus* in terms of hydrological functioning, physicochemical characteristics, resource availability (invertebrate community) and habitat structure (aquatic vegetation).

- To evaluate the short-term response of the new *A. iberus* population after their stocking.

- To analyse whether the water management in the Ter Vell lagoon (restoration measures and agricultural management) has affected the limnological characteristics and the zooplankton community of the lagoon.

- To verify if the drastic reduction in the freshwater inflow to the Ter Vell lagoon, resulting from water management in agriculture, has involved an increase in the ecosystem's degree of confinement.

- To analyse whether the water management actions in the Ter Vell lagoon have affected its ecological status.

- To discuss the suitability of several commonly used physicochemical and biological indicators for the assessment of the ecological status of the Ter Vell lagoon.

SECTION II

La Pletera salt marshes

CHAPTER 5

Nutrients and zooplankton composition and dynamics in relation to the hydrological pattern

5.1. Introduction

Costal wetlands, as a confluence of inland water and marine water, are among the most fluctuating and productive ecosystems in the world (Mitsch and Gosselink, 1993; Gopal et al., 2000). These ecosystems play an important role in coastal defence and wildlife conservation and can act as either sinks or sources of a wide range of substances (mineral nutrients, organic matter, pollutants, etc.; Adam, 1990; Boorman, 1999; Costa et al., 2001). Changes in the water regime in coastal wetlands due to human activities, on all spatial and temporal scales, have caused water quality degradation, wetlands to disappear and invasive species to become established and expand (Crivelli, 1995; Callaway and Zedler, 1998; Oltra and Todolí, 2000; Pérez-Ruzafa et al., 2002; O'Connell, 2003; La Jeunesse and Elliott, 2004). Therefore, an understanding of the hydrological functioning of coastal wetlands is particularly important in the context of conservation and management, especially in those coastal areas where a formerly extensive area has been reduced by human land use (agriculture, industry, fisheries or tourism; e.g. Queen, 1977; Day et al., 2000; Puigserver et al., 2002). The Mediterranean coastal areas are a typical example of this. Water regime in Mediterranean salt marshes is determined by energy inputs, such as pulsing events (sea storms, rainfalls), which determine the material fluxes and the salinity gradients, and are important for delivering nutrients and regulating biological processes (Quintana et al., 1998a; Day et al., 2000). Previous studies on Mediterranean coastal wetlands have already demonstrated the strong influence of the hydrology on the nutrient dynamics, water quality as well as on the zooplankton community (Comín et al., 1987; Oltra and Miracle, 1992; Quintana et al., 1998a,b; Ortega et al., 2000).

Mediterranean salt marshes may be N-limited since the nitrogen inputs, during water entries, are rapidly removed because they stimulate both primary production and denitrification. On the other hand, phosphorus inputs are trapped in the salt marshes, usually as insoluble salts or attached to particles (Gomez et al., 1998; Golterman, 1999; Teal and Howes, 2000; Valiela et al., 2000). In comparison with other salt mashes, the Mediterranean ones usually act as net importers of nutrients and particulate material (organic and inorganic) due to the lack of a tidal influence (Ibáñez et al., 2000) and confinement (Guelorget and Perthuisot, 1983). According to this, a progressive accumulation of phosphorus and organic load would be expected to occur over the years. Therefore, the nutrients and zooplankton composition and dynamics may be related to the variability in the hydrological pattern and, on the other hand, they may also be related to the cumulative processes during successive confinement events.

La Pletera salt marshes are a typical example of confined Mediterranean coastal ecosystems, where freshwater inputs are scarce and waters remain confined most of the year due to the lack of exchanges with the adjacent coastal waters. In these salt marshes, new lagoons have been created in the framework of a LIFE Restoration project and, therefore, there are new and old lagoons subject to the same hydrology. Thus, La Pletera salt marshes are a good example for illustrating the intra-annual variability of the hydrological cycle, but also the cumulative effects of confinement by means of the comparison between nutrient and species composition in old and new lagoons. The aim of the present study is to establish how the variability in the hydrological pattern determines both the nutrients composition and zooplankton dynamics and structure in the lagoons of a selected Mediterranean salt marsh (La Pletera), but also if the cumulative mechanisms associated to confinement (over the years) can explain, at least partially, the differences between old and new lagoons.

5.2. Methods

5.2.1. Field sampling and laboratory analyses

Five sampling points were selected, two of which were located in the ''old'' lagoons of La Pletera, Bassa del Pi (O1) and Fra Ramon (O2), and the other three in the ''new'' constructed system considering the three basins as independent lagoons (N1, N2 and N3) (Fig. 5.1). Sampling was carried out during a complete hydrological cycle, starting in summer 2002, when the water level was at its minimum in all lagoons, and finishing in summer 2003, when the water level had decreased again after the flood events occurred between autumn and spring. Water and zooplankton samples were taken monthly from a central point of these lagoons at a depth of 15-30 cm. The temperature (T), electrical conductivity (EC25), pH and dissolved oxygen (O2, in % of saturation) were measured in situ. Alkalinity (Alk) was measured after a few hours in the laboratory.

The water level (WL) was measured as the height in cm above or below average sea level (over the last 15 years) by means of levels installed in the lagoons. For O2 lagoon, water level data were also available from 1999 to 2003. Data on rainfall series and the average sea level over the last 15 years came from the Meteorological Station of L'Estartit (J. Pascual, pers. comm.).

Ammonium ($NH4^+$), nitrite (NO_2^-), nitrate (NO_3^-) and soluble reactive phosphate (SRP) were analysed from filtered water samples and total nitrogen (TN) and phosphorous (TP) from unfiltered water samples according to Grasshoff et al. (1983) and APHA (1989). Organic matter was measured using a TOC analyser from unfiltered samples previously acidified to eliminate the inorganic dissolved carbon. Chlorophyll-*a* (Chl *a*) was measured by high-performance liquid chromatography (HPLC) using methodology modified from Zapata et al. (2000).

Zooplankton samples were obtained by filtering 5 L of water through a 50-µm mesh-size net and preserved in situ in 4% formalin. An inverted microscope was used to count and identify the zooplankton taxa. Several ciliate taxa were identified by their morphology since species identification is often difficult after the formalin fixation and therefore it is recommended to observe the live specimens or use the specialised silver staining technique (Şenler and Yildiz, 2004, and references therein). When copepods nauplii were not identifiable, species proportions of adults were assigned. In the samples without adults, the species proportions of adults captured using a dip-net (250-µm mesh-size) were used. Some groups of organisms such as amphipods, gastropods and some insects were not taken into account in the later calculations and data analysis since the sampling methodology was not suitable for estimating their abundance correctly. For each zooplankton sample the Shannon-Wiener diversity index (H), taxonomic richness (R) and evenness (E) were calculated.

5.2.2. Statistical analyses

Differences in the environmental variables between the hydrological periods were analysed by means of the non-parametric KruskaleWallis test since the majority of variables showed a lack of normality and heteroscedasticity although they had been previously $\log_{10}(x + 1)$ transformed. Differences between old and new lagoons were also analysed. A Detrended Canonical Correspondence Analysis (DCCA) was performed in order to analyse the relationship between the zooplankton composition and environmental variables. The species abundance was square root transformed and down weighted for rare species according to ter Braak (1986) and Lepš and Šmilauer (2003). All environmental variables used in the analysis showed an Inflation Factor < 10 (ter Braak and Šmilauer, 2002). To test the significance of the ordination axes, 499 unrestricted permutations were conducted using the Monte Carlo test. All calculations and statistical analyses were performed with SPSS 12.0 for Windows, except the DCCA, which was carried out using CANOCO 4.5 for Windows.

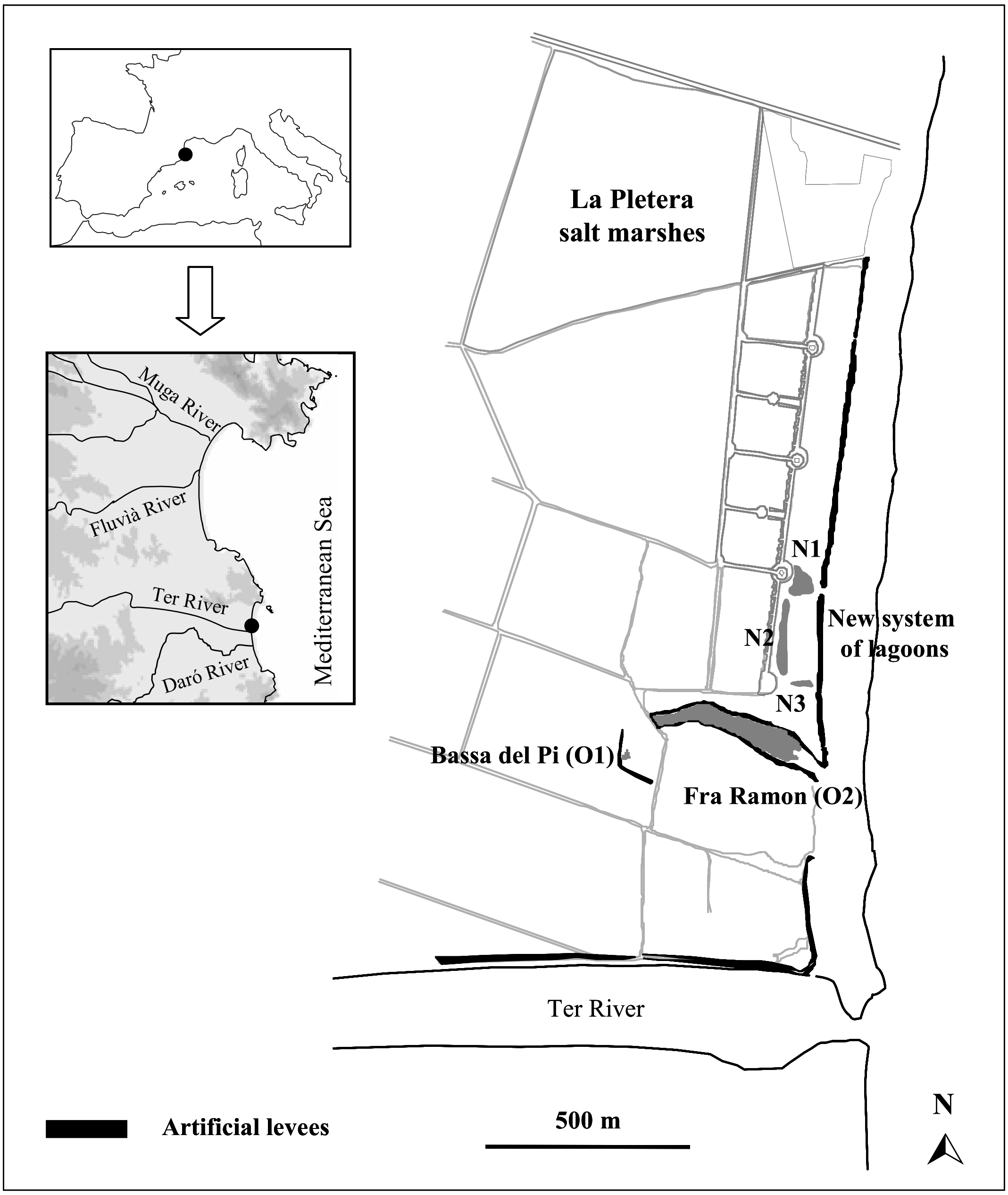


Figure 5.1. Sketch map of La Pletera salt marshes showing the location of the studied lagoons. The artificial levees constructed in the marsh can also be seen.

5.3. Results

5.3.1. Hydrological regime

Data from four consecutive hydrological cycles (July 1999-July 2003) in O2 lagoon (Fig. 5.2) show that the maximum increase in the water level always coincided with marine intrusions during sea storms, when waves came over the sand bar. There was at least one intense sea storm every year and in some unusual years (e.g. during the 2001-2002 cycle) there were more. Sea storms always took place between autumn and spring but the frequency with which they occurred was different each year, and any seasonal pattern was not observed during the four cycles. For example, in the 1999-2000 cycle the biggest sea storm was in October, but in 2000-2001 it was in January and in 2001-2002, there was even an intense sea storm in March (Fig. 5.2). Marine intrusion is usually a sudden event and, in terms of volume, it is the largest input of water into the marsh, since intense rainfalls do not cause large increases in the water level (e.g. July 2000, October 2001 and May 2002). During the rest of the cycle, although the increase in the groundwater level or rainfall often resulted in a water input, the water level gradually decreased mainly due to evaporation and infiltration, and in some summers it even descended below the average sea level (e.g. September 2000, 2001).

Considering only the 2002-2003 hydrological cycle when nutrients and organisms sampling was carried out, the maximum water level, that in all lagoons exceeded 95 cm above sea level, was reached by marine intrusion after a sea storm (Fig. 5.3 A). During the rest of the cycle, rainfall also caused freshwater inputs but the increase in the water level was always lower. In summer 2003, the lack of inputs and an increase in evaporation caused a drastic decrease in the water level, especially in the old lagoons where it decreased below the average sea level. The same seasonal conductivity evolution pattern was observed in all lagoons during the studied period (Fig. 5.3 B). Conductivity increased in the dry seasons reaching and even exceeding the seawater conductivity in the old lagoons. In all lagoons, marine intrusion (December 2002) and intense rainfall (> 65 mm; January 2003) caused a decrease in the conductivity, especially in the second case, when conductivity values reached a minimum because of the freshwater inputs into the salt marshes.

During the entire cycle, the old lagoons always showed the highest conductivity values.

In a hydrological cycle, a dry season and a flooding period could be distinguished. The criterion followed was based on the consideration that the dry season included those months when water level was always lower than 40 cm a.s.l. and the unvegetated bed of the lagoons (a muddy area) was partially exposed to the air. During the flooding period the lagoons bed was completely inundated and water level in most lagoons was higher than 40 cm a.s.l. In the present study the dry season include two periods, at the beginning and at the end of the studied hydrological cycle (Fig. 5.3). In the first one (July 2002-September 2002) water levels in the lagoons were always above the average sea level while in the second one (May 2003-September 2003) lagoons tend to desiccation due to the lack of rainfalls and a high evaporation. During the dry season the average water level and the average conductivity in the studied lagoons was 13.5 cm a.s.l. and 50.39 mS cm $^{-1}$, respectively. During the flooding period (October 2002-April 2003) water level in the lagoons oscillated depending on the precipitations and the maximum water level was reached after the marine intrusion during a sea storm. In this period, the average water level in the lagoons was 50.7 cm a.s.l. and the average conductivity was 30 mS cm^{-1}.

5.3.2. Nutrient composition

Results in Table 5.1 show that in the dry seasons (July 2002-September 2002 and May 2003-September 2003) concentration of nitrate was significantly lower than that in the flooding period (October 2002-April 2003). In all lagoons, nitrate concentration peaked coinciding with the main water inputs and in the rest of the studied period concentrations decreased to undetectable levels (Fig. 5.4 A). Conversely, total nitrogen, total phosphorus, total organic carbon as well as chlorophyll-a reached significantly higher concentration in the dry seasons, when water levels diminished. As an example, the evolution of total nitrogen throughout the hydrological cycle in the studied lagoons is shown (Fig. 5.4 B).

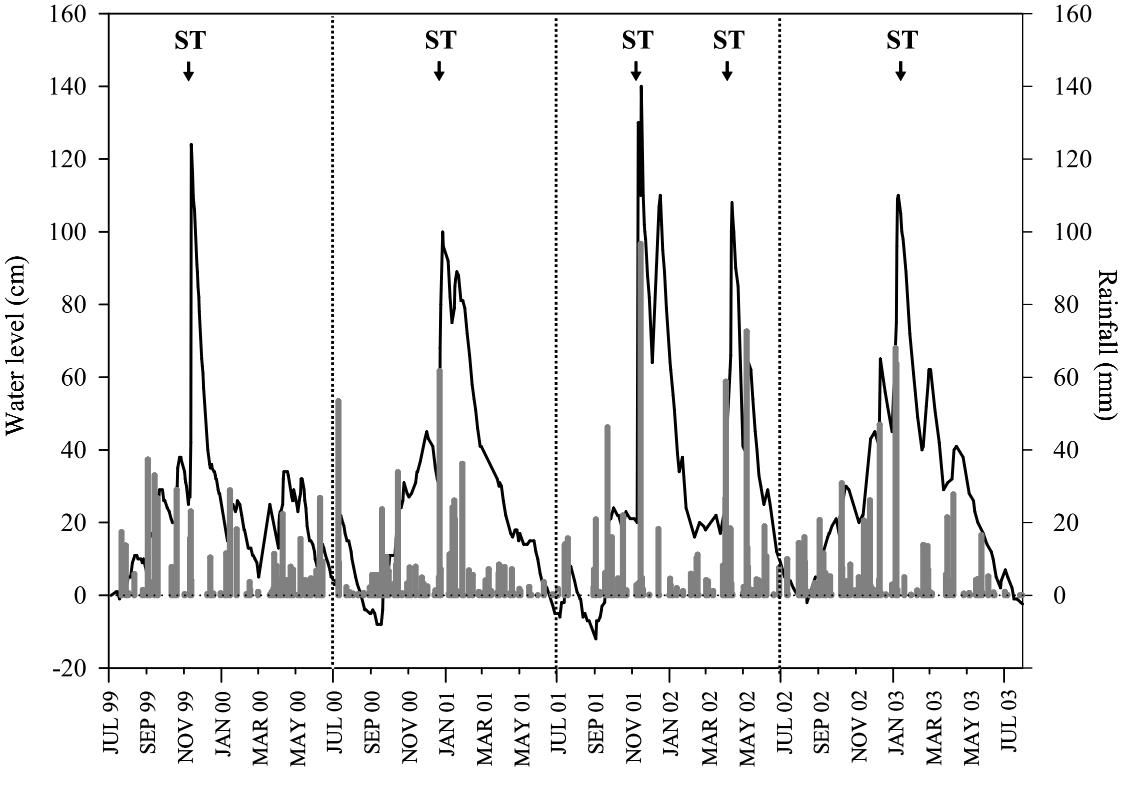


Figure 5.2. Variation in the water level (cm above or below the average sea level) in O2 lagoon during four hydrological cycles (from 1999 to 2003, separated by dotted lines). The bars represent rainfall (in mm). ST indicates sea storm events. The zero water level corresponds to the average sea level over the last 15 years.

Temperature and conductivity were also higher in the dry period whereas alkalinity and water level were higher in the flooding period. Nonsignificant differences with regard to ammonium, nitrite, soluble reactive phosphate, pH and dissolved oxygen were observed between the hydrological periods.

The old lagoons of La Pletera salt marshes had shown significantly higher concentrations of phosphorus (total and inorganic soluble), total nitrogen, total organic carbon and chlorophyll-*a* than the recently built lagoons suggesting a progressive accumulation over the years of the nutrients and the organic content. Conductivity and alkalinity were also significantly higher in the old lagoons. In contrast, nitrate, temperature and water level, directly related with the hydrological regime, did not show significant differences related to the lagoon age since old and new lagoons were subject to the same hydrological pattern.

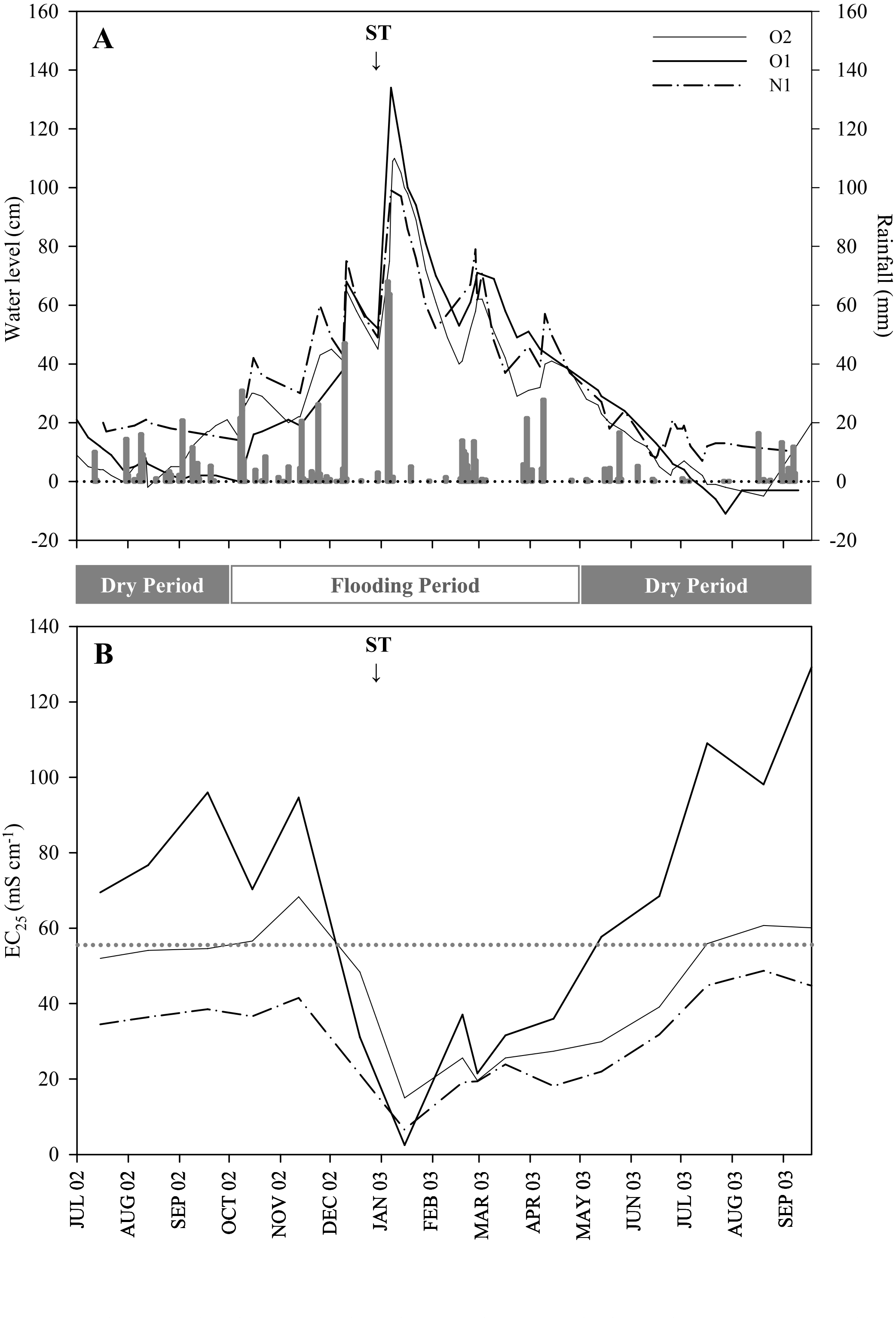


Figure 5.3. Changes in the water level (cm above or below the average sea level) of three studied lagoons during the hydrological cycle 2002-2003. ST indicates the sea storm event. The bars represent rainfall (in mm). The zero water level corresponds to the average sea level over the last 15 years. (B) Changes in Electrical Conductivity (EC_{25}, in mS cm^{-1}) in the same lagoons. The dotted line shows the average seawater conductivity in the Mediterranean Sea. The dry period and the flooding period are shown.

Table 5.1

Mean and standard deviation (in brackets) of the dissolved inorganic nutrients, total nutrients and chlorophyll-*a*. Other physical and chemical variables are also shown. Abbreviations of the variables are given in the text (see Section 5.2.). Significant (**: p < 0.01) and nonsignificant (n.s.: p > 0.05) differences between dry and flooding periods and old and new lagoons are shown (Kruskal-Wallis test).

	Dry		Flooding		Sig.	Old lagoons		New lagoons		Sig.
NH_4^+ (mg N l^{-1})	0.07	(0.13)	0.03	(0.07)	n.s.	0.04	(0.07)	0.07	(0.12)	n.s.
NO_2^- (mg N l^{-1})	0.001	(0.002)	0.01	(0.03)	n.s.	0.01	(0.03)	0.002	(0.003)	n.s.
NO_3^- (mg N l^{-1})	0.01	(0.06)	0.17	(0.38)	**	0.12	(0.39)	0.07	(0.15)	n.s.
SRP (mg P l^{-1})	0.02	(0.03)	0.03	(0.04)	n.s.	0.05	(0.05)	0.01	(0.01)	**
TN (mg N l^{-1})	3.66	(2.45)	2.07	(1.62)	**	4.53	(2.38)	1.67	(0.97)	**
TP(mg P l^{-1})	0.48	(0.67)	0.13	(0.15)	**	0.59	(0.70)	0.10	(0.10)	**
TOC (mg l^{-1})	66.02	(84.07)	21.26	(21.38)	**	78.64	(88.33)	18.43	(15.22)	**
Chl *a* (mg l^{-1})	0.03	(0.04)	0.01	(0.01)	**	0.03	(0.05)	0.005	(0.01)	**
Temperature (ºC)	26.11	(3.74)	12.34	(5.51)	**	20.29	(8.34)	18.94	(8.39)	n.s
EC_{25} (mS cm^{-1})	50.39	(25.77)	30.07	(19.88)	**	56.04	(29.02)	28.85	(12.61)	**
pH	8.00	(0.40)	8.06	(0.67)	n.s.	7.99	(0.54)	8.06	(0.55)	n.s.
O_2 (% sat.)	107.84	(45.91)	99.68	(52.71)	n.s.	115.39	(67.70)	95.13	(25.11)	n.s.
AlK (meq l^{-1})	6.28	(1.80)	5.40	(1.82)	**	6.81	(2.25)	5.13	(1.00)	**
WL (cm a.s.l.)	13.54	(11.12)	50.69	(23.45)	**	26.20	(28.27)	35.24	(23.58)	n.s.

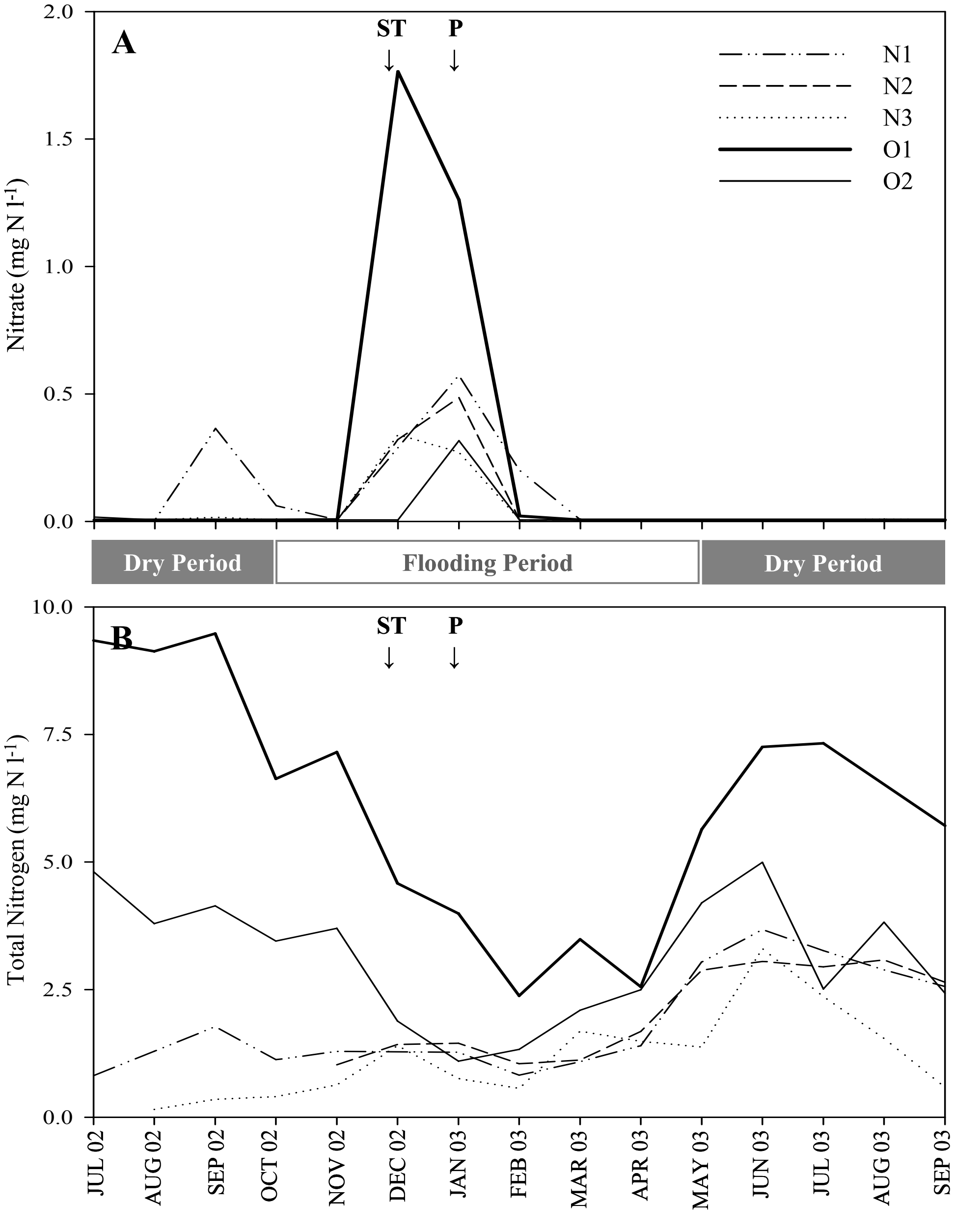


Figure 5.4. Seasonal changes in the concentration (in mg N l^{-1}) of nitrate (A) and total nitrogen (B) in the studied lagoons during the hydrological cycle 2002-2003. The main disturbances are indicated as ST (sea storm) and P (precipitation > 65 mm). The dry period and the flooding period are shown.

5.3.3. Zooplankton community structure and composition

Forty-three taxa belonging to 6 different Phyla were distinguished in the zooplankton samples. The most represented phyla were Ciliates, Rotifera and Arthropoda (mainly copepods) that included 14, 12 and 9 taxa, respectively. Only 19 taxa coincided in both types of lagoons (Table 5.2). Among them, only the following were found in the five lagoons: the ciliate *Fabrea* gr. salina, the rotifers *Brachionus plicatilis* and *Testudinella clypeata*, the calanoid *Eurytemora velox* and the harpacticoids *Canuella perplexa* and *Mesochra lilljeborgi*. The rotifer *B. plicatilis* was the most abundant taxa in all studied lagoons since population explosions were usual, mainly in summer 2003, when this species reached more than 30 $\times 10^3$ ind. l^{-1} and dominated the community. Among the taxa that did not occur in both the old and new lagoons (Table 5.2) it is necessary to point out the absence of the harpacticoid *Tisbe longicornis* in the new lagoons and the absence of the calanoid *Calanipeda aquaedulcis* in the old ones. The oligochaete *Nais* sp., 6 rotifer taxa and 4 undetermined ciliate taxa were also only found in the new lagoons. Cumulative taxonomic richness in the new lagoons (Table 5.2) was higher than that in the old ones but the average richness was not significantly different in the old and new lagoons (Table 5.3). The evenness and Shannon-Wiener diversity averages were significantly higher in the new ones but no significant differences were observed when comparing the dry and the flooding periods.

In all lagoons, the maximum zooplankton abundance, which ranged from 1800 ind. l^{-1} (in O1) to 34,000 ind. l^{-1} (in N1), was reached during summer 2003, while the minimum values were recorded in December and January, after the marine intrusion during a sea storm (Fig. 5.5 and 5.6). Regarding the zooplankton temporal pattern, in the new lagoons the calanoid *Eurytemora velox* and the cyclopoid *Diacyclops bicuspidatus odessanus* dominated the community during the months that followed the sea storm (Fig. 5.6) but in the old ones the cyclopoid was not very abundant and calanoid was the dominant taxa (Fig. 5.5). Later, when water levels were lower, as well as in the months before the marine intrusion, zooplankton was mainly dominated by the rotifer *Brachionus plicatilis* and the harpacticoids *Mesochra lilljeborgi* and *Canuella perplexa*. In the same period in the old lagoons the presence of ciliates became more significant, especially in the most eutrophic lagoon (O1), in which the ciliate *Fabrea* gr. *salina* dominated the community during the months with the lowest water levels.

Table 5.2

Number of taxa of each Phylum found in old and new lagoons. The number of taxa that occur in both types of lagoons and cumulative richness (R_c) are also shown.

Phylum	Total	Old lagoons	New lagoons	Coincident taxa
Ciliates	14	10	9	5
Rotifera	12	6	10	4
Platyhelminthes				
Cl. Turbellaria	1		1	
Nematoda				
Cl. Nematoda	5	3	4	2
Annelida				
Cl. Oligochaeta	1		1	
Cl. Polychaeta	1	1	1	1
Arthropoda				
Cl. Ostracoda	1	1	1	1
Cl. Copepoda				
O. calanoida	2	1	2	1
O. cyclopoida	2	2	2	2
O. harpacticoida	4	4	3	3
R_c	43	28	34	19

Table 5.3

Mean and standard deviation (in brackets) of the taxonomic richness (*R*), evenness (*E*) and Shannon-Wiener diversity (*H*). The total average of all studied lagoons is also shown. Significant (*: $p < 0.05$, **: $p < 0.01$) and nonsignificant (n.s.: $p > 0.05$) differences between dry and flooding periods and old and new lagoons are shown (Kruskal-Wallis test).

	Total	Dry	Flooding	Sig.	Old lagoons	New lagoons	Sig.
R	4.79 (2.36)	4.59 (1.99)	5.00 (2.70)	n.s.	4.86 (2.64)	4.74 (2.15)	n.s.
E	0.43 (0.28)	0.39 (0.29)	0.47 (0.26)	n.s.	0.31 (0.25)	0.52 (0.27)	**
H	0.96 (0.73)	0.90 (0.76)	1.01 (0.70)	n.s.	0.75 (0.75)	1.12 (0.69)	*

The results of the DCCA ordination are summarized in Fig. 5.7. The first two axes together accounted for 35.6% of the total variance. The first canonical axis and the overall test were significant (Monte Carlo test, p < 0.01). The first axis (23.8%) was related with the hydrological gradient. Samples with the lowest coordinates, dominated by *Eurytemora velox* and *Diacyclops bicuspidatus odessanus*, were collected after the flooding event when the water level was highest. On the other hand, the samples with the highest coordinates, dominated by ciliates or *Brachionus plicatilis*, were collected when the temperature and conductivity were at their highest, during the driest periods, also coinciding with the maximum values of total nutrients and chlorophyll-*a*. On the second axis (11.8%) samples were situated according to the lagoon age. The upper part of this axis, where the old lagoons were located, indicated situations of high nutrient concentrations. The main taxon related with this situation was the harpacticoid *Tisbe longicornis*. Samples from the new lagoons, located in the lower part, were characterized by higher values of richness and evenness.

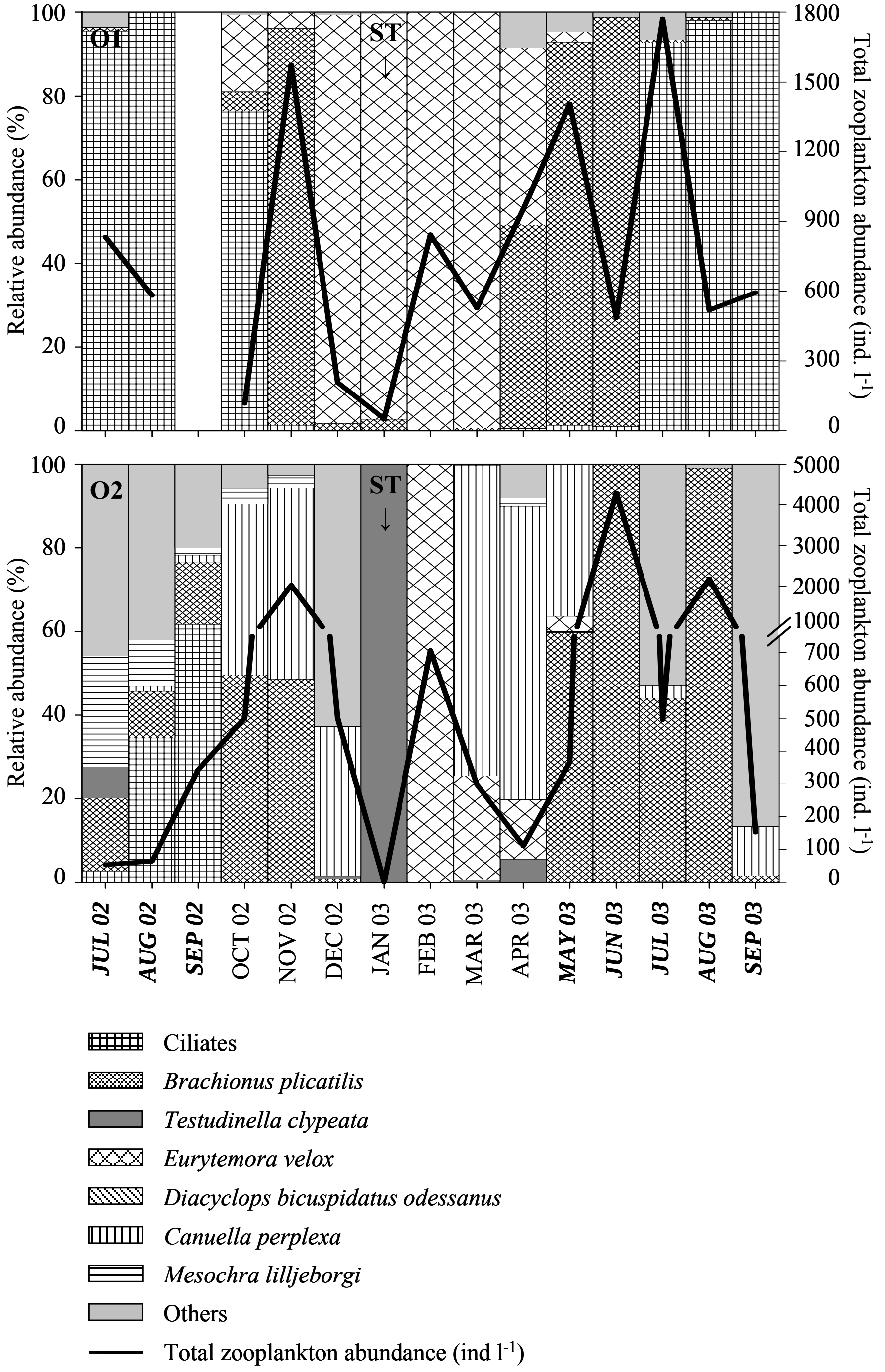


Figure 5.5. Relative abundance (in %) of the zooplankton species during the hydrological cycle 2002-2003 in the old lagoons. The represented species dominated the community in some samples (> 75% of the total individuals). Total zooplankton abundance evolution (ind. l^{-1}) is shown on the right axis of the plots. In September 2002 in the O1 lagoon (white bar) zooplankton sampling was carried out but no individual was found. ST indicates sea storm events. Months belonging to the dry seasons are indicated by bold and italic letters.

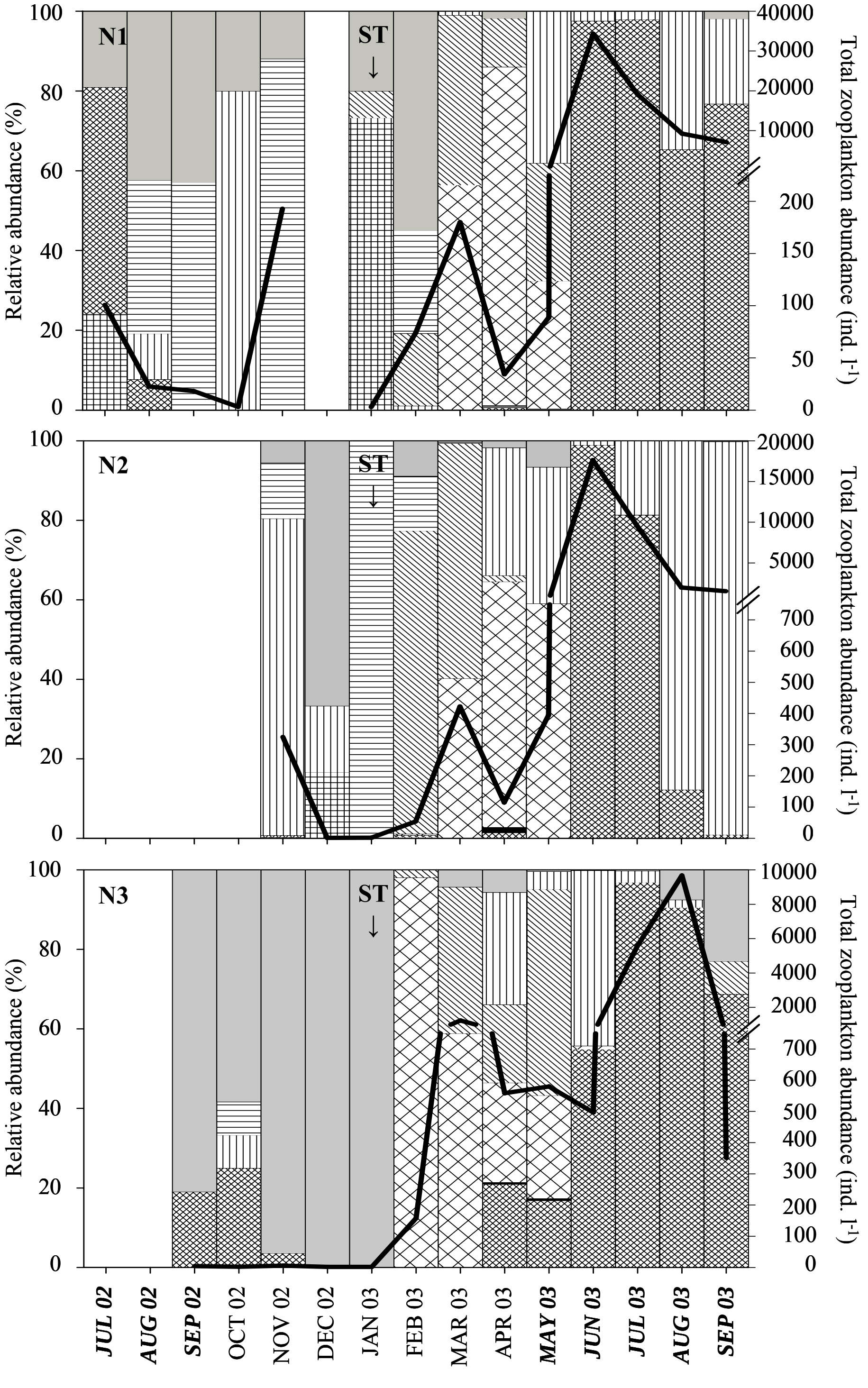


Figure 5.6. Relative abundance (in %) of the zooplankton species during the hydrological cycle 2002-2003 in the new lagoons. The represented species dominated the community in some samples (> 75% of the total individuals). Species legend is given in the Fig. 5.5 caption. Total zooplankton abundance evolution (ind. l^{-1}) is shown on the right axis of the plots. White bars represent those months in which no zooplankton sample was taken. ST indicates sea storm events. Months belonging to the dry seasons are indicated by bold and italic letters.

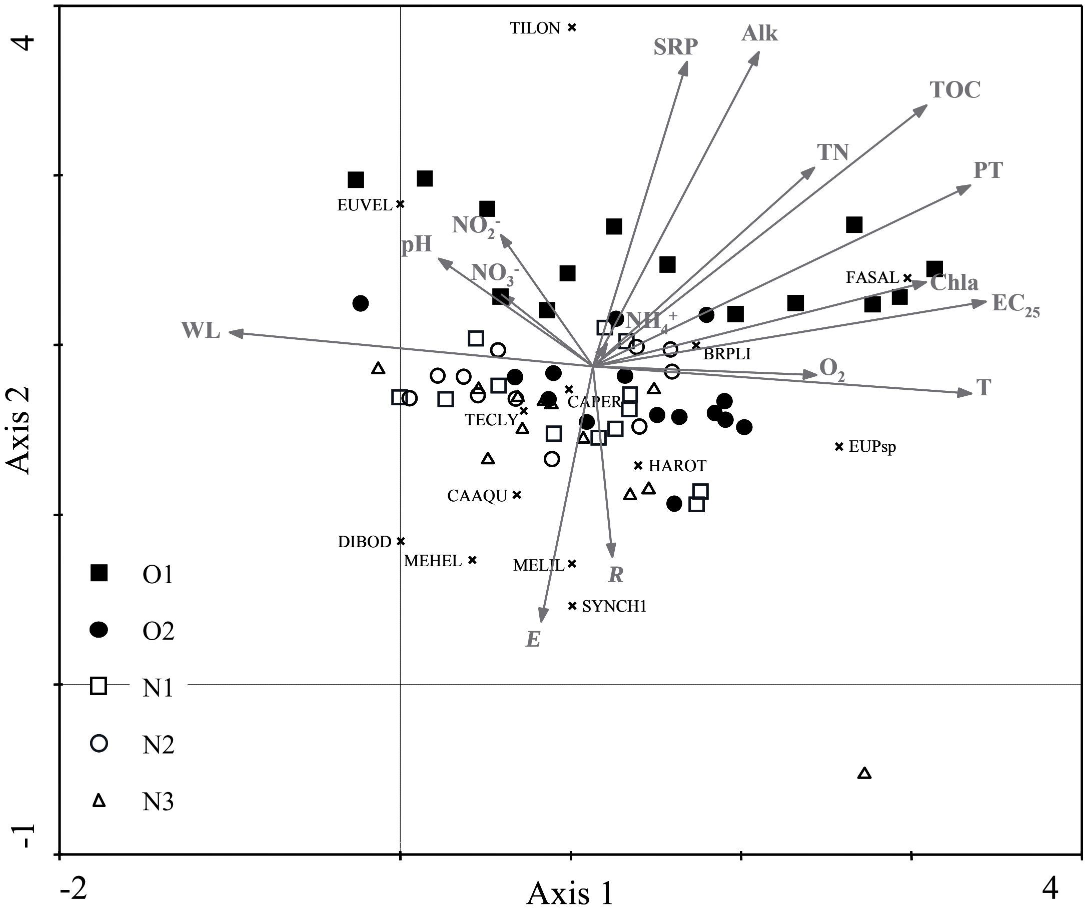


Figure 5.7. DCCA ordination diagram: triplot showing the samples and the zooplankton taxa position in relation to some environmental and community variables (arrows) in the space created by the first two axes. Abbreviations of the variables are given in the text (see section 5.2.1). Only species with more than 5% weight in the analysis are shown: *Fabrea* gr. *salina* (FASAL), *Euplotes* sp. (EUPsp), *Brachionus plicatilis* (BRPLI), *Testudinella clypeata* (TECLY), Synchaetidae undet. sp.1 (SYNCH1), *Eurytemora velox* (EUVEL), *Calanipeda aquaedulcis* (CAAQU), *Halicyclops rotundipes* (HAROT), *Diacyclops bicuspidatus odessanus* (DIBOD), *Mesochra heldti* (MEHEL), *Mesochra lilljeborgi* (MELIL), *Canuella perplexa* (CAPER) and *Tisbe longicornis* (TILON).

5.4. Discussion

In La Pletera salt marshes, the water inputs determine the entries of the dissolved inorganic nitrogen into the lagoons whereas total phosphorus, total nitrogen, organic matter and chlorophyll-*a* increased in the dry periods due to confinement. The lack of water inputs and the high evaporation cause a progressive decrease of the water level and, therefore, there is a progressive accumulation of the salts, total nutrients and organic content, as well as the organism densities. In this kind of coastal lagoons, which remain confined during a prolonged period without water inputs, a differential confinement of nutrients has been described. Phosphorous tends to accumulate progressively in the sediment, and nitrogen (the oxidized compounds) rapidly diminishes mainly due to denitrification, becoming the limiting factor of the primary producers (Quintana et al., 1998a; Frascari et al., 2002; Mendoza-Salgado et al., 2005). The fact that the dissolved inorganic nitrogen is usually related to water inputs, either marine or freshwater, and phosphorus depends more on the internal recycling processes (e.g. release from sediment, mineralisation of organic matter), is a common feature in most coastal lagoons (Souchu et al., 1997; Victor et al., 1997; Kormas et al., 2001; Lucena et al., 2002; Pérez-Ruzafa et al., 2002; Serrano et al., 2004).

Lagoon age appears to be related with differences in total nutrients and organic matter. Concentrations were always higher in the old lagoons than those in the recently built ones, suggesting that a progressive accumulation of the organic content takes place during the successive confinement events over the years. This is expected to occur in these coastal wetlands without continuous freshwater inputs and with low and irregular rainfall, where inflowing waters remain confined. In fact, the Mediterranean salt marshes usually have an endorheic character, acting as a sink for nutrients and organic matter (Ibáñez et al., 2000; Cotner et al., 2004; Mendoza-Salgado et al., 2005). However, this hydrology based on sudden flooding events and prolonged confinement situations may be less determinant for the nutrients cycle in those Mediterranean salt marshes or coastal lagoons where other factors such as human regulation, riverine inputs or a permanent connexion with the sea are more relevant (Heurteaux, 1992; Comín and Valiela, 1993; Frascari et al., 2002; Pérez-Ruzafa et al., 2002; Villena and Romo, 2003).

The zooplankton composition in La Pletera salt marshes was common to those Mediterranean coastal environments characterized by unstable chemical and physical parameters. Dominant species are very euryhaline, adapted to the high salinity fluctuations that characterized these brackish water ecosystems (Cognetti and Maltagliati, 2000; Ramdani et al., 2001). The zooplankton temporal pattern found in La Pletera, related to the hydrological variability, was similar to that of other Mediterranean coastal wetlands where, depending on the hydrology, there is a series of situations in which the community is dominated by characteristic taxa (Brucet et al., 2005a). While calanoids dominated during the winterespring hydrological stability, harpacticoids dominated when the water level was low and *Brachionus plicatilis* during the summer hypertrophy, due to its tolerance to low oxygen concentrations (Miracle et al., 1987; Esparcia et al., 1989). The ciliate *Fabrea* gr. *salina* dominated in higher salinity conditions, and it has also been described to dominate when temperatures and organic matter levels are high (Moscatello and Belmonte, 2004; Toumi et al., 2005). In spite of the similarities, it is worth pointing out that the 'Synchaeta situation', described immediately after the marine intrusion by Quintana et al. (1998b) has not been observed in La Pletera salt marshes due to the lower frequency of sampling. The intra-annual variability of the hydrological cycle and the ecological changes that it induces in the planktonic community have already been reported in other coastal lagoons (Victor and Victor, 1997; Quintana et al., 1998b; Gilabert, 2001) as well as in benthic (Trobajo et al., 2004; Gascón et al., 2005) and fish communities (Poizat et al., 2004).

The lagoon age also affects the zooplankton composition since cumulative mechanisms over years associated to the confinement determine the higher nutrient load and salinity of the old lagoons. Thus, the harpacticoid *Tisbe longicornis*, which has been associated with eutrophic conditions in some Mediterranean thalassohaline wetlands, appears only in the old lagoons while the calanoid *Calanipeda aquaedulcis*, related with more oligotrophic conditions, was only found in the new ones (Boix et al., 2005). Differences in taxonomic composition due to the ‘‘site age’’ have also been detected in other aquatic macro-invertebrates communities (Barnes, 1983; Talley and Levin, 1999; Fairchild et al., 2000). Lower diversity in the old lagoons could be expected since it has already been described that species diversity of aquatic animals decreases in more hyperhaline conditions (Britton and Johnson, 1987; Therriault and Kolasa, 1999; Therriault, 2002; Toumi et al., 2005). Furthermore, the impoverishment of fauna in this kind of coastal lagoons can also be attributed to confinement according to Guelorget and Perthuisot (1983). Other processes such as colonization could explain some of the differences between the old lagoons and the recently built ones, such as the exclusive presence of the opportunist Naidid oligochaetes and the higher evenness in the new lagoons. Naidid oligochaetes have already been described to be the earliest colonists in recently created habitats in other salt marshes (Talley and Levin, 1999; Moseman et al., 2004). Higher evenness in the recently created habitats could be expected at the beginning of colonization since there is more habitat available, less competition for food and fewer predators (Wiggins et al., 1980; Lake et al., 1989).

CHAPTER 6

Influence of hydrology, nutrient composition, food resource availability and predation on the zooplankton taxonomic and size diversity

6.1. Introduction

Predation by planktivorous fishes plays a key role in structuring the zooplankton community, especially with respect to the size structure, since prey selection is typically size-dependent. Thus, when fish predation pressure increases, the largest zooplankters are removed from the community allowing the smallest ones to dominate (Brooks and Dodson, 1965; Hall et al., 1976; Vanni, 1986). In shallow waters, fish predation usually exerts a strong influence on the size structure but also on the taxonomic composition of the zooplankton (Jeppesen et al., 1997; Zimmer et al., 2001; Jakobsen et al., 2003). Macroinvertebrate predation may also significantly affect the zooplankton species composition and size distribution, especially when planktivorous fishes are reduced or removed (McQueen et al., 1986; Hampton et al., 2000; Hampton and Gilbert, 2001). However, in contrast with fish predation, a decrease of the small zooplankters will be expected when the macroinvertebrate predation pressure increases (Lynch, 1979; Arnott and Vanni, 1983; Hanazato and Yasuno, 1989).

In addition to predation, the zooplankton community structure also depends on food abundance and/or quality and, in turn, on competition for this food (Benndorf and Horn, 1985; Vanni, 1987; Kerfoot et al., 1988). Competition for food is also size-dependent since, under food limitation, the largest species or organisms are usually better competitors and the smallest ones are excluded (Brooks and Dodson, 1965; Declerck et al., 1997; Kreutzer and Lampert, 1999). Competitive interactions for food could be reduced if there is resource partitioning among the coexisting species or, in the case of competitive exclusion, among different development stages of the same species (Hall et al., 1976; Werner and Gilliam, 1984).

Hydrology and nutrient enrichment may also exert a strong influence on the species composition and size distribution of the zooplankton, as shown in other shallow Mediterranean waters (e.g. Quintana et al., 1998b; Gilabert, 2001; Pérez-Ruzafa et al., 2002). Hydrology is usually the main driver of nutrient supply in these systems. An increase in the more rapidly-growing and more edible phytoplankton forms, which are often of smaller cell size, is typically observed after a nutrient perturbation. Zooplankton community structure might shift because small bodied zooplankters with short generation times will probably be the first to respond to a shift in prey availability (Romo et al., 2004; Buyukates and Roelke, 2005).

Traditionally, community structure has been analyzed by means of a taxon-based approach (Tilman, 1982; Giller, 1984; Margalef, 1991), and the Shannon species diversity index has been widely used as an integrating measure of this structure. However, since energy fluxes through the food web and feeding ecology are more dependent on body size, patterns in size distribution would provide a more functional perspective of the community structure (Sprules and Holtby, 1979; Stein et al., 1988; Gaedke et al., 2004). In that sense, Margalef (1991) suggested that an ataxonomic diversity descriptor based on the organisms' size distribution would be helpful to understand the community organization and functioning. Thus, a measure of size diversity, as proposed by Lurie and Wagensberg (1983, 1984), might better reflect some functional aspects of the community structure than a measure of species diversity. Nevertheless, several authors have recommended the simultaneous use of taxon and size-based approaches since they became complementary in studies of aquatic communities (Rodríguez and Magnan, 1993; Boix et al., 2004; Jennings, 2005).

There are few studies dealing with the combination of taxon and size-based approaches and the knowledge about how these approaches respond to different community structuring factors is still very scarce. The objectives of this study were to establish whether both the taxonomic and the size structure of a zooplankton community are affected by the following factors: predation, food resource availability, nutrient composition and hydrology. The Shannon species diversity and a size diversity measure were used as taxon and size-based approaches to the study of the community, respectively. For this purpose, we selected a group of coastal lagoons in a Mediterranean salt marsh (La Pletera) where zooplankton predator density, as well as food resource, nutrient composition and water level varied over a wide range (Table 6.1).

Table 6.1

For each studied lagoon, mean of all monthly samples averaged and coefficient of variation (%; in brackets) of several environmental and biological variables, taxonomic diversity (*H*) and size diversity ($\mu_{s'}$) are shown. (Details about the computation of Food Resource availability, Fish density and Macroinvertebrate predator densiy will be given in the section 6.2.4).

	N1	N2	N3	O1	O2
Water level	32.71	36.75	36.57	26.67	25.73
(cm a.s.l.)	(72)	(68)	(64)	(113)	(106)
Electrical Conductivity	31.94	32.20	23.13	67.20	44.89
(mS cm^{-1})	(38)	(51)	(29)	(51)	(36)
Dissolved Inorganic Nitrogen	0.16	0.14	0.11	0.27	0.04
(mg N l^{-1})	(141)	(128)	(148)	(243)	(203)
Soluble reactive phosphate	0.01	0.01	0.005	0.08	0.02
(mg P l^{-1})	(161)	(108)	(145)	(72)	(88)
Total nitrogen	1.88	2.03	1.19	6.05	3.12
(mg N l^{-1})	(52)	(43)	(74)	(39)	(39)
Total phosphorus	0.12	0.14	0.05	0.86	0.32
(mg P l^{-1})	(88)	(76)	(91)	(104)	(59)
Chlorophyll-*a*	4.59	5.62	4.27	44.78	19.75
(µg l^{-1})	(177)	(191)	(200)	(127)	(182)
Bacterial biovolume	59.94	68.87	10.62	701.6	61.43
(μm^3 l^{-1}) $\times 10^7$	(181)	(246)	(118)	(190)	(87)
Phytoplankton biovolume	543.5	34.68	195.2	636.8	477.2
(μm^3 l^{-1}) $\times 10^7$	(161)	(142)	(198)	(197)	(234)
Zooplankton biomass	412	212.1	3987	260.7	107.2
(µg-DW l^{-1})	(205)	(154)	(342)	(160)	(153)
Food Resource availability	132.9	13.09	114.5	29.77	132.5
(μm^3 µg-DW^{-1}) $\times 10^7$	(276)	(144)	(275)	(179)	(328)
Macroinvertebrate predator density	18.43	99.32	1.67	8.58	73.67
(ind. m^{-2})	(317)	(278)	(224)	(163)	(245)
Fish density		0.60			11.93
(ind. m^{-2})	——	(123)	——	——	(104)
Shannon-Wiener diversity (*H*)	1.13	0.77	1.38	0.36	1.11
	(57)	(67)	(55)	(115)	(72)
Size diversity ($\mu_{s'}$)	4.46	4.41	4.62	5.55	5.30
	(42)	(50)	(26)	(33)	(40)

6.2. Methods

6.2.1. Field sampling and laboratory analyses

The five permanent lagoons of La Pletera salt marshes were sampled: the two ‘‘old’’ lagoons, Bassa del Pi (O1) and Fra Ramon (O2), and the three ‘‘new’’ constructed ones (N1, N2 and N3) (Fig. 5.1, pg. 45). All sampled lagoons are shallow. The water column depth can reach up to about 2.5 m in the flooding periods and it commonly falls to 30 cm during the prolonged confinement situations. Sampling was carried out during a complete hydrological cycle, from July 2002 to September 2003 except in the N2 lagoon where it started in November. Water and zooplankton samples were taken monthly from a central point of the lagoons at a depth of 15-30 cm. The water level and electrical conductivity were measured *in situ*. Dissolved inorganic nitrogen (ammonium + nitrite + nitrate) and soluble reactive phosphate were analysed from filtered water samples and total nitrogen and total phosphorus from unfiltered water samples following Grasshoff et al. (1983) and APHA (1989). Organic matter was measured using a TOC analyser from unfiltered samples previously acidified to eliminate the inorganic dissolved carbon. Chlorophyll-*a* was extracted using 95% methanol after filtering 500 ml of water (GF/C Whatman filters) and its concentration was measured by HPLC using a methodology modified from Zapata et al. (2000).

6.2.2. Zooplankton sampling and biomass assessment

Zooplankton samples were obtained by filtering 5 L of water through a 50-µm mesh-size net and preserved in situ in 4% formalin. An inverted microscope was used to count and identify the zooplankton taxa. Several ciliate taxa were identified by their morphology since species identification is often difficult after formalin fixation (Şenler and Yildiz, 2004, and references therein). When copepods nauplii were not identifiable, species proportions of adults were assigned. In the samples without adults, the species proportions of adults from the macroinvertebrate samples (see below in this section) were used. Some groups of organisms that appeared in the zooplankton samples such as amphipods, gastropods and insects were not taken into account in the calculations of the zooplankton diversities and data analysis since the sampling methodology was not suitable for estimating their abundance correctly. Following Brucet et al. (2006), biomass dry weight was estimated, for ostracods and copepods, from the allometric relationship between the weight and the body length. For other invertebrates, it was obtained by converting biovolume into dry weight. Biovolume was estimated by the approximation of the body shape to geometric figures (see Appendix II for more details about zooplankton biomass estimates).

6.2.3. Diversity calculations

For each zooplankton sample taxonomic diversity was obtained by means of the Shannon-Wiener index (*H*) using the numerical abundance of each identified taxon.

Size diversity (μ) was calculated using an index proposed by Lurie and Wagensberg (1983, 1984), which is analogous to *H* but appropriate for continuous variables and is based on the function of probability density of individuals with respect to size ($p_\mathrm{i}(s)$):

$$\mu = -\int p_i(s)\log_2 p_i(s)ds$$

The use of a continuous distribution avoids the arbitrariness introduced when using size classes since size diversity for a given sample can vary depending on the chosen number of size classes (Ruíz, 1994). In the present study, the non-linear Pareto type-II distribution has been used as the probability density function ($p_i(s)$) according to Brucet et al. (2006) and the individual biomass dry weight has been used as the individual size (s). Since when using the Pareto type-II distribution it is assumed that this function adequately represents the size distribution of individuals within a functional group (Vidondo et al., 1997), size diversity ($\mu_{s'}$) was only measured for those zooplankton samples that showed a good fit ($r^2 > 0.8$) to this distribution. For this reason, only 77% of the samples were used in the posterior analyses and calculations (see Appendix III for more details about Pareto type-II distribution and size diversity computation).

6.2.4. Assessing the zooplankton's food resource availability and predator density

As an approximation of the zooplankton's potential food resource availability (FR_a) the quotient between the bacterial + phytoplankton biovolume (in µm^3) and the zooplankton biomass (in µg dry weight) was calculated. Resource-consumer ratios have been recommended when applying theoretical models for studying the whole community, since all prey species available are contained in the numerator, and all consumer species in the denominator (Berryman, 1992). In fact, studies in natural systems have suggested that the predator's functional response is a function of the number of prey per predator and that this ratio dependency would be more representative in heterogeneous environments (Matson and Berryman, 1992; Akçakaya et al., 1995). Flow cytometry was used to assess the bacterial and phytoplankton biovolume. Samples were collected from 50 µm filtered water, fixed with 1% paraformaldehyde and 0.05% glutaraldehyde (final concentration), deep frozen in liquid nitrogen and stored frozen at -20 ºC. For the phytoplankton, biovolume estimations were obtained from the correlation between FSC signals of the flow cytometer and cell volume according to López-Flores et al. (2006). The bacterial biovolume estimations were performed following Gasol and Del Giorgio (2000).

Fish density was estimated following Seber (1982) from a bimonthly sampling carried out using throw enclosure traps (Alcaraz and García-Berthou, in press). Some marine fishes such as eels (*Anguilla anguilla*) and several mullet species (Mugilidae) were occasionally observed but they were not captured in any sampling period. Only *Aphanius iberus* presented stable populations in the lagoons O2 and N2 and, therefore, density of this species was used as fish density (FD; ind. m^{-2}) in posterior analyses. Furthermore, in coastal wetlands, eels have generally been described as piscivorous or infaunal feeders (Elliott et al., 2002; Maes et al., 2003) and the mullet's diet usually focuses on detritus and phytobenthos (Laffaille et al., 1998, 2002; Almeida, 2003). On the other hand, the fact that the diet of *A. iberus* in the O2 lagoon's population was more related with zooplanktonic organisms (Alcaraz and García-Berthou, in press) suggests that it is probably the only fish species with the greatest potential to influence the zooplankton community. The *A. iberus* population of the O2 lagoon is not able to colonise the other lagoons during the flooding periods since O2 is isolated from the marsh by artificial levees (Fig. 5.1, pg. 45). Although the three new lagoons were connected during the flooding periods, the fish sampling did not detect the presence of *A. iberus* in the N1 lagoon or in the N3 lagoon during the study period (Pou-Rovira, pers. comm.).

The macroinvertebrate predator density (MPD; in ind. m^{-2}) was obtained from a monthly macroinvertebrate sampling using a 20 cm diameter dip-net (250-µm mesh-size). One sweep per lagoon, consisted of 20 dip-net ''pushes'' in rapid sequence, was carried out in order to cover all the different habitats in the lagoon. Each dip-net ''push'' swept a distance of half a meter. Macroinvertebrate taxa were counted and identified by means of a stereomicroscope. The dominant species were the gastropod *Hydrobia acuta*, the amphipod *Gammarus aequicauda* and the chironomids *Chironomus salinarius* and *Halocladius varians*. All these taxa are basically deposit feeders or grazers and, except for *G. aequicauda*, they do not include animal prey in their diet (Levinton and Bianchi, 1981; MacNeil et al., 1997). In addition to *G. aequicauda*, three more potential zooplankton predator taxa were found, the dytiscid *Nebrioporus ceresyi* and two corixid species *Sigara selecta* and *Sigara lateralis*. Adult and immature individuals of the genus *Nebrioporus* are well known as aquatic predators (Kehl and Dettner, 2003 and references therein). Although having been considered herbivorous or detritivorous (Hungerford, 1948; Popham et al., 1984), nymphs and adults of the genus *Sigara* have been reported as potential zooplankton predators as well (Suton, 1951; Murillo and Recasens, 1986). Therefore, densities of *G. aequicauda* and densities of larvae and adults of *N. ceresyi* and the two *Sigara* species were pooled together to get a total density of potential macroinvertebrate predators.

6.2.5. Statistical analyses

Generalized Additive Models (GAMs) were used to test the relationship between the taxonomic (*H*) and the size diversity ($\mu_{s'}$), as response variables, and environmental variables as predictors (water level, electrical conductivity, total and dissolved nutrient concentrations, chlorophyll-*a*). Another two GAMs were performed to fit the response of the two diversity indexes to food resource availability. A GAM extends the generalized linear model by fitting non-parametric functions to estimate relationships between the response and the predictors. The non-parametric functions are estimated from the data using smoothing operations, which are fitted using a range of different error structures (Hastie and Tibshirani, 1990). A Poisson error structure was used for the response variables (taxonomic and size diversity) since it ensured that all fitted values were positive, and the *s* (spline) smoother was used as the smoothing function. The *s* function introduces non-parametric fitting into the model and exhibits superior properties regarding testing for goodness of fit than other non-parametric fitting procedures (Raventos and Macpherson, 2005). The selected variables included in the final model were obtained by performing an automatic stepwise selection, and the Akaike Information Criterion (AIC) was used to select the best model with increasing complexity (degrees of freedom equal to 1, 2, and 3). AIC not only considers models for goodness of fit but also ‘‘parsimony’’, penalising very complex models (Hastie and Tibshirani, 1990; Crawley, 2003). However, automatic stepwise procedures are rather generous about leaving terms in the model. Thus, the increase in deviance caused by each variable included in the model and obtained with the stepwise selection was tested. Only those variables that caused a significant increase were retained in the final model (Hastie and Tibshirani, 1990). GAMs were performed on the entire data matrix (all samples from all lagoons) and were carried out using the S-PLUS software. However, when differences between lagoons were obtained by means of ANOVA and Games-Howell post-hoc tests, GAMs were repeated without the significantly different sampling site in order to analyse if the GAM results varied.

In addition, lagoons were classified according to their fish density (fishless, low fish density and high fish density). Linear regression and correlation analyses were also performed to assess the relationship between the diversity indexes and food resource availability, macroinvertebrate predator density and fish density in such separated data sets. These analyses were performed with SPSS 13.0 for Windows.

All explanatory variables were previously $\log_{10}(x + 1)$ transformed in order to obtain a more homogeneous spread for calculating the response curves in the regression models and correlation analyses.

6.3. Results

In the GAM carried out for the H as a response variable and environmental variables as predictors, the AIC stepwise approach only retained the SRP in the regression model (Chi-test, $p = 0.004$). SRP was incorporated as a linear function (d.f. = 1 for the spline smoother) explaining 8.39% of the total deviance of taxonomic diversity. This relationship is shown in Fig. 6.1, which illustrates that SRP had a general negative influence on H. It is worth noting that samples from O1 lagoon, in relation to the other lagoons, appeared displaced towards the right side of the plot. Results of ANOVA indicated that in O1 lagoon H was significantly lower ($F_{4,66} = 4.882$, $p < 0.01$, Games-Howell post-hoc tests) and SRP was significantly higher ($F_{4,68} = 16.727$, $p < 0.01$, Games-Howell post-hoc tests) than in the other studied lagoons. GAM was repeated excluding all samples from O1 lagoon and the same response function was obtained, although the type error I probability (p) has varied from 0.05 to 0.10. No variable was selected in the GAM carried out with $\mu_{s'}$ as a response variable and environmental variables as predictors.

No relationship was found between water level, as a predictor variable directly related to the hydrology and the two diversity indexes since it was not retained in any of the GAMs. The evolution of both diversities and water level throughout the hydrological cycle in one lagoon is shown as an example in Fig. 6.2. No temporal pattern coincided between water level and either of the diversity indexes and, while water level peaked following the flooding events and decreased in the dry seasons, both diversity indexes were very variable.

GAMs performed to test the relationship between the taxonomic and size diversity and the zooplankton food resource availability (FR_a) had significant results (Chi-test, $p = 0.038$ for H; Chi-test, $p = 0.008$ for $\mu_{s'}$). In both cases FR_a was incorporated as a linear function (d.f. =1 for the spline smoother). The relationships obtained showed that the diversity indexes had inverse responses to FR_a (Fig.6.3 A, B). While H responded positively to FR_a, the response of $\mu_{s'}$ was negative. Thus, the higher values of H were found at high FR_a and the higher values of $\mu_{s'}$ were found at low FR_a.

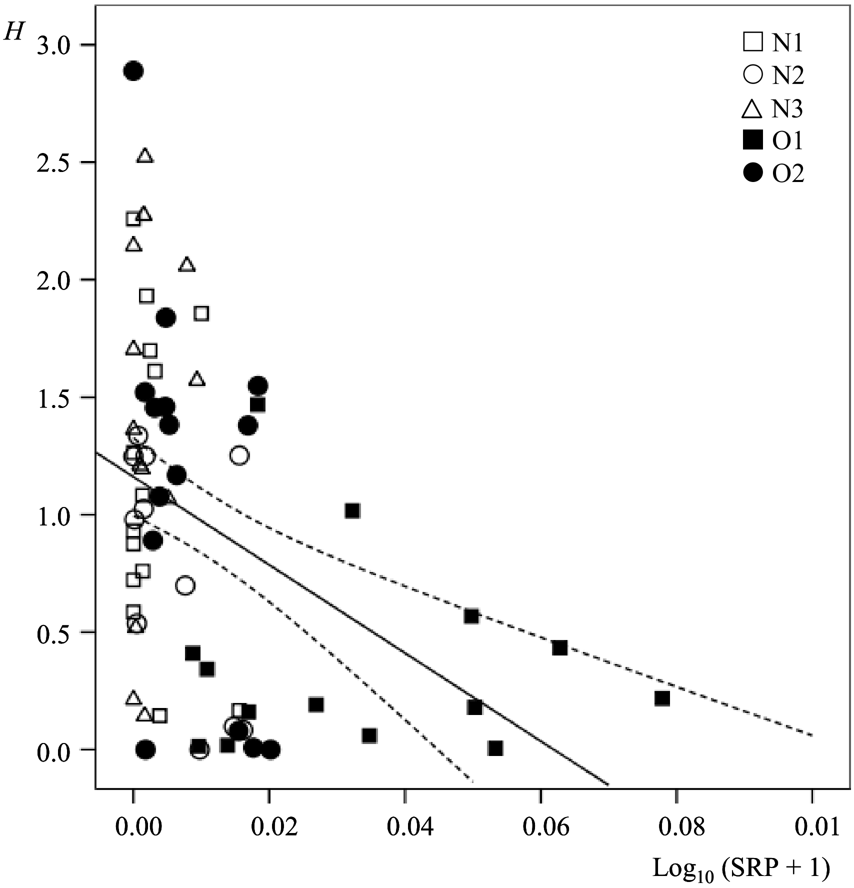


Figure 6.1. Linear relationship between taxonomic diversity (H) and soluble reactive phosphate (SRP). The dashed lines are the 95% confidence intervals. Data points represent monthly samples and they have been differentiated according to the lagoons.

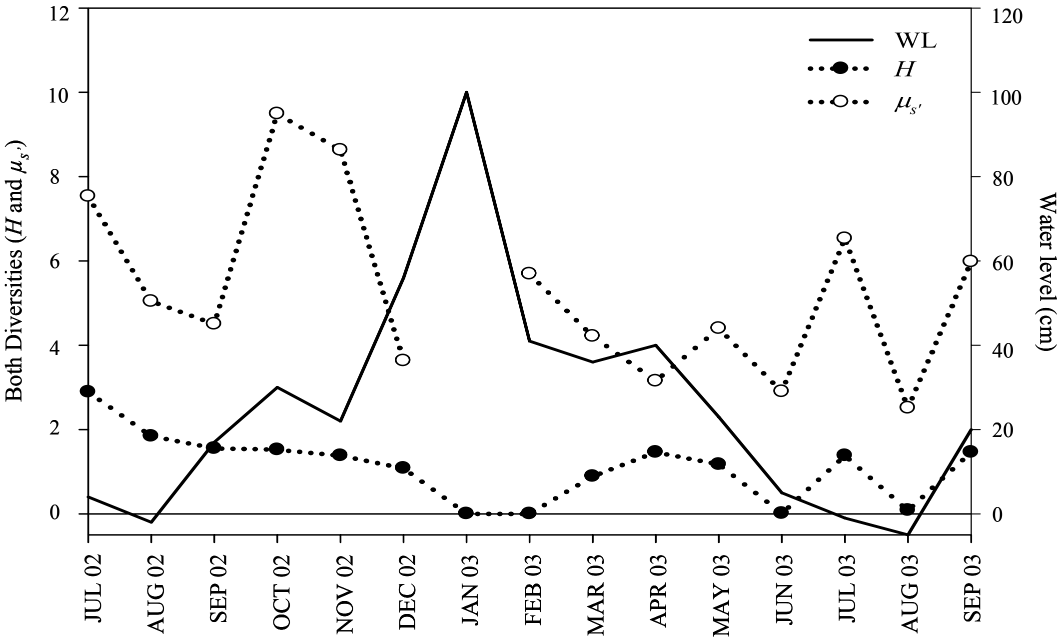


Figure 6.2. Changes in taxonomic (*H*) and size diversity ($\mu_{s'}$) and water level (WL; cm above or below the average sea level) in the O2 lagoon during the hydrological cycle 2002-2003.

As an example, two monthly biomass-size distributions of the zooplankton community in situations of high and low food resource availability are represented in Fig. 6.3 C, D respectively. In the first one, *H* showed one of its highest values with the presence in similar abundances of nematodes, several rotifers species and copepods nauplii. However, all the species found were of a similar size and consequently $\mu_{s'}$ was low. In the second one, *H* decreased because the calanoid *Eurytemora velox* widely dominated the community. In this case, small naupliar stages, copepodites and large adult individuals of that calanoid species coexisted, leading to a high value of $\mu_{s'}$.

When lagoons were separated according to their fish density, no significant correlation was observed between macroinvertebrate predator density and the two diversity indexes (Table 6.2). Separately, for each predator taxon (*G. aequicauda*, *N. ceresyi* and the *Sigara* species), correlations between individual density and the two diversities were also calculated and in no case significant relationships were obtained. On the other hand, there was a strong correlation between $\mu_{s'}$ and fish density in the high fish density lagoon (Table 6.2 and Fig. 6.4 B). This strong relationship was not a side effect of the seasonal pattern of the *A. iberus* population since the lowest fish densities were detected in both winter and summer samplings and therefore the fish density data in the regression were not ordered according to seasonality. Neither in the high fish density nor in the low fish density lagoons *H* correlated with fish density. In Table 6.2 and Fig. 6.4 A, C it can also be observed that FR_a was positively correlated with *H* but negatively correlated with $\mu_{s'}$ in the fishless lagoons. A negative relationship between FR_a and $\mu_{s'}$ was also reported in the low fish density lagoon.

Fig. 6.5 shows the zooplankton biomass-size distribution in the high fish density lagoon for the months when the *A. iberus* density was estimated. When fish density was low, in February and July 2003, $\mu_{s'}$ reached maximum values but the size range enlargement showed the opposite pattern in these two months. In February 2003, when the community was exclusively composed of the calanoid *Eurytemora velox*, the size distribution was enlarged towards the largest sizes, ranging from -5 to 4 (0.03-15.55 µg-DW). In fact, the largest individuals recorded in the whole zooplankton community during the present study belonged to this calanoid species. In July 2003, when $\mu_{s'}$ increased again, the enlargement of the size range was towards the smallest sizes of the distribution, which ranged from -9 to 0 (0.003-0.77 µg-DW). At that time, the community was formed by small zooplankters such as ciliates, copepods nauplii and rotifers. Thus, high $\mu_{s'}$ is not a derived side effect of having large species with a big size distribution range since high $\mu_{s'}$ was also achieved when the species involved were of smaller sizes. *A. iberus* density was highest in August 2003, when the juvenile fish density was at its maximum (Fig. 6.5), coinciding with the minimum value of $\mu_{s'}$. In that situation, ciliates and copepods nauplii disappeared and the zooplankton community was dominated by the medium-size rotifer *Brachionus plicatilis*, whose population showed a narrow size distribution.

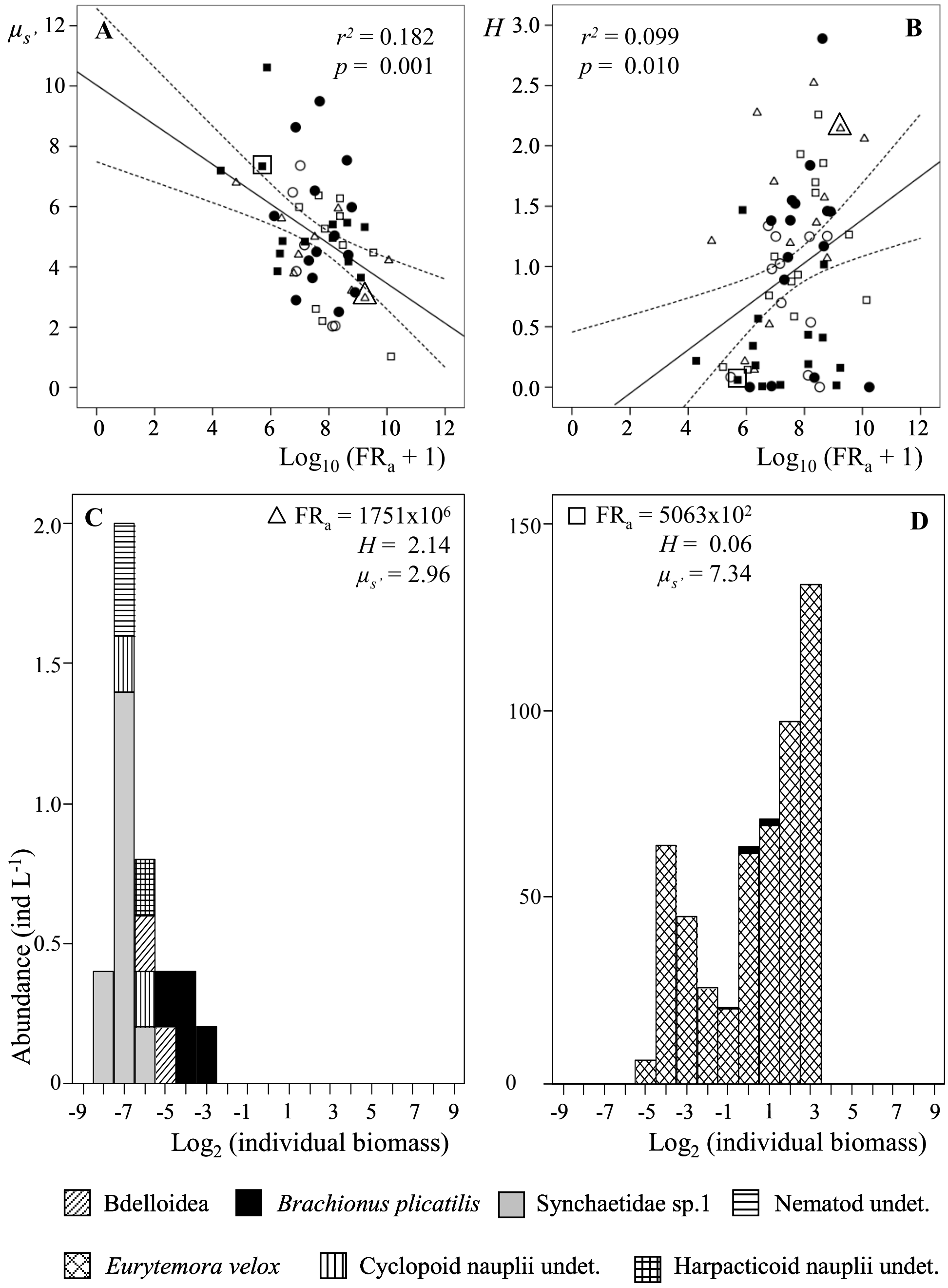


Figure 6.3. Linear relationship between size ($\mu_{s'}$) and taxonomic diversity (H) and food resource availability (FR_a) (plots A and B, respectively). The dashed lines are the 95% confidence intervals. See Fig. 6.1 for lagoons legend. Two monthly biomass size-distributions of the zooplankton community are represented as examples of situations of high and low FR_a (plots C and D, respectively). The respective values of FR_a, H and $\mu_{s'}$ are given. Both situations are located in the upper figures with an empty triangle for the high FR_a situation and an empty square for the low FR_a situation.

Table 6.2

Range of values (in italic letters) of fish density (FD, in ind. m^{-2}) and macroinvertebrate predator density (MPD, in ind. m^{-2}) and Pearson correlations between the two diversity indexes (*H*: taxonomic diversity and $\mu_{s'}$ size diversity) and FD, MPD and FR_a (food resource availability). Results are shown for the lagoons which are grouped according to their fish density. Number of cases (in brackets), significant correlation coefficients (*: $p < 0.05$, **: $p < 0.01$) and non-significant relationships are also shown (n.s.: $p > 0.05$).

	Fishless (O1, N1,N3)			Low fish density (N2)			High fish density (O2)		
FD	—			*0.13 – 1.70*			*2.33 – 32.38*		
MPD	*0.5 – 221*			*0.5 – 925*			*1.5 – 704*		
	FD	MPD	FR_a	FD	MPD	FR_a	FD	MPD	FR_a
H	—	n.s. (29)	0.383* (41)	n.s. (4)	n.s. (11)	n.s. (11)	n.s. (5)	n.s. (15)	n.s. (15)
$\mu_{s'}$	—	n.s. (22)	- 0.544** (33)	n.s. (4)	n.s. (6)	- 0.826* (6)	- 0.986** (5)	n.s. (14)	n.s. (14)

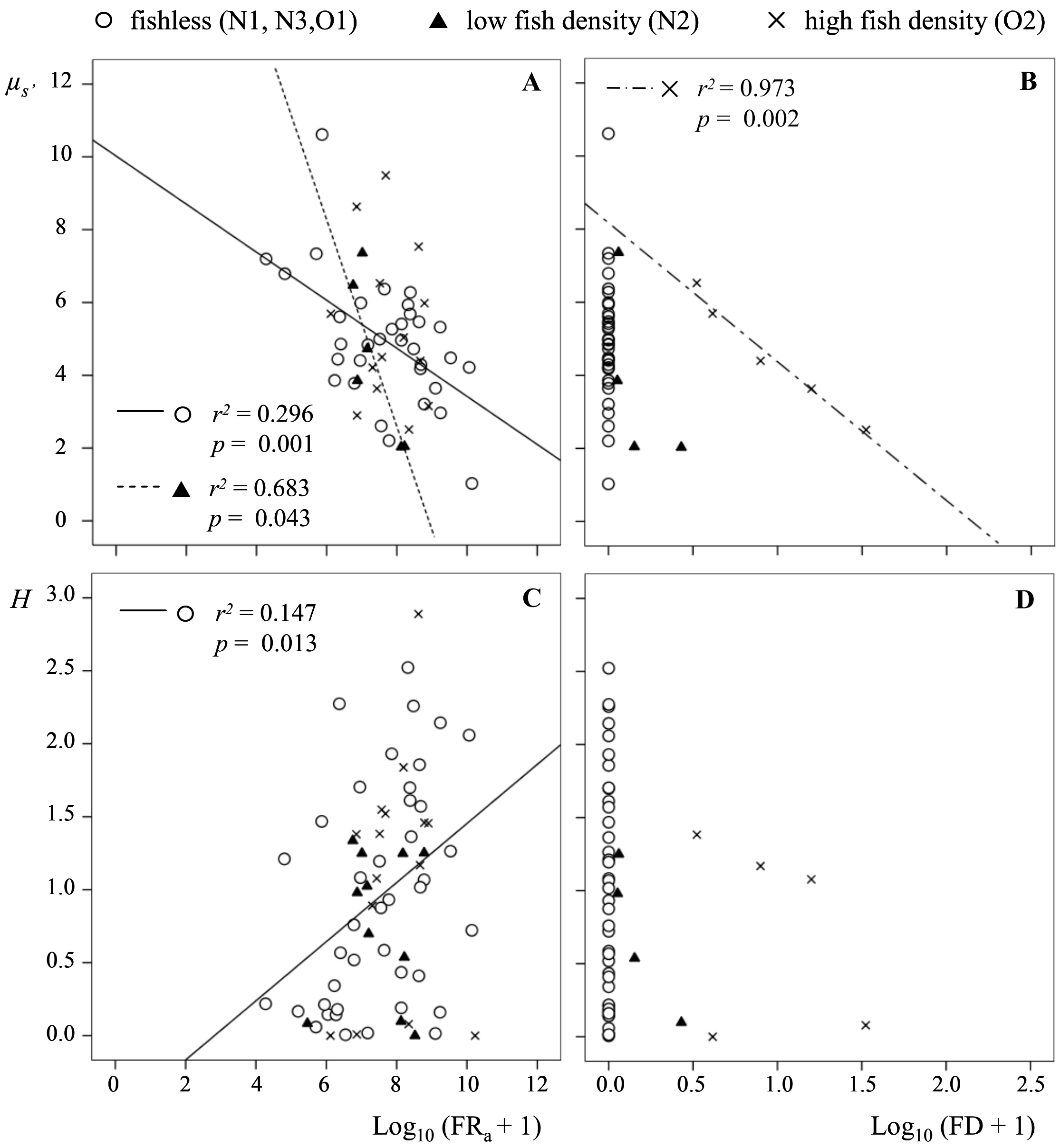


Figure 6.4. Relationships between size ($\mu_{s'}$) and taxonomic diversity (H) and food resource availability ($\mathrm{FR_a}$; plots A and C, respectively) and fish density (FD; plots B and D, respectively). Lagoons are grouped according to their fish density. Data points represent monthly samples. Significant coefficients of determination (r^2) and their significance levels (p) are shown.

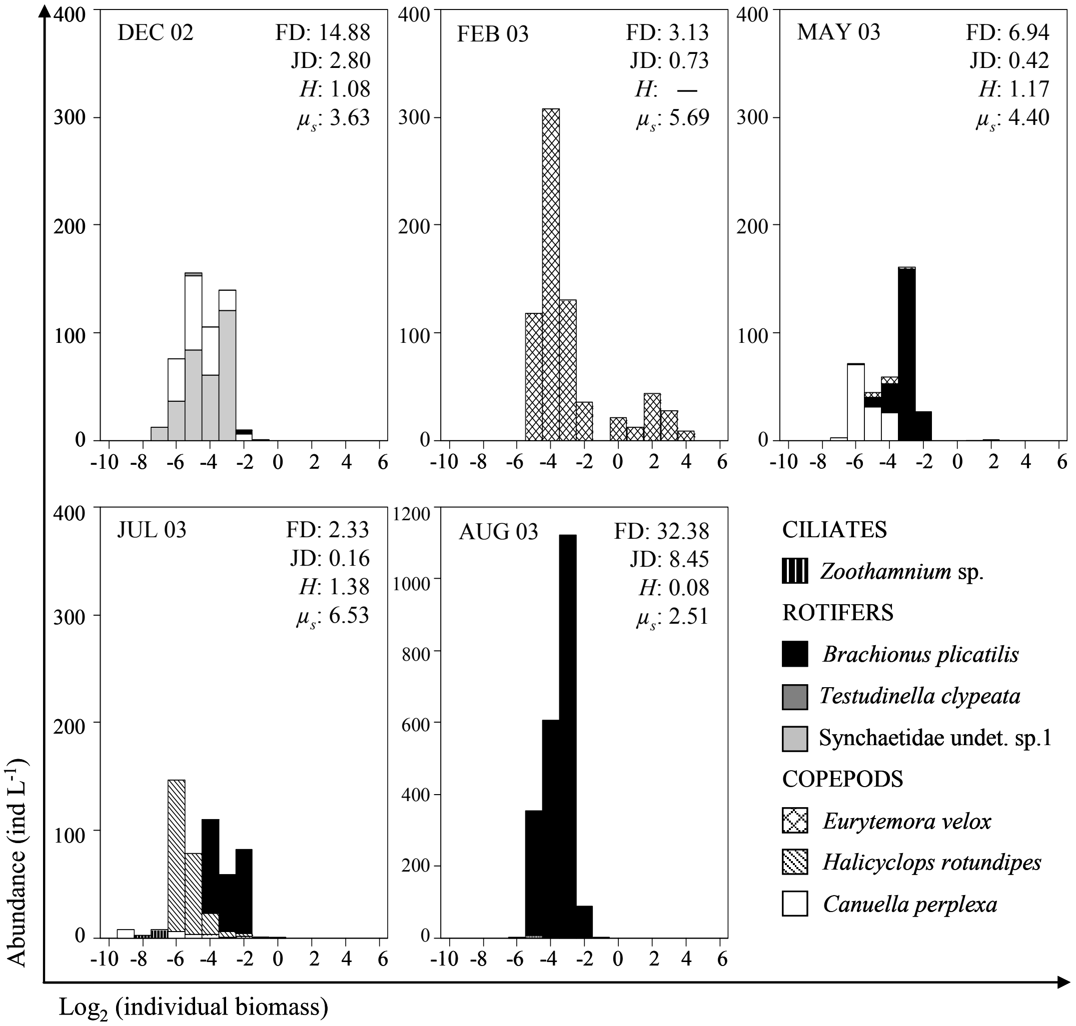


Figure 6.5. Zooplankton size distribution in the O2 lagoon for the months when the *Aphanius iberus* density was estimated. The fish density (FD; in ind. m-2), juvenile fish density (JD; in ind. m-2), taxonomic diversity (*H*) and size diversity ($\mu_{s'}$) values are shown for each month.

6.4. Discussion

Zooplankton size diversity and taxonomic diversity showed different patterns throughout the hydrological cycle of the studied coastal lagoons and had different responses to the environmental conditions. Neither H nor $\mu_{s'}$ was related to water level, suggesting that hydrology did not have any measurable effect on either diversity in these systems. This fact contrasts with previous studies in Mediterranean coastal lagoons, which describe a relationship between the hydrological regime and the zooplankton composition (Quintana et al., 1998b; Gilabert, 2001), although sometimes with a certain temporal delay (Quintana et al., 1998a). A delayed relationship between hydrology and zooplankton taxonomic and size structure cannot be discarded, since in a high frequency study developed in a similar Mediterranean salt marsh, an increase in the zooplankton taxonomic diversity was in fact observed after flooding events (Brucet et al., 2005a).

Taxonomic diversity was negatively related to the soluble reactive phosphate. A negative relationship between nutrients and other taxonomic descriptors of the community (e.g. species richness) has been previously found in other shallow ecosystems (Jeppesen et al., 2000; Declerck et al., 2005). In other studies, however, the effect of nutrient enrichment was more related to changes in species composition or abundance than a decline in species richness (e.g. McCormick et al., 2004; García-Criado et al., 2005).

Taxonomic and size diversity showed inverse responses to zooplankton's food resource availability. These relationships suggest that taxonomic diversity tends to be higher when food availability is high and low competition for food is expected. Greater food availability would reduce the interspecific competition and favour the establishment and coexistence of a larger number of species (Giller, 1984). Coincident results were obtained in other Mediterranean coastal lagoons where a positive relationship between zooplankton species diversity and resource availability was found (Brucet et al., 2006). Conversely, size diversity tends to be higher when food availability is low and high competitive interactions are expected. Food scarcity would increase the interspecific competition, a size-dependent mechanism that generally leads to competitive exclusion by the large bodied zooplankters (Brooks and Dodson, 1965; Hall et al., 1976; Gliwicz, 1990). This situation was clearly apparent during the winter-spring confinement, a period of hydrological stability and low productivity, when the community was almost exclusively formed by a population of a large calanoid species. In this case, the zooplankton size diversity was highest due to the co-occurrence of different developmental stages. A broad size distribution of the planktonic community in situations of calanoids' dominance has also been observed in other estuarine areas (Quintana et al., 2002b; Lawrence et al., 2004). The existence of food resource partitioning among development stages, already reported in calanoids (Maly and Maly, 1974; Meyer et al., 2002; Brucet et al., 2005b), would avoid the intraspecific competition allowing the maintenance of stable populations over time (Werner and Gilliam, 1984).

The measure of zooplankton's food resource availability (FR_a) used in the present study has to be considered as an approximation and it shows some limitations. First, several studies have reported that certain phytoplankton species are better than others in terms of food quality. With regard to this, it is worth pointing out that colonial forms and filamentous phytoplankton, which may become inedible for the zooplankton (Vanni, 1986 and references therein), have been very scarce in these lagoons throughout the study period (López-Flores et al., 2006). Second, omnivory is common and, furthermore, zooplankton does not always directly use the food resource provided by the primary producers since the largest zooplankters can feed on the smallest ones (Dole-Olivier et al., 2000; Hansen, 2000; Jürgens and Jeppesen, 2000). Nevertheless, trophic interactions within the zooplankton would have been scarce since carnivorous zooplankters such as the large copepodites and adults of cyclopoids were not very abundant throughout the study period. Assuming these limitations and the derived errors, the use of this measure of resource availability is justified since it was the only way to get a global estimate of the resource available for the whole zooplankton community.

The lack of relationship between macroinvertebrate predation and both diversity indexes could be explained by the low predation pressure of the potential invertebrate predators found in the studied lagoons. The great majority of them are omnivorous and the animal fraction of their diet is usually small (Popham et al., 1984; MacNeil et al., 1997). Furthermore, although invertebrate predation has been described as playing an important role in the community structure, especially in absence of fish since their densities used to be higher (Wellborn et al., 1996; Zimmer et al., 2001; Tate and Hershey, 2003), this is not the case in the present study where macroinvertebrate predator density was low, and even lower in the fishless lagoons.

Zooplankton size structure was related to fish predation since a decrease in the size diversity was observed when the fish density increased. Conversely, no relationship was found between taxonomic diversity and fish density. These results could be explained on the basis that fish predation is considered size-dependent and, therefore, usually determines the range of individual sizes in the zooplankton communities, not the species composition (Brooks and Dodson, 1965; Hall et al., 1976; Benndorf and Horn, 1985). A decrease in the large zooplankters is usually observed when the fish predation pressure increases (Jakobsen et al., 2003; Romo et al., 2004); however, in the present study the maximum fish density not only coincided with a decrease in the large sizes, but also in the smaller ones. The highest juvenile fish density, found at the moment of maximum fish density, would increase the predation effects on the smaller zooplankton since gape-limitation influences prey-selection in younger fish stages (Mehner and Thiel, 1999 and references therein). This was in agreement with a previous *Aphanius iberus* diet analysis in the same lagoons, which observed that smaller fishes actively select smaller sizes (Alcaraz and García-Berthou, in press). Thus, the resultant zooplankton community has a narrow size distribution coinciding with the minimum of the size diversity. In the absence of fish, or when fish densities were too low to exert a predation effect, the size diversity values ranged widely indicating that the zooplankton size structure may also depend on the other factors. Several studies have experimentally determined a threshold fish density (4 ind. m^{-2}) below which the zooplankton size structure is not affected by fish predation (Jeppesen et al., 1997; Jakobsen et al., 2003). Our results are consistent with such a threshold, since the relationship between fish density and size diversity are only found in the high fish density lagoon. In both the fishless and low fish density lagoons, size diversity seemed to be more affected by the food resource availability since negative correlations were obtained. In fact, in another salt marsh zooplankton community, a negative relationship between zooplankton size diversity and resource availability was also observed in absence of fish predation (Brucet et al., 2006).

Results in the present study have revealed different patterns in zooplankton size and taxonomic diversity. Size diversity better reflected those changes in the community structure derived from trophic species interactions, such as fish predation or competition for food. Taxonomic diversity, however, better showed those changes caused by environmental fluctuations, such as nutrient variability. Brucet et al. (2006) also provided evidence of different patterns in size and taxonomic diversity when studying zooplankton succession in Mediterranean salt marshes. Thus, the combined use of both diversity descriptors will provide a better understanding of ecological factors involved in structuring aquatic communities.

CHAPTER 7

Creation and stocking of new refuges as conservation measure for the Iberian toothcarp *Aphanius iberus* (Valenciennes, 1846)

7.1. Introduction

The Iberian toothcarp *Aphanius iberus* (Valenciennes, 1846) is a small cyprinodont species (< 5cm), with external sexual dimorphism, short longevity (age up to 2+) and strongly euryhaline and eurythermal character (Fernández-Delgado et al., 1988; García-Berthou and Moreno-Amich, 1992; Vargas and Sostoa, 1997). This species is endemic of the Mediterranean coast of the Iberian Peninsula and nowadays is a strictly protected species (Bern Convention of 1979 and EU Habitats Directive 92/43/EEC) considered “threatened with extinction” (Spanish National Catalogue of Threatened Species, R.D. 439/1990). This is due to the number of populations being drastically reduced over the last century to the situation that no more than twenty exist at the present time (Planelles-Gomis, 1999; Oliva-Paterna et al., 2006). It originally inhabited a wide range of lowland waterbodies but its distribution is now estimated in less than 200 Km^2, and is highly fragmented and restricted to brackish and hyperhaline waters in salt marshes and coastal lagoons (Doadrio, 2001). Extensive habitat destruction and water pollution, mainly caused by the massive tourist development of the coastal areas, as well as the introduction of exotic species, mainly the eastern mosquitofish (*Gambusia holbrooki*), have been attributed as the main causes of the decline of *A. iberus* (Planelles-Gomis, 1999; Elvira and Almodóvar, 2001; Rincón et al., 2002).

Baix Ter Wetlands (NE Iberian Peninsula) are a fragmented wetlands area, formed by several fresh and brackish waterbodies (Trobajo et al., 2002; López-Flores et al., 2006) located in a highly altered and frequented tourist coast. In such wetlands, *A. iberus* showed one of the last populations in the north of its distribution (García-Berthou and Moreno-Amich, 1991; Moreno-Amich et al., 1999a). It is found in the Fra Ramon lagoon, a small brackish coastal lagoon located in La Pletera, a confined salt marsh. Consequently, this population is particularly vulnerable to any disturbance that takes place in the lagoon, especially during summer when tourist visitor numbers are highest. The loss of the Fra Ramon population would be irreversible and would involve the loss of the genetic patrimony and the evolutionary history of the species in the area, since high levels of differentiation exists between geographic groups of *A. iberus* populations throughout its distribution (García-Marin et al., 1990; García-Marin and Pla, 1999; Torralva et al., 2001). In order to improve the conservation status of *A. iberus* in the whole Baix Ter Wetlands, an EU LIFE project (LIFE 99 NAT/E/006386) was developed between 1999 and 2003 (Quintana et al., 2002a). The main objective to did its recovery was the restoration of preferred habitat (i.e. brackish or hypersaline coastal lagoons) by means of the habitat creation and stocking of new refuges. This is consistent with accepted conservation practice for threatened fish species which have a focus on *in situ* actions such as the habitat restoration and management, as well as the establishment of new populations by means of the stocking of new habitats (e.g. Maitland, 1995; Philippart, 1995; Winemiller and Anderson, 1997). Several EU LIFE projects have already been carried out in other coastal wetlands of the Iberian Peninsula in order to enhance the conservation status of *A. iberus* (e.g. Llobregat Delta, Albufera de València Natural Park, the Murcia region, Adra Delta). In all these projects, recovery and habitat management as well as the creation and stocking of new habitats have been applied as conservation measures (more details available at http://europa.eu.int/comm/environment/life/project/).

In La Pletera salt marshes criteria for the design of the new refuges were established with the aim that they mimic as close as possible the existing habitat of *A. iberus* in the area (Fra Ramon lagoon). If structure and functioning of the newly created habitats were sufficiently comparable to the natural habitat for this species, the establishment of the new population would be ensure, allowing potentially the long-term recovery of *A. iberus* in the area. Thus, the aim in the present study was to compare the limnological characteristics of the Fra Ramon lagoon (the local population) with those of the created waterbodies to ensure the feasibility of the new refugee. The short-term evolution of the new population during the first years after their stocking was also analysed.

7.2. Methods

7.2.1. Field sampling and laboratory analyses

Four sampling points were selected, one in the Fra Ramon lagoon (current habitat of *A. iberus*) and three in the new refuge lagoons (N1, N2 and N3) (Fig. 5.1, pg. 45). Sampling was carried out during a complete hydrological cycle, from July 2002 to September 2003 except in the N2 lagoon where it started in November. Water and zooplankton samples were taken monthly from a central point of these lagoons at a depth of 15-30 cm. The water level, temperature, electrical conductivity, pH and dissolved oxygen (in % of saturation) were measured *in situ*. Alkalinity was measured after a few hours in the laboratory following APHA (1989). Inorganic nutrients (ammonium, nitrite, nitrate and soluble reactive phosphate) and total nutrients (nitrogen and phosphorus) were analysed from filtered and unfiltered water samples respectively following Grasshoff et al. (1983) and APHA (1989). Organic matter was measured using a TOC analyser from unfiltered samples previously acidified to eliminate the inorganic dissolved carbon. Chlorophyll-*a* was extracted using 95% methanol after filtering 500 ml of water (GF/C Whatman filters) and its concentration was measured by high-performance liquid chromatography (HPLC) using a methodology modified from Zapata et al. (2000).

Flow cytometry was used to assess the bacterial and phytoplankton biovolume according to Gasol and Del Giorgio (2000) and López-Flores et al. (2006), respectively. Samples were collected from 50µm filtered water, fixed with 1% paraformaldehyde and 0.05% glutaraldehyde (final concentration), deep frozen in liquid nitrogen and stored frozen at -20ºC.

Zooplankton samples were obtained by filtering 5 L of water trough a 50-µm mesh-size net and preserved in situ in 4% formalin. An inverted microscope was used to count and, where possible, identify at species level the zooplankton taxa. More details about the zooplankton counting and identification are available in Chapter 5. Zooplankton biomass dry weigth esimations for crustaceans were obtained from the allometric relationship between the weigth and the body length, and for other groups was calculated by converting biovolume into dry weigth (see appendix II for more details about zooplankton biomass estimates).

Macroinvertebrate samples were obtained using a 20 cm diameter dip-net (250-µm mesh-size). One sweep per lagoon, consisted of 20 dip-net “pushes” in rapid sequence, was carried out in order to cover the different habitats of the lagoon. Each dip-net “push” swept a distance of half meter. Macroinvertebrate taxa were counted and, where possible, identified at species levels by means of a stereomicroscope.

The size of *A. iberus* population was estimated following Seber (1982) from a bimonthly sampling carried out during the hydrological cycle 2002-2003 by means of throw enclosure traps (Alcaraz and García-Bethou, in press). This fishing method is particulary useful in estimanting the population density of small fishes in shallow estuarine habitats since its capture efficiency is generally high and consistent (Chick et al., 1992; Raposa et al., 2003 and references therein), especially when submerged vegetation is present and fish cannot easily escape (Hayes et al., 1996). In Fra Ramon, a previous sampling campaign was performed throughout the year 2000. In the new lagoons a punctual sampling was performed in September 2004.

The vegetation mapping was carried out by means of the photointerpretation of both digital orthophotomaps and low-altitude aerial photographs followed by *in situ* validation. The cartography obtained was integrated in a GIS and five categories of maturity of the vegetation were established: 0: without vegetation; 1: anthropic vegetation; 2: opportunistic or pioneering vegetation; 3: immature or degraded vegetation; 4: mature vegetation. The location and design of the new lagoons was based on such vegetation mapping, as well as in the analysis of old aerial pictures which enable the detection of *Ruppia* prairies present and past and, therefore, where they might readily develop in the future.

The criteria for the creation of new refuges and the fish stocking of them are detailed in sections 3.2.1 and 3.2.2, in Chapter 3.

7.2.2. Statistical analyses

Differences in environmental variables between Fra Ramon and the new lagoons were analysed by means of the analysis of variance (ANOVA). All variables except the pH were previously $\log_{10}$ (x +1) transformed to improve linearity, as well as the normality and homogeneity of variances (Quinn and Keough, 2002). All calculations and statistical analyses were performed with SPSS 13.0 for Windows.

7.3. Results

7.3.1. The new refuges: size, shape and vegetation

Three new lagoons with a total surface of 0.88 ha were created in July 2002 by excavation of the terrain down to 1 m below sea level (Photo 5). They were built with the characteristic shape of coastal lagoons in this zone: two elongated lagoons perpendicular to the coastline (N1 and N3) were interconnected by a third lagoon (N2), running parallel to the coast (Fig. 7.1; Photo 4). The minimum depth in the central part of the basins was 1m below the average sea level (coinciding with the phreatic level) thus assuring a permanent flooded area of 0.23 ha, surrounded by 0.65 excavated hectares that experienced temporary flooding (Table 7.1; Photo 6).

Seventy-three percent of the excavated area was previously occupied by opportunistic or pioneering vegetation or by immature or degraded vegetation (maturity degrees = 2 or 3). Only 27 % of the excavated area previously had mature vegetation, representing 3.88 % of the total area with mature vegetation in the whole salt marsh (Table 7.2). Around 30 % of the total excavated surface (~2750 m^2) had a final elevation between -20 to +20 cm (relative to the average sea level) which has been described as the most suitable topographic elevation for the development of *Ruppia cirrhosa* prairies in the zone (Gesti et al., 2005). In fact, in one of the new basins (N2) a small but dense *Ruppia* prairie appeared in late summer, a few weeks after the basin creation. Aerial photographs showed that the location of the prairie exactly coincided with the intersection of the new created lagoon and an ancient lagoon, which disappeared in late 1980s. This fact suggested that the new *Ruppia* population proceeded from the buried seed bank, revealed by the excavation of the new lagoon. The surface occupied by the new *Ruppia* prairie progressively increased after the creation of the new lagoons (Fig. 7.1 B). In the temporary flooded areas, the annual halophytic community *Suaedo-Salicornietum patulae* has been developed.

Table 7.1

Main morphological characteristics of the Fra Ramon and the new lagoons. Topographic elevation has been referred to the average sea level in the zone (over the last 15 years; a.s.l. = above sea level). Surface data in m^2.

	Fra Ramon	New lagoons			
		N1	N2	N3	Total
Minimum topographic level (m a.s.l.)	-1.75	-1.00	-1.00	-1.00	
Total surface	13598	3202	4621	968	8791
Surface permanently flooded (< 0 m a.s.l.)	2530	1071	910	338	2319
Surface temporarily flooded (0-0.5 m a.s.l.)	11068	2131	3711	630	6472
Ratio permanent/total surface	0.19	0.33	0.20	0.35	0.26

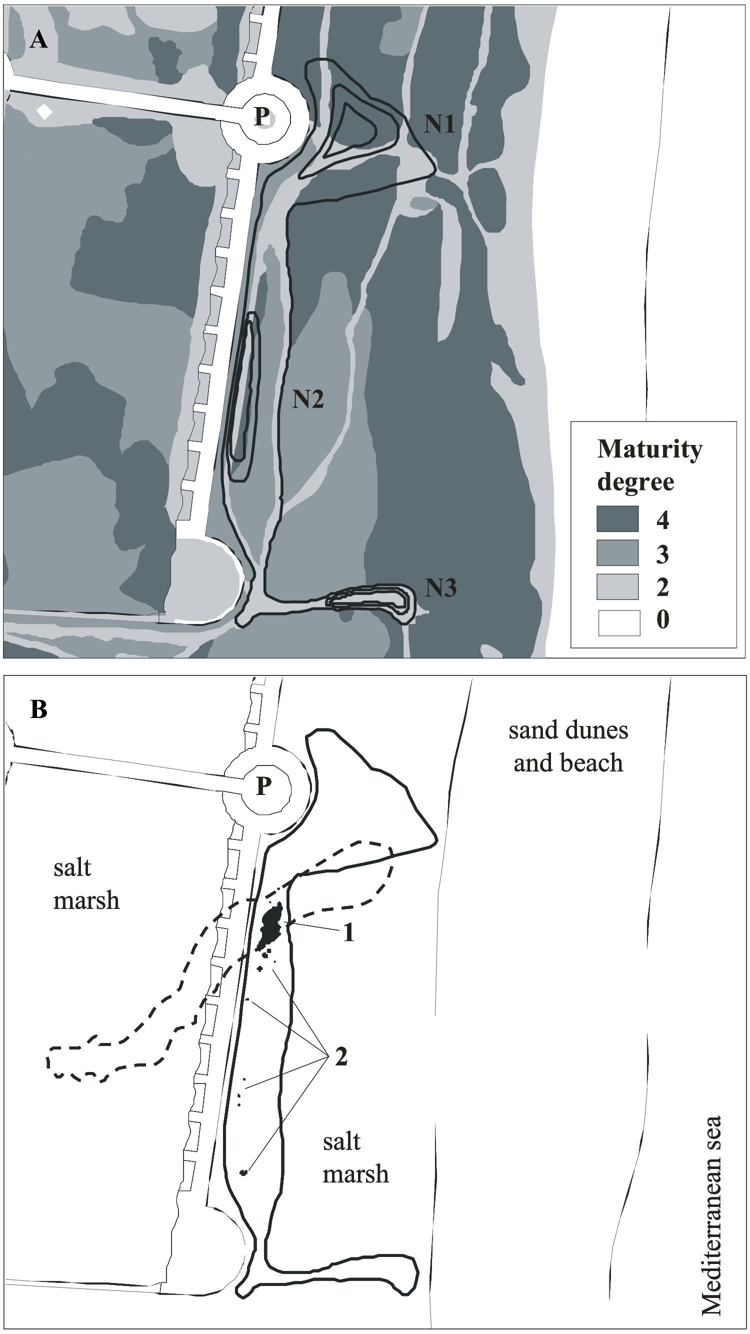


Figure 7.1. A) Location of the three new lagoons (N1, N2 and N3) and maturity of vegetation before their creation. 0: without vegetation; 1: anthropic vegetation 2: opportunistic or pioneering vegetation; 3: immature or degraded vegetation; 4: mature or climax vegetation. B) Appearance of *Ruppia cirrhosa* (black) in the intersection between the new created lagoons and the old one currently filled (dotted line). A first *Ruppia* prairie appeared one month after excavation (1: August 2002) and later on progressively colonized the rest of the lagoon (2: March 2003). P indicates the promenade of the partially urbanized area.

Table 7.2

Vegetation maturity degree (0: without vegetation; 1: anthropic vegetation; 2: opportunistic or pioneering vegetation; 3: immature or degraded vegetation; 4: mature or climax vegetation) in the excavated areas before the creation of the new lagoons compared with that of the whole salt marsh. The ratio between the excavated and the total salt marsh surface is also shown.

Maturity degree	Salt marsh		Excavated areas		Ratio
	Surface (m^2)	%	Surface (m^2)	%	
0	0	0.00	0	0.00	0.00
1	0	0.00	0	0.00	0.00
2	12934	13.75	3729	42.42	0.29
3	20044	21.30	2690	30.60	0.13
4	61121	64.95	2371	26.98	0.04
Total	94099	100.00	8790	100.00	0.09

7.3.2. Comparative limnology of Fra Ramon and the new refuges

All the new lagoons and the Fra Ramon lagoon showed the same hydrological pattern, characterized by sudden water inputs during disturbances (usually between autumn and spring) followed by prolongued periods of confinement without water inputs. During the confinement period, water level gradually decreases and, conversely, water conductivity progressively increases due mainly to the high evaporation rate (Fig. 7.2 A, B, only one of the new lagoons is shown). Since all lagoons were subject to the same hydrology, the average values of those physical and chemical parameters related more to the hydrological regime, such as nitrate, temperature and water level did not show significant differences between the Fra Ramon and the new lagoons (Table 7.3). The highest water level, as well as the highest nitrate concentration was reached after sea storms or intense rainfalls in all studied lagoons (Fig. 7.2 A, D). Conversely, concentrations of phosphorous (total and inorganic), total nitrogen, total organic carbon and chlorophyll-*a* were significantly higher in Fra Ramon lagoon, suggesting a progressive accumulation of nutrients and organic matter during the successive confinement periods over the years (Table 7.3; see also Chapter 5).

Table 7.3

Mean and standard deviation (in brackets) of several physical and chemical variables in the studied lagoons. Significant (*: $p < 0.5$, **: $p < 0.01$) and nonsignificant (n.s.: $p > 0.05$) differences between the new lagoons and the Fra Ramon lagoon are shown.

	New lagoons		Fra Ramon lagoon		Sig.
Water level (cm a.s.l.)	35.24	(23.58)	25.73	(27.32)	n.s.
Termperature (ºC)	18.94	(8.39)	19.80	(8.25)	n.s.
Electrical Conductivity (mS cm^{-1})	28.85	(12.61)	44.89	(16.32)	**
pH	8.06	(0.55)	8.01	(0.54)	n.s.
Dissolved oxygen (% of saturation)	95.13	(25.11)	105.88	(39.90)	n.s.
Ammonium (mg N l^{-1})	0.06	(0.12)	0.02	(0.03)	n.s.
Nitrite (mg N l^{-1})	0.002	(0.003)	0.003	(0.006)	n.s.
Nitrate (mg N l^{-1})	0.07	(0.15)	0.02	(0.08)	n.s.
Soluble reactive phosphate (mg P l^{-1})	0.01	(0.01)	0.02	(0.02)	*
Total nitrogen (mg N l^{-1})	1.67	(0.97)	3.12	(1.23)	**
Total phosphorus (mg P l^{-1})	0.10	(0.10)	0.32	(0.19)	**
Total organic carbon (mg l^{-1})	18.43	(15.22)	31.61	(14.80)	**
Chlorophyll-*a* (µg l^{-1})	4.76	(8.87)	19.75	(35.99)	*
Bacterial biovolume (μm^3 l^{-1}) $\times 10^8$	4.48	(11.13)	6.14	(5.38)	**
Phytoplankton biovolume (μm^3 l^{-1}) $\times 10^8$	36.30	(63.48)	47.72	(111.7)	n.s.
Zooplankton biomass (µg-DW l^{-1})	1577	(8001.71)	107.21	(165.05)	n.s.

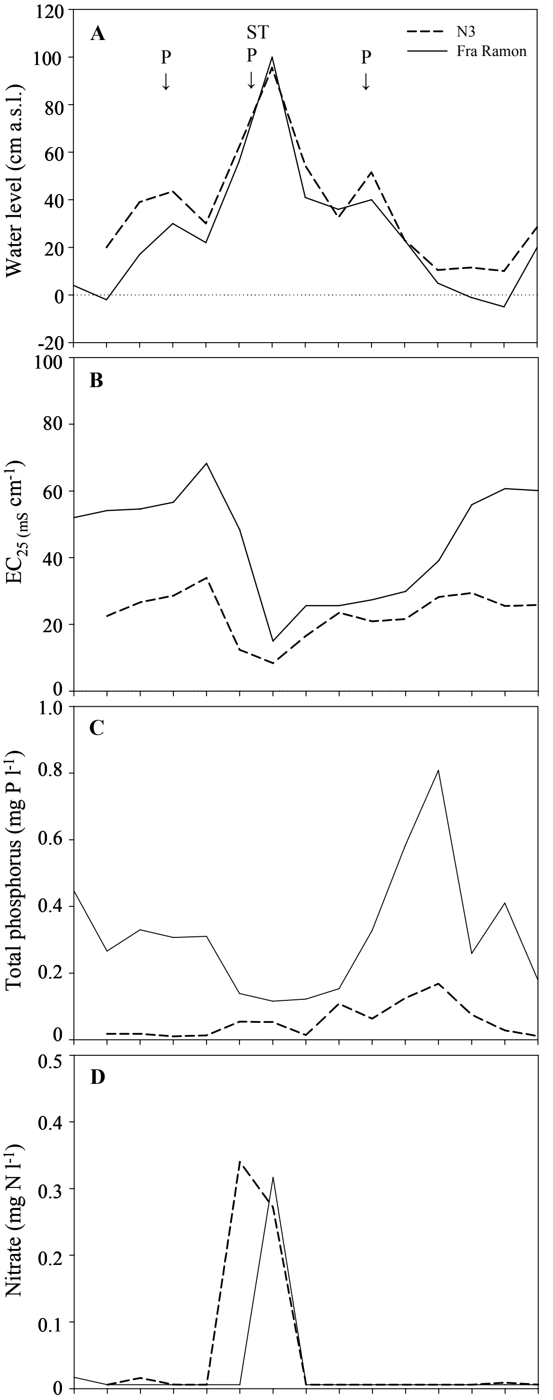


Figure 7.2. Changes in the water level (A; cm above or below sea level), conductivity (B; $EC_{25,}$ in mS cm^{-1}) and concentration of total phosphorus (C; in mg l^{-1}) and nitrate (D; in mg l^{-1}) in the Fra Ramon lagoon (solid line) and in one of the new basins (N3; dotted line) throughout the study period. The zero water level corresponds to the average sea level over the last 15 years (J.Pascual, pers.comm., Meteorological Station of l'Estartit). The main disturbances are indicated as ST (sea storm) and P (precipitation > 40 mm in 24 h), respectively.

Zooplankton dynamics in the new lagoons was similar to that observed in the Fra Ramon lagoon, and in both cases it was related to the hydrological pattern. As an example, the relative abundances of the main taxa throughout the hydrological cycle in Fra Ramon and in one of the new lagoons are shown in Fig. 7.3. The zooplankton temporal pattern in the new lagoons was characterized by the dominance of the calanoid *Eurytemora velox* and the cyclopoid *Diacyclops bicuspidatus odessanus* during the months that followed the sea storm. In Fra Ramon the cyclopoid was absent and the calanoid was the dominant taxa. When water levels were lower, as well as in the months before the marine intrusion, the rotifer *Brachionus plicatilis* and the harpacticoids *Canuella perplexa* and *Mesochra lilljeborgi*, which are particularly abundant in the new lagoons, dominated the community. Differences in the taxonomic composition between the Fra Ramon and the new lagoons may be related to the lagoon age. For example, the exclusive presence in the new lagoons of earliest colonists, such as the naidid oligochaetes, and the calanoid *Calanipeda aquaedulcis*, related to the more oligotrophic conditions in the new lagoons. Conversely, the harpacticoid *Tisbe longicornis* was exclusive of the Fra Ramon lagoon due to its higher eutrophic conditions (see also Chapter 5).

In the macroinvertebrates samples, the gastropod *Hydrobia acuta,* the amphipod *Gammarus aequicauda* and the chironomids *Chironomus salinarius* and *Halocladius varians* were found in Fra Ramon and in each of the new lagoons. The polychaetes *Ficopomatus enigmaticus* and *Nereis diversicolor* were found only in the Fra Ramon lagoon, whilst the heteropter *Sigara lateralis* and the coleopter *Hygrotus impressopunctatus* were found only in the new lagoons (Table 7.4).

Table 7.4

Main invertebrates taxa recorded in the Fra Ramon and in the new lagoons during a sampling carried out in the hydrological cycle 2002-2003 (+: presence, -: absence).

Phylum		Taxa	Fra Ramon	New lagoons
Ciliates		*Fabrea* gr. *salina*	+	+
Rotifera		*Brachionus plicatilis*	+	+
Mollusca	Cl. Gastropoda	*Hydrobia acuta*	+	+
Annelida	Cl. Oligochaeta	*Nais* sp.	-	+
	Cl. Polychaeta	*Nereis diversicolor*	+	-
		Ficopomatus enigmaticus	+	-
Arthropoda	Supercl. Crustacea			
	Cl. Ostracoda	*Cyprideis torosa*	+	+
	Cl. Copepoda			
	O. Calanoida	*Eurytemora velox*	+	+
		Calanipeda aquaedulcis	+	-
	O. Cyclopoida	*Diacyclops bicuspidatus odessanus*	-	+
		Halicyclops rotundipes	+	+
	O. Harpacticoida	*Canuella perplexa*	+	+
		Mesochra lilljeborgi	+	+
		Tisbe longicornis	+	-
	Cl. Malacostraca			
	O. Amphipoda	*Gammarus aequicauda*	+	+
	O. Decapoda	*Carcinus aestuarii*	+	+
	Supercl. Insecta			
	O. Heteroptera	*Sigara selecta*	+	+
		Sigara lateralis	-	+
	O. Coleoptera	*Neobrioporus ceresyi*	+	+
		Hygrotus impressopunctatus	-	+
		Helophorus fulgidicollis	+	+
		Enochrus bicolor	+	+
		Paracymus aeneus	+	+
		Ochthebius dentifer	+	+
	O. Diptera	*Halocladius varians*	+	+
		Chironomus salinarius	+	+
		Nemotelus sp.	+	+
		Ephydra sp.	+	+

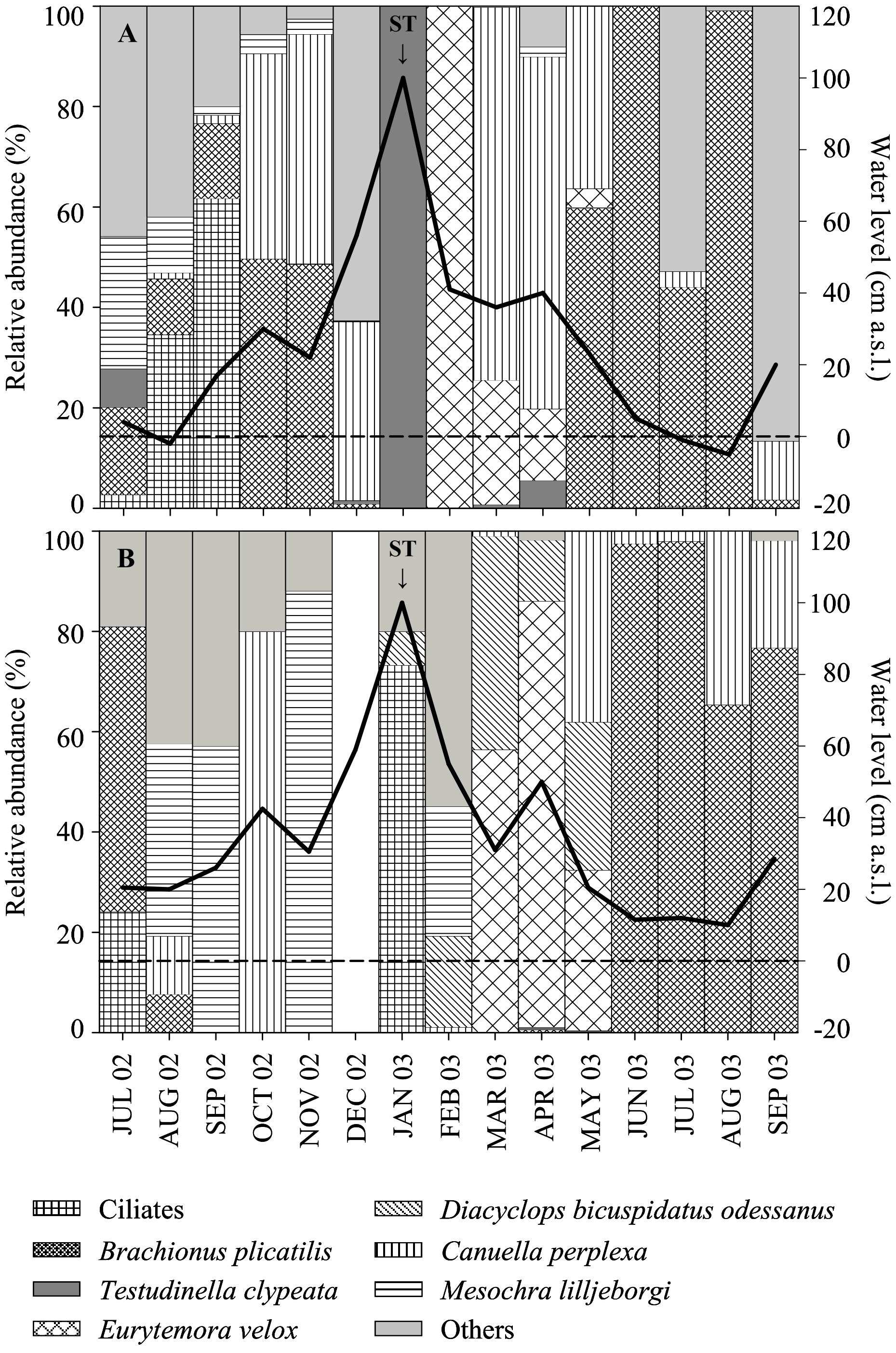


Figure 7.3. Relative abundance (in %) of the zooplankton species during the hydrological cycle 2002-2003 in the Fra Ramon lagoon (A) and in one of the new lagoons (N1, graphic B). The represented species dominated the community in some samples (> 75% of the total individuals). Water level evolution is shown on the right axis of the plots. The zero water level corresponds to the average sea level over the last 15 years (J.Pascual, pers.comm., Meteorological Station of l'Estartit). ST indicates the sea storm event.

7.3.3. A. iberus *population in Fra Ramon and in the new refuges*

The estimated total abundane of *A. iberus* population in the Fra Ramon lagoon showed high seasonal and interannual oscillations. In 2000 (Fig. 7.4 A), the highest value was recorded in the late summer sampling (402×10^3 ind., October) while the lowest was observed in late winter (32×10^3 ind., March). This seasonal pattern was quite different from that observed during the hydrological cycle 2002-2003 (Fig. 7.4 B), where the highest number of individuals only reached up to 14×10^3 and was achieved in the autumn months (November). The abundance decreased throughout the hydrological cycle, reaching the lowest value in summer (8×10^3 ind., July). Extreme low temperatures during the winter 02-03 and the extreme summer drought of 2003 may explain the low records of *A. iberus* in the Fra Ramon lagoon. Coefficients of variation of the total estimated abundance range from 12.7% and 26.5%, indicating a high spatial heterogeneity in fish distribution related not only to the gregarious behavior of this species but also to the microhabitat heterogeneity.

In the first fish stocking campaign (November 2002) a total of 464 individuals, 231 of them directly translocated from the Fra Ramon lagoon, were released in one of the new lagoons (N2; Photo 8), where a small but dense *R. cirrhosa* prairie have developed in a natural way. At that time, the newly created basins were unconnected (Photo 4 A). The stock released in the new lagoons showed a broad size distribution (Fig. 7.5 A), where the smallest individuals came from the Fra Ramon lagoon, and the largests were provided by captive breeding programme. The wide size distribution reflected a heterogeneous age structure for the implanted population which, together with a sex ratio skew towards females (215:109), is expected to maximize the success of the stocking programme. Extraction of individuals, for both the direct translocation and the captive breeding, coincided with the maximum abundance in Fra Ramon lagoon and did not involve any threat since the number of extracted individuals did not exceed 0.2% of the total number estimated in the source population.

During the first year after the stocking, a progressive increase in the number of individuals of *A. iberus* was observed in the new refuges. Thus, total abundance was estimated as 595 ind. in February 2003 (CV = 31.8%), as 705 ind. in July 2003 (CV = 27.2%) and as 4 ×10^3 ind. in September 2003 (CV = 50.1%). The size distribution of individuals in the new population after approximately one year after the stocking was wide and it was skewed towards the smallest sizes suggesting a successful recruitment (Fig. 7.6 A).

The new population was reinforced in March 2004 when 1118 captively-bred individuals were released in N2 lagoon. The size distribution of this second stock was also wide but, in comparison with the first stock (Fig. 7.5 A), small individuals were dominant (Fig. 7.5 B). In September 2004, the estimated total abundance in the new lagoons was high (31 ×10^3 ind., CV = 20.3%) suggesting a further successful recruitment of the new population. The size range of individuals was similar to that observed a year before but mature individuals were more abundant (Fig. 7.6 B).

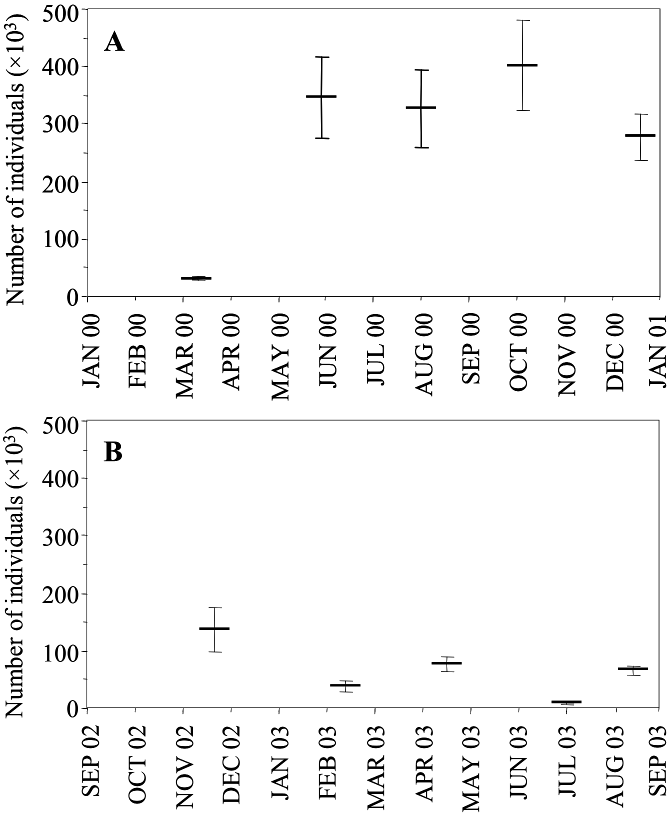


Figure 7.4. Evolution of the estimated total abundance of *A. iberus* individuals in the Fra Ramon lagoon during two annual cycles, 2000 (A) and 2002 (B). Error bars represents the 95% confidence intervals.

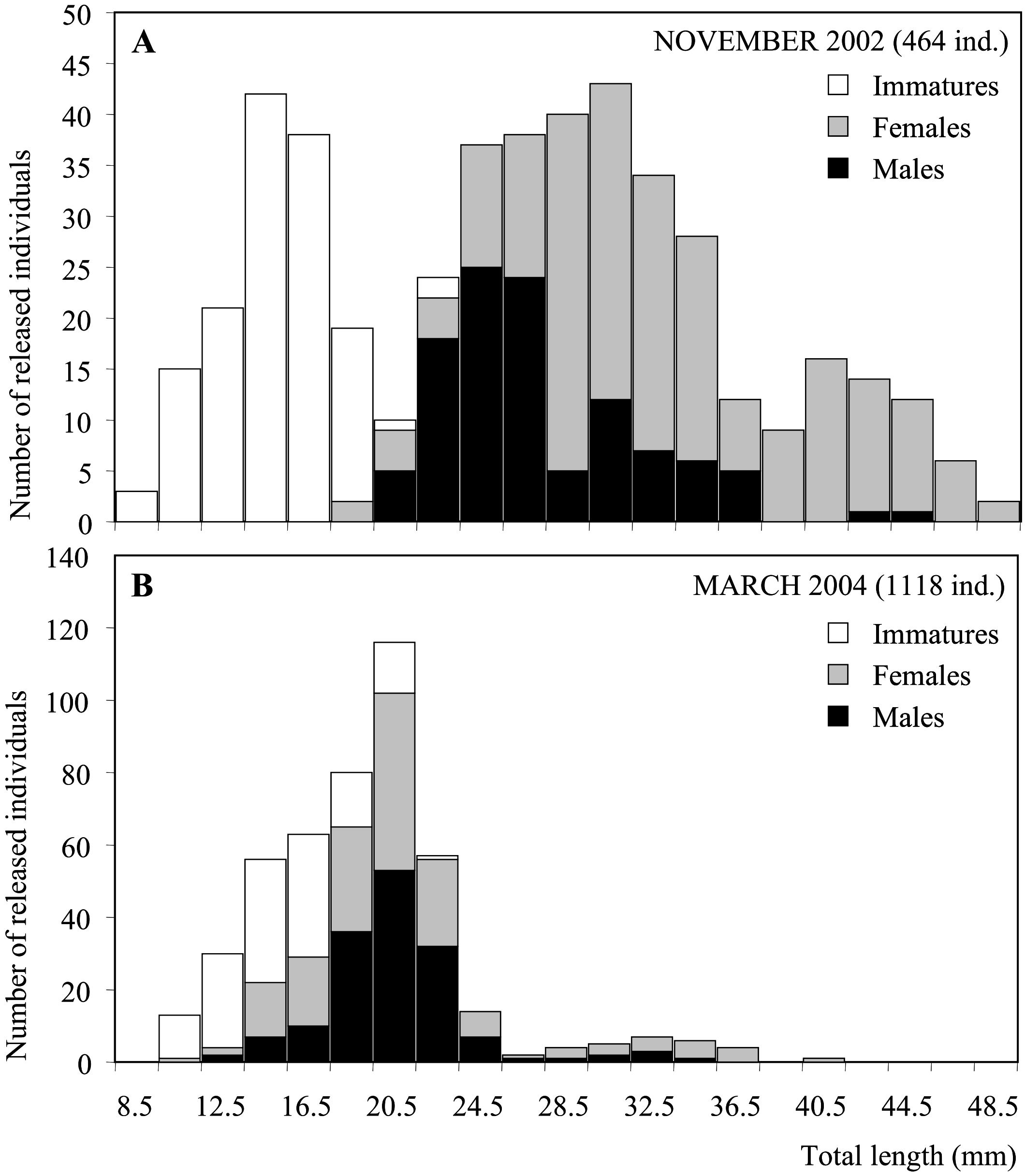


Figure 7.5. Size distribution of *Aphanius iberus* individuals released in the first (A) and in the second (B) stocking campaigns in the new created lagoons. In brackets, the total number of individuals released in each campaign.

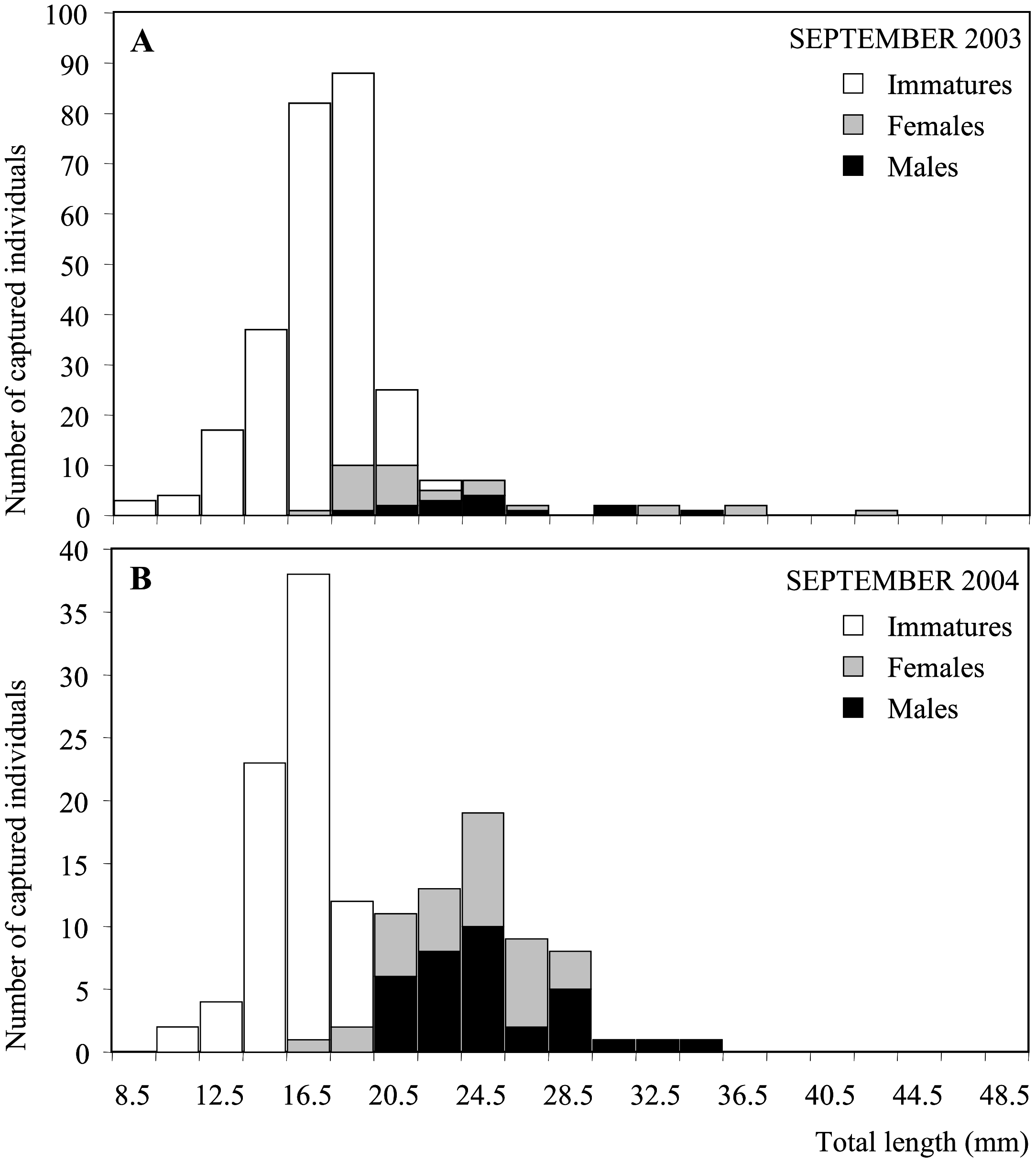


Figure 7.6. Size distribution of the *Aphanius iberus* individuals captured in the new lagoons one year (A) and two years (B) after the first stocking campaign carried out in November 2002.

7.4. Discussion

Creation of new habitats for conservation purposes will be only successful if it recreates the suitable habitat for the protected species. The comparison between the Fra Ramon lagoon, the natural habitat of *A. iberus* in the marshland, and the newly created habitats showed that all these lagoons were subject to the same hydrology, which was based on occasional inputs of fresh and/or marine water, the lack of continous freshwater inflow, infrequent connexions with the sea and a prolongued confinement period. This hydrology is characteristic of those Mediterranean salt marshes that are not disturbed by human activity, and causes a high variability of water level, salinity, temperature and nutrient composition (Quintana et al., 1998a; Trobajo et al., 2002; Gascón et al., 2005).

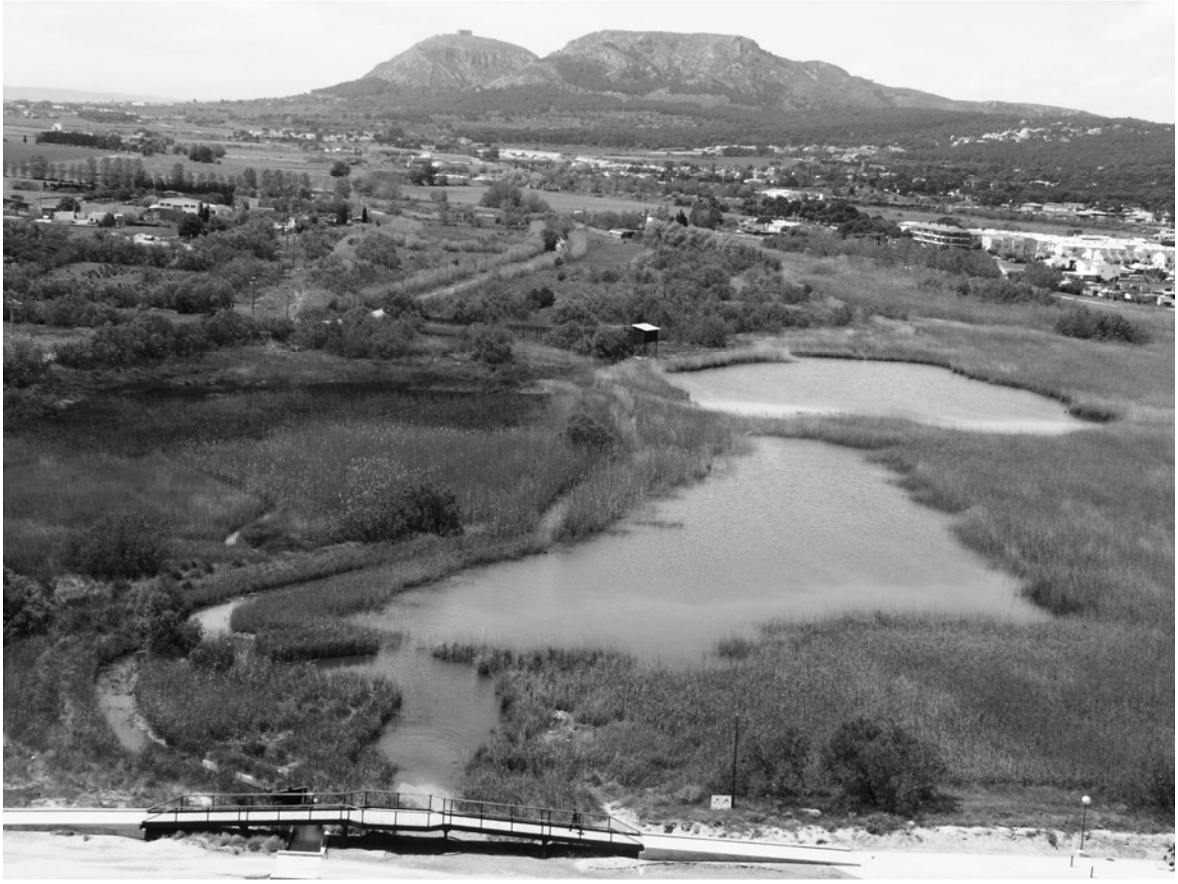
A. iberus is a euryhaline and eurytherm fish species highly adapted to the environmental variability of these brackish-hyperhaline coastal lagoons, which have become its key refuge habitat (Fernandez-Delgado et al., 1988; Garcia-Berthou and Moreno-Amich, 1992; Vargas and Sostoa, 1997). This fact is mainly due to the large salinity fluctuations and the extrem salinity values achieved during the dry season, which benefit *A.iberus* rather than its main competitor, *G. holbrooki* (Chervinski, 1983; Doadrio, 2001; Kandl, 2001). Thus, spatial segregation within coastal wetlands is commonly observed, with the most saline environments preferred by *A. iberus* and the freshwater ones by *G. holbrooki* (Moreno-Amich et al., 1999a, b). This poeciliid was introduced in the Iberian Peninsula at the beginning of the 20th century for mosquito control and has been related with the decline of other endemic toothcarps, *A. baeticus* and *Valencia hispanica* (Elvira and Almodóvar, 2001; Rincón et al., 2002; Schönhuth et al., 2003). In other Mediterranean countries, mosquitofish has also negatively affected the native toothcarps such as *Aphanius fasciatus* or *Valencia letourneuxi* (Crivelli, 1995; Economidis, 1995; Maitland and Crivelli, 1996). Potential predators of *A. iberus* such as the introduced freshwater sunfish *Lepomis gibbosus* or several marine fishes such as the European seabass (*Dicentrarchus labrax)* or the gilthead seabream (*Sparus aurata)* occasionally colonize coastal lagoons (Poizat and Crivelli, 1997; Franco et al., 2006; Pérez-Ruzafa et al., 2006) but they are not able to maintain stable populations because of the high fluctuations in salinity, water level and oxygen concentrations that take place throughout the hydrological cycle. Only eels (*Anguilla anguilla*) tolerate such conditions and, although they have been occasionally observed in La Pletera salt marshes, they maintain very low densities at the lagoons, even to the point of disappearing cyclically. Therefore, it is worth pointing out that fluctuating environmental conditions observed in the new refuges would avoid the establishment of the main competitors and predators of *A. iberus* without implying a threat for such species since its life-strategy is highly adapted to these unstable environments.

Similar physico-chemical conditions between the Fra Ramon and the new lagoons, with only some differences related mainly to the lagoon age, such as the lower total nutrient contents in the new lagoons, lead to similar invertebrate community composition and structure (Chapter 5). This is especially relevant, because *A. iberus* will find similar prey and similar interactions with other species in the new lagoons (Chapter 6). A broad size distribution in the invertebrate community, from small ciliates till more large benthic organisms, has been observed in the new habitats. It is especially important because an ontogenetic shift in the diet of *A.iberus* has previously been observed in the Fra Ramon lagoon, where small individuals showed positive electivity for small planktonic preys (copepod nauplii, the harpacticoids *M. lilljeborgi* and *T. longicornis*, the rotifer *B. plicatilis* and ostracods) while the larger ones elected and preyed on larger zooplankters and/or more benthic prey, such as the harpacticoid *C. perplexa*, polychaetes and ephydrid and stratiomyid dipterans (Alcaraz and García-Berhou, in press). Most of these taxa have been also recorded in the created refuges. Submerged vegetation, mainly the *Ruppia* prairies, has been described as a suitable habitat for *A. iberus* as it provides places of refuge and decreases the predation risk, and increases the food supply (Moreno-Amich et al., 1999b). Nevertheless, Alcaraz (2006) highlighted the role of glasswort, the surrounding habitat of Fra Ramon, as preferred feeding habitat for adult *A. iberus* when inundated during the periods of flooding. In La Pletera salt marshes, the presence of *Ruppia* prairies in the new habitats has been favoured by the design criteria of the new lagoons. However, the conservation of the current mature communities, such as the glasswort community (*Puccinelio-Arthrocnemetum fruticosi*) has so much priority than the recovery of the conditions that existed in the past. The old distribution of vegetation communities should not be the only factor to take into account in the design of new habitats in restoration projects.

The fast growth of *A. iberus* population in the new lagoons during the two years after its initial release suggests a successful fish stocking programme, at least in a short term. *A. iberus* shows a reproductive strategy adapted to unstable environments (Oliva-Patterna et al., 2006) due to its opportunistic life-history pattern characterized by high growth rate, quick maturation (3-4 weeks), high fecundity and a lengthy spawning season (Fernandez-Delgado et al., 1988; García-Berthou and Moreno-Amich, 1992; Vargas and Sostoa, 1997). These traits are the basis of the high productivity typically shown by *A. iberus* and other little minnows that colonize marsh habitats (Poizat and Crivelli, 1997; Planelles-Gomis, 1999). The broad year-to-year fluctuations of the abundance of the *A. iberus* in the Fra Ramon lagoon are an example of the fluctuating characteristics of its demography, already observed in other studies (García-Berthou and Moreno-Amich, 1999). Reductions in population size of brackish-water fauna following unpredictable natural events is a common phenomenon in these unstable environments (Cognetti, 1982, 1994). Therefore, high natural fluctuations in the new *A. iberus* population are also expected to occur irregularly, and this is not necessarily a threat for its maintenance.

SECTION III

The Ter Vell lagoon



CHAPTER 8

Short-term effects of changes in water management on the limnological characteristics and the zooplankton community

8.1. Introduction

In the Mediterranean region, alterations of the hydrological pattern, at all spatial and temporal scales, and water quality degradation are the main impacts of human activities and the main causes of coastal wetland disappearance and degradation (Day et al., 2000; Cloern, 2001; Beklioglu et al., in press). Although anthropic pressure is particularly high in the littoral areas, human land use in the catchments has also had a strong influence on Mediterranean coastal wetlands (Álvarez-Cobelas et al., 2005; La Jeunesse et al., 2002; Serrano et al., 2006). Traditionally, agriculture has been the main reason for the hydrological manipulation of coastal wetlands; however, since the second half of the 20th century it tends to be replaced by housing, industrial or tourism development (Britton and Crivelli, 1993; Pearce and Crivelli, 1994).

Alterations of water regime for agricultural purposes have mainly affected less confined and relatively well-flushed freshwater bodies whose functioning and dynamics depend mainly on the riverine inputs. Consequently, the freshwater flow throughout the ecosystem has become very high, the flooding period has been prolonged and marine influence has decrease (e.g. Herteaux, 1992; Lucena et al., 2002; Chauvelon, 1998). Therefore, variations in the hydrological pattern have become more predictable and controlled than natural, climate-dependent fluctuations.

This is the case, among numerous examples along the Mediterranean coast, of La Albufera de Valencia (Spain), the Vaccarès lagoon (Rhone Delta, France), L'Encanyissada lagoon (Ebro Delta, Spain) and several lagoons in the Nile Delta (Egypt) whose surroundings and catchments are severely affected by agricultural activities (Vicente and Miracle, 1992; Comín and Valiela, 1993; Chauvelon, 1998; Flower, 2001). The most widespread consequences of the alterations of the freshwater flow are a decline in salinity but also an increase in both eutrophy and environmental pollution as a result of increased nutrient and chemical contaminant inputs (Cloern, 2001; de Jonge et al., 2002; López-Flores et al., 2003; Carafa et al., 2006). Subsequent alterations in planktonic (Oltra and Miracle, 1992; Pérez-Ruzafa et al., 2002; Lenzi et al., 2003), benthic (Cognetti and Maltagliati, 2000; Marchini et al. 2004; Lloret et al., 2005) and fish communities (Blanco et al., 2003; Poizat et al., 2004) are commonly observed.

Recovering the ecological value of coastal wetlands is particularly important in the Mediterranean region, where losses exceeding 50% of the original coastal wetland areas in many countries (e.g. France, Greece, Italy, Spain; WWF, 2004) were recorded in the last century. Since salinity gradients, water quality and biological processes are closely related to the amount and timing of water flow as well as to the flow pathways within the ecosystem (Day et al., 2000; Becklioglu et al., in press), an understanding of hydrological functioning is necessary to restore and manage coastal wetlands.

Ter Vell (NE of Iberian Peninsula) is a typical example of a eutrophic freshwater coastal lagoon which has experienced the effects of increased agricultural and tourism pressure, especially during the second half of the 20th century. Recently, the hydrological pattern of the lagoon was significantly altered as a consequence of changes in water management related to agricultural and restoration activities (more details available in section 2.1.2, Chapter 2 and sections 3.3 and 3.5, Chapter 3). The present study aims to analyse how these changes have affected the limnological characteristics and the zooplankton community of the lagoon. Since the main consequence of the management activities was a drastic reduction of the freshwater inflow to the ecosystem, it is expected that the Ter Vell lagoon has become a more confined ecosystem. To test this hypothesis, limnological characteristics and the zooplankton community of the lagoon were compared with those of a nearby confined coastal ecosystem (La Pletera salt marshes) whose hydrology is not subject to human regulation.

8.2. Methods

8.2.1. Field sampling and laboratory analyses

In Ter Vell lagoon, three basins differently situated with respect to the main freshwater flow through the ecosystem were selected for sampling. One of them was located in the western part of the lagoon, where it received direct freshwater inflow (hereafter Inflow), and another was located near the drainage channel to the sea (hereafter Outflow). Freshwater flux through the lagoon takes place preferentially through a main channel, which flows in a NW to SE direction and connects these two basins (Fig. 8.1). The third sampled basin was situated in the most confined area (NE section of the lagoon) where the effect of the freshwater inputs was smaller and the water flowing toward it does so in a diffuse way (hereafter Confined). Sampling was performed during two hydrological cycles, one before changes in water management (from July 1999 to June 2000) and the other one after those changes (from July 2002 to June 2003). During the second cycle, the inflowing and outflowing waters of the constructed wetlands were also sampled to assess the concentration of total nutrients (N and P).

Five sampling points were selected in La Pletera salt marshes, three of which were located in three recently created lagoons (N1, N2 and N3) and the other two in older lagoons (O1 and O2) which have their origin in an old river distributarie. The new lagoons were created in the summer of 2002 in the framework of the EU LIFE project to increase the number of refuges for an endangered endemic fish species (Quintana et al., 2002a). The two old lagoons show higher nutrient loads and salinity than the new ones (see Chapter 5). Sampling was carried out from July 2002 to June 2003, coinciding with the second hydrological cycle studied in the Ter Vell lagoon.

Water and zooplankton samples were taken monthly from a central point of these basins at a depth of 15-30 cm. Temperature (t), electrical conductivity (EC_{25}), pH and dissolved oxygen (O2, in % of saturation) were measured in situ. The water level (WL) was measured as the height in cm above or below average sea level (over the last 15 years) by means of levels installed in the lagoons. Water flow in the runoff channel that supplies the Ter Vell lagoon was measured with a flowmeter. Data on rainfall series and the average sea level over the last 15 years came from the Meteorological Station of L'Estartit, 1 km from the lagoon (J. Pascual, pers. comm.).

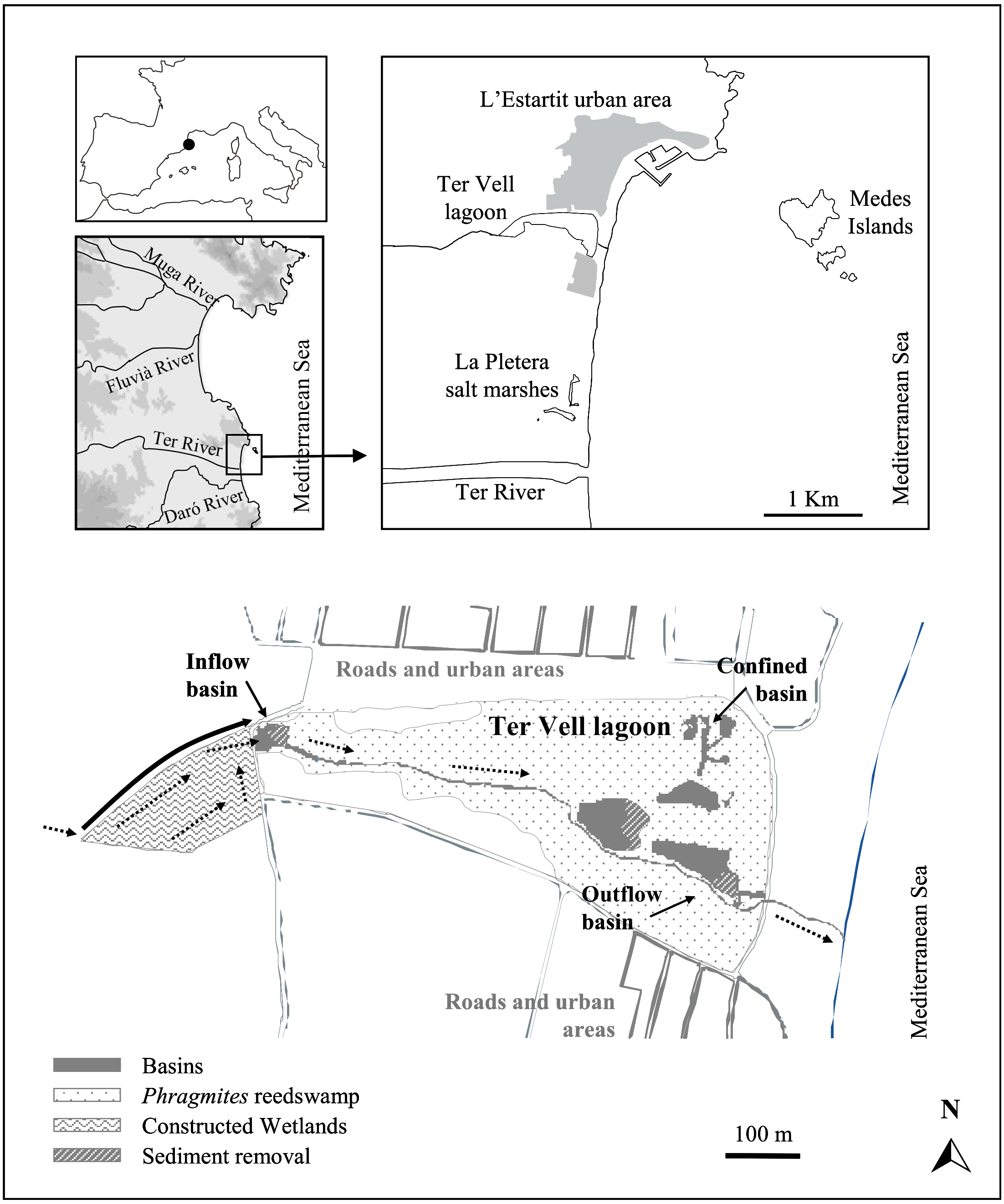


Figure 8.1. Sketch map of the Ter Vell lagoon showing the location of the three sampled basins: Inflow, Outflow and Confined. The constructed wetlands and the points where sediment was removed are shown. Dotted arrows indicate the current freshwater flux throughout the ecosystem. The location of the irrigation channel before the creation of the constructed wetlands is indicated by a curved black arrow.

Ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-) and soluble reactive phosphate (SRP) were analysed from filtered water samples and total nitrogen (TN) and phosphorous (TP) from unfiltered water samples according to Grasshoff et al. (1983) and APHA (1989). Organic matter was measured using a TOC analyser from unfiltered samples previously acidified to eliminate the inorganic dissolved carbon. Chlorophyll-*a* (Chl-*a*) was extracted using 95% methanol after filtering 500 ml of water in the lab (GF/C Whatman filters) and its concentration was estimated spectrophotometrically following Talling and Driver (1963).

Zooplankton samples were obtained by filtering 5 L of water through a 50-µm mesh-size net and preserved *in situ* in 4% formalin. An inverted microscope was used to count and, when possible, identify the zooplankton taxa at species level. Several ciliate taxa were identified by their morphology since species identification is often difficult after formalin fixation (Şenler and Yildiz, 2004 and references therein). When copepod nauplii were not identifiable, species proportions of adults were assigned. In the samples without adults, the species proportions of adults captured using a dip-net (250-µm mesh-size) were used. Only ciliates, rotifers and microcrustaceans (cladocera, copepoda and ostracoda) were taken into account in the later calculations and data analysis. For each zooplankton sample, the following community parameters were calculated: the Shannon-Wiener diversity index (*H*), taxonomic richness (*R*), evenness (*E*) and the Berger-Parker dominance index (*D*).

8.2.2. Statistical analyses

MANOVA analyses were performed considering simultaneously (1) all environmental variables and (2) all community parameters to test for significant temporal and spatial differences. The factors considered were hydrological cycle (99/00 and 02/03) and basin (Inflow, Outflow and Confined). The multivariate test statistic Pillai's Trace was used to detect significance because it is more robust to heterogeneity of variance and to deviations from multivariate normality (Quinn and Keough, 2002). The effects of factors on each environmental variable were assessed by means of analysis of variance (ANOVA). Post-hoc comparisons using the Games-Howell test at the 0.05 significance level let us analyse differences between the Ter Vell basins. Games-Howell tests are among the most powerful and most robust to unequal variances post-hoc multiple comparison methods (Day and Quinn, 1989).

In order to compare the Ter Vell lagoon with the nearby lagoons of La Pletera salt marshes, records of some environmental variables and the zooplankton composition of the five salt marsh lagoons, obtained during the 02/03 hydrological cycle, were included in the Ter Vell data matrix. A principal component analysis (PCA) was used to examine the variability of the physical and chemical composition of the water and to identify the relationships between study sites and environmental variables. This ordination technique is adequate when linear relationships between environmental variables are expected (Quinn and Keough, 2002). A canonical correspondence analysis (CCA) was performed to analyse the relationship between the zooplankton composition and environmental conditions. CCA is a modification of correspondence analysis that selects a linear combination of environmental variables to maximise the dispersion of species scores. It is an efficient ordination technique because living organisms often show unimodal responses to environmental gradients (ter Braak, 1986). According to Lepš and Šmilauer (2003), the species abundance was square root-transformed and downweighted for rare species. All environmental variables used in the analysis showed an Inflation Factor < 10. Variables were selected manually and, to test for the statistical significance of each selected variable ($p < 0.05$), 499 unrestricted permutations were conducted using the Monte Carlo test.

All statistical analyses were done with $\log_{10}(x + 1)$ transformed variables (except water level, pH, temperature, dominance, Shannon diversity and evenness) in order to improve linearity, as well as the normality and homogeneity of variances (Quinn and Keough, 2002). Calculations and statistical analyses were performed with SPSS 13.0, except PCA and CCA, which were carried out using CANOCO 4.5.

8.3. Results

8.3.1. Changes in the hydrological regime

A comparison of freshwater inflow between the 99/00 and 02/03 hydrological cycles, when nutrient and organism sampling was carried out, showed that in the first cycle the highest values, with a maximum of 13 $\times 10^4$ m^3 day^{-1}, were recorded during the spring-summer of 2000, coinciding with a high agricultural demand for water. Throughout this cycle the freshwater inflow was very high and irregular and the recorded average was 2 $\times 10^4$ m^3 day^{-1}, seven times higher and significantly different ($F_{1,729} = 175.07$, $p < 0.001$; one-way ANOVA) from the average of the 02/03 cycle (0.30 $\times 10^4$ m^3 day^{-1}). This was because in November 2001 the irrigation system changed and since then, except in the summer-autumn of 2002 when the surface channel worked again, the excess irrigation water has not fed the lagoon. In contrast to the first cycle, in the second one the freshwater inputs were recorded mainly after rainfall events and the highest freshwater inflow, with a maximum of 5 $\times 10^4$ m^3 day^{-1}, was observed in winter, during intense precipitation (Fig. 8.2 A).

A comparison of the temporal pattern of the water level between one basin of Ter Vell and one lagoon (O2) of La Pletera salt marshes without a human-regulated hydrology (Fig. 8.2 B), corroborated that in the 99/00 cycle the Ter Vell showed an artificial hydrological regime. In the dry seasons, high and irregular water levels in Ter Vell were related to freshwater inflow and contrasted with the low levels observed in the salt marsh lagoon. In the latter, the water level gradually decreased during the summer drought mainly due to evaporation, and in some summers it even descended below the average sea level (e.g. September 2000 and 2001). Conversely, in the dry seasons of the 02/03 cycle the water level in Ter Vell was lower and less variable than in the first cycle, and its temporal pattern tended to be more similar to that in the salt marsh lagoon. In the two cycles, the highest water level in both the Ter Vell lagoon and La Pletera salt marshes was reached by marine intrusion after sea storms, which usually took place between autumn and spring (Fig. 8.2 B).

The low and irregular freshwater inflow throughout the 02/03 cycle meant that the constructed wetlands were fed intermittently only during rainfalls, except in August- September 2002 when agriculture surface irrigation took place again.

8.3.2. Nutrient composition and dynamics

Considering simultaneously all environmental variables, significant differences were found between the studied basins (MANOVA Pillai's trace $F_{26,96} = 3.81$, $p < 0.01$) and between the hydrological cycles (MANOVA Pillai's trace $F_{13,47} = 8.11$, $p < 0.01$). Interaction between factors was not significant (MANOVA Pillai's trace $F_{26,96} = 1.12$, $p = 0.335$). The effects of both factors were significant for conductivity, dissolved oxygen, ammonium, nitrate, total nitrogen and total organic carbon. Differences in water level, nitrite and total phosphorus were only significant between basins, whereas differences in pH were only significant between cycles. For temperature, soluble reactive phosphate and chlorophyll-*a* no differences were found (Table 8.1).

With regard to the spatial variability within the Ter Vell lagoon, in the basin situated in the most confined area (Confined) water level and concentrations of ammonium, nitrite and nitrate, total nitrogen and total phosphorus were significantly lower than in the basin more affected by the freshwater inputs (Inflow). Conversely, the concentration of total organic carbon was significantly higher. No differences were found between the Inflow and the Outflow basins with regard to those variables highly conditioned by the freshwater flow (nitrite, nitrate and total nitrogen). In the two basins more subject to marine influence (Outflow and Confined) water conductivity was significantly higher than in the other one (Inflow). Dissolved oxygen in the Outflow basin was significantly higher than in the other two basins. Although non significant differences were found in the post-hoc comparisons, in the Outflow basin the average values of water level, total phosphorus and ammonium were more similar to the Confined basin, whereas the average values of total nitrogen were more similar to the Inflow basin.

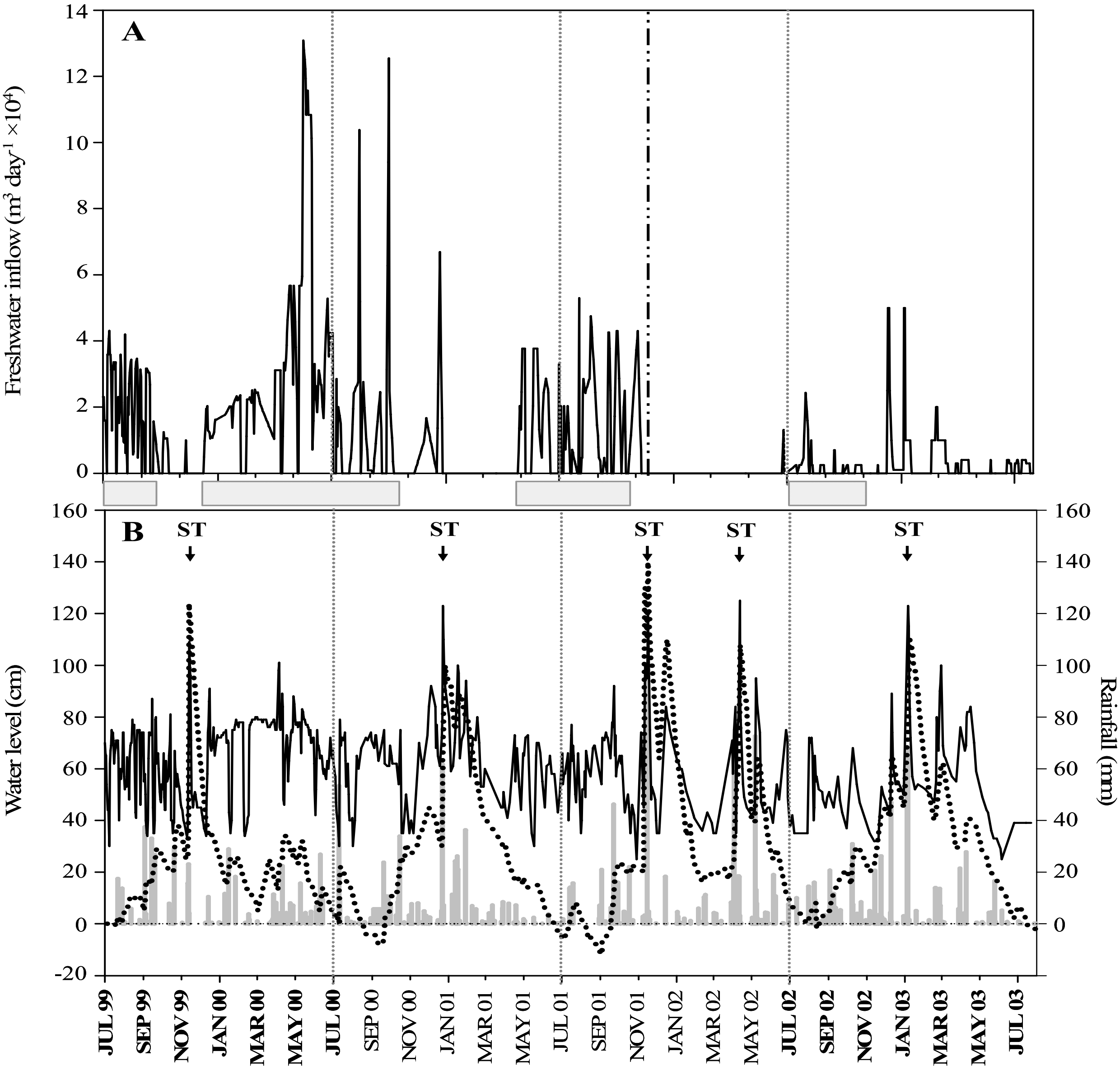


Figure 8.2. A) Freshwater inflow to the Ter Vell lagoon during four hydrological cycles from 1999 to 2003 (separated by grey dotted lines). The dash-dotted line in November 2001 indicates the beginning of the change in the irrigation system. Periods of freshwater irrigation for agricultural use are shown (grey boxes).

B) Variation in the water level (cm above or below the average sea level) in the Inflow basin of the Ter Vell lagoon (solid line) and in the O2 lagoon of La Pletera salt marshes (dotted line). The bars represent rainfall (in mm). ST indicates sea storm events. The zero water level corresponds to the average sea level over the last 15 years. The 99/00 and 02/03 hidrological cycles, when sampling was carried out, are indicated by bold letters.

Regarding temporal variability (Table 8.1), in the 99/00 cycle when freshwater entries were more abundant and prolonged, the average concentrations of nitrate and total nitrogen were significantly higher, but only in those basins most affected by freshwater circulation (Inflow and Outflow) (Table 8.2). Throughout the cycle, concentrations of nitrate and total nitrogen were very irregular and followed the same temporal pattern since large fraction of total nitrogen (> 80%) was in inorganic form. This pattern coincided in all basins (Fig. 8.3 A, only nitrate in the Inflow basin is represented). In the 02/03 cycle, temporal pattern of nitrate and total nitrogen was also similar in all basins, but in contrast with the first cycle, concentrations peaked coinciding with the main water inputs (meteorological disturbances) and the rest of the cycle concentrations were low. This nitrogen dynamics was similar to that found in the confined lagoon of La Pletera salt marshes (Fig. 8.3 A). The change of the hydrological regime also affected the organic content of the basins since total organic carbon was significantly higher in all of them in the 02/03 cycle (Table 8.2). In the 99/00 cycle, in those basins more affected by freshwater flow (Inflow and Outflow) the temporal pattern of total organic carbon was similar and did not vary appreciably throughout the study period (Fig. 8.3 B, only the Inflow basin is shown). On the other hand, in the Confined lagoon, total organic carbon was more variable and its temporal dynamics was similar to that observed in the lagoon of La Pletera. Concentration increased in the summer months coinciding with a decrease in water level (Fig. 8.3 B, only the salt marsh lagoon is shown). In the 02/03 cycle, the average concentration of total organic carbon, as well as its temporal pattern, was similar in all basins (Table 8.2). Concentration values increased during the spring-summer drought in 2003 when water levels were very low in all basins (Fig. 8.3 B). The average conductivity was also significantly different between cycles, but only in the Confined basin, where it increased in the second cycle. In the basins with more marine influence (Outflow and Confined), the average values of pH and ammonium concentration decreased significantly in the 02/03 cycle.

Table 8.1

Two-way ANOVAs testing for significant spatial (between basins) and temporal (between cycles) differences (*: $p < 0.05$). Significant differences between basins obtained in the post-hoc comparisons (Games-Howell tests, $p < 0.05$) are shown (I: Inflow basin; O: Outflow basin; C: Confined basin). Abbreviations of the environmental variables are given in the text (section 8.2.1). No significant interactions were found between factors.

	Basin			Cycle	
	$F_{2,59}$	*p-value*	*Post-hoc comparisons*	$F_{1,59}$	*p-value*
WL (cm a.s.l.)	3.87	0.026*	$I \neq C$	0.00	0.983
T (ºC)	0.22	0.803		0.20	0.656
EC_{25} (mS cm^{-1})	11.76	< 0.001*	$I \neq O$; $I \neq C$	5.82	0.019*
pH	1.23	0.301		16.05	< 0.001*
O_2 (% sat.)	4.34	0.017*	$O \neq I$; $O \neq C$	5.40	0.024*
NH_4^+ (mg N l^{-1})	5.21	0.008*	$I \neq C$	11.94	0.001*
NO_2^- (mg N l^{-1})	9.38	< 0.001*	$C \neq I$; $C \neq O$	2.21	0.143
NO_3^- (mg N l^{-1})	10.18	< 0.001*	$C \neq I$; $C \neq O$	17.33	< 0.001*
SRP (mg P l^{-1})	2.11	0.130		1.23	0.273
TN (mg N l^{-1})	5.28	0.008*	$I \neq C$	21.45	< 0.001*
TP (mg P l^{-1})	4.23	0.019*	$I \neq C$	3.75	0.058
TOC (mg l^{-1})	6.31	0.003*	$C \neq I$; $C \neq O$	18.19	< 0.001*
Chl-*a* (µg l^{-1})	0.33	0.720		2.52	0.118

During the first year of functioning of the constructed wetlands, only 6 measures of total nutrients were obtained, coinciding with those situations when a continuous freshwater flow through the system was observed. No reduction in nutrients was detected since no significant differences in the average concentrations of TN and TP were found between the inflowing (3.50 ± 1.76 mg N l^{-1} and 0.19 ± 0.21 mg P l^{-1}) and the outflowing waters (2.79 ± 1.53 mg N l^{-1} and 0.13 ± 0.10 mg P l^{-1}) of the constructed wetlands ($F_{1,10} = 0.65$, $p = 0.440$ for TN and $F_{1,10} = 0.33$, $p = 0.578$ for TP; one-way ANOVA).

8.3.3. Zooplankton community structure and composition

When considering simultaneously all community parameters (diversity, taxonomic richness, dominance and evenness), significant differences were found among the studied basins of the Ter Vell lagoon (MANOVA Pillai's trace $F_{8,128} = 2.18$, $p < 0.05$) but not between the hydrological cycles (MANOVA Pillai's trace $F_{4,63} = 2.39$, $p = 0.060$). Interaction between factors was not significant ($F_{8,128} = 1.60$, $p = 0.131$). Shannon-Wiener diversity and evenness were the community parameters that most differ between basins, although statistical significance was marginal ($F_{2,66} = 2.773$, $p = 0.070$ and $F_{2,66} = 2.693$, $p = 0.075$ respectively, two-way ANOVA). In the basin which directly received the freshwater inputs (Inflow) their average values were higher than in the Confined basin (Games-Howell post-hoc test $p < 0.05$). No significant differences were found with regard to the average richness and dominance ($F_{2,66} = 1.298$, $p = 0.280$ and $F_{2,66} = 1.191$, $p = 0.0310$ respectively, two-way ANOVA).

Table 8.2

Mean of monthly samples and standard deviation (in brackets) of physical and chemical variables in each hydrological cycle for the three basins of the Ter Vell lagoon. Abbreviations of the environmental variables are given in the text (section 8.2.1). Significant (*: $p < 0.5$, **: $p < 0.01$) and nonsignificant (n.s.: $p > 0.05$) differences between cycles within each basin are shown (one-way ANOVA).

	Inflow			Outflow			Confined		
Cycle	99/00	02/03		99/00	02/03		99/00	02/03	
	(N=12)	(N=12)	Sig.	(N=12)	(N=12)	Sig.	(N=12)	(N=12)	Sig.
WL	61.25	56.00	n.s.	47.08	46.92	n.s.	43.17	44.42	n.s.
(cm a.s.l.)	(15.90)	(19.85)		(11.12)	(25.76)		(17.36)	(30.42)	
T	15.22	16.91	n.s.	15.35	17.62	n.s.	16.22	17.34	n.s.
(ºC)	(7.50)	(5.73)		(7.68)	(6.39)		(7.85)	(6.71)	
EC_{25}	1.66	1.27	n.s.	5.50	9.64	n.s.	4.39	5.99	*
(mS cm^{-1})	(2.80)	(0.46)		(8.70)	(8.75)		(8.29)	(3.03)	
pH	7.58	7.33	n.s.	7.84	7.40	**	7.75	7.24	**
	(0.50)	(0.47)		(0.21)	(0.46)		(0.25)	(0.44)	
O_2	52.93	47.62	n.s.	63.43	59.17	n.s.	56.53	43.02	n.s.
(% sat.)	(14.84)	(43.53)		(13.44)	(20.49)		(27.21)	(23.21)	
NH_4^+	0.76	0.29	n.s.	0.47	0.08	**	0.28	0.02	**
(mg N l^{-1})	(0.89)	(0.24)		(0.50)	(0.12)		(0.21)	(0.03)	
NO_2^-	0.03	0.02	n.s.	0.02	0.01	n.s.	0.005	0.004	n.s.
(mg N l^{-1})	(0.03)	(0.02)		(0.02)	(0.02)		(0.01)	(0.01)	
NO_3^-	1.64	0.42	**	1.33	0.29	**	0.43	0.10	n.s.
(mg N l^{-1})	(1.13)	(0.67)		(1.00)	(0.61)		(0.82)	(0.25)	
SRP	0.19	0.17	n.s.	0.10	0.20	n.s.	0.06	0.12	n.s.
(mg P l^{-1})	(0.16)	(0.16)		(0.06)	(0.32)		(0.03)	(0.11)	
TN	3.19	1.35	**	2.47	1.30	*	1.69	1.08	n.s.
(mg N l^{-1})	(1.40)	(0.86)		(1.36)	(0.76)		(1.18)	(0.33)	
TP	0.27	0.40	n.s.	0.18	0.31	n.s.	0.12	0.20	n.s.
(mg P l^{-1})	(0.14)	(0.24)		(0.05)	(0.40)		(0.07)	(0.14)	
TOC	4.94	10.62	**	4.60	10.08	**	7.86	12.97	*
(mg l^{-1})	(0.99)	(8.64)		(0.81)	(8.27)		(3.26)	(7.08)	
Chl-*a*	22.90	13.56	*	15.28	15.01	n.s.	13.38	16.59	n.s.
(µg l^{-1})	(22.36)	(22.98)	(0.042)	(10.86)	(9.64)		(10.26)	(14.45)	

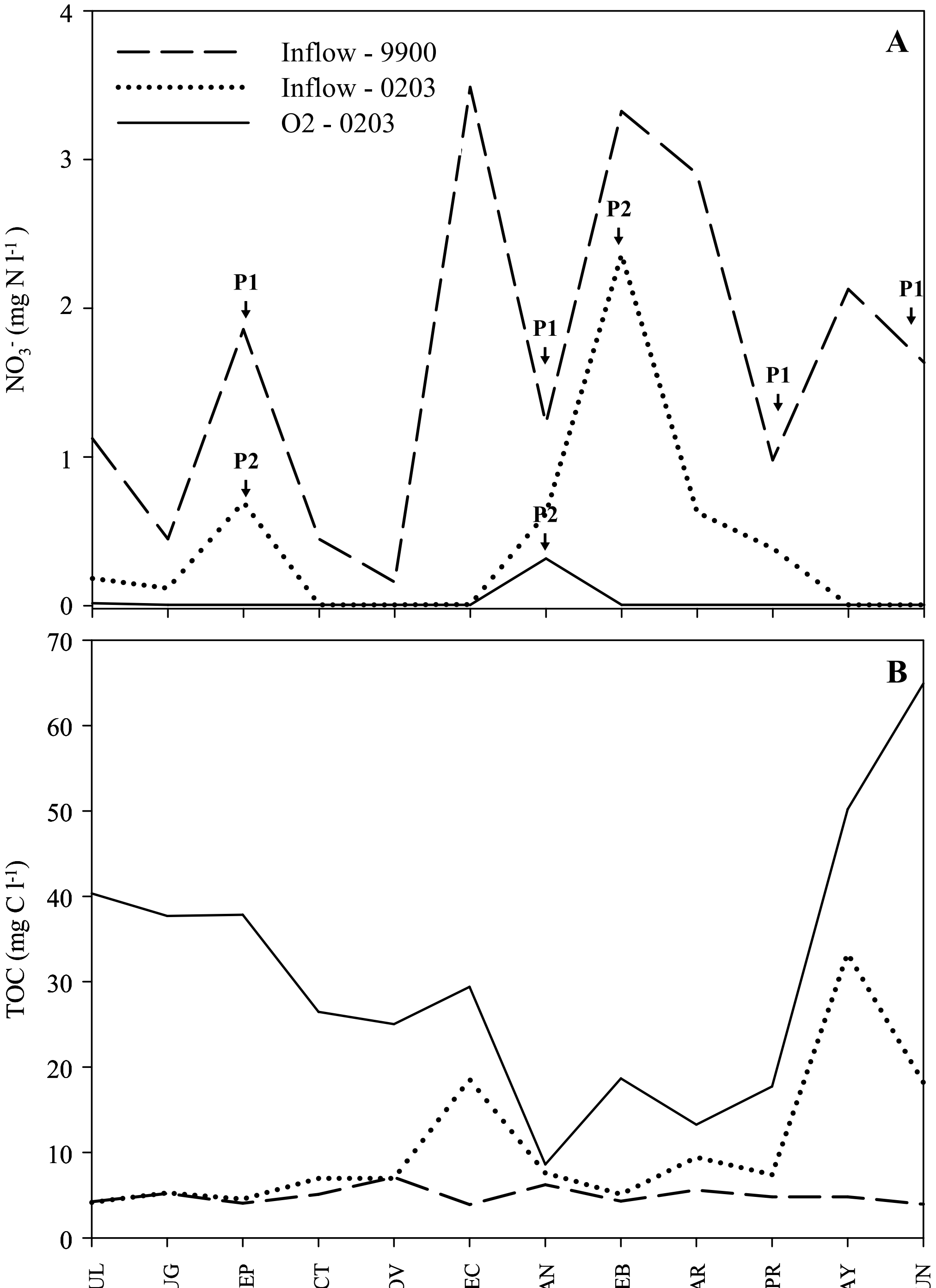


Figure 8.3. Concentration of (A) nitrate (NO_3^-, in mg N l^{-1}) and (B) total organic carbon (TOC, in mg N l^{-1}) in the Inflow basin of the Ter Vell lagoon and in the O2 lagoon of La Pletera salt marshes throughout the hydrological cycle. The precipitations events are indicated as P1 for the 99/00 cycle and P2 for the 02/03 cycle.

A total of 122 taxa were distinguished in all zooplankton samples, with Ciliates and Rotifera being the most represented phyla with 18 and 63 taxa, respectively. Only 29 taxa coincided in the studied basins: 5 ciliates, 21 rotifers and 3 crustaceans (Table 8.3). Among the ciliates, the most abundant taxa were *Strombidium* sp. and *Euplotes* sp. The dominant rotifer species were *Notholca squamula*, Synchaetidae undet. sp.1, *Brachionus angularis* and *B. calyciflorus*. Among the crustaceans, the 3 coincident taxa were also the most abundant: the branchiopod *Bosmina longirostris*, the calanoid *Calanipeda aquaedulcis* and the cyclopoid *Acanthocyclops* gr. *robustus*. Among the taxa that did not occur in all basins, it is worth pointing out the presence in high abundance of the rotifer *Brachionus plicatilis*, which reached more than 16 $\times 10^3$ ind. l^{-1} due to population explosions in summer 2003, and the cyclopoid *Diacyclops bicuspiatus odessanus* in those basins with more marine influence (Outflow and Confined). The branchiopod *Chydorus sphaericus* were almost exclusive of the basin directly affected by the freshwater inputs (Inflow) since its presence in the Outflow basin was sporadic and it was absent in the Confined one. Harpacticoids were not very abundant in the Ter Vell lagoon since they never exceeded 6% of the total individuals in any zooplankton sample.

3.4. Comparison between the Ter Vell lagoon and La Pletera salt marshes

Principal Components Analysis determined that the first two axes accounted together for 52.1% of the total variance. Sample ordination in Axis I (34%) responded to a hydrological gradient (Fig. 8.4). Samples from La Pletera salt marshes were mainly located on the left side, and the samples from the old lagoons (O1 and O2) showed the lowest coordinates. Samples from the Ter Vell lagoon were located on the right side, and those samples recorded in the 99/00 cycle in the basins most affected by the freshwater flow (Inflow and Outflow) showed the highest coordinates. Axis 1 was associated with a water level and a dissolved inorganic nitrogen (ammonium, nitrite and nitrate) gradient in the positive direction, and with an organic content (total organic carbon, Chl-*a*) and a conductivity gradient (EC_{25}) in the negative.

Table 8.3

Number of taxa of each Phylum found in the three basins of the Ter Vell lagoon. The total number of taxa for each Phylum (Total), the number of taxa that occur in all basins (Coincident) and cumulative richness ($R_{c.}$) are also shown.

Phylum	Total	Inflow	Outflow	Confined	Coincident
Ciliates	18	9	9	11	5
Rotifera	63	44	41	34	21
Arthropoda					
Cl. Branchiopoda	14	7	5	8	1
Cl. Ostracoda	3	2	1	2	0
Cl. Copepoda					
O. Calanoida	1	1	1	1	1
O. Cyclopoida	16	8	11	6	1
O. Harpacticoida	7	1	2	5	0
R_c	122	72	70	69	29

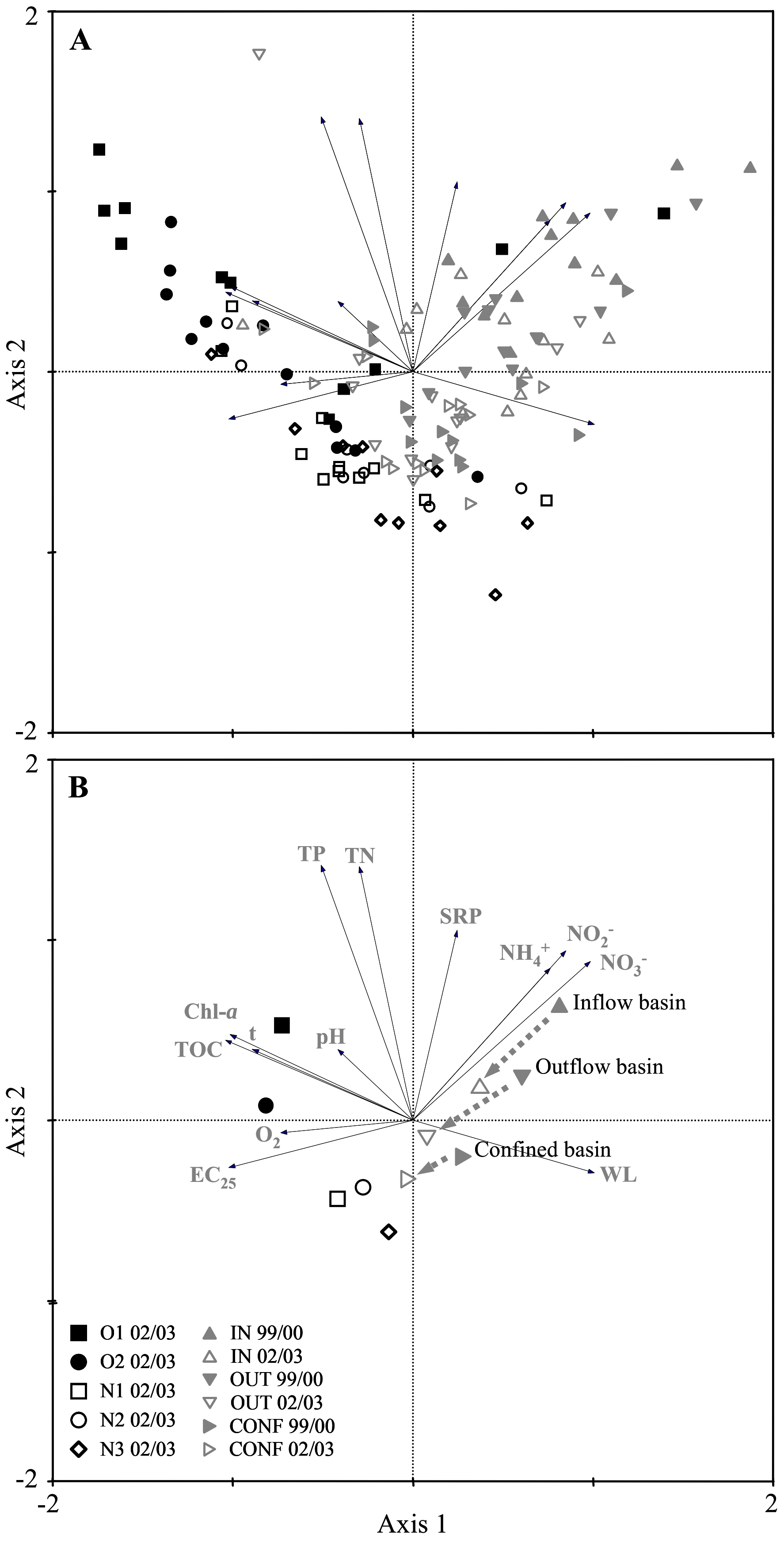
Sample ordination in Axis 2 (18.1%), which was mainly associated with a total nutrient (TN and TP) gradient in the positive direction, responded to a eutrophic gradient. Thus, samples from the old lagoons of La Pletera and the Inflow and Outflow basins of the Ter Vell lagoon in the 99/00 cycle, with high nutrient content, showed the highest coordinates. Conversely, samples from the new salt marsh lagoons and the Ter Vell lagoon in the 02/03 cycle showed the lowest ones. In both axes, the position of the centroids of each basin in the 02/03 cycle was displaced toward lower coordinates, that is, to a situation of lower water flow and lower nutrient content. This displacement was more apparent in those basins most affected by the freshwater flow (Inflow and Outflow).

In the Canonical Correspondence Analysis (Fig. 8.5) the first two axes together accounted for 62.9% of the total variance. Coinciding with the PCA, sample ordination in the first axis (42.2%) was related with a hydrological gradient: prolonged water flow situations (Ter Vell lagoon in the 99/00 cycle) with the lowest coordinates and extreme confinement situations (old lagoons of La Pletera) with the highest coordinates. In this axis, the zooplankton communities of both kinds of ecosystems were completely differentiated since almost all samples from the Ter Vell lagoon showed negative coordinates and, conversely, almost all samples from La Pletera salt marshes showed positive coordinates. Samples with the lowest coordinates were dominated by rotifers, mainly species of the genus *Brachionus* and *Polyarthra vulgaris*, and the cyclopoid *Acanthocyclops* gr. *robustus*. Increasing values of taxonomic richness, as well as concentrations of ammonium and nitrate, were characteristic of these situations. On the other hand, samples with the highest coordinates were dominated by the ciliate *Fabrea* gr. *salina*, the rotifer *Brachionus plicatilis*, several harpacticoids and the calanoid *Eurytemora velox*. High values of conductivity, pH and total nitrogen were characteristic of these situations. On the second axis (20.7%) samples were situated according to temperature. Samples collected in the cold season, situated in the upper part, were characterized by high abundances of the rotifer *Notholca squamula* and the cyclopoid *Diacyclops b. odessanus* in the Ter Vell basins and the calanoid *E. velox* in the salt marsh lagoons. Samples from the hot season, located in the lower part, were dominated by the ciliate *F.* gr. *salina* and *B. plicatilis* in the salt marsh lagoons and by *Brachionus angularis* and *B. quadridentatus* in the Ter Vell lagoon. The displacement of the basin centroids between cycles was almost insignificant for both axes.

In the next two pages, respectively:

Figure 8.4. PCA ordination model: biplots showing the environmental variables dispersion (arrows) and the position of (A) all samples and (B) the centroids of the Ter Vell basins and La Pletera lagoons in the plane of the first two axes. Dashed arrows indicate the displacement of centroids of each Ter Vell basin from 99/00 cycle to 02/03 cycle. Abbreviations of the environmental variables are given in the text (section 8.2.1). The sampling site abbreviations for the Ter Vell lagoon are: IN, Inflow basin; OUT; Outflow basin; CONF, Confined basin; and for La Pletera salt marshes: O1, O2, the old lagoons; N1, N2, N3, the new lagoons.

Figure 8.5. CCA ordination diagram: triplot showing the position of the zooplankton taxa and (A) all samples and (B) the centroids of every sampling site in relation to the environmental and community variables (arrows) in the plane of the first two axes. Abbreviations of the variables are given in the text (section 8.2.1) and the sampling sites abbreviations are as in Fig. 8.4. Only species with more than 10% weight in the analysis are shown. Zooplankton species codes are: ACROB: *Acanthocyclops* gr. *robustus*; BDEsp: Bdelloidea sp.; BRANG: *Brachionus angularis*; BRCAL: *B. calyciflorus*; BRPLI: *B. plicatilis*; BRQUA: *B. quadridentatus*; BRURC: *B. urceolaris*; CAAQU: *Calanipeda aquaedulcis*; CAPER: *Canuella perplexa*; COADR: *Colurella adriatica*; DIBOD: *Diacyclops bicuspidatus odessanus*; EUPsp: *Euplotes* sp.; EUVEL: *Eurytemora velox*; FASAL: *Fabrea* gr. *salina*; HAROT: *Halicyclops rotundipes*; LECsp: *Lecane* sp.; MELIL: *Mesochra lilljeborgi*; NOSQU: *Notholca squamula*; POVUL: *Polyarthra vulgaris*; SYNCH1: Synchaetidae undet. sp.1; TEPAT: *Testudinella patina.*


A
B
2
-2
Axis 2
TP
TN
SRP
NH$_4^+$
NO$_2^-$
NO$_3^-$
Inflow basin
Outflow basin
Confined basin
WL
Chl-*a*
t
TOC
pH
O$_2$
EC$_{25}$
O1 02/03
O2 02/03
N1 02/03
N2 02/03
N3 02/03
IN 99/00
IN 02/03
OUT 99/00
OUT 02/03
CONF 99/00
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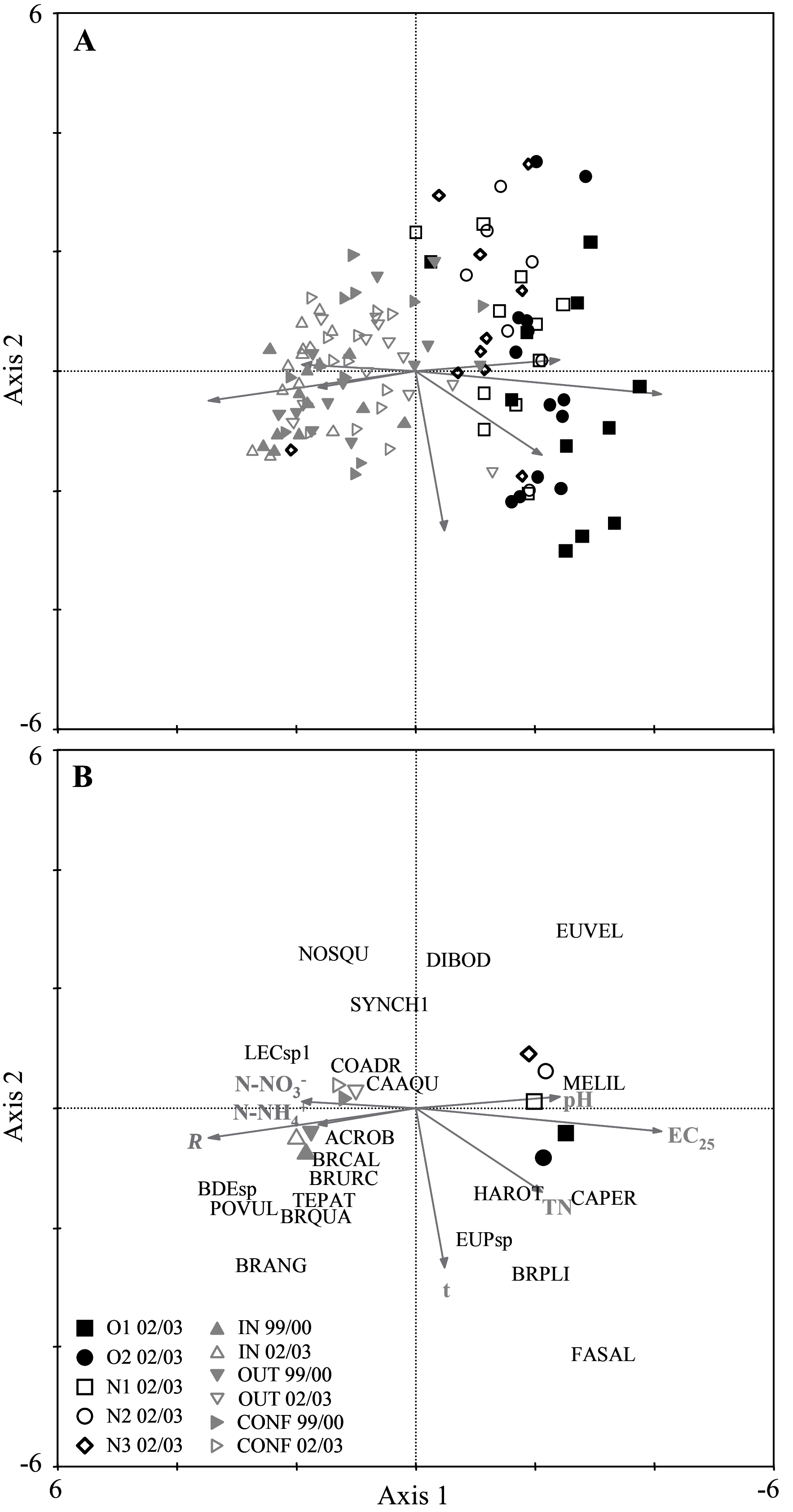
-2
2
Axis 1

Figure 8.4


A
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6
-6
6
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Axis 2
Axis 2
Axis 1
6
-6
EUVEL
NOSQU
DIBOD
SYNCH1
LECsp1
COADR
CAAQU
MELIL
N-NO₃⁻
N-NH₄⁺
pH
R
EC₂₅
ACROB
BRCAL
BRURC
BDEsp
POVUL
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▲ IN 99/00
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▽ OUT 02/03
▶ CONF 99/00
▷ CONF 02/03

Figure 8.5

8.4. Discussion

Hydrological functioning of the Ter Vell lagoon in the 99/00 cycle was not characteristic of a coastal lagoon in a Mediterranean climate since the highest freshwater inputs were recorded during the dry seasons, when the lowest inputs were expected. The freshwater-flooding phase was prolonged due to continuous surface inputs and the lagoon had an exorheic character, similar to some lakes in northern temperate regions (e.g. Liere and Gulati, 1992) or the Mediterranean ones subjected to a great deal of human pressure (e.g. Heurteaux, 1992; Vicente and Miracle, 1992). During the 02/03 cycle, after the change in the water regime, a more natural hydrology was observed in Ter Vell. The lagoon had a more endorheic character since the water inputs, more scarce and related to meteorological disturbances, were followed by prolonged periods of confinement without inputs, as is typical in coastal wetlands of the Mediterranean region with a climate-dependent hydrology (e.g. Quintana et al., 1998a; Serrano et al., 2006; see Chapter 5).

Nutrient composition and dynamics of the Ter Vell lagoon were affected by the change in the hydrological functioning, and in the 02/03 cycle they tended to be more similar to those of confined coastal ecosystems with an endorheic character, as shown by the comparison of the Ter Vell lagoon and La Pletera salt marshes. The higher nitrogen inputs (mainly as oxidized compounds) found in the Ter Vell lagoon throughout the 99/00 cycle, especially in those basins more affected by the freshwater flow, were expected since in coastal ecosystems affected by agricultural activities, high nitrogen supplies (mostly as nitrate) are usually related to freshwater inputs (Chapelle et al., 2000; Lucena et al., 2002; Pérez-Ruzafa et al., 2005). In the 02/03 cycle nitrogen inputs diminished and, as is typical of confined coastal ecosystems with a climate-dependent hydrology, they were detected only after meteorological disturbances and then rapidly diminished. Inorganic nitrogen reductions caused by denitrification are common in Mediterranean lagoons when water inputs cease (Comín and Valiela, 1993; Quintana et al., 1998a; Sylaios and Theocharis, 2002). The lack of differences in phosphorus content between hydrological cycles would agree with the fact that phosphorus dynamics in most coastal wetlands does not depend as much on water inputs as nitrogen dynamics does, but rather on the internal recycling processes (e.g. release from sediment, mineralisation of organic matter, etc.; Gomez et al., 1998; Frascari et al., 2002), which become more relevant in the confined ecosystems. In fact, the phosphorus content in the Confined basin was always higher than in the basin directly receiving the freshwater inputs. The organic content and conductivity of the Ter Vell lagoon increased in the 02/03 cycle, especially in the Outflow and Confined basins, probably resulting from a concentration effect derived from the prolonged period without water inputs and with low water turnover rate. The accumulation of salts and organic matter is expected to occur in Mediterranean coastal wetlands without continuous freshwater inputs and with low and irregular rainfall. These ecosystems which usually act as a sink for organic matter and nutrients due to their endorheic character (Ibáñez et al., 2000; Cotner et al., 2004; see Chapter 5). Moreover, an increase in organic content and nutrients due to internal loading and recycling processes could be enhanced in shallow ecosystems like the Ter Vell lagoon, where external loading has been prolonged over the years and a high pool of nutrients and organic matter has accumulated (Søndegaard et al., 1999, 2003).

The zooplankton community structure in the Ter Vell lagoon was not significantly affected by changes in the water regime, at least in the short term, but did differ between the basins in terms of Shannon-Wiener diversity and evenness. The lower values found in those basins with more marine influence, especially in the most confined one, were expected since it has been previously described that diversity decreases in the more hyperhaline conditions (Britton and Johnson, 1987; Therriault, 2002; Toumi et al., 2005). Furthermore, confinement situations usually involve an impoverishment of aquatic fauna (Guelorget and Perthuisot, 1983). This trend was also observed when comparing the zooplankton of the Ter Vell lagoon with that of La Pletera salt marshes since the lowest richness was recorded in the confined and hyperhaline salt marsh ecosystems. No similarities were found when comparing zooplankton communities of both kinds of ecosystems due basically to large differences in salinity. In fact, salinity and its range were among the most important factors accounting for variability in aquatic communities (Basset et al., 2006b and references therein). Zooplankton in the salt marshes was dominated by few euryhaline species of copepods, mainly harpacticoids and calanoids adapted to the high salinity fluctuations of these confined ecosystems (Cognetti and Maltagliati, 2000; Ramdani et al., 2001; Brucet et al., 2005a) whereas ciliates, rotifers and cladocerans were the most representative taxonomic groups in Ter Vell. The dominance of several species of the genus *Brachionus* as well as the cladocerans *Bosmina longirostris* and *Chydorus sphaericus* and the cyclopoid *Acanthocyclops* gr. *robustus* have previously been related with eutrophic conditions (Gannon and Stemberger, 1978; Miracle et al., 1987; Caramujo and Boavida, 2000). The zooplankton composition found in the Ter Vell lagoon was common to those freshwater Mediterranean coastal ecosystems receiving high amounts of nutrients due to agricultural runoff such as La Albufera de Valencia (Oltra and Miracle, 1992) or some lagoons in the Ebro and Nile deltas (Menéndez and Comín, 1986; Ramdani et al., 2001). It is worth noting that the prolonged confinement situations in the 02/03 cycle would lead to episodes of hypertrophy in the basins of the Ter Vell lagoon less affected by the freshwater inputs (Outflow and Confined), as suggested by the increase in *B. plicatilis* abundance. In fact, due to its tolerance to low oxygen concentrations, this species has already been related with summer hypertrophy in La Pletera salt marshes (see Chapter 5) as well as in other Mediterranean coastal wetlands (Esparcia et al., 1989; Quintana et al., 1998b).

In summary, differences in nutrient composition and dynamics between the hydrological cycles in the Ter Vell lagoon were observed after the management actions. Specifically, the actions derived from water management in agriculture, which lead to a change in the hydrological regime. The drastic reduction of the freshwater inflow and the unpredictability of the freshwater inputs in the 02/03 cycle prevented the correct working of the constructed wetlands. The restoration of a wetlands area often depends on what is happening in its surroundings, and an integrated management of the wetlands and the adjacent land is desirable to achieve a successful recovery (Hodge and McNally, 2000 and references therein). Thus, agricultural water management in the surroundings of Ter Vell should adapt to the restoration measures carried out in the lagoon. Since the lagoon is originally a deltaic ecosystem highly conditioned by the riverine inputs, it is recommended that there be a continuous and regular flow in the surface irrigation channel to feed the constructed wetlands and the lagoon, at least during the winter season. It would lead to a reduced water level variation and allow the maximization of hydraulic residence time and thus overall treatment efficiency of constructed wetlands (Kadlec and Knight, 1996). In turn, a regular and continuous flow to the lagoon would sustain a sufficient water turnover to avoid an increase in the nutrients and organic content due to internal loading or concentration effects.

CHAPTER 9

Short-term changes in the ecological status after water management actions

9.1. Introduction

Eutrophication and the associated deterioration of water quality in Mediterranean coastal wetlands are the main consequences of human activities within the ecosystems, their surroundings and catchments. They are mainly caused by increased nutrient loading through agriculture, aquaculture, industrial and domestic wastewaters discharges (de Jonge et al., 2002; Álvarez-Cobelas et al., 2005; Beklioglu et al., in press). Nowadays, anthropogenic eutrophication is already considered one of the leading forces in the structuring of European shallow lakes ecosystems (Nõges et al., 2003). To protect and enhance the ecological status of European waterbodies is the key purpose of the EU Water Framework Directive (WFD 2000/60/EC). The definition of wetland ecotypes, the determination of their reference conditions and the assessment of their ecological status are required to comply with the WFD. Ecological status should be assessed on the basis of physicochemical, hydromorphological and biological ‘quality elements’ together, but giving priority to the latter. While the concept of ‘ecological status’ really focuses on the condition of biological communities, the physicochemical and hydromorphological elements basically refer to the factors determining the 'health' of these communities (Pollard and Huxham, 1998; Elliott et al., 1999; Moss et al., 2003).

Physicochemical parameters (e.g. total phosphorus, chlorophyll-*a*, Secci depth) have been the basis for the most common indicators of trophic state in lentic ecosystems (e.g. OECD; Carlson’s Trophic State Index), which were mainly developed for northern temperate lakes (Havens, 2004 and references therein). Most of the first biological indicators were also performed for the same ecosystems, and were commonly based on zooplanktonic groups such as crustaceans or rotifers (e.g. Gannon and Stemberger, 1978; Gulati, 1983; Mäemets, 1983; Sládeček, 1983).

In Mediterranean aquatic ecosystems, it is still not well established which physicochemical and/or biological indicators should to be used for the assessment of the ecological status. In fact, the major handicap to the implementation of the WFD is probably the development of suitable biological indicators for a defined wetland ecotype (Basset et al., 2006a, b). In that sense, several attempts have recently been made in some Mediterranean waterbodies to assess the potential of several biological groups, such as zooplankters or macroinvertebrates, as indicators of ecological status (e.g.; Bianchi et al., 2003; de Eyto et al., 2003; Boix et al., 2005; García-Criado et al., 2005).

Ter Vell (NE of Iberian Peninsula) is a eutrophic freshwater coastal lagoon which has recently been subject to restoration management to improve its water quality. Nevertheless, changes in water management in agriculture altered the hydrological pattern of the lagoon and partially obstructed the restoration progress (see Chapter 8). According to the WFD, European waterbodies have to achieve ‘good’ or ‘high’ ecological status by 2015 and any water management actions, related or not to restoration, must take this into account.

The aims of this study were (1) to analyse whether such management actions have affected the ecological status of the Ter Vell lagoon and (2) to discuss the suitability of several commonly used physicochemical and/or biological indicators for the assessment of the ecological status of Mediterranean shallow water ecosystems.

9.2. Methods

9.2.1. Sampling design

To take into account the spatial variability within the Ter Vell lagoon, three basins situated differently with respect to the main freshwater flow through the ecosystem were selected to be studied (Fig. 8.1, pg. 104). One of them was located in the part of the lagoon directly receiving the freshwater inflow (NW section; hereafter Inflow) and another one was located near the drainage channel to the sea (hereafter Outflow). Freshwater flux through the lagoon takes place preferentially through a main channel, which flows in a NW to SE direction and connects these two basins (Fig. 8.1, pg. 104). The third basin studied was situated in the most confined area (NE section of the lagoon) where the effect of the freshwater inputs was smaller since the water flows toward this basin in a diffuse way (hereafter Confined).

9.2.2. Assessment of the ecological status

The Carlson's Trophic State Index (TSI; Carlson, 1977), which uses algal biomass as the basis for trophic state classification, was calculated. According to Carlson (1977), three variables are used to independently estimate algal biomass: the Secchi depth and concentrations of chlorophyll-*a* and total phosphorus. Since these variables are interrelated by linear regression models, the three TSIs should yield the same value regardless of which type of measurement is used. Given that Carlson's TSIs are based on the assumption that phosphorus is the major limiting factor for algal growth, Kratzer and Brezonik (1981) also proposed a TSI based on total nitrogen concentrations to be used under nitrogen limitation. In this situation the values of the nitrogen index should coincide with those of the chlorophyll-*a* (Kratzer and Brezonik, 1981). Values of the TSI range from approximately zero to 100 and are categorized in the classic terms of oligotrophy ($TSI < 40$), mesotrophy ($40 < TSI < 50$), eutrophy ($50 < TSI < 70$) and hypertrophy ($TSI > 70$) according to Carlson and Simpson (1996). In the present study, the TSI has been obtained from the concentrations of chlorophyll-*a* (TSI_{Chla}), total nitrogen (TSI_{TN}) and total phosphorus (TSI_{TP}). Total nitrogen and phosphorus were analysed following Grasshoff et al. (1983) and APHA (1989) and chlorophyll-*a* was determined spectrophotometrically after methanol extraction (90%) following Talling and Driver (1963).

Limnetic rotifer species indicative of eutrophy have been considered to assess the ecological status of the Ter Vell lagoon since rotifers are particularly sensitive to water quality changes and have previously been used as indicators of the trophic state (Gannon and Stemberger, 1978; Sládeček 1983; Attayde and Bozelli, 1998). This phylum is well represented in the zooplankton community of the lagoon with more than 50% of all zooplankton taxa recorded belonging to it. Furthermore, rotifers were present in all the zooplankton samples recorded. Thus, the ratio (in %) of abundance of all species indicative of eutrophy (pooled together) to total abundance of rotifers was calculated (% RSI, Rotifer Species Indicators). Taking into account quantitative data (abundances) rather than simply the presence or absence of these taxa would yield more indicator value, and it is especially recommended in warm-temperate regions where waters are more productive in nature (Gannon and Stemberger, 1978). According to previous studies, the species considered in the present study as indicators of eutrophy were *Keratella cochlearis, Polyarthra vulgaris* and several species of the genus *Brachionus* such as *B. angularis, B. bidentata, B. calyciflorus, B. quadridentatus and B. urceolaris* (Gannon and Stemberger, 1978; Gulati, 1983; Mäemets, 1983; Sládeček, 1983; Pontin and Langley, 1993; Attayde and Bozelli, 1998; Duggan et al. 2001). In addition, another percentage of the rotifer species indicative of eutrophy was calculated by including abundances of the rotifer *Brachionus plicatilis* (% RSI+) although this species is not considered an indicator of eutrophy in the aforementioned references. *B. plicatilis* is typically from brackish waters and has been related with summer hypertrophy in Mediterranean coastal wetlands (Miracle et al., 1987; Esparcia et al., 1989; Quintana et al., 1998b; Brucet et al., 2005a). The percentage of rotifers indicative of eutrophy was calculated from zooplankton samples obtained by filtering 5 L of water trough a 50-µm mesh-size net and preserved *in situ* in 4% formalin. Counting and identification were performed using an inverted microscope.

The water quality index (*QAELS*) developed by Boix et al. (2005) and based on crustacean and insect assemblages was also used in the present study. This index was obtained by the combination of (1) the *ACCO* index: the relative abundance of several indicative microcrustacean taxa (Cladocera, Copepoda and Ostracoda) weighted by an ecological quality requirement coefficient, obtained by the same authors through partial CCA; and (2) the *RIC* index: an estimator of the crustaceans and aquatic insect taxonomic richness. Prior to computing the *QAELS* and following Boix et al. (2005), the typology of the Ter Vell lagoon was taken into account since the quality coefficient for each microcrustacean taxon varies among wetland ecotypes (brackish, permanent or temporary freshwater wetlands). A categorization of the *QAELS* values was proposed by these authors to assign the water quality categories proposed by the WFD: High: $QAELS \geq 8$; Good: $6 \leq QAELS < 8$; Moderate: $4 \leq QAELS < 6$; Poor: $2 \leq QAELS < 4$ and Bad: $QAELS < 2$. The *QAELS* index was calculated from macroinvertebrate samples obtained using a 20 cm diameter dip-net (250-µm mesh-size) and preserved *in situ* in 4% formalin. One sweep per basin, consisted of 20 dip-net "pushes" in rapid sequence, was carried out in order to cover all the different habitats in the lagoon. Counting and identification were performed under a stereomicroscope.

Additionally, several microcrustacean ratios already used as indicators of trophic status were calculated to obtain complementary information about the ecological status of the Ter Vell lagoon: (1) the ratio of abundances of calanoid copepods to abundances of cyclopoid copepods and cladocerans (Cal:Cyc+Cla; Gannon and Stemberger, 1978), (2) the ratio of abundances of large species of cladocerans (in the present study only *Daphnia* and *Simocephalus*) to total abundances of cladocerans (LargeCla:TotalCla; Moss et al., 2003) and (3) the ratio of the abundance of the cladocer *Chydorus sphaericus* to the total abundance of all chydorid species (CHSPH:TotalChy; de Eyto et al., 2003). Nevertheless, it is worth mentioning that a problem emerged when calculating these ratios, especially the most specific ones (2 and 3) since the absence of chydorids or even the total absence of cladocerans impeded obtaining a value for the ratios for a large fraction of the samples. These ratios were calculated from the macroinvertebrate samples (dip net, 250-µm mesh-size).

For each zooplankton sample (50-µm mesh-size), the Shannon-Wiener diversity index (H), the taxonomic richness (R), the evenness (E) and the Berger-Parker dominance index (D) were also calculated taking into account only ciliates, rotifers and microcrustaceans (cladocera, copepoda and ostracoda). These descriptors of the community have also been used in environmental monitoring to assess changes in water quality (Attayde and Bozelli, 1998; Bianchi et al., 2003; de Eyto et al., 2003; Fano et al., 2003; García-Criado et al., 2005).

The assessment of the ecological status of the Ter Vell lagoon was performed before and after the water management actions to analyse their effects. In the three selected basins, water, zooplankton and macroinvertebrate samples were taken monthly from November to June during three hydrological cycles: one before the water management actions (99/00) and the other two after them (02/03 and 03/04). As suggested by Boix et al. (2005), the most extreme confinement periods (e.g. July to September-October) were excluded for the *QAELS* index computation, and sampling campaigns on days following intense flooding events were also avoided.

9.2.3. Statistical analyses

Two-way ANOVA analyses were performed to test for significant temporal and spatial differences in the different measures of ecological status: (1) the TSI indexes, (2) the % of rotifer species indicative of eutrophy with (% RSI+) and without (% RSI) abundances of *B. plicatilis* and (3) the *QAELS* index. The factors considered were hydrological cycle (99/00, 02/03 and 03/04) and basin (Inflow, Outflow and Confined). Post-hoc comparisons were performed using the Games-Howell tests at the 0.05 significance level in order to analyse differences between hydrological cycles and basins. Games-Howell tests are among the most powerful and the most robust to unequal variances of post-hoc multiple comparison methods (Day and Quinn, 1989). A correlation analysis was also performed on the overall values of the indicators of ecological status. In order to improve the linearity as well as the normality and homogeneity of variances, the percentage of the rotifer species indicative of eutrophy and the microcrustacean ratios have been arcsin transformed, with the exception of the ratio Cal:Cyc+Cla, which has been fourth-root transformed. Calculations and statistical analyses were performed with SPSS 13.0.

9.3. Results

The average values of the TSI_{TP} classified the basins of the Ter Vell lagoon as ‘hypereutrophic’ in the three hydrological cycles, except for the Confined basin in the 99/00 cycle, which it was classified as ‘eutrophic’ (Fig. 9.1). Significant differences were found between the hydrological cycles; although in the post-hoc comparisons statistical significance was marginal, in the 02/03 cycle the average value of the TSI_{TP} was significantly higher than in the 99/00 (Games-Howell tests, $p = 0.060$) and 03/04 (Games-Howell tests, $p = 0.068$) cycles. Significant differences were also found between basins. The average value of the TSI_{TP} in the basin most affected by the freshwater inputs (Inflow) was higher than in the Confined one (Table 9.1 and Fig. 9.1). Over the three hydrological cycles, the Outflow basin always showed intermediate values between those of the other two basins. However, according to the average value of the TSI_{TN}, the three basins were each classified as ‘eutrophic’ in the three hydrological cycles, except for the Inflow basin in the 99/00 cycle when it was considered ‘hypereutrophic’. The average value of the TSI_{TN} before the hydrological change, when freshwater inputs were greater and more prolonged (i.e. 99/00 cycle), was significantly higher than after the change (02/03 and 03/04 cycles). In the Inflow basin the average value of the TSI_{TN} was significantly higher than in the Confined one (Table 9.1 and Fig. 9.1). For the TSI_{Chla}, the average value classified all basins as ‘eutrophic’ in all three hydrological cycles with no significant differences found between any of the basins or cycles (Table 9.1). The TSI_{Chla} and TSI_{TN} indexes coincided in their classification of the trophic status but differences between them were more marked before the hydrological change (99/00 cycle). The decrease in the TSI_{TN} after the hydrological change (02/03 and 03/04 cycles) reduced the differences and their values tended to be similar to those of the TSI_{Chla}. In all cycles TSI_{TP} values were markedly higher than the other two indexes, especially after the hydrological change (02/03 and 03/04 cycles).

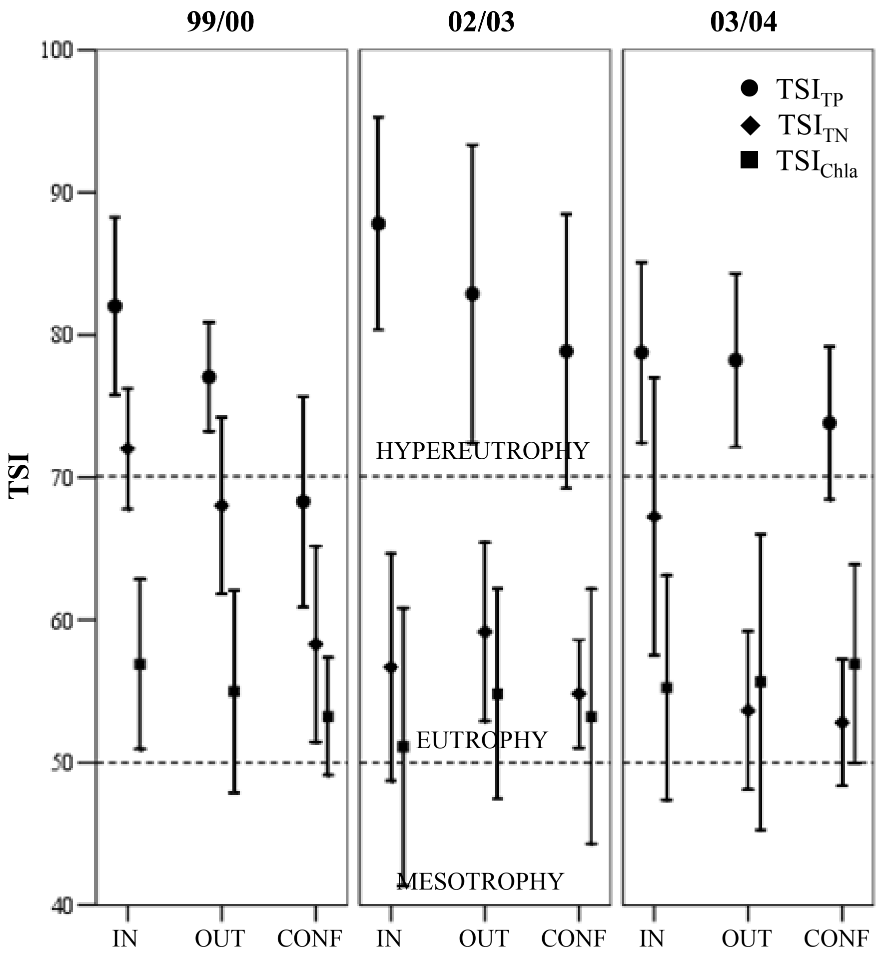

Figure 9.1. Mean values of TSI_{PT}, TSI_{NT} and TSI_{Chla} in the studied basins for each hydrological cycle. Error bars plot the 95% confidence intervals of the means. Abbreviations for the basins are: IN, Inflow basin; OUT, Outflow basin and CONF, Confined basin.

The two calculated percentages of rotifer species indicative of eutrophy, excluding (% RSI) and including *Brachionus plicatilis* (% RSI+), showed significant differences between cycles but not between basins (Table 9.1). In the 99/00 cycle, when freshwater inputs were greater and more prolonged, the average values were significantly higher than in the 03/04 cycle. In the 02/03 cycle, the averages values of both percentages were intermediate (Fig. 9.2). The deviation of the % RSI+ from the % RSI found in the basins located far from the freshwater inputs and, therefore, more confined (Confined basin and Outflow) was due to an increase in the proportional abundance of *B. plicatilis*.

The average value of the *QAELS* index in the 99/00 cycle was significantly lower than in the 03/04 cycle. In the 02/03 cycle values of the index were intermediate between those of the other two cycles (Table 9.1 and Fig. 9.3). In those basins most affected by the freshwater inputs (Inflow and Outflow) the average value of the index was significantly lower than in the most confined basin (Confined). Within each hydrological cycle, the Inflow basin always showed the lowest average value and the Confined one always showed the highest one. According to the average value of the index, water quality status before the hydrological change (99/00 cycle) was 'moderate' in those basins most affected by the freshwater inflow (Inflow and Outflow) and 'good' in the Confined basin.

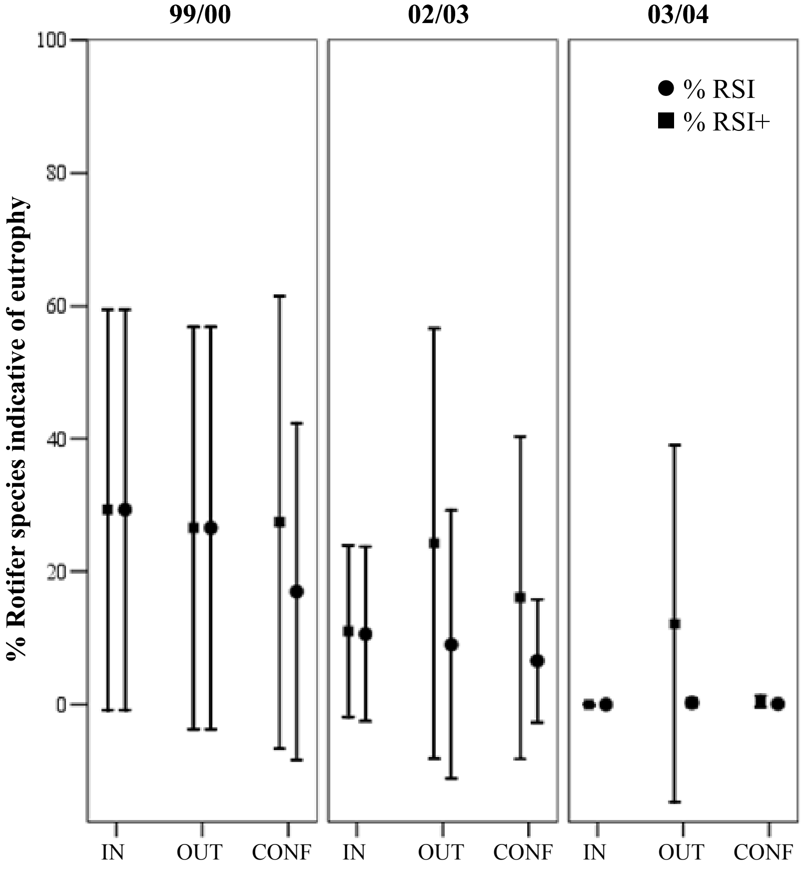

Figure 9.2. Mean values of the percentage of rotifer species indicative of eutrophy, with (% RSI+) and without (% RSI) abundances of *B. plicatilis*, in the studied basins for each hydrological cycle. Error bars plot the 95% confidence intervals of the means. Abbreviations for the basins are given in the Fig. 9.1 caption.

Table 9.1

Two-way ANOVAs testing for significant differences between basins and cycles in (1) the TSIs, (2) the % RSI and % RSI+ (including *Brachionus plicatilis*) and (3) the *QAELS* index. Abbreviations for the basins are: I, Inflow basin; O, Outflow basin and C, Confined basin, and for cycles: 1, 99/00 cycle; 2, 02/03 cycle and 3, 03/04 cycle. Significant results obtained in the post-hoc comparisons (Games-Howell test at the 0.05 significance level) are shown.

	TSI_{TP}	TSI_{TN}	TSI_{Chla}	% RSI+	% RSI	*QAELS*
Basin	$F_{2,63} = 6.60$	$F_{2,63} = 9.30$	$F_{2,60} = 0.05$	$F_{2,63} = 0.375$	$F_{2,63} = 0.428$	$F_{2,61} = 9.20$
	$p = 0.002$	$p < 0.001$	$p = 0.955$	$p = 0.689$	$p = 0.654$	$p < 0.001$
Post-hoc comparisons	I ≠ C	I ≠ C				I ≠ C; O ≠ C
Cycle	$F_{2,63} = 4.64$	$F_{2,63} = 13.85$	$F_{2,60} = 0.60$	$F_{2,63} = 5.00$	$F_{2,63} = 9.44$	$F_{2,61} = 3.79$
	$p = 0.013$	$p < 0.001$	$p = 0.550$	$p = 0.010$	$p < 0.001$	$p = 0.028$
Post-hoc comparisons		1 ≠ 2; 1 ≠ 3		1 ≠ 3	1 ≠ 3; 2 ≠ 3	1 ≠ 3
Basin × Cycle	$F_{4,63} = 0.476$	$F_{4,63} = 2.83$	$F_{4,60} = 0.30$	$F_{4,63} = 0.257$	$F_{4,63} = 0.231$	$F_{4,61} = 0.867$
	$p = 0.753$	$p = 0.032$	$p = 0.874$	$p = 0.904$	$p = 0.920$	$p = 489$

After the hydrological change, the average value of the index increased in all basins achieving ‘good’ water quality in the last studied cycle (03/04), where values of the index reduced their interannual variability (Fig. 9.4). Throughout the hydrological cycles, values of this index fluctuated widely but a general trend of increasing values from autumn to summer was observed (Fig. 9.4). Nevertheless, in the basins located far from the freshwater inputs (Confined and Outflow), values of the index decreased toward the summer season (e.g. June of 02/03 and 03/04 in Confined and Outflow, respectively; Fig. 9.4).

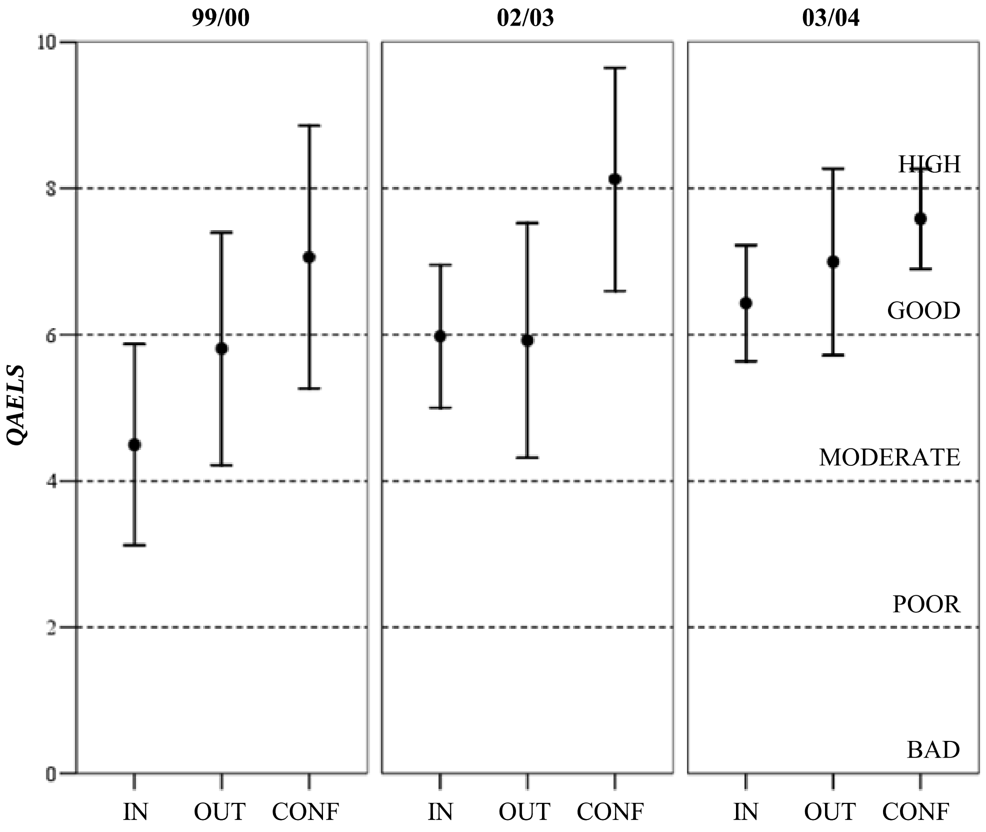

Figure 9.3. Mean of the *QAELS* values in the sampling basins for each hydrological cycle. Error bars plot the 95% confidence intervals of the means. Abbreviations for the basins are given in the Fig. 9.1 caption.

Significant results obtained in the correlation analysis are shown in Table 9.2. A decrease in the TSI_{TN} (after the hydrological change) coincided with an increase in the water quality index *QAELS* and in the zooplankton dominance (*D*) and with a decrease in the zooplankton diversity (*H*) and evenness (*E*). Similarly, the decrease in the abundance of the rotifer species indicative of eutrophy (% RSI, without *B. plicatilis*) after the hydrological change was also coincident with an increase in zooplankton dominance and with a decrease in diversity, richness and evenness. The decrease in the % RSI was also related with an increase in the proportional abundance of the large cladoceran species (high values of the ratio LargeCla:TotalCla). These relationships changed when the abundance of *B. plicatilis* was taken into account and the % RSI+ was positively related only with the TSI_{TP}. While the increase in the TSI_{TP} after the hydrological change was also related with a decrease in the proportional abundance of the cladoceran indicative of eutrophy, *Chydorus sphaericus* (low values of the ratio CHSPH:TotalChy), it was related, on the other hand, with a decrease in the proportional abundance of the copepod group most related with oligotrophy, the calanoids (low values of the ratio Cal:Cyc+Cla).

Finally, the increase in the water quality index *QAELS* after the hydrological change coincided with the increased in the proportional abundance of calanoids (high values of the ratio Cal:Cyc+Cla) and also of the large cladoceran species (high values of the ratio LargeCla:TotalCla), both groups more abundant in oligotrophic conditions. The TSI_{Chla} was not significantly related with any of the other indicators used (Table 9.2).

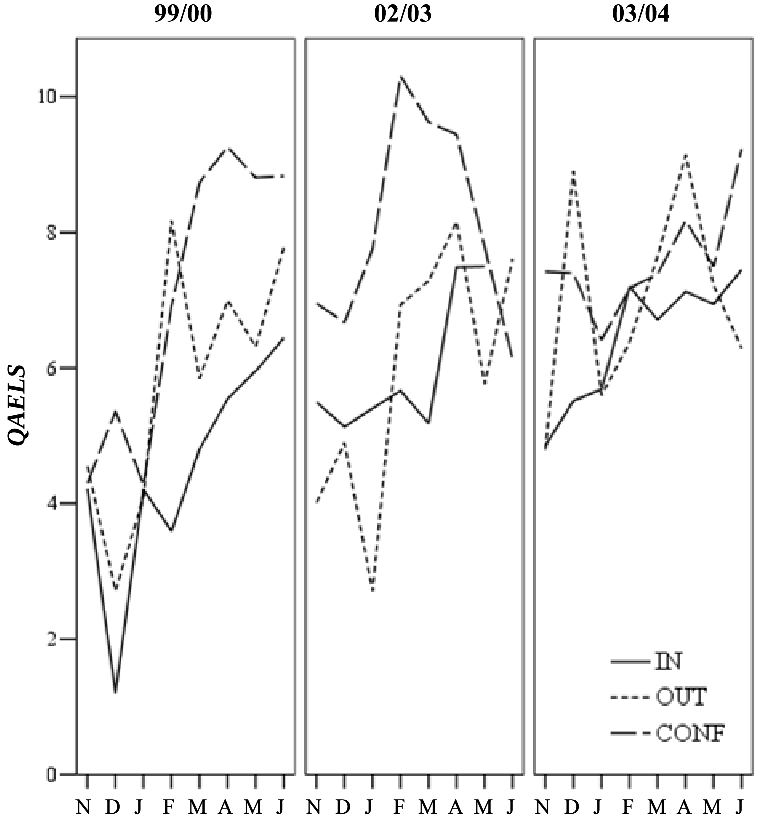

Figure 9.4. Temporal evolution of the monthly values of the *QAELS* index throughout the hydrological cycle in the studied basins. Abbreviations for the basins are given in the Fig. 9.1 caption.

Table 9.2

Significant Pearson correlations between the indicators of the ecological status (Acronyms and abbreviations are given in the text, section 9.2.2). Significant results are indicated as $^{*}p < 0.05$, $^{**}\ p < 0.01$.

	TSI_{TP}	TSI_{TN}	*QAELS*	% RSI+	% RSI
TSI_{PT}	1				
TSI_{NT}		1			
QAELS		- 0.386**	1		
% RSI+	0.321**			1	
% RSI				0.801**	1
H		0.301*			0.326**
R					0.305*
E		0.310**			0.239**
D		- 0.234*			- 0.262**
Cal: Cyc+Cla	- 0.276*		0.293*		
CHSPH:TotalChy	- 0.479*				
LargeCla:TotalCla			0.551**		- 0.394*

9.4. Discussion

After the change in the hydrological regime when the freshwater inflow and, therefore, the nutrient inputs (mainly nitrogen) were drastically reduced, a general improvement of the ecological status of the Ter Vell lagoon was observed, especially in those basins most affected by the freshwater inputs. This was revealed by the significant decrease in the TSI_{TN} and in the percentage of rotifers indicative of eutrophy, and by an increase in the *QAELS* index. According to the latter, the water quality in the Ter Vell lagoon achieved 'good status' in all basins after the hydrological change. Within the Ter Vell lagoon, spatial differences in ecological status corresponded to the location of each basin with respect to the preferential freshwater flow through the ecosystem. In general, the basin directly receiving the freshwater inputs showed the worst ecological status whereas the basin located in the most confined area showed the best one. Nevertheless, some specific results as well as the relationships found between the overall indicators raised doubts about the suitability of some of the indicators used.

After the hydrological change, the increase in the TSI_{TP} and the decrease in the TSI_{TN} are compatible with an increase in the P content due to internal loading (e.g. sediment release) and with a decrease in the N content due to the reduction of the freshwater inputs. The TSI_{TN} tended to coincide with the TSI_{Chla} indicating situations of N limitation (Kratzer and Brezonik, 1981; Carlson and Simpson, 1996). In fact, Mediterranean coastal wetlands with scarce water inputs and low water turnover, like Ter Vell after the hydrological change, are naturally P-enriched and N-limited. A differential confinement of nutrients takes place in these ecosystems, and whereas phosphorus tends to accumulate progressively in the sediment, nitrogen diminishes mainly due to denitrification (Gomez et al., 1998; Quintana et al., 1998a). Thus, primary producers are generally stimulated by the nitrogen entries suggesting a key role of N in the eutrophication process. In these P-enriched ecosystems the TSI_{TP} could consistently overestimate the lagoon's trophic state compared to the TSI_{TN} (Hilbright-Ilkowska et al., 1984; Goldyn et al., 2003; Hoyer et al., 2005). Nevertheless, using only the TSI_{TN} could also lead to erroneous results, since the alternation of N and P limitation has sometimes been reported in coastal wetlands (Comín and Valiela, 1993). For this reason, Carlson and Simpson (1996) recommended the simultaneous use of all indexes in order to analyse the deviations among them, which will truly indicate the functioning of the ecosystem. Thus, the deviations of the TSI_{TP} and TSI_{TN} from the TSI_{Chla} before the hydrological change might indicate that algal production in the Ter Vell lagoon was limited by some other factors. Light limitation by turbidity due to a mixing regime (e.g.wind-induced sediment resuspension, flushing), zooplankton grazing and the presence of mixotrophic phytoplankton, typical in organic-enriched ecosystems, may cause the chlorophyll-*a* to fall below that expected from the nutrient concentrations (Carlson and Simpson, 1996; Isacksson, 1998; Mazumder and Havens, 1998; Quintana and Moreno-Amich, 2002; Moss et al., 2003).

The significant decrease in the percentage of rotifer species indicative of eutrophy after the reduction in the freshwater inflow and, hence, in nitrogen inputs would support the use of this measure as an indicator of the ecological status in the Ter Vell lagoon. Furthermore, its decrease was coincident with an increase in the large species of cladocerans, which are more abundant at high ecological status (Moss et al., 2003). However, the increase of the proportional abundance of the species *B. plicatilis* after the hydrological change would likely suggest contradictory results. Actually, the increment of this species was related with the fact that salinity and the organic content of the Ter Vell lagoon increased after the drastic reduction in the freshwater inputs. In confined Mediterranean ecosystems, this species has been related with natural hypertrophy episodes that are very usual during the confinement periods in summer when salinity and the organic content reach high levels due to a concentration effect (Quintana et al., 1998b, Brucet et al., 2005; see Chapter 5).

The relationships found among some indicators of ecological status indicated that zooplankton diversity, richness and evenness increased when the ecological status of the Ter Vell lagoon worsened, while zooplankton dominance increased when it improved. These results do not agree with the general assumption that in polluted or enriched environments species diversity and richness decrease while dominance increases (Magurran, 1988; de Eyto et al., 2003; Pinto-Coelho et al., 2005), and they are conditioned by the natural impoverishment of the aquatic fauna in confined Mediterranean coastal wetlands (Guelorget and Perthuisot, 1983). In these kinds of ecosystems with scarce freshwater inputs and prolonged confinement periods, like Ter Vell after the hydrological change, low diversity and the dominance of a few species are characteristic of the natural organization of the zooplankton community (Quintana et al., 1998b; Brucet et al., 2005a; Toumi et al., 2005). In fact, species diversity increases after a hydrological perturbation and reaches its minimum during hydrologically stable periods when oligotrophic conditions prevail and the community is usually dominated by one single calanoid species (Brucet et al., 2006). The proportional abundance of calanoid copepods, commonly related with oligotrophic conditions (Gannon and Stemberger, 1978; Pinto-Coelho et al., 2005), tended to increase in the Ter Vell lagoon after the hydrological change coinciding with an increase in the *QAELS* index.

Thus it is suggested that a correct assessment of the ecological status requires a previous deep knowledge of the ecosystem structure and functioning, as well as of the organization and composition of aquatic communities. In this way, a misunderstanding of the results will be avoided, especially when using common indicators developed for another type of ecosystem, such as the TSIs (northern temperate region), or when the indicators show different behaviour depending on the wetlands ecotype (e.g. indexes of zooplankton community structure). The *QAELS* index is the only ecotype-specific biological indicator used in the present study because a prior classification of the wetlands typology is required to obtain the index (Boix et al., 2005). This is because the sensitivity to water quality at the species level (quality coefficient of each taxon) is different in each kind of wetland ecotype and, as suggested by other authors (Attayde and Bozelli, 1998; Basset et al., 2006a, b; Tagliapietra and Volpi Ghirardini, 2006), it has been taken into account. In the Ter Vell lagoon, the *QAELS* index seemed to be sensitive to changes in ecological status. An increase in the index after the hydrological change coincided with a decrease in the TSI_{TN} indicating an improvement of the water quality when freshwater inputs and, hence, nitrogen inputs were reduced. In turn, the increase in the *QAELS* index coincided with the increase in the proportional abundance of the large cladocerans and of the calanoid copepods, both groups more abundant in oligotrophic conditions (Gannon and Stemberger 1978; Moss et al., 2003). Nevertheless, the correct use of the *QAELS* index is nowadays only guaranteed on a regional scale, in the lentic shallow waters in Catalonia (NE Iberian Peninsula), where this index has been developed. Therefore, additional studies and intercalibration procedures in other areas are called for in order to correctly define the ecological status categories and to generalize the application of this index to other Mediterranean shallow waters.

SECTION IV

General Discussion and Conclusions

CHAPTER 10
GENERAL DISCUSSION

10.1. Hydrological functioning of Mediterranean coastal wetlands

The functioning of Mediterranean coastal wetlands is mainly determined by the confinement. Guelorguet and Perthuisot (1983) introduced this term in relation to the low hydrodynamic energy that governs these coastal ecosystems due to the lack of tidal fluxes, the altered river dynamics and the restricted connections to the sea. Thus, the degree of confinement of an ecosystem, reflecting its water turnover, basically depends on connections to the sea and continental inputs.

Modifications of the degree of confinement due to alterations in the hydrological regime are one of the main impacts of human activities on Mediterranean coastal wetlands. An extremely low or high degree of confinement involves two opposite situations in the hydrological functioning of coastal wetlands. In the present study, La Pletera salt marshes and the Ter Vell lagoon were illustrative of two such situations. In La Pletera salt marshes, an artificial impoundment (1970s) resulting from physical modifications (canalisation, levee construction, partial urbanization, etc.) isolated the marshland from the river and sea, increasing the degree of confinement of the salt marsh waterbodies. As in other Mediterranean salt marshes (e.g. Aiguamolls de l'Alt Empordà), the hydrology has been characterized by prolonged periods without water inputs (winter anticyclone and summer drought) irregularly interrupted by punctual, climate-dependent flooding events (Quintana et al., 1998a; Gascón et al. 2005). Therefore, water turnover is low and the degree of confinement is high. Since precipitation events are very scarce in the Mediterranean climate and, moreover, there is a lack of a continuous freshwater inflow, marine influence in these confined ecosystems is particularly high because surface marine intrusion during sea storms is, in terms of volume, the main water input. On the other hand, the Ter Vell lagoon showed a low degree of confinement since the hydrology, before the management actions, was characterized by high water turnover and low residence time. The freshwater flooding period had been artificially prolonged due to agricultural activities and, as a consequence, freshwater influence was more significant than the marine influence and variations in the hydrological pattern became artificial and more predictable and controlled than natural, climate-dependent fluctuations. Water regime manipulation for agriculture has been especially observed in those coastal ecosystems of riverine origin that are less confined in nature (e.g. Albufera de Valencia; L'Encanyissada lagoon in the Ebro Delta; The Vaccarès lagoon in the Rhone Delta) and whose functioning is determined by continuous freshwater inputs, at least during a certain period of time (Vicente and Miracle, 1992; Comín and Valiela, 1993; Chauvelon, 1998).

10.2. Nutrient dynamics in Mediterranean coastal wetlands

Mediterranean coastal wetlands have generally been considered as net importers of nutrients and organic matter due to their low hydrodynamic energy and the long residence time (Ibáñez et al., 2000; Cloern 2001). Confined and poorly flushed ecosystems, like La Pletera salt marshes, show endorheic behaviour and the retention mechanisms prevail in front of the exportation mechanisms, which become more relevant in highly flooded ecosystems with exorheic behaviour and low residence time, like the Ter Vell lagoon. There is a concentration effect of the salts, nutrients (mainly in organic form) and organisms, especially during the confinement and, therefore, a progressive accumulation occurs along successive confinement events over the years, as has been observed in La Pletera. Nevertheless, total nutrient concentrations in the extremely confined old lagoons of these salt marshes were similar to those found in the Ter Vell lagoon but, whereas nutrients were mainly in organic form in the former ecosystems, inorganic compounds dominated in the latter. In fact, in those coastal lagoons where hydrology has been greatly altered for agricultural purposes, high levels of inorganic nutrient supplies (mostly as oxidized nitrogen compounds) have commonly been observed (Chapelle et al., 2000; Lucena et al., 2002; Pérez-Ruzafa et al., 2005). In confined ecosystems like La Pletera salt marshes, inorganic nitrogen inputs were scarce and nitrogen concentration diminished rapidly, mainly due to denitrification (Comín and Valiela, 1993; Quintana et al. 1998a, Frascari et al.

2002). Conversely, phosphorus tends to accumulate progressively in the sediment and its dynamics depends more on the internal recycling processes (e.g. release from sediment, mineralisation of organic matter, etc.; Gomez et al., 1998; Quintana et al., 1998a; Frascari et al., 2002) than on the hydrology. For this reason this kind of ecosystems is considered naturally nitrogen-limited but phosphorus- and organic-enriched (Ibáñez et al. 2000, de Jonge et al. 2002). In spite of the accumulation of nutrients, La Pletera salt marshes would be less productive and, therefore, less eutrophic than the Ter Vell lagoon since nutrients in the salt marshes appear mainly in organic form and are less available to primary producers. In that sense, Margalef (1980) defined eutrophy as "the capacity of production", giving more importance to ecosystem productivity than to nutrient concentrations, contrasting with common definitions of eutrophy (Lampert and Sommer, 1997; Lincoln et al., 1998; Wetzel, 2001)

10.3. Zooplankton community in Mediterranean coastal wetlands

The degree of confinement, and the subsequent effect on salinity and nutrients, is decisive in the structure of invertebrate aquatic communities, since a natural impoverishment of the fauna is usually observed in the most confined ecosystems (Guelorget and Perthuisot, 1983). In the present study, this impoverishment was especially evident in the extremely confined old lagoons of La Pletera where zooplankton diversity and richness were lower than in either the new lagoons or, also, the Ter Vell lagoon. Nevertheless, in the Ter Vell lagoon, a tendency towards impoverishment of the zooplankton community was observed when, after the hydrological change, nutrient inputs were reduced and the ecological status improved. Thus, low diversities and high dominances appeared to be related to more oligotrophic conditions. Conversely, when freshwater and nutrient inputs were prolonged and conditions were more eutrophic (before the hydrological change), zooplankton diversity and richness tended to be higher. Then, the community was mainly formed by small bodied zooplankters with short generation times, such as ciliates, rotifers and small cladocerans, which would probably be the first to respond to hydrological and nutrient perturbations (Romo et al., 2004; Buyukates and

Roelke, 2005). As in other Mediterranean coastal wetlands subjected to agriculture activities (e.g. Menéndez and Comín, 1986; Miracle et al., 1987; Ramdani et al., 2001), the community was dominated by several rotifer and cladoceran species indicative of eutrophy (Gannon and Stemberger, 1978; Miracle et al., 1987). On the other hand, the zooplankton community in confined ecosystems not subject to human regulation, such as in La Pletera salt marshes, was characterized by the prolonged dominances of few euryhaline species of larger zooplankters, mainly harpacticoid and calanoid copepods (Quintana et al., 1998b). Taxonomic diversity only increases after flooding disturbances and the subsequent fertilization, and then progressively decreases throughout the confinement period when conditions are more oligotrophic (Brucet et al., 2005a, 2006).

Zooplankton structure in Mediterranean coastal wetlands has traditionally been analysed using only taxon-based approaches (e.g. Menéndez and Comín 1986; Pretus 1989; Oltra and Miracle, 1992) but the combined use of taxon- and size-based approaches in the present study has provided complementary information and a better understanding of zooplankton structure. In La Pletera salt marshes taxonomic and size diversity measures showed opposite responses to several community structuring factors: whereas the size diversity was mainly related to biotic interactions (fish predation or competition for food), the taxonomic diversity appears to be more sensitive to abiotic factors (nutrient variability). In similar confined salt marsh ecosystems both diversity measures showed opposite temporal patterns throughout the hydrological period (Brucet et al., 2006).

After flooding and nutrient perturbations, an increase in zooplankton food resource availability reduces the interspecific competition favouring the establishment and coexistence of a larger number of species (Giller, 1984), which leads to a high taxonomic diversity. Nevertheless, size diversity is low since similar sizes are found among the present species. During the subsequent periods of confinement and food scarcity, interspecific interactions lead to competitive exclusion and, while taxonomic diversity progressively decreases, the size diversity increases (Brucet et al., 2006). Dominances of a single, large-bodied calanoid species are usually observed in situations of competitive exclusion (Brucet et al., 2005a), where taxonomic diversity is low but size diversity reaches high values. Such community structure based on the organism's body size responds to a food resource partitioning among co-occurrent development stages of the calanoid species (Poulet, 1977; Brucet et al., 2005b; Brucet et al., submitted), allowing stable populations to be maintained over time (Werner and Gilliam, 1984; Brucet et al., 2005a). In La Pletera salt marshes, the role of competitive interactions for food in zooplankton structure becomes less relevant under high fish predation pressure. The Iberian toothcarp (*Aphanius iberus*) played a key role in structuring the zooplankton community since decrease in the size diversity was closely related with an increase in the fish densities.

10.4. Ecological functioning of the Baix Ter Wetlands in relation to the confinement

A confinement gradient is established within all waterbodies of the Baix Ter Wetlands when ordering them according to their salinity distribution (Fig. 10.1), which reflects the degree of freshwater influence. In Fig. 10.1, not only the waterbodies of La Pletera salt marshes and the Ter Vell basins are included, but also another freshwater lagoon found in the Baix Ter Wetlands, Basses d'en Coll lagoon, not subjected to the Life project. This lagoon shows an artificial hydrology with a prolonged freshwater flooding period due to the rice crops irrigation (Badosa et al., 2006). Its hydrology is similar to that of the Ter Vell lagoon before the water management actions. The old lagoons of La Pletera salt marshes, which exhibit the highest salinity fluctuation and the highest salinity values, are the most confined ecosystems due to the low freshwater inputs. Conversely, the well-flooded and, therefore, the less confined ecosystems such as Basses d'en Coll and the Inflow basin of the Ter Vell are located at the opposite end of the salinity gradient. Salinity values, and also their variability, decrease in these waterbodies. Along the established gradient of confinement, concentration of organic matter tends to increase while the dissolved inorganic nitrogen tends to decrease. Thus, an increased degree of confinement is related with an organic enrichment and an impoverishment of inorganic nitrogen. Nevertheless, total nitrogen increases in both ends of the gradient and similar concentrations are achieved in both the most and the less confined ecosystems. Thus, in the former ecosystems nitrogen appears mainly in organic form and it is related with accumulation processes over the years (e.g. the old saltmarsh lagoons), whereas in the latter ecosystems it appears mainly in inorganic form and is related with the greater freshwater inputs (e.g. Inflow basin of Ter Vell lagoon, Basses d'en Coll lagoon). On the other hand, phosphorus concentrations for each waterbody do not appear ordered along the confinement gradient. Concentrations are higher in the most confined waterbodies, where phosphorus tends to be trapped in the sediment. The impoverishment of inorganic nitrogen and enrichment of phosphorus in confined Mediterranean salt marshes is known as differential confinement of nutrients (Quintana et al., 1998a).

According to Guelorget and Perthuisot (1983), the confinement can affect the taxonomic and size structure of the aquatic communities by a reduction in the species richness and diversity and in the organisms' body size. In the Baix Ter Wetlands, a decrease in zooplankton species richness and diversity has been observed when increasing the degree of confinement but patterns of the size structure along the confinement gradient have not still been analysed. It would be probably expected a decrease in the zooplankton size diversity in the less confined and well-flushed ecosystems since flooding and nutrient disturbances lead to an increase in the food resource availability and to a decrease in the zooplankton size diversity. In fact, in the Ter Vell lagoon when water inputs were greater and prolonged, the zooplankton community was dominated by small species with short generation times, all them of similar sizes. This fact would not agree with Guelorget and Perthuisot (1983) which assumed a decrease in the organisms' size but in the confinement situations. Nevertheless, the confinement should not to be considered as the unique determinant community structuring factor, since biotic factors such as competition and predation are also very important (Pérez-Ruzafa and Marcos, 1993; Brucet et al, 2006). In La Pletera salt marshes the zooplankton size structure is mainly determined by biotic factors (e.g. competition and predation) while taxonomic structure appears to be more sensitive to abiotic factors (nutrient variability).

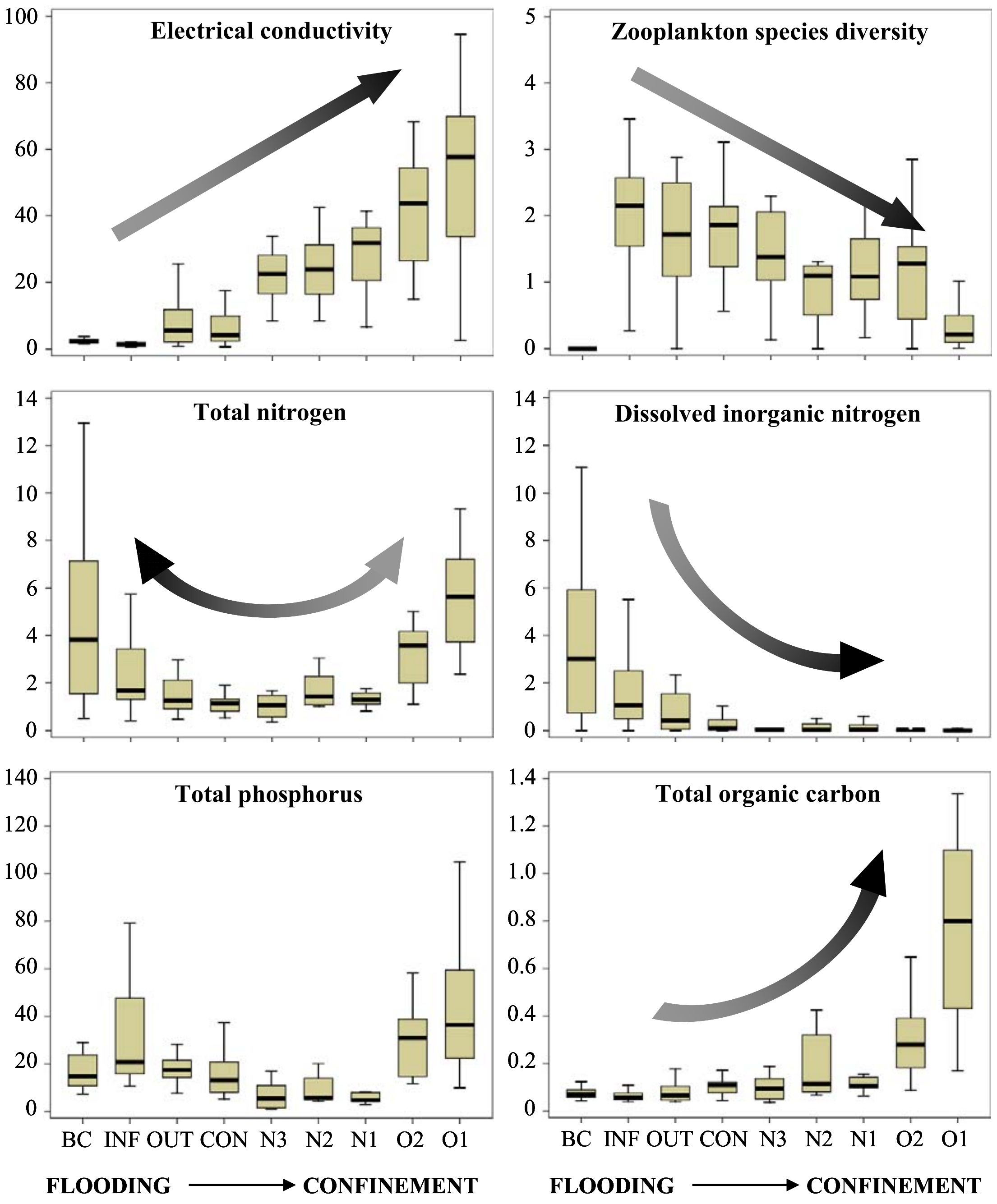

Figure 10.1. Box plots with median and percentiles (10-90%) for several variables in the waterbodies of the Baix Ter Wetlands. The abbreviations are for Basses d'en Coll lagoon: BC; for the Ter Vell lagoon: IN, Inflow basin; OUT; Outflow basin; CONF, Confined basin; and for La Pletera salt marshes: O1, O2, the old lagoons; N1, N2, N3, the new lagoons.

As a synthesis of the ideas and concepts discussed in the above sections, a description of the main characteristics of the ecological functioning of the Baix Ter Wetlands is shown in the following Table.

LA PLETERA SALT MARSHES
Confined Ecosystems

a) Occasional climate-dependent flooding events (rainfall, sea storms)
b) Absence of surface outlets/outflow (evaporation and/or infiltration)
c) Low water turnover (~ months)*
d) Endorheic character (concentration effects of salts, nutrients, organisms, etc.)
e) Internal loading and accumulation effects are more relevant
f) High salinity fluctuation
g) High salinity values, exceeding even that of the seawater
h) Inorganic compounds are scarce: they are rapidly consumed by the primary producers. Nutrients mainly in organic form. Differential confinement of nutrients: phosphorus accumulates progressively in the sedimend and nitrogen rapidly diminishes mainly due to denitrification
i) Heterotrophic metabolism usually dominates (production ≈ respiration)
j) Reduction in the zooplankton specific richness and diversity; prolonged dominances of few species

TER VELL LAGOON, LES BASSES D'EN COLL LAGOON
Flooded Ecosystems

a) Freshwater inputs prolonged in time (riverine inputs, irrigation water)
b) Active surface outflows
c) High water turnover (~ days)*
d) Exorheic character (exportation mechanisms prevail)
e) External loading of nutrients are greater (always related with water inputs)
f) Low salinity fluctuation
g) Low salinity values
h) Inorganic compounds are dominant (mostly as oxidized nitrogen compounds)
i) Autotrophic metabolism usually dominates (production > respiration)
j) Increase in the zooplankton specific richness and diversity

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* Water turnover is highly dependent of the lagoons' volume

10.5. Environmental problematic of the Baix Ter Wetlands in relation to the confinement

In the Baix Ter Wetlands, the degree of confinement has also been related to the presence of invasive species and, in turn, to the survival of the autochthonous ones. This is the case of the endangered cyprinodont Iberian toothcarp (*Aphanius iberus*) and one of its main competitors, the invasive mosquitofish (*Gambusia holbrooki*). Hydrological alterations may increase the ecosystem's susceptibility to invasions by non-native species (Saunders et al., 2002) and in the Baix Ter Wetlands mosquitofish abundance is, in fact, higher in the freshwater ecosystems. The high degree of confinement of La Pletera salt marshes has impeded the arrival of mosquitofish and allowed the survival of the Iberian toothcarp. Nevertheless, such extreme confinement has become, in turn, a threat for its long-term survival. The artificial impoundment imposed in these salt marshes (1970s) involved the loss of this species' habitat and restricted its presence to only one lagoon, increasing its vulnerability to any disturbance. Creating and stocking new refuges (permanent brackish coastal lagoons) were the main restoration actions carried out in La Pletera, where the conservation of this target species had become the main environmental problematic. The degree of confinement of the new refuges should be sufficiently high to reduce the possibility of mosquitofish arrival and to guarantee suitable environmental conditions but, in turn, it should be sufficiently low to allow communication between new refuges and the surrounding marshland during the flooding periods. High abundances of Iberian toothcarp in the new population two years after the first stocking of the new refuges would suggest that the degree of confinement in the new refuges was sufficient, at least in the short-term.

In the Ter Vell lagoon, environmental problems were related to its low degree of confinement, derived from an artificially prolonged freshwater inflow and the subsequent nutrient inputs. After the hydrological change, an improvement of the ecological status was related to an increase in the degree of confinement. A decrease in the Trophic State Index based on total nitrogen (Kratzer and Brezonik, 1981) suggested a lower trophic state and a nitrogen limitation after the hydrological change. Nevertheless, if the index based on total phosphorus was taken into account, an overestimation of the Ter Vell's trophic state would be obtained. When increasing the ecosystem's degree of confinement, the phosphorus content tends to increase due to the enhanced accumulation processes and internal recycling (Quintana et al., 1998a). The Trophic State Index based on phosphorus was developed for ecosystems in northern temperate regions where confinement does not govern hydrological functioning, phosphorus is mainly in inorganic form and algal biomass is phosphorus-limited (Carlson 1977). Thus, a previous deep knowledge of the natural functioning of an ecosystem is absolutely necessary when evaluating its ecological status. Obviously, it should also be considered when using biological indicators. In the Ter Vell lagoon, an improved ecological status and a higher degree of confinement after the hydrological change lead to low diversities and high dominances in the zooplankton community. These results do not agree with the general assumption that low diversities and richness are characteristic of the more eutrophic conditions (e.g. Magurran, 1998; de Eyto et al., 2003; Pinto-Coelho et al., 2005) and suggest that a deep knowledge of the natural structure of the community is necessary before applying criteria developed for other types of ecosystems. This has been highlighted in the EU Water Framework Directive which requires taking into account the wetlands typology before obtaining biological indexes since they are known to be highly ecotype-specific (Basset et al., 2006a, b).

CHAPTER 11
CONCLUSIONS

1. The hydrology of La Pletera salt marshes is typical of a Mediterranean salt marsh without continuous freshwater inflow and restricted connection with the sea. Scarce and irregular water inputs due to punctual meteorological disturbances (sea storms and intense rainfall) are followed by prolonged periods of confinement without water inputs (winter anticyclone and summer droughts).

2. The hydrology has a direct effect on water nutrient compositon and dynamics. While nitrogen (mainly oxidized inorganic compounds) depends on external water inputs, phosphorus depends mainly on accumulation processes and internal recycling, which become more relevant during the confinement periods.

3. The old lagoons of La Pletera exhibited higher concentrations of phosphorus, total nitrogen, total organic carbon and chlorophyll-*a* than the new lagoons. The lack of water inputs and the high evaporation during confinement periods cause a progressive accumulation of the salts, total nutrients (mainly in organic form) and organic matter. Therefore, successive confinement events over the years, in addition to the impoundment of the lagoons, bear a progressive accumulation of total nutrients and organic matter.

4. The zooplankton temporal pattern in both the new and the old lagoons is determined by the hydrology and is characterized by the dominance of: (1) the calanoid *Eurytemora velox* during the winter-spring hydrological stability; (2) several harpacticoid species (mainly *Canuella perplexa* and *Mesochra lilljeborgi*) when the water level is low; (3) the rotifer *Brachionus plicatilis* during the summer confinement; and (4) the ciliate *Fabrea* gr. *salina* under the highest salinity and temperature conditions. Differences in community structure between old and new lagoons are more related to the degree of confinement, the

nutrient concentrations and the colonization mechanisms, which explain the higher diversity and evenness found in the new lagoons.

5. Zooplankton community structure in the old and new lagoons, studied by means of the combined use of a taxonomic and a size diversity measure, was determined by fish predation, food resource availability and nutrient concentrations. Hydrological variability and macroinvertebrate predation appear to have no effect on either diversity measure.

6. Taxonomic diversity correlated with nutrient concentrations, whereas size diversity was more sensitive to fish predation, but only when fish (the Iberian toothcarp *Aphanius iberus*) reached high densities. Under low fish densities, the role of food resource availability (FR_a) appeared to be more relevant in structuring the zooplankton community. Nevertheless, the two diversity indexes showed opposite responses to this factor. With increasing FR_a the taxonomic diversity increased and the size diversity decreased.

7. The correlations between both diversities and environmental parameters suggest that size diversity is mainly related to biotic interactions, such as fish predation or inter/intraspecific competition, while taxonomic diversity appears to be more sensitive to abiotic factors such as the nutrient composition.

8. The hydrological functioning, the physical and chemical characteristics, the vegetation community and the invertebrate community composition and structure of the new lagoons, created as refuges for *A. iberus*, have been comparable to the natural habitat of this species in the marsh (the old lagoon Fra Ramon). A quick growth of the new population was recorded two years after the first fish stocking, suggesting that new refuges have provided a favourable habitat for this species.

9. In the Ter Vell lagoon, water management actions in agriculture caused a drastic reduction of the freshwater inflow to the ecosystem and, therefore, a change in the hydrological regime. Unfortunately, such a reduction in the freshwater inflow also impeded the correct functioning of the constructed wetlands, the main restoration action carried out in the lagoon to improve water quality.

10. The hydrology of the Ter Vell lagoon before and after the hydrological change was illustrative of two opposite situations in the hydrological functioning of coastal wetlands. Before the change, the hydrology was artificial since the freshwater flooding period was prolonged and the highest water inputs were recorded during the dry season coinciding with a high agricultural demand. Thus, the lagoon had an exorheic character with a high water turnover rate. After the change, the lagoon had a more natural and climate-dependent hydrology and, therefore, a more endorheic character with low water turnover due to scarce water inputs and more prolonged confinement periods.

11. In the Ter Vell lagoon the hydrological change had a direct effect on the nutrient composition and dynamics, which tended to resemble those observed in the confined lagoons of La Pletera salt marshes. Nutrient inputs (mainly nitrogen) diminished after the change and were punctually detected only after meteorological disturbances. The organic load and salinity of the lagoon increased because internal loading and accumulation effects became more relevant after the hydrological change, when confinement periods were prolonged.

12. The zooplankton community of the Ter Vell lagoon was not significantly altered by the hydrological change and did not resemble the community of La Pletera salt marshes, at least in the short-term. Several taxa related with eutrophic conditions such as some species of the rotifer *Brachionus*, the

cladoceran *Bosmina longirostris* and the cyclopoid *Acanthocyclops* gr. *robustus* were dominant.

13. After the hydrological change, an improvement of the ecological status of the Ter Vell lagoon was detected, at least in the short-term. This improvement was related to the decrease in the Trophic State Index obtained for total nitrogen (TSI_{TN}) and in the percentage of rotifers indicative of eutrophy, and to the increase in the water quality index *QAELS*.

14. Contradictory results emerged when additional measures of water quality change were applied. In contrast to general assumptions, in the Ter Vell lagoon the improvement in the ecological status after the hydrological change was related to a decrease in the zooplankton taxonomic diversity and richness and to an increase in the dominance of few species. This zooplankton community structure is characteristic of Mediterranean coastal wetlands where the confinement leads to an impoverishment of the aquatic fauna.

CHAPTER 11

CONCLUSIONES (in Spanish)

1. La hidrología de las marismas de La Pletera es típica de las marismas mediterráneas que no presentan entradas continuas de agua dulce y cuya conexión con el mar es restringida. Las pocas e irregulares entradas de agua dulce se deben a perturbaciones meteorológicas puntuales (temporales de mar y precipitaciones intensas) que vienen seguidas por largos períodos de confinamiento sin entradas de agua (anticiclón invernal y sequía estival).

2. La hidrología tiene un efecto directo sobre la composición y la dinámica de los nutrientes. Mientras que el nitrógeno (principalmente compuestos inorgánicos oxidados) depende de las entradas de agua externas, el fósforo depende básicamente de los procesos de acumulación y del reciclado interno, que adquieren más relevancia durante los períodos de confinamiento.

3. Las lagunas antiguas de La Pletera presentan concentraciones de clorofila-*a,* fósforo, nitrógeno y carbono orgánico totales más elevadas que las nuevas lagunas. La falta de entradas de agua y la elevada evaporación durante los períodos de confinamiento causan una progresiva acumulación de las sales, de los nutrientes totales (principalmente en forma orgánica) y de la materia orgánica. Así pues, sucesivos períodos de confinamiento a lo largo de los años, junto con el aislamiento físico de las lagunas, conllevan una progresiva acumulación de los nutrientes totales y de la materia orgánica.

4. El patrón temporal del zooplancton en las nuevas y las antiguas lagunas viene determinado por la hidrología y se caracteriza por la dominancia: (1) del calanoide *Eurytemora velox* durante el período de estabilidad hidrológica de invierno-primavera; (2) de varias especies de harpacticoides (principalmente *Canuella perplexa* y *Mesochra lilljeborgi*) cuando los niveles de agua son bajos; (3) del rotífero *Brachionus plicatilis* durante el confinamiento estival; y (4) del

ciliado *Fabrea* gr. *salina* cuando las salinidades y temperaturas son máximas. Las diferencias en la estructura de la comunidad entre las lagunas antiguas y las nuevas están relacionadas con el grado de confinamiento, las concentraciones de nutrientes y los mecanismos de colonización, que explicarían la mayor diversidad y equitabilidad observadas en las lagunas nuevas.

5. La estructuración de la comunidad zooplanctónica en las lagunas nuevas y las antiguas, estudiada por medio del uso combinado de medidas de diversidad taxonómica y de tamaños, ha sido afectada por la depredación por peces (el fartet *A. iberus*), la disponibilidad de recurso y las concentraciones de nutrientes. La variabilidad hidrológica y la depredación por invertebrados parecen no tener efecto sobre ambas medidas de diversidad.

6. La diversidad taxonómica se correlacionó con las concentraciones de nutrientes, mientras que la diversidad de tamaños fue más sensible a la depredación por peces aunque solamente cuando los peces alcanzaron altas densidades. Cuando las densidades fueron bajas, el papel de la disponibilidad de recurso (FR_a) en la estructuración de la comunidad pareció ser más relevante. No obstante, los dos índices de diversidad presentaron respuestas opuestas frente a este factor. Al incrementarse FR_a, se incrementó la diversidad taxonómica y disminuyó la diversidad de tamaños.

7. Las correlaciones encontradas sugieren que la diversidad de tamaños se relaciona principalmente con las interacciones bióticas, como la depredación por peces o la competencia inter e intraespecífica, mientras que la diversidad taxonómica parece ser más sensible a factores abióticos como la composición de nutrientes.

8. El funcionamiento hidrológico, las características físicas y químicas, las comunidades vegetales y la composición y estructura de la comunidad de invertebrados de las nuevas lagunas, creadas como refugios para *A. iberus*, han

sido comparables a los del hábitat natural de la especie en la marisma (la laguna antigua Fra Ramon). Un rápido crecimiento de la población ha sido registrado dos años después de la primera liberación de individuos en los nuevos refugios sugiriendo que éstos han proporcionado el hábitat adecuado para esta especie.

9. En la laguna de Ter Vell, la gestión del agua en la agricultura causó una drástica reducción del caudal de entrada de agua dulce a la laguna y, por tanto, un cambio en el régimen hídrico. Desafortunadamente, esta reducción del caudal de entrada impidió el correcto funcionamiento de los humedales construidos, la principal acción de restauración realizada en la laguna para mejorar la calidad del agua.

10. La hidrología de la laguna de Ter Vell antes y después del cambio hidrológico fue ilustrativa de dos situaciones opuestas del funcionamiento hidrológico de los humedales costeros. Antes del cambio, la hidrología era artificial ya que el período de inundación se había prolongado y las máximas entradas de agua se registraban durante la estación seca, coincidiendo con una mayor demanda agrícola. Así pues, la laguna presentaba un comportamiento exorreico con una elevada tasa de renovación del agua. Después del cambio, la laguna presentó una hidrología más dependiente de la climatología y, por consiguiente, un comportamiento más endorreico con baja renovación del agua debido a las escasas entradas de agua y a los largos períodos de confinamiento.

11. En la laguna de Ter Vell el cambio hidrológico tuvo un efecto directo sobre la composición y dinámica de los nutrientes, que tendieron a parecerse a las observadas en las lagunas confinadas de la marisma de La Pletera. Las entradas de nutrientes (principalmente nitrógeno) disminuyeron después del cambio y sólo se detectaron de manera puntual después de perturbaciones meteorológicas. La carga orgánica y la salinidad de la laguna aumentaron porque el reciclaje interno y los efectos de acumulación se hicieron más relevantes después del cambio hidrológico, cuando los períodos de confinamientos fueron prolongados.

12. La comunidad de zooplancton de la laguna de Ter Vell no fue alterada significativamente por el cambio hidrológico y no se pareció a la comunidad de la marisma de La Pletera, al menos a corto plazo. Varias especies de rotíferos del género *Brachionus* y el ciclopoide *Acanthocyclops* gr. *robustus* dominaron la comunidad.

13. Después del cambio hidrológico se detectó una mejora del estado ecológico de la laguna de Ter Vell, al menos a corto plazo. Esta mejora se vió reflejada en una bajada del Trophic State Index obtenido para el nitrógeno total (TSI_{TN}) y del porcentaje de rotíferos indicadores de eutrofia, y con un aumento del índice de calidad del agua *QAELS*.

14. Se obtuvieron resultados contradictorios cuando se utilizaron medidas adicionales del estado ecológico. Al contrario de lo que se asume generalmente, en la laguna de Ter Vell la mejora del estado ecológico después del cambio hidrológico fue relacionada con una disminución de la diversidad taxonómica y la riqueza del zooplancton, y con un aumento de la dominancia de pocas especies. Esta estructura de la comunidad zooplanctónica es característica de los humedales costeros mediterráneos dónde el confinamiento conlleva un empobrecimiento de la fauna acuática.

CHAPTER 11
CONCLUSIONS (in Catalan)

1. La hidrologia de la maresma de La Pletera és típica de les maresmes mediterrànies que no reben entrades contínúes d'aigua dolça i que presenten una restringida connexió amb el mar. Les poques i irregulars entrades d'aigua dolça són degudes a pertorbacions meteorològiques puntuals (temporals de mar i precipitacions intenses) que vénen seguides de llargs períodes de confinament sense entrades d'aigua (anticicló hivernal i sequera estival).

2. La hidrologia té un efecte directe sobre la composició i dinàmica dels nutrients. El nitrogen (principalment les formes inorgàniques oxidades) depèn de les entrades d'aigua externes, mentre que el fòsfor depèn bàsicament dels processos d'acumulació i del reciclatge intern, que prenen més rellevància durant els períodes de confinament.

3. Las llacunes antigues de La Pletera presenten concentracions de clorofil.la-*a*, fòsfor, nitrogen i carboni orgànic totals més elevades que les llacunes noves. La manca d'entrades d'aigua i l'elevada evaporació durant els períodes de confinament provoquen una progresiva acumulació de les sals, dels nutrients totals (principalment en forma orgànica) i de la matèria orgànica. Així doncs, successius períodes de confinament al llarg del anys, juntament amb l'aïllament físic de les llacunes, suposen una progressiva acumulació dels nutrients totals i de la matèria orgànica.

4. El patró temporal del zooplàncton a les noves i les antigues llacunes ve donat per la hidrologia i es caracteritza per la dominància: (1) del calanoid *Eurytemora velox* durant el període d'estabilitat hidrológica d'hivern-primavera; (2) de diverses espècies d'harpacticoids (principalment *Canuella perplexa* i *Mesochra lilljeborgi*) quan els nivells d'aigua són baixos; (3) del

rotífer *Brachionus plicatilis* durant el confinament estival; i (4) del ciliat *Fabrea* gr. *salina* quan les salinitats i les temperatures són màximes. Les diferències en l'estructura de la comunitat trobades entre les llacunes antigues i les noves estan relacionades amb el grau de confinament, les concentracions de nutrients i els mecanismes de colonització, que explicaríen la major diversitat i equitativitat observades a les noves llacunes.

5. L'estructuració de la comunitat zooplanctònica en les llacunes noves i les antigues, estudiada per mitjà de l'ús combinat de mesures de diversitat taxonòmica i de mides, s'ha vist afectada per la predació per peixos (el fartet *Aphanius iberus*), la disponibilitat de recurs i les concentracions de nutrients. La variabilitat hidrològica i la predació per invertebrats semblen no tenir efecte sobre ambdues mesures de diversitat.

6. La diversitat taxonòmica es va correlacionar amb les concentracions de nutrients, mentre que la diversitat de mides fóu més sensible a la predació per peixos, però només quan les densitats del peix eren elevades. Quan les densitats eren baixes, el paper de la disponibilitat de recurs (FR_a) en l'estructuració de la comunitat zooplanctònica va semblar prendre més rellevància. No obstant, les dues mesures de diversitat van presentar respostes oposades davant aquest factor. Al incrementar FR_a, la diversitat taxonòmica també ho va fer però la diversitat de mides va disminuir.

7. Les correlacions obtingudes suggereixen que la diversitat de mides es relaciona principalment amb les interaccions biòtiques, com la predació per peixos o la competència inter i intraespecífica, mentre que la diversitat taxonòmica sembla ser més sensible a factors abiòtics com la composició de nutrients.

8. El funcionament hidrològic, les característiques físiques i químiques, les comunitats vegetals i la composició i estructura de la comunitat

d'invertebrats de les noves llacunes, creades com a refugis per *A. iberus*, han sigut comparables a les de l'hàbitat natural de l'espècie a la maresma (la llacuna antiga Fra Ramon). Un ràpid creixement de la població fóu registrat dos anys després del primer alliberament d'individus en els nous refugis, suggerint que aquests han proporcionat l'hàbitat adequat per aquesta espècie.

9. En la llacuna de Ter Vell, la gestió de l'aigua en l'agricultura va causar una dràstica davallada del cabal d'entrada d'aigua dolça a la llacuna i, per tant, un canvi en el règim hídric. Malauradament, aquesta reducció del cabal d'entrada va impedir el correcte funcionament dels aiguamolls construïts, la principal acció de restauració realitzada a la llacuna per millorar la qualitat de l'aigua.

10. La hidrologia de la llacuna de Ter Vell abans i després del canvi en el règim hídric fóu il.lustrativa de dues situacions oposades del funcionament hidrològic dels aiguamolls costaners. Abans del canvi, la hidrologia era artificial ja que el període d'inundació s'havia allargat i les màximes entrades d'agiua s'enregistraven durant l'estació seca, coincidint amb una major demanda agrícola. Així doncs, la llacuna presentava un comportament exorreic amb una elevada taxa de renovació de l'aigua. Després del canvi, la llacuna va presentar una hidrologia més depenent de la climatologia i, per tant, un comportament més endorreic i una baixa renovació de l'aigua degut a les escasses entrades d'aigua i als llargs períodes de confinament.

11. En la llacuna de Ter Vell el canvi hidrològic va tenir un efecte directe sobre la composició i dinàmica dels nutrients, que van tendir a semblar-se a les observades a les llacunes confinades de la maresma de La Pletera. Les entrades de nutrients (principalment nitrogen) van disminuir després del canvi i només es van detectar de manera puntual després de pertorbacions meteorològiques. La càrrega orgànica i la salinitat de la llacuna van augmentar perquè el reciclatge intern i els efectes d'acumulació es van fer

més rellevants després del canvi hidrològic quan els períodes de confinament foren prolongats.

12. La comunitat de zooplàncton de la llacuna de Ter Vell no es va veure alterada significativament pel canvi en el règim hídric i no s'assemblà a la comunitat de la maresma de La Pletera, almenys a curt termini. Diverses espècies de rotífers del gènere *Brachionus* i el ciclopoid *Acanthocyclops* gr. Robustus van dominar la comunitat.

13. Després del canvi en el règim hídric es va detectar una millora de l'estat ecològic de la llacuna de Ter Vell, almenys a curt termini. Aquesta millora es va relacionar amb la baixada del Trophic State Index obtingut pel nitrogen total (TSI_{TN}) i del percentatge de rotífers indicadors d'eutrofia, i amb un augment de l'índex de qualitat de l'aigua *QAELS*.

14. Es van obtenir resultats contradictoris quan es van utilitzar mesures adicionals de l'estat ecològic. Al contrari del que s'assumeix generalment, a la llacuna de Ter Vell la millora de l'estat ecològic després del canvi en el règim hídric es va relacionar amb una davallada de la diversitat taxonòmica i la riquesa del zooplancton, i amb un augment de la dominància de poques espècies. Aquesta estructura de la comunitat zooplanctònica és característica dels aiguamolls costaners mediterranis on el confinament comporta un empobriment de la fauna aquàtica.

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APPENDICES

Appendix I

Characteristics of the five sampling points selected in the present study
(*The zero water level corresponds to the average sea level over the last 15 years)

		Ter Vell lagoon			**La Pletera salt marshes**				
Sampling points		Outflow	Inflow	Confined	O1	O2	N1	N2	N3
					Bassa del Pi	Fra Ramon	New lagoons		
Origin		Natural	Artificial (1993)		Natural		Artificial (2002)		
UTM coordinates	x	516.218	515.576	516.256	515.686	515.861	516.066	516.038	516.085
(square 1 Km^2)	y	4655.06	4655.25	4655.27	4653.14	4653.23	4653.53	4653.44	4653.29
Longitude	E	3º11’46”	3º11’17”	3º11’47”	3º11’22”	3º11’29”	3º11’38”	3º11’37”	3º11’39”
Latitude	N	42º2’48”	42º2’54”	42º2’54”	42º1’46”	42º1’49”	42º1’58”	42º1’55”	42º1’50”
Total flooded surface (m^2)		5925	2600	4985	436	15713	3202	4621	968
(topographic level, cm a.s.l.)		(25)	(75)	(25)	(50)	(100)	(50)	(50)	(50)
Minimum topografic level (m b.s..l)		-0.75	- 0.25	-1.25	-0.75	-1.75	-1.00	-1.00	-1.00
Surface permanently flooded (m^2 at the zero water level*)		2772	402	2565	214	2530	1071	910	338
Surface temporarily flooded (m^2)		3153	2198	2420	222	13183	2131	3711	630
Main vegetation communities:									
Helophytic		*Typho-Schoenoplectetum tabernaemontani* subas. *phragmitetosum australis*							
Halophytic					*Puccinellio-Arthrocnemetum fruticosi; Suaedo-Salicornietum patulae*				
Hydrophtyic					*Chaetomorpho-Ruppietum* (only in O2 and N2 lagoons)				

Appendix II

Biomass estimates of the zooplankton taxa. *F*: conversion factor; V: volume (in µm^3), *WW*: Wet Weight; *W*: biomass (in µg dry weight) already including the conversion factor *F*; *a* and *Lt* : maximum body length (in mm); *b* and b_2: width or diameter (in mm) and ds_{IX}: the diameter of the ninth segment (in mm). *R*, references : Br, Brucet, 2003; Hi, Hillebrand et al., 1999; Hu, Hullé, 1981; Je, Jensen, 1983; Jo, Johnston, 1995; La, Lawrence et al., 1987; Ma, Malley et al., 1989; Ru, Ruttner-Kolisko, 1977; Sc, Schönborn, 1992; Sm, Smit et al., 1993; Wa, Warwick and Price, 1979; Wi, Wieser, 1960. For the colonial ciliate (*) biomass was estimated for each individual.

	F	R	WW	R	W	Geometric figure		R
Ph. Ciliates							Note	
Strombidium sp.	0.11V	Sc	V	La*	$41.6\cdot a\cdot b^2$	ellipsoid	b=c	Sc
Euplotes sp.	0.11V	Sc	V	La*	$41.6\cdot a\cdot b^2$	ellipsoid	b=c	Sc
Didinium sp.	0.11V	Sc	V	La*	$28.80\cdot a\cdot(b^2+b\cdot b_2+b_2^2)$	truncated cone		Hi
Tintinid undet.	0.11V	Sc	V	La*	$44\cdot a\cdot b^2$	cylinder		
Zoothamnium sp.*	0.11V	Sc	V	La*	$57.2\cdot a\cdot b^2$	ellipsoid		
Undetermined	0.11V	Sc	V	La*	$57.2\cdot a\cdot b^2$	ellipsoid		
Ph. Rotifera				La*				
Fam. Bdelloidea	0.07V	Ma	V	La*	$36.4\cdot a\cdot b^2$	ellipsoid	b=c	Ru
Brachionus plicatilis	0.07V	Ma	V	La*	$(36.4\cdot a\cdot b^2)+(3.64\cdot a\cdot b^2)$	ellipsoid	b=c	Ru
Notholca spp.	0.07V	Ma	V	La*	$(5.46\cdot a^2\cdot b)+(0.29\cdot a^3)$	segment of ellipsoid	c=0.2a	Ru
Colurella adriatica	0.07V	Ma	V	La*	$(12.38\cdot a^2\cdot b)+(1.38\cdot a^3)$	ellipsoid + conical tail	c=0.34a	Ru
Fam.Synchaetidae	0.07V	Ma	V	La*	$36.4\cdot a\cdot b^2$	ellipsoid	b=c	Ru
Asplanchna sp	0.04V	Ma	V	La*	$20.8\cdot a\cdot b^2$	ellipsoid	b=c	Ru
Testudinella clypeata	0.07V	Ma	V	La*	$(5.6\cdot a^2\cdot b)+(0.56\cdot a^2\cdot b)$	cylinder	c=0.2a	Ru
Undetermined	0.07V	Ma	V	La*	$36.4\cdot a\cdot b^2$	ellipsoid	b=c	Ru
Ph.Platyhelminthes								
Cl. Turbellaria	0.20WW	Sm	1070V	Sm	$167.99\cdot a\cdot b^2$	cylinder		Sm
Ph. Nematoda								
Cl. Nematoda	0.25WW	Wi;Je	1.13V	Wi;Je	$149.725\cdot a\cdot b^2$			Wa
Ph. Annelida								
Cl. Oligochaeta	5WW	Hu	1070V	Sm	$45903\cdot ds_{IX}^{3.07}$			Sm
Cl. Polychaeta (larvae)	0.07V	Ma	V	La*	$54.95\cdot a\cdot b^2$			Br
Ph. Arthropoda								
Cl. Ostracoda					$27.9383\cdot Lt^{2.8}$			Jo
Cl. Copepoda								
O. Calanoida					$2.9946\cdot Lt^{2.1951}$			Ma
O. Harpacticoida					$1.8504\cdot Lt^{2.0340}$			Ma
O. Cyclopoida					$1.8504\cdot Lt^{2.0340}$			Ma

* Volume was equal to wet weight by assuming that 1 mm^3 of animal biomass = 1 mg.

Appendix III

Size diversity was calculated using Lurie and Wagensberg's (1983, 1984) index, which is analogous to *H* but appropriate for continuous variables and is based on the function of probability density of individuals with respect to size (s):

$$\mu = -\int_{s_i}^{s_{i+1}} p_i(s)\log_2 p_i(s)ds \qquad (1)$$

The non-linear Pareto type-II distribution has been used as the probability density function p_i *(s)* according to Brucet et al. (2006) and the individual biomass dry weight has been used as the individual size (*s*). The Pareto type-II function is given by the equation (2) with a cumulative distribution of probability defined in equation (3):

$$p_i(s) = c(K + D)^c (s + D)^{-(c+1)} \qquad (2)$$

$$prob(s \geq S) = (K + D)^c (s + D)^{-c} \qquad (3)$$

where prob($s \geq S$) represents the probability that the size (*s*) of an individual taken at random was bigger or equal to a threshold size (*S*) and is calculated for each individual as the fraction of all individuals larger than or equal to itself ($N_s \geq S / N_t$). Previously, the size of each organism (*s*) was standardised by dividing it by the minimum size of each sample ($s_{\min}$) following Winiwarter and Cempel (1992):

$$s' = s/s_{\min}$$

In this case, μ is independent from the units in which size is expressed and $K = 1$.The equation parameters *c* and *D* can be estimated when fitting the size distribution of each zooplankton sample to Pareto type-II function (2) by means of an iterative nonlinear regression following Brucet et al. (2005a).

The standardised size diversity index ($\mu_{s'}$) has been obtained by integrating the equation (1) after substituting p_i (s) in the equation (1) for the Pareto type-II function (2) following Brucet et al. (2006). The resulting equation is:

$$\mu_{s'} = (A_1 \cdot A_2 - A_3) \cdot \log_2 e$$

where:

$$\text{A1} = \left(\frac{1+D}{s'_{\max}+D}\right)^c$$

$$\text{A2} = \ln\left[\left(\frac{c}{s'_{\max}+D}\right)*\left(\frac{1+D}{s'_{\max}+D}\right)^c\right] - \frac{c+1}{c}$$

$$\text{A3} = \left(\ln\frac{c}{1+D}\right) - \left(\frac{c+1}{c}\right)$$

$s'_{\max}$ is the maximum size of each sample.

PHOTOS

Photo 1. A) Aerial view of La Pletera salt marshes (partially urbanized) and the Ter River mouth. The 1 km long promenade and the network of roads can be seen. B) Aerial view of the Ter Vell lagoon. L'Estartit urban area, several urbanizations and the Medes Islands can be seen.
(Photos by Bon Vent de l'Empordà S.L.).

Photo 2. Aerial photographs of the Ter Vell lagoon and La Pletera salt marshes from 1956 and 1973 (on this page) and from 1984 and 1997 (the next page). Dotted lines indicates the free-water surface (modified from Gesti, 2003).


1984
⇧
N


1997
⇧
N

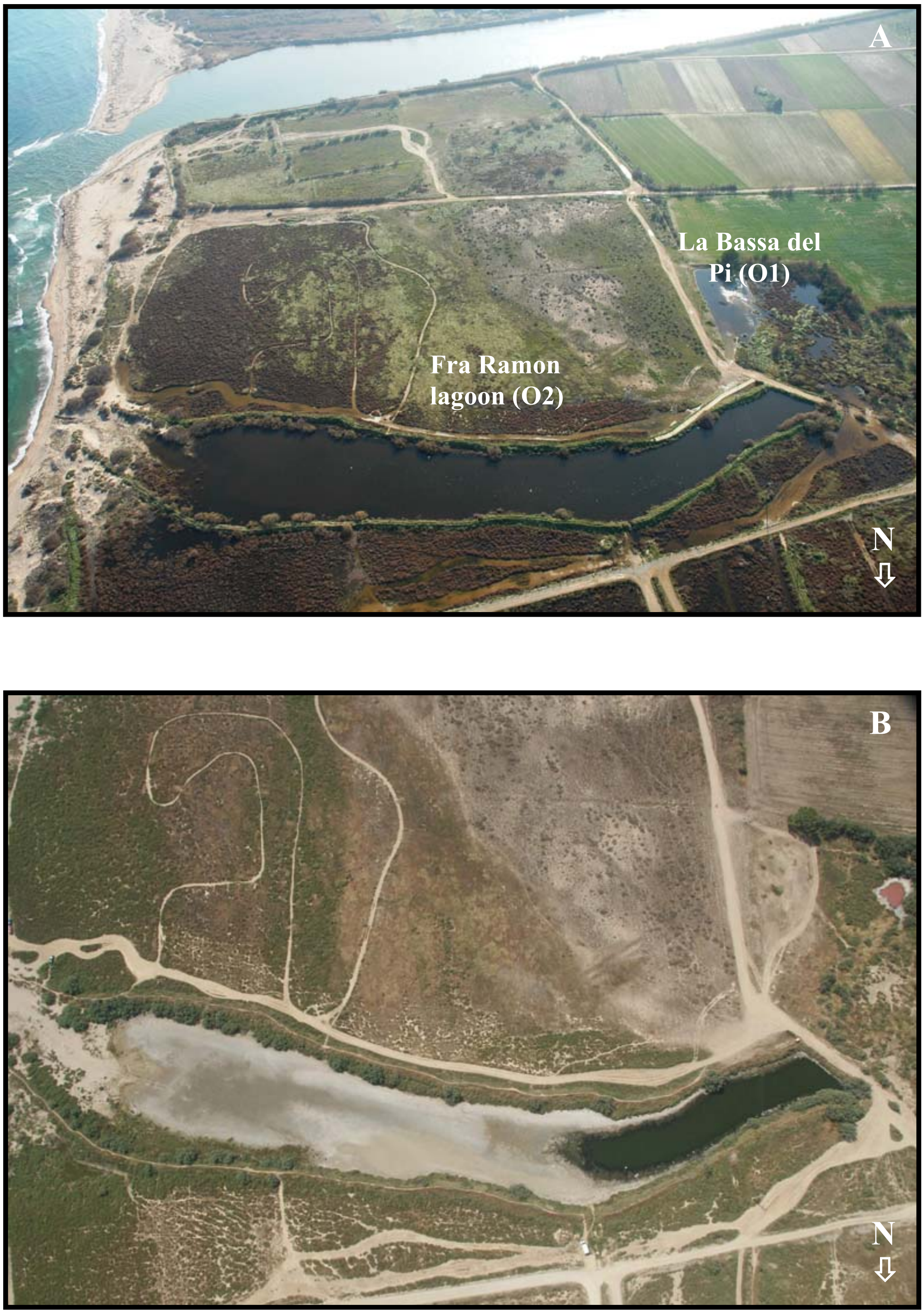

Photo 3. Aerial view of the old lagoons after a flooding period (A) and during an extreme confinement period (B). Note that the water colour is pink in the O1 lagoon during the confinement due to the presence of *Chromatium*-like phototrophic bacteria.
(Photos by Bon Vent de l'Empordà S.L.).

Photo 4. A) Aerial view of the new system of lagoons (N1, N2 and N3) created in La Pletera salt marshes as refuge for the Iberian toothcarp. They remain isolated during the confinement periods. B) Aerial view of the old and new lagoons after a flooding event. Note that the new lagoons are connected.

(Photos by Bon Vent de l'Empordà S.L.).

Photo 5. A) Construction of one of the new lagoons (N2) in La Pletera salt marshes. B) The N2 lagoon after its construction.
(Photos by Servei de Control de Mosquits de la Badía de Roses i el Baix Ter).

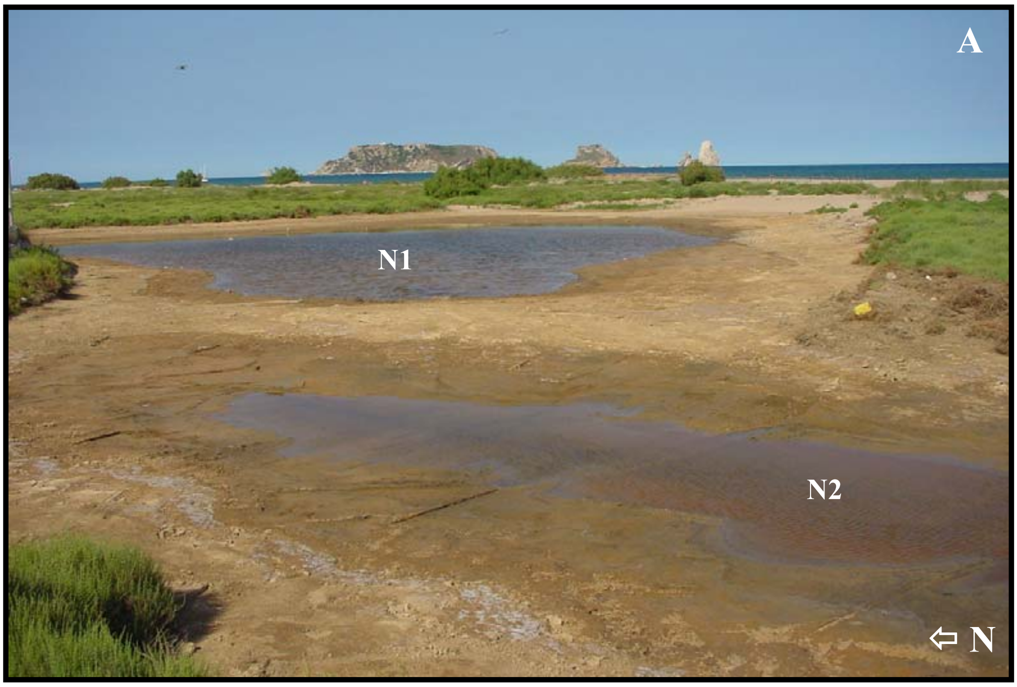

Photo 6. The new lagoons of La Pletera salt marshes (N1 and N2 in this picture) remain isolated during the dry seasons (A) but they connect after flooding events (B).
(Photos by Servei de Control de Mosquits de la Badía de Roses i el Baix Ter).

Photo 7. The Iberian toothcarp (*Aphanius iberus* Valenciennes, 1846) is a small cyprinodont (total length usually < 5cm) endemic of the Mediterranean coast of the Iberian Peninsula which is threatened with extinction. Sexual dimorphism in the pigmentation pattern can be seen. (Photos by Josep Armengol).

Photo 8. During the fish stocking of the new lagoons, scholars from L'Estartit participated in the release of the *Aphanius iberus* individuals.
(Photo by Servei de Control de Mosquits de la Badía de Roses i el Baix Ter).

Photo 9. A) Aerial view of the constructed wetlands. The freshwater flux through the treatment system is indicated with solid arrows. The last section of the irrigation channel which fed the lagoon before the construction of wetlands is indicated by dashed arrows. B) Aerial view of the Ter Vell lagoon. The three studied basins (Inflow, Outflow and Confined) and the constructed wetlands are indicated.
(Photos by Bon Vent de l'Empordà S.L.).

Photo 10. A) Aerial view of the dredged area: a common reed extension between the central basin of artificial origin and the Outflow basin. B) Picture of the dredged sediment from the bird observatory.
(Photos by Servei de Control de Mosquits de la Badía de Roses i el Baix Ter).

Photo 11. In the Inflow basin, where the sediment dredging was not feasible or involved high habitat perturbation, the surface layer of mud sediment was removed by aspiration.
(Photos by Servei de Control de Mosquits de la Badía de Roses i el Baix Ter).

Photo 12. A) Artificial mechanisms to retain sand were installed throughout the littoral zone to recover the sand dune vegetation.
B) Once sand had been accumulated due to the wind action, vegetation recovery took place mainly by spontaneous colonization.
(Photos by Servei de Control de Mosquits de la Badía de Roses i el Baix Ter).

Photo 13. A) Artificial mechanisms to retain sand were formed by a succession of square cells which were grouped in modules running parallel to the coast (Photo by Bon Vent de l'Empordà S.L.).
B) In order to guide the visits and sensitize the visitors, several informative posters explaining the main restoration activities were incorporated along the itineraries. (Photo by Servei de Control de Mosquits de la Badía de Roses i el Baix Ter).

..... això d’haver de fer, a consciència, memòria retrospectiva dels darrers anys, sincerament, em feia més por que goig.....i és que són molts anys, i mentre es viuen no s’és del tot conscient de que cada detall, historieta, aventura o coneixença influirà (bé o malament) en el resultat final, en aquesta tesi....a més, us seré franca, la meva memòria ja no és el que era, l’estrès dels darrers mesos l’ha vençuda, cada cop m’és més fàcil oblidar....però sé que seria molt lleig per part meva no afrontar aquest repte, que de desagraïts el infern n’és ple! (i no és pas que vulgui anar al cel jo!), que no costa gens reconèixer el que altres fan o han fet per a tu (sovint sense ser-ne conscients)...a més, no m’agradaria decebre a aquells que el primer que faran quan obrin aquesta tesi serà llegir-ne els **AGRAÏMENTS ;-)**

.... (*inicis*) Començaré doncs per donar les gràcies a en Xavier Quintana i a en Ramon Moreno, per confiar en mi i obrir-me les portes de l’Ecologia aquell estiu del 97. Les inacabables sessions al 225 fent alkalinitats i matèries orgàniques, que malgrat l’esforç no van servir per res (ho vaig saber al cap d’uns anys), no van aconseguir desmitificar-me l’ecologia, ans al contrari, vaig voler continuar i al 98 en Xavi ja em presentava, ‘formolitzats’ això sí, els primers rotífers i copèpodes. Al 99 ja me’ls recollia jo mateixa en els aiguamolls del Baix Ter.... (*mestre i director*) Va ser aquell 97 quan en Xavier Quintana em va parlar per primer cop de la limnologia, caòtica però genial assignatura que em va convèncer el suficient com per decidir dedicar-hi el meu futur (i encara no me’n penedeixo!!).... gràcies Xavi, pel teu coneixement, per la teva intuïció, pel caos, per parir el projecte LIFE, per les correccions de les correccions de les correccions... en definitiva, gràcies per ser el meu mestre!!.... (*mestre i director II*) És d’agrair que en Dani Boix també es volgués implicar en aquesta tesi (malgrat tractar-se de basses costaneres, brutes... i permanents!!!)....gràcies Dani per l’esforç que hi has dedicat i la feina feta, pel teu coneixement de ‘bichos’ i basses, per l’energia inesgotable (això no s’encomana?), i per la dosi de realisme justa per contrarestar l’excessiu optimisme d’en Xavi i l’extremat pessimisme d’una servidora.... (*des de dins*) Evidentment que no n’hi ha prou amb la feina de dos directors i una doctoranda... aquesta tesi no hagués arribat a la fi sense el suport físic i moral de la resta dels ‘Aiguamullerus’: Sandra, Rocío, Stéphanie, Jordi S., Jordi C., Rosa, Lluís, Cristina, el recent incorporat Albert...Gràcies per les estones de bar, sopars i dinars, excursions i escalades; amb vosaltres he ampliat horitzons....fitoplàncton, bentos, estadística, taxonomia, metodologia, anglès, etc....què més vull?? I què dir de la nostra Queen of lab.? Merci altre cop Cristina per la pesada i avorrida (en sóc molt conscient) analítica d'aigües i sediment, per la teva eficiència i perseverança, sense tu les coses no haguessin ni la meitat de bé... Agrair també a l'Esther i la Gemma la santíssima paciència, la bona cara, aquell material que mai ens ha faltat...Tampoc voldria deixar d'agrair la feina, sovint mecànica i poc agraïda, feta per els alumnes de crèdits per equivalència durant tots aquests anys, i que no ha estat poca!! (conscient de la meva mala memòria m'estimo més no dir noms)....Les interaccions fora del grup també han estat igualment profitoses e imprescindibles: gràcies a la Mònica per compartir mostreig i coneixement; a en Pep per la botànica i els mapes, pel bon humor; a en J. Pascual per la meteorologia, per la topografia interminable... He d'agrair especialment l'ajuda (i paciència) constant d'en Carles, es pot dir que quasi tot el que sé de peixos, informàtica, així com bona part de l'estadística li dec a ell. Merci!!.... No seria just oblidar la resta de companys de l'àrea d'Ecologia, amb els que no comparteixo temàtica però sí laboratoris, menjadors, estones vàries, penes i glòries. A tots i cada un d'ells, als passats, als presents, als més recents (als de peixos, Ramon, Emili, Anna V., Lluís Z., Lluís B., Quim P., Carles F., Quim C., Josep, Miguel, Pablo (i Silvie), als de rius, Sergi, Anna R., Elena, Joan, Sandra, Elisabet, Gemma U., Anita, Gemma V., Irene, Marta) gràcies pels ànims, els consells, les estones de menjador, els cafès, les ganes de fer i de riure. Per compartir espai vital, radiacions informàtiques i calor humà, especial agraïment pels companys/es del 231.... (*des de fora*) No voldria deixar d'agrair a la gent que em va acollir durant les meves estades pel sud, especialment a en Jaime Rodríguez de la Universitat de Málaga i a en Carlos García de la Universitat de Cádiz, gràcies ambdós per guiar-me en el treball de Màster, pels diferents punts de vista, per una altra visió de l'ecologia. Un record també per a tots aquells que van fer de la meva estada a les terres andaluses una experiència molt enriquidora: M^{a} del Mar, Francis, Pablo, María, Raquel, Laura i Rafa, gràcies per la vostra hospitalitat, per tantes tapes, finos i somriures!!!.... Molt agraïda també a en Michael Fox pels consells i correccions d'un dels capítols d'aquesta tesi.... Gràcies també als revisors 'anònims' que han millorat el resultat final... (*amics*)....A l'amiga Sílvia, la de sempre i per sempre, la incondicional, també va per a tu Aldo. A la Sandra, amiga i confident dins i fora de la UdG, un exemple a seguir, i de retruc, a la franquesa i entusiasme d'en Pere. A en Carles, pel que hem viscut plegats, per entendre'm i sobretot, per fer-me riure tant!!! (també va per la seva mare, que sense ser-ne conscient i amb el seu afany d'omplir els "tàpers" del seu fill fins al capdamunt ha enriquit substancialment la meva dieta). A la Penya de l'Espardenya & Co. (Sandra, Pere, Núria, Dolors, Àlex, Sandra, Josep, Alba, Carles, Rakel-In, Edu, Rakel-Out, Maite, Alfons, Elena, David, Olga, Francesc, Montse, Miquel, Mireia, Kiko...) per acollir-me, per tot el

que m'heu ensenyat i el que hem viscut (amb vosaltres tot esdevé tant fàcil!!). A la complicitat dels del komando Eiximenis: palamosins, Uli, Mercè, Bruna, gironins, Glòria, Toni, Martina i Èlia, i belgues, Sandra i Arvid...que per molts anys podem gaudir-nos uns als altres!!. A la Mª del Mar, per les escapades a Almería, per la seva franquesa, per ser com és.... A en Miquel, la Neus, l'Enric, l'Imma (també biòlegs), per les trobades esporàdiques i nostàlgiques... Als banyolins Salvador, Mane i Jepi, per les converses profundes, per les preguntes incòmodes, per les farres intenses... A tots plegats, moltíssimes gràcies per compartir amistat, farres i fugides, pels consells i els ànims, per les rialles, pel suport logístic (Internet dels veïns) pel suport alimentari (neveres del veïns, horts diversos, etc.), pel suport psicològic, pel suport musical, pels inicis al kun-fu etc., etc., etc... en definitiva, per ser al meu costat sempre que us he necessitat!!.... (*matèria gris*) Sé que us semblarà estrany i poc usual, però aquests darrers anys de tesi he agraït profundament, sense saber a qui dirigir-me, la meva enteresa mental, signes d'estrès els que vulguis, ansietat, urticàries, herpes, llengua geogràfica, etc., etc., però el meu cervellet encara no ha dit prou, no s'ha rendit i li ho agraeixo moltíssim.... (*els de casa*) Finalment, què dir dels que m'aguanten la mala lluna sense saber d'on baixa, dels que m'alimenten, dels que m'escolten molt i pregunten poc, dels que em consolen i m'animen, dels que intenten entendre'm, dels que es desviuen...i és que mai he gaudit d'un suport tant INCONDICIONAL!! Mama, papa, germanet....gràcies per tot, aquesta tesi va per a vosaltres!!!

....I per acabar, donar les gràcies als qui van fer possible l'existència i el desenvolupament del projecte LIFE "Restauració i ordenació de les llacunes i dels sistemes costaners del Baix Ter" en el qual s'ha emmarcat aquesta tesi i, en definitiva, tot el meu treball des del 99: a l'Ajuntament de Torroella de Montgrí, a la Universitat de Girona, al Servei de Control de Mosquits de la Badia de Roses i del Baix Ter, a l'Escola Taller de les Gavarres, a la Fundació Territori i Paisatge de la Caixa de Catalunya, a la Direcció General de Costes del Ministeri de Medi Ambient, al Departament de Medi Ambient de la Generalitat de Catalunya i a la Diputació de Girona. M'ha encantat (i alhora desesperat) participar en aquest projecte, en 'l'intent' d'arreglar allò que amb els anys l'home ha espatllat, i dic intent sense malícia, simplement se'm fa difícil creure en l'èxit a curt termini de la restauració ambiental, hi haurem de continuar insistint....