Ecohydrology of Temporary Ponds

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I, Sara Duarte Varandas Martins, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated.

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Abstract

Temporary ponds (TP) comprise a complex ecohydrology and are extremely vulnerable to habitat degradation. These ecosystems contain species rarely found elsewhere but the environments are highly exposed to human and climate change pressures. TP have been recognised as priority habitats for conservation in Europe by the Habitats Directive, and work towards improved knowledge, understanding and conservation has increased since. This thesis focuses on the ecohydrological controls on the cladoceran species and communities in coastal temporary ponds in two geographic regions along a northwest Europe latitudinal gradient: the northwest coast of Ireland and the southwest coast of Portugal. It explores local time-variance in two dune slack ponds at Sheskinmore, Co. Donegal, Ireland, local spatial-variance across 24 ponds at the same site, and regional controls on temporary pond Cladocera ecohydrology at two sites in southwest Portugal (Malhão (MAL) and Vila do Bispo (VB)). The focus of investigation here is the seasonal hydrological balance and dependence of this dynamic on the ecology and biology of the habitats. This research aims to achieve a better understanding of individual, local and regional ecohydrological dynamics of TP's and characterise the drivers of ecosystem variation within.

Results uncover that ponds and slacks of Sheskinmore are highly dependent on rainfall and their vulnerability is confirmed when precipitation levels are not met. Increased variability inter-annually of cladocerans as opposed to intra-annual seasonal variation showed this. Weather changeability is the most important aspect of future climate change but there is a complex interaction between weather variability, local hydroregime and topography of a waterbody, impacting species' temporal and spatial assemblages varyingly. Spatial dynamics of these dune ponds evaluated that within a rather small area, cladoceran composition differs, mainly according to hydrological categories, which are then linked to proximity of each site to the water table. Water chemistry was not so relevant to the spatial patterns in species. At a regional scale, sediment composition and water chemistry were better predictors of differentiation of the cladoceran composition within the Portuguese ponds, rather than geomorphological differentiations between regions. MAL ponds contained higher cladoceran abundance, whereas VB ponds contained higher cladoceran richness. Regionally, cladoceran communities are shaped by a combination of important factors and explanations vary according to the individual characteristics and to the collective groups of ponds. Results corroborate the importance of hydrology, topology and climatic factors as ecological drivers of ecosystem functioning in TP. This work demonstrates the uniqueness and sensitivity of TPs, therefore supporting the need to conserve these habitats.

Impact statement

Temporary ponds exhibit a high biodiversity value and often comprise unique species shaped by the seasonal water level fluctuations and the necessity of keeping this balance is essential for the long-term sustainability and conservation of temporary waterbodies. But these ecosystems are also vulnerable and exposed to the pressures of anthropogenic activities and climate change, leading to significant challenges for habitat and species conservation. This thesis explores microcrustacean zooplankton Cladocera (commonly known as water fleas) in two European countries with different climatic scenarios, and aims to gain a new perspective on the ecological knowledge of temporary ponds in two different habitat settings: humid dune slacks (specifically in Ireland) and Mediterranean temporary ponds (in Portugal). This is the first study undertaken on zooplankton in dune ponds in Ireland, and it builds on previous work in Portugal, but extends the knowledge and understanding of Cladocera and hydrology in Portuguese temporary ponds.

Until relatively recently, temporary ponds have been largely overlooked due to their small size and seasonal nature. Nonetheless, in recent years research has been carried out in small water bodies since they have become recognised by European legislation including the WFD and Habitats Directive. The present work is proof that temporary ponds contain high biodiversity value, including localised endemic species such as the ones found in southwest Portugal. Furthermore, the thesis also demonstrates the role of hydrology in the function and vulnerability of temporary pond habitats, particularly relative to inter-annual climate variability, thereby providing some insight in terms of how these systems will respond to future climate change. This work contributes knowledge and understanding at local and European level of the importance and threats that these ecosystems face in terms of climate forcing of hydrological regime.

Academia and research community, local management authorities (National Parks and Wildlife Service (NPWS) – Ireland, and Parque Natural do Sudoeste Alentejano e da Costa Vicentina (PNSACV) – Portugal) and general public, all benefit from the work presented here. The direct benefits to academia and research are the incorporation of an ecohydrological approach to the understanding of temporary waterbodies with the use of Cladocera, and the inclusion of more sites, regions and countries to the list of sensitive and conservation-relevant habitats, some of them unexplored so far (Ireland). Furthermore, the geographical species distribution of cladocerans in European temporary ponds is updated in this work, contributing to a larger spectrum than previously recorded in the literature. The study sites are within natural park areas but not limited or controlled, therefore with open access to the general public. As an overall conservation project, it is important to reach out to the broader public, and the knowledge and understanding delivered in this thesis can inform the environmental education activities of the local natural parks to improve the awareness and sustainable use of the ponds and their adjacent landscape, and eventually integrate some of the strategies presented in order to protect these sites from the present anthropogenic impacts.

Table of Contents

List of Figures

List of Tables

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Chapter 1 - Introduction

1.1 Overview

Freshwater systems are fundamental to terrestrial life, not only as a source of water for land organisms, but also for the direct biodiversity support function which they provide in terms of food, shelter and breeding sites for aquatic species (Grillas *et al*., 2004). Still, they are under severe pressure due to human activities and the rate of their disappearance is a cause of concern (Rhazi *et al.*, 2012). Most limnological research has focused on large and permanent water bodies such as lakes and rivers (Cobelas, Rojo and Angeler, 2005; EPCN, 2008). Ponds, on the other hand, have been understudied even though they represent 30% of the freshwater surface area reserve across the globe (EPCN, 2008; Oertli *et al.*, 2009). The importance of ponds as "hotspots" for freshwater biodiversity has more recently gained recognition (Céréghino *et al.*, 2008; Miracle *et al.*, 2010; Rhazi *et al.*, 2012). Although human activities have heavily affected ponds, they collectively support a high biodiversity compared with larger systems (EPCN, 2008; Miracle *et al.*, 2010; Bouahim *et al.*, 2014).

Within small lentic systems, temporary ponds are one category that has been seriously overlooked (Williams *et al.*, 2001; Grillas *et al.*, 2004). As the name implies, these aquatic ecosystems are not flooded year-round, and tend to be only active in a flooded aquatic state in the winter and/or spring and remain dry throughout summer and beginning of autumn months (Rhazi *et al*. 2001). For this reason they are highly dependent on the local geomorphological context and weather conditions. Temporary ponds are defined as <10ha shallow wetlands with visible alternating phases of drought and flooding with local specific hydrology (Silva *et al.*, 2007). However, a functional feature common to all temporary ponds that defines them without size limitation is the substantial hydrological fluctuations (ibid). Because temporary ponds represent a significant freshwater feature in the landscape and are extremely vulnerable to climatic shifts and human activities (Rhazi *et al.*, 2012; Bouahim *et al.*, 2014), they are of high conservation importance (Grillas *et al.*, 2004; EPCN, 2008; Amami *et al.*, 2013).

Water is the most fundamental control on temporary pond ecology and function. The hydrological regime of a temporary pond is dependent on its water source, the duration of flooding and desiccation frequency which, from a conservation perspective, makes these systems totally dependent on climate forcing and water management (Brooks, 2004; Lumbreras *et al.*, 2012). Natural or anthropogenic pressures have numerous effects on the biodiversity of temporary ponds and ultimately affect to a great extent the hydrological regime of which the whole ecosystem functioning depends upon.

Within a temporary pond, species have a distinct ecological functioning that copes with a strongly seasonal and changeable hydrological regime and have adapted to an ephemeral life form (Grillas *et al.*, 2004; Bouahim *et al.*, 2014). Zooplankton, unlike vegetation, amphibians and some invertebrate species, have received little attention in temporary pond habitats (Caramujo and Boavida, 2010), despite the Alonso's thorough taxonomical study on ecology and distribution of cladocerans in Spain since 1985 (Alonso, 1985). Furthermore, although specialised to produce drought-resistant eggs, consequence of a strong effect of hydrological variability (Serrano and Fahd, 2005; Hand *et al.*, 2016), the hydroregime influence on zooplankton is still poorly understood (Girdner and Larson, 1995).

In the last decade references of temporary ponds in the literature started to appear more frequently, nevertheless the limited understanding of these systems still exists (Nicolet *et al.*, 2004). Despite the increasing public awareness around freshwater ecosystems, temporary ponds recognition is still in its early stages, and their vulnerability largely exposed due to lack of information available from scientific basis to enforce appropriate management and conservation for these valuable ecosystems (Grillas *et al.*, 2004; EPCN, 2008; Rhazi *et al.*, 2012).

1.2 Temporary ponds

Temporary waterbodies can originate from natural processes, when a depression in the landscape leads to the formation of a basin where water accumulates seasonally; or can be formed artificially by humans serving different purposes such as livestock watering, crop irrigation or clay/marl extraction (Grillas *et al.*, 2004; EPCN, 2008; Ruiz, 2008). Despite their usual small size they can persist for hundreds or thousands of years (Williams *et al.*, 2001; Amami *et al.*, 2013).

Temporary ponds comprise a variety of shapes and sizes (Williams *et al.*, 2001; EPCN, 2008) and are well spread within the landscape, from coastal areas (Pinto-Cruz, 2010) to woodlands (Brooks, 2009), across very different precipitation and temperature conditions (Deil 2005). The designations of temporary ponds are vast throughout the world, given according to specifications in hydrology, morphology, geography and cultural features (Grillas *et al.*, 2004). Some examples are: 'turloughs' in Ireland; 'bilabongs' and 'gnammas' in Australia; 'vernal' and 'autumn pools' in North America (spring and summer fed ponds); 'dayas', 'rain pools' or 'vleis' in Africa (depending on the region); ‘polje’ in Slovenia (karstic basins); and seasonal/ephemeral/temporary ponds or pools used generally worldwide (Keeley and Zedler, 1998; Williams *et al.*, 2001; Grillas *et al.*, 2004).

Since the 1920’s North America and particularly California have pioneered the detailed study of vegetation communities in vernal pools’ (Deil, 2005), compared to other parts of the world. Europe’s interest in temporary ponds has been acknowledged since the 20’s and 30’s but has risen largely since the late 90’s (ibid), with topics related to vegetation conservation and ecology of Mediterranean temporary ponds. In Britain however, temporary ponds were not considered a freshwater habitat until 1996, when Biggs *et al*. acknowledged that not only were they very common in agricultural lowland areas, but also that those habitats were likely to exist even more of such habitats in semi-natural areas. Before then, it was thought that the amount of rain experienced in the country would allow only small pools to dry out in the summer (Williams *et al.*, 2001), hence not making it an important waterbody type.

Figure 1.1 The two extreme ecophases of dry (September 2015) and flooded (March 2016) of a temporary pond in northwest Ireland

1.2.1 European conservation context

The first major international wetland conservation agreement appeared in 1971 with the Ramsar Convention. After that, the European Union wetland legislation and policy started to progress with the Birds Directive in 1979 (79/409/EEC) and the Habitats Directive in 1992 (Silva *et al.*, 2007). The Natura 2000 (the European ecological network of protected areas), is the core of EU nature and biodiversity policy, generated by the Habitats Directive to ensure the conservation of Europe’s unique and endangered species and habitats (EU, 2007; Silva *et al.*, 2007). Having created

Special Areas of Conservation (SACs) and Special Protection Areas (SPAs), freshwater Natura 2000 sites are highly represented across Europe (Silva *et al.*, 2007; Houston, 2008; Ruiz, 2008).

The growing awareness of the value of ponds in Europe led to recent efforts in order to understand their ecological dynamics, as a means to better conserve them (Williams *et al.*, 2004; Biggs *et al.*, 2005; EPCN, 2008). Ponds have been included under the Ramsar Convention for wetland conservation since 2002, and in 2007 they were added to the list of priority habitats in the UK Biodiversity Action Plan (BAP) (Keeble *et al*., 2009). Water Framework Directive (WFD) recognises water bodies irrespective of their size or depth, as long as they are dependent on groundwater bodies, part of surface waters, or part of Protected Areas (Directive 2000/60/EC).. In addition, the European Pond Conservation Network defines a pond as a temporary or permanent small water body (EPCN, 2008), without specifying the size limits.

Temporary ponds are known to be very particular and important habitats for aquatic species in Europe (Céréghino *et al.*, 2008; Ferreira and Beja, 2013), since they detain the settling of upland species due to its wetness, at the same time they keep fully aquatic species away during the dry period (Keeley and Zedler, 1998). Nowadays, the classification used as a base for grouping temporary ponds into categories is defined by the specific vegetation that they support (Grillas *et al.*, 2004), such as employed by the Habitats Directive (EU, 2007).

Annex I of the Habitats Directive (European Union Council legislation (92/43/EEC)) describes more than two hundred different European natural habitat types for protection, including over seventy classified as ‘priority’ (signalled by an asterisk), which are habitat types in danger of disappearance (EU, 2007). Standing water habitats appear classified into ten different categories varying according to the trophic status, water physico-chemistry, climate and geomorphology. These habitats are:

- (3110) Oligotrophic waters containing very few minerals of the sandy plains (*Littorelletalla uniflorae*);
- (3120) Oligotrophic waters containing very few minerals generally on sandy soils of the West Mediterranean with *Isoetes spp*;
- (3130) Oligotrophic to mesotrophic standing waters with vegetation of the *Littorelletalia uniflorae* and/or *Isoeto-nanojuncetea*;
- (3140) Hard oligo-mesotrophic waters with benthic vegetation of *Chara spp*;
- (3150) Natural eutrophic lakes with *Magnopotamion* or *Hydrocharition* type vegetation;

- (3160) Natural dystrophic lakes and ponds;
- (3170*) Mediterranean Temporary Ponds;
- (3180*) Turloughs;
- (3190) Lakes of gypsum karst;
- (31A0*) Transylvanian hot-spring lotus beds.

Aquatic systems in coastal dune settings (within the category of ‘Sea dunes of the Atlantic, North Sea and Baltic coasts’) are also recognised in the Natura 2000 habitat network as ‘Dunes with *Salix repens ssp. argentea* (*Salicion arenariea*)’ (Habitat 2170) and ‘Humid Dune Slacks’ (Habitat 2190) (Houston, 2008), in which some freshwater habitats can in fact also exist (e.g. Habitat 3130) (EU, 2007).

Mediterranean Temporary Ponds and Humid Dune Slacks will be the main habitats present in this study and a brief description is given below:

Mediterranean Temporary Ponds are defined as very shallow ponds which exist only in winter or early spring, with a rare flora assemblage composed of Mediterranean species such as *Isoetes*, *Marsilea*, *Pilularia* (EU, 2007). These habitats are in a steep decline either due to habitat loss or severe damage caused by anthropogenic impacts (Canha and Pinto-Cruz, 2010; Rhazi *et al.*, 2012). They are generally winter-flooded water bodies that give rise to annual, pioneer, rare ephemeral plants and animal species within a restricted geographic distribution, usually in arid and semi-arid areas within the Mediterranean climate region (Ruiz, 2008; Canha and Pinto-Cruz, 2010; Bouahim *et al.*, 2014). The hydrological regime, and in particular phases in wetting and drying, makes them disguised during the dry season, leading to transformation, destruction or sometimes even irreversible collapse. This is due to the lack of awareness of their ecological functions in combination with overall poor conservation and management of the landscape (Ruiz, 2008; Canha and Pinto-Cruz, 2010; Rhazi *et al.*, 2012).

Humid Dune Slacks are an extremely rich and specialised environment, but very threatened by the lowering of water tables (EU, 2007). The hydrological regime of dune slacks also leads to phases of drying (in the summer) and flooding (in the winter), regulated by water-table fluctuations within the surrounding sand dune system (Davy *et al.*, 2006). They have a unique biodiversity and are important niche habitats within the wider dune landscape (Houston, 2008). More often than not, they occur in lulls between dune ridges but can also form in depressions formed through erosional processes, both natural and anthropogenic (Davy *et al.*, 2010). The floristic diversity of dune slacks is very high, holding a variety of species such as the Red Data Book petalwort (*Petalophyllum ralfsii)* (Jones *et al.*, 2006), as well as species that are specifically associated with the early stages of succession in dune slacks (e.g. *Salix repens* (creeping willow), which can be followed rapidly by *Liparis loeselii* (fen orchid), *Dactylorhiza incarnata* (early marsh-orchid) and *Epipactis palustris* (marsh helleborine) (Smith, 2007; Houston, 2008).

1.2.2 Threats to the temporary pond ecosystem

Similarly to other freshwater systems, temporary ponds face numerous threats, but their vulnerability is greater than other small waterbodies specially because of their shallow nature and unpredictable water regime (EPCN, 2008). Most of the threats that ponds face nowadays are related to human interventions and alterations in the neighbouring landscape (Ruiz, 2008; Canha and Pinto-Cruz, 2010). Historically though, this has not always been the case. Artificial ponds have been created to provide humans with assistance to extensive agricultural practises amongst other functions. However the economic development in the past decades has led to their abandonment or destruction (Grillas *et al.*, 2004). Still, in Morocco they are an essential part of the population's daily activities (Rhazi *et al*. 2001) used as a source of water for cattle, agriculture and personal use.

Some of the most serious pressures on temporary ponds are:

- Habitat destruction
- Alterations to the hydrological regime
- Sediment disturbance
- Terrestrialisation
- Climate change

Habitat destruction is related to agricultural intensification (which may increase pollution and ground water usage) and to touristic development and urbanisation (Grillas *et al.*, 2004; Kløve *et al.*, 2011). Hydrological regime can be affected by extraction of water for human use, land drainage (especially in the summer to water crops and livestock), and artificial recharge of temporary ponds in order to create reservoirs for permanent water sources (Zacharias *et al.*, 2007). This leads to a shortening of the hydroperiod and opens the potential for species invasion (such as fish and crayfish species) that exist in the irrigation channels used to connect the reservoirs (Grillas *et al.*, 2004; Oertli *et al.*, 2005). Sediment disturbance may affect the morphology of the bed of the pond which can be caused by cattle and wild boar trampling, but it can also be an issue during the dry season, when human activities such as horseback riding, 4x4 tours and walks are increased (Grillas *et al.*, 2004; Zacharias *et al.*, 2007). Terrestrialisation of temporary ponds by woody species becomes serious when it decreses the likelihood of typical species to exist, such as typical annual plant species and amphibians that require light and open areas to thrive (Grillas *et al.*, 2004; Sayer *et al.*, 2012). This happens when extensive grazing is eliminated and ponds are abandoned (Marty, 2005; Bouahim *et al.*, 2010). Climate change is expected to cause major alterations in temporary ponds' hydrological regime by modifying the biogeographical range of pond species and the overall environment surrounding the habitat due to different temperature and precipitation conditions (Céréghino *et al.*, 2008; Brooks, 2009; Curreli *et al.*, 2013; Trigo *et al.*, 2013).

1.3 Hydrology of temporary ponds

The hydrological dynamic of a temporary pond is considered the most important driving factor, and is characterised by the hydroregime (the temporal pattern of inundation, drying, and water-level change at any location in the pond environment) and hydroperiod (the duration of the inundation phase) that together dictate the ecological cycle within a season (Leibowitz and Brooks, 2008; Brooks, 2009). Because the water level fluctuation is variable and unpredictable throughout the year, significant shifts exist between wet and dry phases as shown in Figure 1.1.

Water (hydro) is the most important factor in shaping the surface of the earth (geomorphology) (Scheidegger, 1973), and therefore hydrogeomorphology in temporary ponds is a major influence in both internal and external pond characteristics. The form and shape of the basin of a pond are very variable, and particularly related to the 3D structure and the ground/surface water function and interchange (Rheinhardt and Hollands, 2008; Zacharias and Zamparas, 2010). The hydrodynamics are thus linked to physical and biotic features (geological and soil structure below the pond bed, surrounding topography, plant community composition and assemblage) which command the duration of the hydroperiod; climatic factors (precipitation, temperature and losses from evapotranspiration); and connection to surface and ground water resources, which determine the inter-annual variation of both hydroregime and hydroperiod (Leibowitz and Brooks, 2008; Camacho *et al.*, 2009; Bouahim *et al.*, 2014). Within a temporary pond, the volume of water derives from inflow (inputs from rainfall, rise of the underground water-table or possibly runoff from surrounding higher topography) and outflow (primarily loss by evapotranspiration and infiltration, though possibly also overflow) (Grillas *et al.*, 2004; Leibowitz and Brooks, 2008) (Figure 1.2).

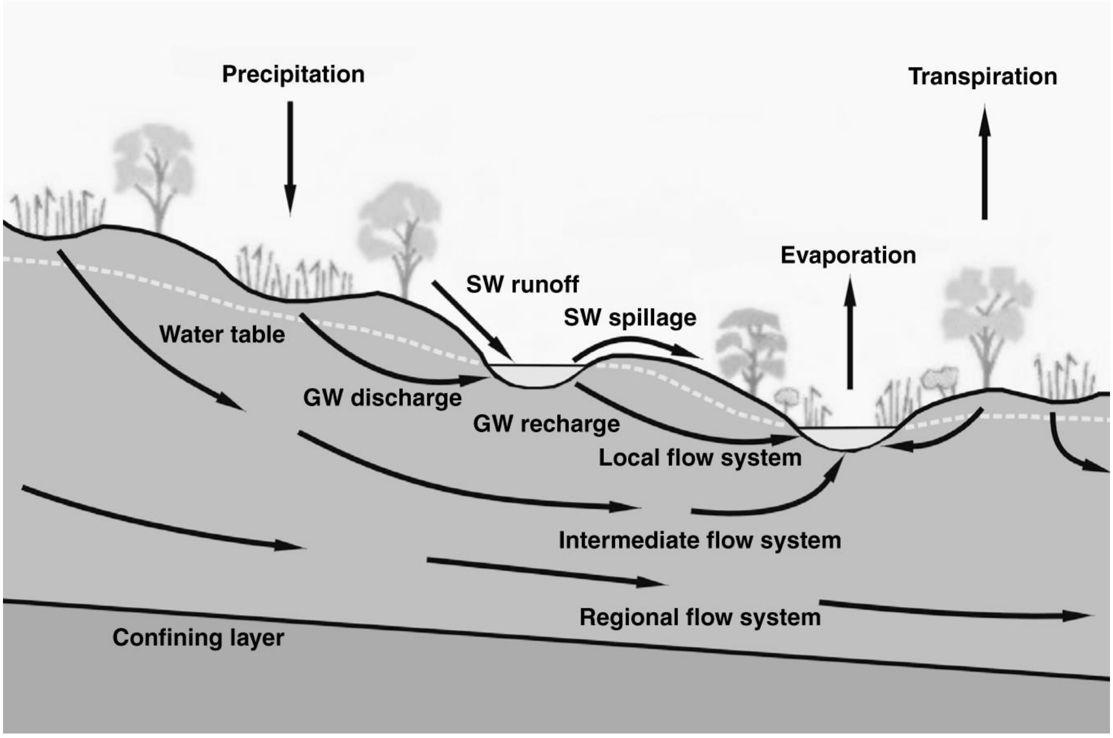


Figure 1.2 Hydrological cycle of a temporary pond. Source: Leibowitz & Brooks 2008

Typically temporary ponds only have permanent open water for a few months of the year and for this reason they often support rare specialist species adapted to dealing with major water level variations and drying periods (Grillas *et al.*, 2004; Pinto-Cruz *et al.*, 2009; Rhazi *et al.*, 2012; Amami *et al.*, 2013; Bouahim *et al.*, 2014). Hydroregime therefore plays a fundamental part in achieving optimal breeding conditions for the species that live in these ponds (Bauder, 2005), by influencing their ability to complete their life cycle within the appropriate flooding and drying phases (NPWS, 2008; Ruiz, 2008). Longer hydroperiods improve the likelihood that amphibians, for instance, achieve an adult phase (Canha and Pinto-Cruz, 2010). Consequently, the potential impacts of climate change, which will drive shifts in the duration and intensity of rainy and dry seasons, might be considerable (Sarma *et al.*, 2005; Brooks, 2009; Dimitriou *et al.*, 2009) and this modification can impose a significant risk to the maintenance of the biological and ecological characteristics of the temporary pond habitat.

1.4 Biological communities in temporary ponds

Biodiversity within temporary ponds is directly linked to the annual supply of seeds and eggs to the soil during periods of desiccation (Ruiz, 2008). When a pond dries out, normally during periods where evapotranspiration is greater than the rainfall (Brooks, 2009), flowers release their seeds, some of which enter the pond's sediment propagule bank (Amami *et al*. 2010; Rhazi *et al*. 2001). A similar mechanism occurs with fauna, where species such as small crustaceans and some invertebrates produce eggs and assume resting stages (diapause) that are buried in the sediment until the next rainy season (Ruiz, 2008; Waterkeyn *et al.*, 2008; Hand *et al.*, 2016). This period is crucial since the biological cycle restricts survival and persistence to species specifically adapted to these conditions (Zacharias and Zamparas, 2010). When favoured precipitation, photoperiod and temperature conditions are met, seeds and eggs start to flourish and hatch out of the sediment and a new cycle begins (Ruiz, 2008; Paes *et al.*, 2016). This phenomenon sets the base for the appearance of life in temporary ponds, thus generating a rich and diverse habitat in which also birds and mammals can rely on for their survival (EU, 2007; NPWS, 2008). These species not only use the pond as a breeding and feeding area (Waterkeyn *et al.*, 2008), but in addition to other mammals, as well as wind, are important to the passive dispersal of invertebrates' eggs and vegetation seeds across the landscape (Vanschoenwinkel *et al.*, 2008; Waterkeyn *et al.*, 2008; Rhazi *et al.*, 2012; Horváth *et al.*, 2016; Pinceel *et al.*, 2016).

Species that inhabit and colonise temporary ponds have a very variable, but often short life cycle. An example of this is the prehistoric tadpole shrimp (*Triops* sp.), which is known to have survived under these conditions for more than 200 million years (Collinson *et al.*, 1995). As an example, in the Iberian Peninsula exists a species of Branchiopod, *Triops vicentinus*, which has a very limited geographic distribution (Machado *et al*. 1999; Grillas *et al*. 2004) confined to no more than twenty temporary ponds in the most southwestern tip of Europe, the Vincentina coast. Some faunal species remain in the same temporary pond throughout their complete life cycle, whereas other more mobile amphibians and invertebrate species (such as dragonflies and beetles) may use more than one breeding site in the same season (Ruiz, 2008; Caramujo *et al.*, 2013). Temporary ponds are a preferred breeding location and feeding ground for some species of amphibians and invertebrates due to the absence of egg-predating permanent fish populations (Brooks, 2000; Grillas *et al.*, 2004; Canha and Pinto-Cruz, 2010). In Ireland and Portugal some species like *Bufo calamita* (Natterjack toad) are facing serious conservation challenges due to habitat loss (Canha and Pinto-Cruz, 2010). In fact, seasonal coastal ponds in Ireland are the preferred habitat for this species to breed (Houston, 2008).

Although some studies of aquatic vegetation (Bagella & Caria, 2012; Biggs *et al*., 2005; DeKeyser *et al*., 2003; Lumbreras *et al*., 2012; Pinto-Cruz, 2010; Rhazi *et al*., 2006), invertebrate and amphibian communities exist (Blackstock *et al.*, 1993; Brooks, 2000;

Biggs *et al.*, 2005; Fonseca *et al.*, 2008), most other categories of biota are relatively poorly known and new species are regularly found (Biggs *et al.*, 2005; Cobelas *et al.*, 2005; Canha and Pinto-Cruz, 2010; Dudgeon, 2010).

1.4.1 Vegetation

As part of a complex habitat structure, aquatic vegetation plays an active role in aquatic ecosystems (Cook, 1974; Carpenter and Lodge, 1986) by increasing habitat complexity in which a wide range of species of plants, aquatic vertebrates and invertebrates, as well as birds and mammals depend direct or indirectly on (Heegaard *et al.*, 2001; Katende, 2004; Ali, Mageed and Heikal, 2007; Ngari *et al.*, 2008). In the aquatic environment, plants are in close contact with the water through their leaves which interact with chemicals and nutrients in solute, as well as through their roots from which they attach to the soil, and hence interact with and influence a wide range of environmental conditions within the aquatic system (Carpenter and Lodge, 1986; Heegaard *et al.*, 2001). By absorbing nutrients and minerals from the water, plants are not only useful in cleaning the water, but also important in providing oxygen to fish and other living organisms, when performing photosynthesis (Cook, 1974; Carpenter and Lodge, 1986).

Aquatic vegetation is divided into submerged, floating-leaved and emergent species. Floating and emergent plants are essential for birds that use them to feed and also for fish that hide and reproduce underneath them (Katende, 2004; Kateyo, 2006; Ngari *et al.*, 2008). Other species, such as insects, invertebrates (Carpenter and Lodge, 1986; Heegaard *et al.*, 2001) and grazers (Carpenter and Lodge, 1986; Bouahim *et al.*, 2010) also depend on the vegetation to a greater or less extent. Therefore, aquatic plants act as a source for food as well as an important shelter and protection from predation for a wide range of wildlife that live, whether in the water or in the surrounding environment (Heegaard *et al*. 2001; Katende 2004;). On the other hand, submerged vegetation is crucial for promoting the quality of the water by uptaking its nutrients, both from the water column as well as from the decomposed material from the sediment (Kateyo, 2006; Ngari *et al.*, 2008), and the main source of refuge and sometimes food for microcrustaceans (Peretyatko *et al.*, 2009).

Aquatic plants are very good bioindicators since most are sessile, providing a better understanding of past changes in environmental conditions that occurred in the water where they were surveyed (Haury, 1996; Dallas *et al.*, 2010). Other important factors for using this type of vegetation for monitoring aquatic ecosystems’ health is the fact that they are widely distributed along different abiotic and biotic conditions and are relatively easy to collect (Dallas *et al.*, 2010; Sousa *et al.*,2011; Sayer *et al.*, 2012).

The occurrence and distribution of aquatic vegetation is driven by a combination of biotic and/or abiotic factors (Sabbatini *et al.*, 1998; Fernnández-Aláez *et al.*, 1999; Murphy *et al.*, 2000; Li *et al.*, 2006; Ngari *et al.*, 2008), which operate at various degrees from microhabitat conditions up to landscape scale. In the water, physico-chemical characteristics such as depth, availability of light, conductivity, pH, alkalinity and nutrients have been widely studied and are usually among the most important factors that affect the spatial distribution and occurrence of aquatic vegetation (Sabbatini *et al.*, 1998; Murphy, 2002; Murphy *et al.*, 2003; Machena, 2010; Sousa *et al.*, 2011) in several types of aquatic ecosystems, along a wide range of latitudes (Chambers *et al.*, 2008). The presence and availability of nutrients is one of the most relevant factors that influence the distribution of macrophytes (Carpenter and Lodge, 1986; Robach *et al.*, 1996) and these are directly influenced by pH and conductivity. Nutrients in the water are related to hydrological conditions and also to sediment characteristics (Bako *et al.*, 2007). Some nutrients, particularly phosphorus and nitrogen, are essential to plants and manipulate the distribution of the species according to their availability (Duarte, 1992; Ngari *et al.*, 2008). However, increased concentration of nutrients may also cause disruptions and increased productivity, creating uncontrolled growth situations (eutrophication) that can consequently cause constraints at several levels, such as impacts on oxygen availability, with negative effects on both fauna and flora (Bako *et al.*, 2007; Ngari *et al.*, 2008). Another pressure on macrophyte distribution and abundance is the presence of alien species (Willis *et al.*, 2007; Oertli, *et al.*, 2009) due to the competitive oppression exerted on the local endemics. Finally, the impacts caused by humans are also relevant to the sustainability of an aquatic ecosystem, as already mentioned above.

Plant species in temporary ponds depend on soil type and humidity conditions, as well as water depth and topography (Ruiz, 2008). Some of them are called amphibious species because they can live in both wet and dry soil conditions (Ruiz, 2008; Camacho *et al.*, 2009; Bagella and Caria, 2012), (e.g. *Mentha* sp., *Ranunculus* sp., *Isoetes* sp. and *Littorella* sp.). The shift from flood to drought phases is important to allow germination when water is available and to produce flowers and release seeds once the temperatures start to rise and the pond begins to dry (Grillas *et al.*, 2004). Usually, vegetation distribution along a pond is concentric, describing a visible zonation with species more adapted to drought located on the outer edge of the circle, followed by species adapted to water environments up till the centre of the basin, where usually full aquatic species occur (Rhazi *et al*. 2001; Camacho *et al*. 2009; Amami *et al*. 2010).

An example of the vegetation zonation of a temporary pond can be seen in Figure 1.3. Aquatic vegetation create the opportunity for a wide diversity of species to live, but particularly vital to invertebrates that live associated to their substrates (Carpenter and Lodge, 1986; Katende, 2004; Ali *et al.*, 2007; Peretyatko *et al.*, 2009).

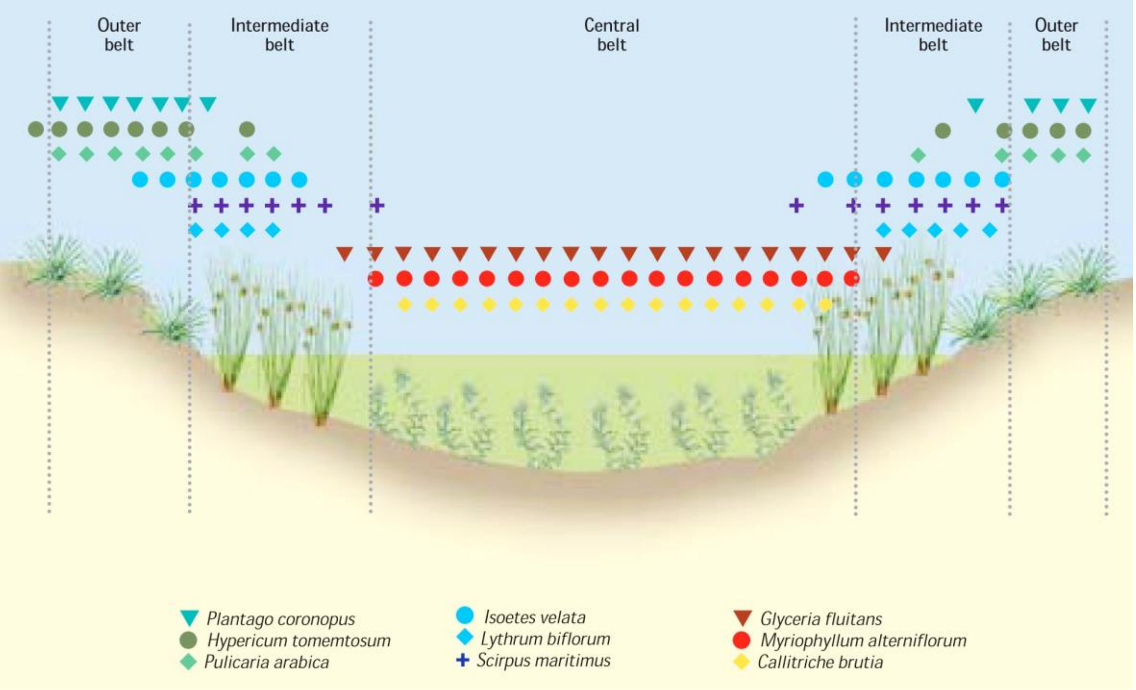


Figure 1.3 Vegetation zonation of a temporary pond in the Mediterranean area. Source: Grillas *et al.* 2004

1.4.2 Cladocera

Microcrustaceans contain many features that make them useful as biological indicators (Boix *et al.*, 2005): they are very rich and dynamic in freshwater systems (Fahd *et al.*, 2000) and easy to collect (Lamotte and Bourlière, 1971); the community structure reacts to trophic status and environmental changes and their high taxonomic resolution allows thorough ecological assessments (Eggermont and Martens, 2011; van den Broeck *et al.*, 2015). Microcrustaceans (Class Crustacea) are the most abundant crustaceans in ponds (Caramujo *et al.*, 2013). They are divided into six major subclasses: Branchiopoda, Remipedia, Cephalocarida, Maxillopoda, Ostracoda and Malacostraca (Witty, 2004; Caramujo *et al.*, 2013). The first subclass, Brachiopoda, contains the Cladocera families like Chydoridae and Daphniidae (the latter commonly recognised as ‘water flea’).

Cladocerans are good bioindicators in temporary ponds because they are very sensitive to aquatic environmental changes (Crosetti and Margaritora, 1987; Caramujo *et al.*, 2013), they have a rapid life-cycle (Fahd *et al.*, 2000) and have developed mechanisms that allow their survival in extreme environmental conditions, such as two reproduction phases, one characterised by parthenogenesis (asexual) and other characterised by production of resting eggs (sexual) (Schön *et al.*, 2009). Physical aspects of the environment directly influence cladoceran populations and the impact of this is seen in a wide range of biogeographical regions (Lynch, 1980; Crosetti and Margaritora, 1987; Alonso, 1991; Sarma *et al.*, 2005). They can be found in all kinds of freshwater environments (Lynch, 1980; Amoros, 1984; Crosetti and Margaritora, 1987), differentiating themselves according to specific habitat types, depending on hydrology, vegetation, salinity and water trophic status (Alonso, 1991; Sarma *et al.*, 2005; Waterkeyn *et al.*, 2008), but nevertheless, very well adapted to temporary ponds (Crosetti and Margaritora, 1987). For this reason, Cladocera was chosen as the main group to be analysed in this study.

Cladocerans include planktonic species (like *Daphnia* spp.) that swim freely in the water column, as well as benthic species (e.g. *Chydorus* spp. and *Simocephalus* spp.) that live on the bottom of the ponds or associated with submerged vegetation (Caramujo *et al.*, 2013). Apart from three marine genera, cladocerans live exclusively in freshwater habitats (Scourfield and Harding, 1941). Cladocerans can be herbivores and detrivores (up to 5 mm long) feeding on phytoplankton and serving as food for larger organisms, thus playing a very important part in the energy transference of the food chain of freshwater ecosystem (Amoros, 1984; Sarma *at al.*, 2005). They use their antennas both as generator of water current to filter suspended nutrients inside their body (fine organic matter, unicellular algae/bacteria), as well as for respiration and swimming (Lynch, 1980; Amoros, 1984; Caramujo and Boavida, 2010). The main predators are vertebrates (fish and amphibious) that prey on large visible sized cladocerans, as well as large invertebrates (copepods and midge larvae) that prefer smaller ones (Vijverberg and Boersma, 1997). *Daphnia* (a large-sized group of species) presence is usually an indicator of the absence of fish populations (Amoros, 1984; Davidson *et al.*, 2007). One of the strategies for the offspring success against mortality is the production of large size descendants that reach maturity at an early stage. Reproduction is predominantly by ameiotic parthenogenesis (Figure 1.4), where eggs are developed into miniature adult females only, morphologically similar to the mother (Scourfield and Harding, 1941; Amoros, 1984). After the release of the young cladocerans, a new set is placed in what is called the brood-chamber, and the life cycle repeats itself. Males are smaller than females and extremely rare in samples, only appearing when environmental conditions are deteriorating (Amoros, 1984), as in the case of a temporary pond environment towards its dry phase (Scourfield and Harding, 1941), or when temperature or daylight shifts (Błędzki and Rybak, 2016; Hand *et al.*, 2016; Paes *et al.*, 2016). When this happens, sexual reproduction occurs and the eggs develop a different shape with a specific protection called *ephippial* (Amoros, 1984). This egg structure remains unhatched (enduring severe environments such as drying and freezing) until the conditions are again favourable (Scourfield and Harding, 1941; Witty, 2004).

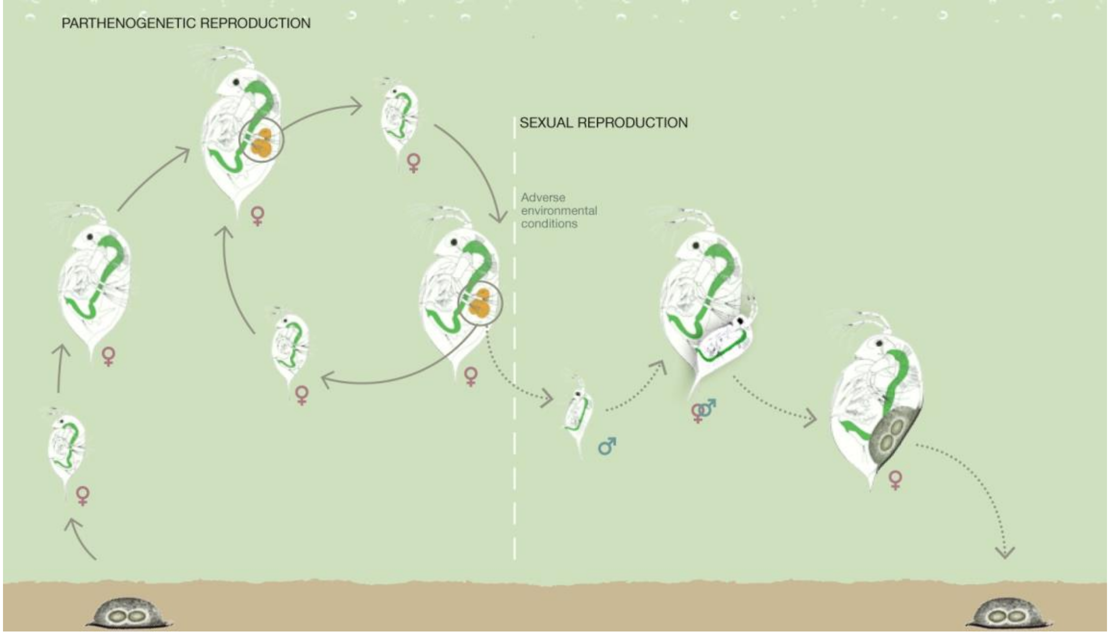


Figure 1.4 Cladocera reproduction cycle in a temporary pond. Source: Caramujo *et al*. 2013

1.5 Aims

This thesis intends to describe and unravel the ecohydrological processes influencing coastal ponds within varying water level regimes at a range of temporal and spatial scales. Cladocera and plant species assemblage and composition together with environmental habitat characteristics and hydrological regime are examined in two study regions: west Donegal, northwest Ireland (Sheskinmore dune system) and southwest Portugal (Malhão dune system and Vila do Bispo in Southwest Alentejo and Vicentina Coast Natural Park). The research focuses on coastal temporary waterbodies (described within the EU Habitats Directive context as Humid Dune Slacks and Mediterranean Temporary Ponds respectively). The reason for including different geographic locations and climates (Temperate and Mediterranean) lies in the need to understand temporary ponds' behaviour according to different hydroregimes and eventually different geomorphological contexts, at the same time that basic features of geographical positioning (Atlantic coastal facing) and intrinsic pond characteristics (seasonally flooded habitats) remain the same. In order to explore these within a framework, a set of different waterbodies will be studied in the two locations. This enables a number of ecological questions to be explored simultaneously, many of which have, until now, remained unaddressed within coastal pond ecology. The main questions to be addressed in the chapters of the thesis are:

- **How are seasonal and climatic controls shaping the species dynamics in coastal ponds? (Chapter 3)**

  The focus of this chapter is to understand the subtleties in the variations in environment and species over time and how the sequence of ecological and hydrological events in the pond takes place, with particular emphasis on climatic forcing. To achieve this, the two largest ponds of the Sheskinmore Nature Reserve, Ireland, were analysed twice a year for two consecutive hydrological years. This chapter aims to identify which factors, external and internal to pond dynamic, are driving seasonal variation in biological communities.

- **How are ecohydrological and physical (topographical) factors influencing cladoceran assemblages at a landscape scale? (Chapter 4)**

  This chapter explores the spatial variation in cladoceran and plant communities across the mosaic of ponds within Sheskinmore Nature Reserve. This is investigated in the context of physical and environmental characteristics to understand the factors that regulate species diversity. This chapter tries to comprehend if cladoceran communities are directly related to water level differences and uses a wide range of hydrological regimes in coastal ponds to:

  - understand how environmental, hydrological and spatial conditions surrounding the ponds affect species distribution in the landscape;
  - determine the relationship between different levels of temporariness and associated cladoceran communities, with a view to classifying different types of hydrological regimes.

- **Is there a geomorphological control on pond hydrological regime? (Chapter 5)**

  This chapter explores the variability and influence of geomorphological setting across sites within two different regions in SW Portugal in order to understand how these waterbodies are shaped and what physical characteristics control their hydrology. By studying the influence of the physical setting (topography, sediment) and the environmental (hydrology and climate), the research will:

  - evaluate the role of precipitation in maintaining the hydroperiod;
  - explain the likely hydromorphological causes for controls on pond ecology.

Chapter 2 - Research Design

2.1 Study area

2.1.1 Sheskinmore Nature Reserve, Ireland

In many of the extensive Irish west coast dune systems, ponds develop within blowouts, depressions formed as a result of erosion and denudation processes, which has lead to distinct isolated pond systems within steep-walled depressions (Fossitt, 2000; Houston, 2008; Delaney *et al.*, 2013). Some of the depressions are classified by the Habitats Directive as 2190 Humid Dune Slacks (Houston, 2008). The conservation status of Humid Dune Slacks in Ireland was assessed as Unfavourable-Inadequate (deteriorating), with an increasing rate of habitat loss since the past 10 years (Delaney *et al.*, 2013), despite harbouring a diverse biological assemblage of species, including a number of rare aquatic plants, amphibians and birds (Grillas *et al.*, 2004; NPWS, 2008; Davy *et al.*, 2010). Threats to this ecosystem are similar to those in SW Portugal, where overgrazing and physical disturbances, mainly related to groundwater extraction, are a major issue (Delaney *et al.*, 2013). These are related to increased human development, poor management of the land and intensive agriculture practices (EPCN, 2008; NPWS, 2008; Amami *et al.*, 2010; Bouahim *et al.*, 2010, 2014).

To date, these water bodies have received little attention in the scientific literature, with very few studies done on coastal sand dune pond ecology in the UK and Ireland, and even less on temporary pond species dynamics and hydrology function (Davy *et al.*, 2006; Smith, 2007; Houston, 2008). Until very recently, there was no record of any literature concerned with temporary ponds in the Donegal coastal region (Fossitt, 2000), but some work by the National Park and Wildlife Service of Ireland (NPWS) has started to emerge (Delaney *et al.*, 2013; NPWS, 2013b, 2015).

In west Donegal a plethora of freshwater ponds occupy the dunes at Sheskinmore Nature Reserve in a range of different local geomorphological contexts (Figure 2.1). Ponds across Sheskinmore lie within Special Areas of Conservation (West of Ardara/Maas Road SAC and Slieve Tooey/Tormore Island/Loughros Beg Bay SAC) designated part of the Sheskinmore Lough Special Protection Area (SPA) and nature reserve (http://sheskinmore.wordpress.com).

Figure 2.1 Samping sites location (Sheskinmore dunes), northwest Ireland

The dune system within the Sheskinmore Nature Reserve is located in a coastal area surrounded by metamorphic bedrock (NPWS, 2015). It is topographically varied and dune slacks are formed within both constructional and erosional depressions in the dunes. These dunes contain a variety of blowouts in which water accumulates to give way to a mosaic of water bodies with different sizes, shapes and hydrological regimes that comprise a rich floristic community (Delaney *et al.*, 2013), as well as rare and endangered species protected under EU legislation (Fossitt, 2000; EPCN, 2008). From an hydrological perspective, the presence of water in dune slacks has an impact on the vegetation communities, and is likely supporting different invertebrate communities within temporary water systems (NPWS, 2015). At this location the evidence of human impacts affects less than 1% (essentially due to walking tracks and occasional litter dumping) and the area was assessed as Favourable (stable) in a recent evaluation by the NPWS (Delaney *et al.*, 2013).

2.1.2 Malhão and Vila do Bispo regions, Portugal

Portuguese sites are located on the southwest coastal plain, along the littoral area of Natural Park of southwest Alentejo and the Vicentina Coast (PNSACV) that extends across 100km long and 5 to 15km wide. Most of the coast is characterised by cliffs reaching up to 150m above sea level. A sedimentary rock layer forms the coastline where sand deposits have settled to form a complexity of dune systems ranging from established solidified dunes to mobile sand structures (Pinto-Cruz, 2010). Two clusters of ponds were selected from the PNSACV (Figure 2.2): Malhão, a coastal dune system in which ponds settle on top of sandy substrate; and Vila do Bispo, a solid sand plateau further south with more clayey substrate. The reason for this is to cover a wide range of potentially different geomorphological and hydrological regimes with varying hydroperiod within the same climatic region.

Malhão is a coastal sandy plain in which several water bodies are scattered across a cliff-top dune system that stretches about 10km north-south, and extends up to 1.8km landward of the shoreline. The ponds within this setting lay approximately 60 m above sea level. They are separated from the agricultural landscape from a stand of pine trees and eucalyptus that form the eastern boundary of the dune system. Hydrologically it is a region characterised by high permeability with no surface runoff, leading to the consideration that the water systems are fed mainly by rainwater (Alves, 1998). Vila do Bispo is located in the most southwestern tip of Portugal, on a plateau positioned over 140m above sea level. Ponds in this location are scattered, situated in isolated areas, next to national walking paths, namely the ‘Rota Vicentina, SW Portugal’ (http://en.rotavicentina.com) and hunting reserves. They sit on top of a sandy sediment underneath which a layer of clay and subsequently schist occur (Canha and Pinto-Cruz, 2010; Pinto-Cruz, 2010), leading to water accumulation due to inability of subsurface drainage.

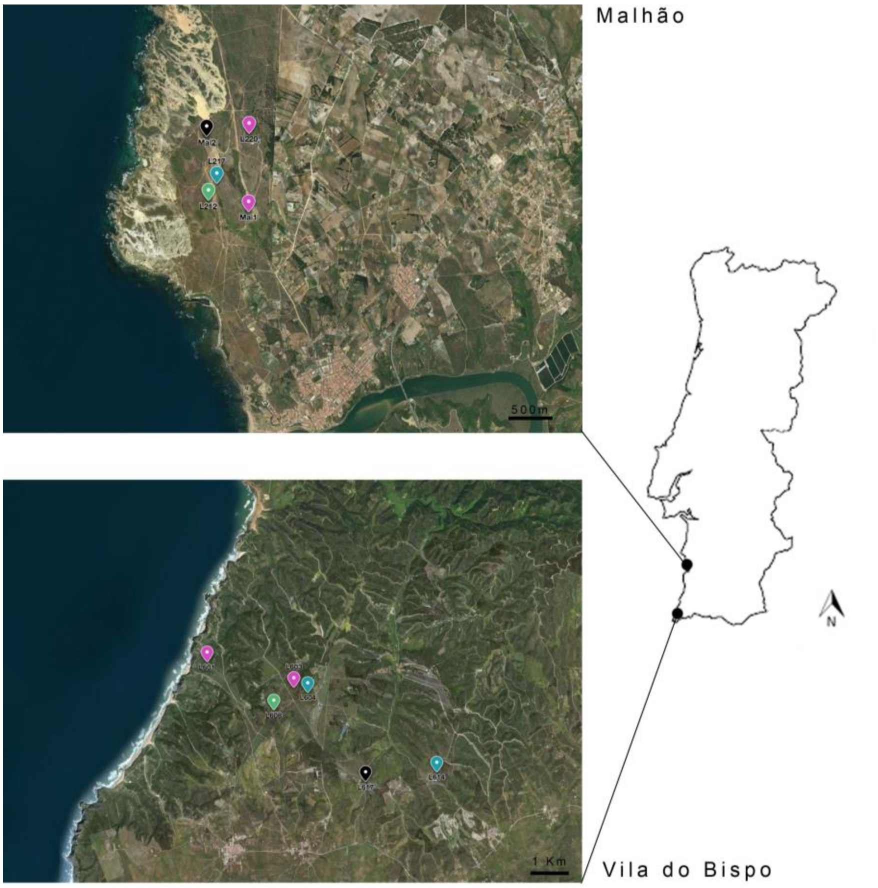


Figure 2.2 Samping sites location (Malhão and Vila do Bispo) on PNSACV, southwest Portugal

A wide number of ponds are located along the southwest coast, classified under the Natura 2000 Network as Site of Community Importance - SCI Costa Sudoeste (Ruiz, 2008; LIFE Charcos, 2013). Some of the ponds in the region however, are considered priority habitats for conservation in Europe, listed under the EC Habitats Directive as Mediterranean Temporary Ponds (Habitat 3170*) (Ruiz, 2008). Although under the protection of the Natural Park (PNSACV) and the Habitats Directive, ponds in this territory still face substantial threat due to increased human development and practices that change the landscape (Beja and Alcazar, 2003; Canha and Pinto-Cruz, 2010; Pinto-Cruz, 2010; LIFE Charcos, 2013). In some areas more than 50% of the temporary ponds have disappeared in the last decade (Canha and Pinto-Cruz, 2010; LIFE Charcos, 2013). However, this area has been monitored and studied thoroughly since 2013 by the on-going project (LIFE12 NAT/PT/000997 Temporary Ponds Conservation in the Southwest Coast of Portugal), aimed at supporting decision-making and management strategies concerned with the restoration of damaged ponds (http://lifecharcos.lpn.pt/en/ctm_intro.php).

A recent shift from traditional non-intensive grazing to intensive regimes (Ruiz, 2008; Pinto-Cruz, 2010) and consequent severe physical disturbance of the local landscape is thought to threaten habitat structure and species diversity in these ponds (Marty, 2005; Ferreira and Beja, 2013), but more information on pond ecology and hydrology is needed in order to inform further consideration of disturbance responses and conservation strategies (Miracle *et al.*, 2010). Other threats to this ecosystem include: alterations to the hydrological regime such as draining, dredging, and modifications to the overall shape of the pond (Beja and Alcazar, 2003; Fonseca *et al.*, 2008; Ruiz, 2008); pond abandonment or refill with waste and pollution resulting from the surrounding agricultural areas (Machado *et al.*, 1999); transformation into permanent ponds for irrigation purposes (LIFE Charcos, 2013); and invasive species either terrestrial such as *Acacia* sp. or aquatic like *Azolla* sp. (Canha and Pinto-Cruz, 2010). In spite of these facts, this region covers a wide variety of hydrological, water chemistry and soil characteristics (Pinto-Cruz *et al.*, 2011), with ideal climatic, environmental and edaphic (influenced by the soil) conditions that promote the existence of this type of freshwater habitat (Canha and Pinto-Cruz, 2010; Pinto-Cruz *et al.*, 2011).

2.2 Sampling strategy

This PhD employs a multidisciplinary approach to facilitate an integrated analysis and examination of temporary ponds. The research focuses on temporary ponds located along a latitudinal gradient (northwest – southwest Europe) along the eastern margin of the North Atlantic Ocean, encompassing sites in northwest Ireland and southwest Portugal (Figure 2.3). The chosen study areas host a large number of temporary ponds and each site is considered of high importance for conservation (Houston, 2008; Ruiz, 2008; Pinto-Cruz, 2010). Although these temporary ponds vary biogeographically and climatically, they have common ecohydrological characteristics; specifically they experience phases of flooding and drying. The sampling strategy was designed to enable comparable applications in the study areas and to maximise the key structural drivers that are likely influencing the hydrology and ecology of the ponds.

Figure 2.3 Location of sampling sites in Ireland and Portugal

The research includes a total of 35 ponds, 24 in Ireland and 11 in Portugal, selected on the grounds of having known different hydrological conditions, with a diversified cover of plant communities, reflecting the range of environmental conditions known to occur across each area (Table 2.1). The choice intended to obtain a representative sample at each geographic region, covering dune slacks in Ireland and Mediterranean Temporary Ponds in a dune system in Malhão and consolidated sand region at Vila do Bispo. The 24 dune ponds selected in Ireland cover all the dune scape and these ranged from temporary to permanent. The 11 Portuguese ponds were chosen to incorporate a diversity of sediment structures (dune sand at Malhão and more compacted sand bedding at Vila do Bispo), to address the different substrate controls on hydrological forcing and ecology on the temporary pond ecosystem. A total of 8 piezometers were placed in both locations (6 in Portugal and 2 in Ireland) to monitor in-pond hydrology.

Table 2.1 Sampled ponds' codes and GPS location

Ireland	Latitude	Longitude	Portugal	Latitude	Longitude
b1	54°48'11.7"N	8°29'35.5"W	mal1	37°44'54.1"N	8°47'34.0"W
b2	54°48'20.1"N	8°29'38.1"W	mal2	37°45'19.0"N	8°47'53.6"W
c4	54°48'25.8"N	8°29'09.1"W	L212	37°45'20.8"N	8°47'32.7"W
ml1	54°48'41.3"N	8°29'29.8"W	L217	37°45'01.2"N	8°47'50.5"W
ml2	54°48'41.6"N	8°29'26.0"W	L220	37°44'54.1"N	8°47'52.6"W
r1	54°48'13.3"N	8°29'08.6"W	L601	37°07'43.4"N	8°55'09.2"W
r2	54°48'14.8"N	8°29'08.5"W	L603	37°07'18.7"N	8°53'35.2"W
r3	54°48'17.4"N	8°29'13.6"W	L604	37°07'16.5"N	8°53'19.7"W
d2	54°48'31.1"N	8°29'25.1"W	L608	37°07'01.2"N	8°53'57.5"W
d3	54°48'31.3"N	8°29'20.2"W	L614	37°06'02.9"N	8°50'58.8"W
d4	54°48'25.6"N	8°29'24.9"W	L617	37°05'58.4"N	8°52'12.5"W
d5	54°48'23.6"N	8°29'33.6"W			
d7	54°48'14.8"N	8°29'36.4"W			
d8	54°48'14.7"N	8°29'36.8"W			
d9	54°48'13.4"N	8°29'36.3"W			
d10	54°48'10.8"N	8°29'36.4"W			
d11	54°48'11.8"N	8°29'34.2"W			
d13	54°48'12.3"N	8°29'32.4"W			
d14	54°48'14.8"N	8°29'13.6"W			
d15	54°48'15.2"N	8°29'13.6"W			
d21	54°48'39.2"N	8°29'51.8"W			
d23	54°48'28.5"N	8°29'16.5"W			
d24	54°48'28.9"N	8°29'14.2"W			
d25	54°48'29.1"N	8°29'13.4"W			

The ponds vary in size, from a few metres to 10s metres wide. Although generally shallow in both regions, pond depth (during the wet phase) varies between 30cm to < 1.5m depth, and more detailed information on individual characteristics will be given throughout the chapters. The sediment coverage of the ponds contains patches of vegetation that develop rapidly when conditions are favourable. These can reach very high densities of *Agrostis sp.* in Portugal, and mosses in Irish ponds where they frequently cover the whole ground.

Two seasonal surveys were undertaken in each region, covering early and late stages of development (winter and spring for Mediterranean ponds and early spring and early summer in Temperate ponds) (Figure 2.4). Hydrologically, these time frames approximate to the maximum flood extent and mid-way through the drying phase, enabling sampling of species that have early and late hatching and flowering timings (Amami *et al.*, 2010).

Biodiversity of ponds was analysed and characterised according to different hydrological regimes. Linking the physical and the biological variables of the ponds, the hydrological dynamics were recorded, in which data on water levels was monitored for the duration of the PhD. The integration of these variables expects to improve the knowledge of temporary ponds at a local individual, local and regional scale, not only by understanding the relationship between biological communities and hydrology, but also by shedding light onto the specificities of the controlling factors of hydrology dynamics for conservation and management practices.

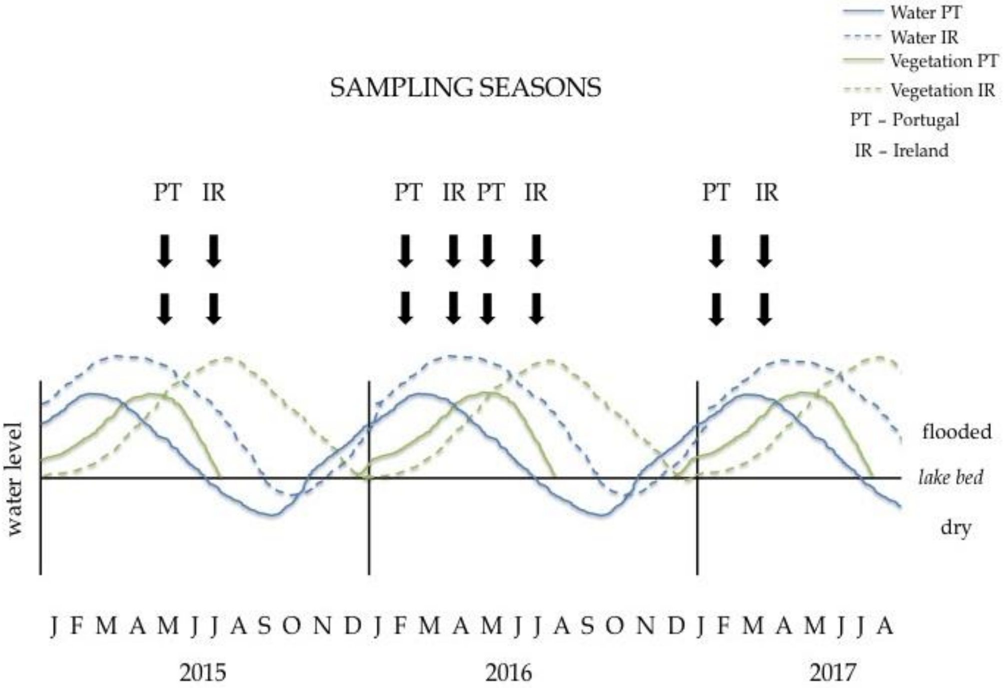


Figure 2.4 Sampling seasons within basic hydrology of flooding and drying and vegetation growth and decay in temporary ponds in Portugal and Ireland.

2.3 Monitoring and data collection

2.3.1 Hydrology

Hydrological regime was monitored in 8 of the 35 ponds (see Table 2.1). For this purpose, 2 piezometers were installed in Ireland and 6 in Portugal with two additional baraloggers (one at each region) to compensate for local atmospheric pressure. Where possible, piezometers were established in the deepest part of the pond depressions. However, since in both regions ponds are located close to walking paths, an important criterion was to hide them as much as possible to avoid violation of the equipment. A hand auger was used to drill a core in the sediment bed. The depth at which the piezometers were laid was dependent on the state of moisture of the sediment, water table height and depth of bedrock, but all were installed during the dry season. The piezometers comprised a vertically installed PVC tube (diameter 5cm) within which an autonomous pressure sensor was deployed (suspended) at depth (i.e. below the water table). The PVC tube was pre-drilled with holes to allow free water movement, and wrapped in weed control membrane to prevent siltation inside the tube. The pressure sensor was programmed to monitor total pressure and temperature every 15 minutes for the duration of the study. Pond bed and piezometer elevations were measured using a dGPS (Leica GS15) and offsets between ground, tubing and sensor captured with tape measure.

2.3.2 Climatic data

Weather data were obtained from local meteorological stations, located as close as possible from the sampling sites. In Ireland, a Davis Vantage Pro2 weather station was installed by UCL in June 2012, located within the Sheskinmore Lough Nature Reserve, and hence just 1-2km from the Magheramore-Sheskinmore ponds. The weather station measures a full suite of climate variables at an hourly interval (Table 2.2). In Portugal, climatic information for Malhão sites was gathered from Sines meteorological station (located 28km from the sites) and Vila do Bispo sites was obtained from Aljezur weather station (33km distance). Main values being measured are temperature, precipitation, relative humidity, wind direction and speed and solar radiation.

Table 2.2 Installed sensors and metric specification ranges recorded by the Sheskinmore Davis weather station.

Installed sensors	
Wind speed	0.5 - 89 ms^{-1} [± 1 ms^{-1}]
Wind direction	0 - 360 ° [± 3 °]
Temperature	-40 - +65 °C [± 0.5 °C]
Relative humidity	1 - 100 % [± 3 %]
Rainfall	0 - 999.8 mm dy^{-1} [± 0.2 mm]
Solar radiation	0 - 1800 Wm^{-2} [± 5 %]
Additional derived measures	
Windchill	-79 - +57 °C [± 1 °C]
Dew point	-76 - +54 °C [± 1.5 °C]
Heat index	-40 - +74 °C [± 1.5 °C]
Evapotranspiration	0 832.1 mm dy^{-1} [±0.25 mm]

2.3.3 Cladocera

There are a number of techniques used to sample cladoceran communities varying according to the specific characteristics of the site and purpose of the work (Vuorinen, 1993; Serrano and Fahd, 2005; Caramujo and Boavida, 2010). All of them however, consist on the principle of filtering a known volume of water through a filter/mesh (Lamotte and Bourlière, 1971; Amoros, 1984), followed by preservation of the collected sample in ethanol or other similar preserving liquid.

In this work samples were collected using a tube-sampler with Ø 7.5cm; 60-100cm. Due to the shallow nature of the majority of the pond habitats, cladoceran samples from the margins and dense vegetation patches were collected using a 5L container. Both the tube length and volume collected were recorded, and water was filtered through a 53µm mesh trawling plankton net. The amount of tube/Litres sampled was proportional to the size (area) of the pond and captured the whole variety of microhabitats and depths present within each pond (margin and open water). Total water volume sampled was added together. For each site, the zooplankton was combined into one single composite sample in a bottle and immediately preserved with 95% ethyl alcohol. To avoid contamination between sites, the zooplankton net was rinsed with pond water three times after each sampling and before any collection with water from the new site.

Samples were later examined in the laboratory, most without the need of dividing the main sample (usually done when samples are dense). From the main sample, 5ml at a time was placed in a Bogorov counting chamber and analysed using a stereomicroscope (10x) until 500 individuals in total or 200 individuals from one species were counted. All cladocerans were picked and counted under a compound microscope (100x) and identified to species using temperate (Scourfield and Harding, 1941; Amoros, 1984; Flobner, 2000) and Mediterranean (Alonso, 1985a, 1996) ID guides, as well as expertise help when appropriate.. Final count numbers were adjusted to provide number of individuals per litre.

2.3.4 Aquatic vegetation

Only vegetation that was rooted in water was surveyed to agree with a direct comparison of zooplankton communities' composition from the same site. Vegetation abundance measures were taken by visual observation using the DAFOR scale: Dominant (5), Abundant (4), Frequent (3), Occasional (2), and Rare (1). This method is used when small areas are studied as a whole (Kent and Coker, 1992; Sayer *et al.*, 2012) and was therefore the most frequently used in the study, as it described the aquatic vegetation composition of the pond, including rare and localised species, in combination with the zooplankton species composition assemblage in different years.

Plant samples were collected within reach, by wading along the perimeter of the pond. If inaccessible, aquatic plants were sampled using a grapnel. When local identification was not possible, plant specimens were collected, dried and pressed for later ID using appropriate keys (Rose 1989; Rose 2006; www.brc.ac.uk; www.flora-on.pt) and expert help was sought when required.

2.3.5 Environmental variables

Water samples were collected at each pond to measure an assortment of chemical parameters. Dissolved oxygen concentration (mg/L), conductivity (µs/cm), temperature and pH were measured using Hach 1001 Intellical probes and a HQ30d Hach meter. For each record, three separate readings were made to account for the average value within each site. Temperature was always read from the pH probe to be consistent throughout the study. Total alkalinity (mg/L $CaCO_3$) was measured on site using the Hach test kit model AL-DT and Hach method 8203. Titration of 1.6N H_2SO_4 was performed to a single endpoint of pH 4.5 using Bromocresol-green Methyl-red indicator as described in Hach (2015). Chlorophyll *a* was collected using a 1.2μm pore size GF/C filter paper in a 500ml Nalgene filter holder with receiver using a hand operated vacuum pump (DWQ, 2011). When enough water had passed through the filter to stain the paper, the volume was recorded. The stained filter was then folded into aluminium foil and frozen for later analysis. Water samples were also gathered in 60ml acid-washed bottles. Both filtered and unfiltered samples were obtained to determine total phosphorus (TP), soluble reactive phosphorus (SRP) and nitrate/nitrite (NO_x^-) later in the laboratory. Bottles were rinsed up to three times before filled (directly with pond water for the unfiltered and with filtered water for the other) and frozen back in the field station.

2.3.6 Laboratory analysis

TP, SRP and nitrate/nitrite (NO_x^-) were determined in the laboratory using the filtered and unfiltered water samples, which were analysed immediately after thawing to avoid contamination from bacteria. SRP analysis will provide information on what is soluble and available to plants in the water at that point in time (Johnson, 2006). TP analysis will tell how much organic and inorganic forms of phosphorus exists in the aquatic system, including ortho-phosphates, complex condensed phosphates and organic phosphates (Johnson, 2006). Nitrates analysis reveals the amount of ions that exist in the water solution (D’Elia *et al.*, 1977).

SRP was determined by the standardised method described in American Public Health Association (1999), using the filtered water sample. To determine TP, unfiltered water samples were digested (hydrolysed) in a CEM Mars Xpress microwave after the persulfate method described in D’Elia *et al*. (1977). This digestion facilitates the colourimetrical reading by breaking down strong chemical bonds in the particles. After this, the same protocol for the SRP analysis was executed. For the nitrogen analysis, spongy cadmium was used as a catalyst for the reduction to nitrite (Elliott and Porter, 1971). A Hach CAMLABDR/4000U spectrophotometer with a 1 cm light path was used to analyse the SRP, TP and Nitrate samples against replicate blanks to compare the results alongside a known value calibrated curve, at a wavelength of 885nm for TP and SRP and 543 nm for nitrates.

Chlorophyll *a* samples were analysed using standard procedures as described in DWQ (2011). Samples were transferred into a mortar with a pinch of sand and 1ml of acetone and grinded into a paste. The mixture was washed into a centrifuge tube and made up to 10ml. All samples were centrifuged for 10 minutes at high speed and the liquid transferred to a 1cm spectrophotometer cell using a Pasteur pipette. The absorbance was measured against an acetone blank at 750nm, 663nm, 480nm, 430nm, and 410nm on a Hach CAMLAB DR/4000U.

2.3.7 Statistical analysis

Statistical analyses were performed using the R 3.1.1 software (R Core Team, 2014) with complementary use of PAST (Hammer *et al.*, 2001) to assess normality and correlation between variables, as well as value scores and graphical representation of ordinations. Environmental and biological variables were tested for normality using Shapiro-Wilk test and logarithmic or square root transformation of the data was performed where required. Environmental variables, including water chemistry and hydrological data were standardised through linear transformation of the data with Z-scores due to high variability within the variables analysed. This was undertaken using the following formula:

$$Zx = \frac{Xi - X}{Sx}$$

where Zx is the standardised score, $Xi\text{-}X$ the mean and Sx the standard deviation from all scores. Even after log transformation, some of the variables were still non-normal and non-parametric tests were used thereafter. Correlations between variables were performed using a Pearson correlation index when data were normally distributed and the Spearman's correlation test when they were not. Kruskal-Wallis non-parametric tests or ANOVA (if normal) were performed to assess the difference between variables and a level of $p<0.05$ used to determine the significance of the variance. Ordinations were performed with Principal Component Analysis (PCA) using the vegan package in R (Oksanen, 2015) and also using non-metric multidimensional scaling (NMDS) ordination to explore similarities among biological samples (Kruskal, 1964).

Shannon diversity index (Colwell, 1988) was chosen as the most appropriate measure according to the type of biological data collected, for being more sensitive to evenness than to species richness alone. The Shannon diversity was generated using PAST3 software (Hammer *et al.*, 2001):

$$H = -\sum_{i}^{S} pi \ln pi$$

where i = individual species; pi = the proportion of species i; and S = total number of species in the community.

Chapter 3 - Seasonal and climatic influences on dune slack ecohydrology

3.1 Introduction

Dune slacks and dune systems are present throughout the coast of Ireland. On the west coast the influence of strong onshore winds promotes a range of dune morphologies due to erosion, shaping the dunes in varied different ways (Mckenzie & Cooper 2001; Barrett-Mold & Burningham 2010). Dune formation in western Ireland is still on-going but in most cases it involves the reworking of existing sediments, due to a limited supply of new sediment material. In much of the west Irish coastal dunes, which accounts for around 20% of the total coastline (Power *et al.*, 2000), aquatic systems occur in lulls between dune ridges, but also develop within blowouts.

There is often a clear distinction between the aquatic environments of inter-dune ridges i.e. classic 'slacks' and those found within blowouts, which due to being more topographically constrained, tend to be hydrologically isolated from each other. The vast majority of Irish coastal dune wet depressions are classified by the EU Habitats Directive as humid dune slacks (HDS; habitat code 2190) (Houston, 2008). Understanding the factors that control the hydrological regime is essential for assessing likely future impacts on the ecosystem functioning and species composition and diversity in dunes slack systems (Robins and Wilson 2017).

Anthropogenic activities have had significant impacts on the dune systems over recent centuries with the development of machair (coastal grassy plain strongly associated with agriculture), grazing and rabbit management, each exerting localised controls on geomorphological processes, vegetation growth and species occurrence (Fossitt, 2000; Mckenzie and Cooper, 2001; NPWS, 2008). It is thought that the near-future climate will drive shifts in water availability within dune systems through the lowering of the groundwater levels (Clarke and Na Ayutthaya, 2010; Curreli *et al.*, 2013; Rhymes *et al.*, 2016).

The hydrological regime (water permanence timings, depth, frequency and seasonality) of temporary freshwater systems plays a crucial role in shaping the dynamics of the different biological communities that inhabit them (Brooks, 2004, 2005; Bauder, 2005; Sim *et al.*, 2013; Camacho *et al.*, 2016). In dune slacks, this leads to phases of drying in summer and flooding in the winter, regulated by water-table fluctuations within the surrounding sand dune system (Davy *et al.*, 2006), and this has a major influence on biological assembly species, by influencing their life cycles (Bauder, 2005; NPWS, 2008; Ruiz, 2008).

The community of aquatic organisms inhabiting dune wetland environments, similar to other temporary ponds, includes many species groups of potential use as biological indicators, including zooplankton and aquatic plants (as described by Boix *et al.* 2005). The crustacean zooplankton (Cladocera and Copepods) are a dynamic and species-rich group in freshwater systems (Fahd *et al.*, 2000). They are easy to sample (Lamotte & Bourlière 1971) and their community structure reacts to trophic status and environmental changes allowing thorough ecological assessments (Eggermont and Martens 2011; van den Broeck *et al*. 2015). Within the zooplankton community, Cladocera species are very sensitive to aquatic environmental changes (Crosetti & Margaritora 1987; Caramujo *et al*. 2013) and are especially well adapted to temporary ponds. Environmental aspects such as hydroperiod (length of the water phase), salinity, biotic and abiotic factors, pond morphology, water chemistry, temperature and overall climatic conditions, directly influence cladoceran populations as seen in studies undertaken in a wide range of biogeographical regions (Crosetti & Margaritora 1987; Alonso 1991; Sarma *et al*. 2005).

Unlike the cladocerans that undergo rapid seasonal change (Crosetti and Margaritora, 1987; Vandekerkhove *et al.*, 2005), vegetation needs to experience prolonged periods of wet or dry to drive community variation. In dune slacks, aquatic vegetation follows patterns of zonation within the waterbody, where species occupy different positions along gradients in relation to water availability and hydroregime (Blackstock *et al.*, 1993; Curreli *et al.*, 2013). Dune slacks are colonised by specialised plant species and in many cases support rare and protected species such as Natterjack toad (*Bufo calamita*), as well as important plant species such as stoneworts (Characeae) and a range of rare mosses and liverworts, including *Petalophyllum ralfsii* (petalwort) and several species of *Bryum* (thread mosses) (Houston, 2008; Stratford *et al.*, 2013), creating a unique biodiversity and important habitat within the wider dune landscape (Jones *et al.*, 2011).

Seasonal and inter-annual variability of biological communities in temporary freshwater bodies as a response to hydroregime has been studied broadly (Mengestou and Fernando, 1991; Brooks, 2000; Creek *et al.*, 2003; Sahuquillo and Miracle, 2010), but research on temporal changes to the inundation of ponds in response to weather variations is lacking (Jeffries, 2016). In the dune slacks at Sheskinmore, Ireland, groundwater variability seen year to year is likely imposing changes on aquatic biological communities and in this study, hydrological influences on seasonal cladoceran community composition is explored for the first time. The aim of this chapter is to assess the impact of hydrological seasonality on cladoceran community dynamics for two dune slacks in western Ireland (Sheskinmore Nature Reserve, Co. Donegal). It describes seasonal and inter-annual variability in hydrology and weather, with a particular consideration of the influences of these on Cadocera. Furthermore, it aims to increase awareness of the importance of water dependent habitats in dune systems as biodiversity pools, through an integrated analysis of ecology, hydrology and climatology. Three questions are explored: 1 – Is hydroperiod directly influenced by climate variability at Sheskinmore dune slacks?; 2 – Is hydrology imposing a stronger control on cladoceran species than other factors (e.g. water chemistry) within the same dune system?; 3 – How are plant species framing the Cladocera habitat?

3.2 Site description and methodology

In west Donegal, freshwater slacks occupy the dunes within the Sheskinmore Nature Reserve. Co. Donegal accommodates HDS, but also only sites where the extremely rare ‘Decalcified Empetrum Dunes’ (EU Habitat code 2140) and ‘Decalcified Dune Heath’ (2150) habitats occur (Delaney *et al.*, 2013). The Tramore-Magheramore dune system at Sheskinmore is located on the north margin of the Loughros More estuary. The dunes comprise a range of local geomorphological contexts, set between two rocky headlands, but also forming a large barrier that obstructs a small valley, leading to the development of the large, shallow freshwater lake, Sheskinmore Lough (Burningham, 2008). The dune system is protected within a Special Area of Conservation (West of Ardara/Maas Road SAC and Slieve Tooey/Tormore Island/Loughros Beg Bay SAC) and Special Protection Area (Sheskinmore SPA) (NPWS, 2015). The site is topographically varied and slacks are formed within both constructional and erosional depressions in the dunes, in troughs between successive dune ridges, and also within blowouts. The underlying bedrock comprises a metamorphic granodiorite and pelite fringe associated with the Ardara pluton (Burningham, 1999). Variations in geomorphological context, depression size, shape and vertical position, and subsurface structural geology have led to the development of a variation of hydrological regimes and wetland types. Permanent ponds, temporary waterbodies, and wetlands comprising no open-water, are all present within the dune system.

To the rear of the westerly Tramore dunes lay two of the largest blowouts and dune slack systems at Sheskinmore named c4 and ml2 (Figure 3.1). C4 blowout has a wide, flat bed (c. 120m x 85m) where topography varies by less than 1m. The rim of the blowout rises up to 17m above this and the lowest point of the slack is at 6.75m OD (Ordnance Datum, c. mean sea level); mean high water springs is locally c. 2m OD, and the slack is therefore well above the tidal frame. Ml2 has a more rounded shape (c. 90m x 75m), and the rim rises up to 19m above the bed; the lowest measured elevation of the slack is 7.85m OD. The shallow bed topography means that the shift between dry, damp and flooded phases can occur with just a small change in the water table. At c4, when the water depth exceeds 1.4m (at the deepest point), the slack overtops the low topography in a narrow neck along the south rim, and connects to a series of slacks to the south. This connection does not arise every flood season, but does present a physical threshold on the maximum water depth that can be achieved. Similarly, ml2 also connects to other slacks and ponds to the south and southeast when flooded to a depth of more than 1.5m (above the deepest point). Blowouts within this, and other dune systems in northwest Ireland, are forced by the predominately southerly and southwesterly winds. A consequence of this is that they comprise steep and tall rims to the north and east, and low rims to the south/southwest. The low rim that acts as a physical limit to the depth of flooding and overflow connection to adjacent low areas on the south side of these blowouts is hence associated with their geomorphological - wind erosion - origins.

Figure 3.1 Location of c4 and ml2 blowout ponds within the Tramore-Magheramore dune system at Sheskinmore.

Several other slacks in the dune system have comparable geomorphological properties, but these two particular slacks are the largest ones in the dune system and were therefore chosen to assess and address how hydrological dynamics are shaping and influencing the ecological habitats within the two largest seasonal freshwater systems. In addition to this, slack c4 has the longest monitoring record having been incorporated into a broader study of the ecohydrology of Sheskinmore Lough to the east of the dunes (Gardner, 2016).

Figure 3.2 Flooded condition within c4 slack in June 2016, from the northeast rim looking southwest. The rim on the north side varies between 14 and 24m above mean sea level; the bed is around 7m above mean sea level and the south rim is around 8-10m OD.

Figure 3.3 Flooded condition within ml2 slack in May 2016, from the west rim looking east. The rim on the north side varies between 17 and 28m above mean sea level; the bed is around 8m above mean sea level and the south rim is around 9-12m OD.

3.2.1 Site monitoring

As mentioned, the research presented here draws from a wider study of ecohydrology within the Sheskinmore Nature Reserve incorporating a range of surveying and monitoring undertaken over a period of 6 years. To examine the hydrological regime of the slacks, an In-Situ Rugged TROLL Data Logger was installed in the deeper part of the slack depression, during dry conditions in summer 2012 (Gardner, 2016) for c4 and in September 2015 for ml2, using the procedure described in Chapter 2 - . Dune topography, slack bed and piezometer elevations were surveyed using a dGPS (Leica GS15). Positions were referenced to Irish Grid and elevations to mean sea level (Ordnance Datum Malin). A Davis Vantage Pro2 weather station was also installed in summer 2012, with detailed information described also in Chapter 2 - (section 2.3.2).

Seasonal surveys of water chemistry, cladocerans and plants were undertaken from summer 2015 to winter 2017, covering two hydrological years and four singular sampling events: June 2015 (which will be referred to here as S15), March 2016 (W16), June 2016 (S16) and April 2017 (W17). Survey timing approximated the maximum flood extent (late winter) and mid-way through the drying phase (early summer) (see Figure 2.4 in Chapter 2 -), enabling sampling of species with different life cycles and hatching or flowering times (Amami *et al.*, 2010). Many studies centre themselves on large areas, with surveys covering a wide range of ponds within the landscape (Sahuquillo and Miracle, 2010; Florencio *et al.*, 2014), but surveys of one or a few ponds embracing multiple seasons provide an accurate assessment of species richness and diversity (Fernnández-Aláez *et al.*, 1999; Fahd *et al.*, 2007). The present study aims at filling this gap on the Irish coastal pond ecology, by looking in detail at the two largest dune slacks in the Sheskinmore Nature Reserve, over a 2-year seasonal monitoring.

3.2.2 Data analysis

Normality distribution of the data was tested using a Shapiro-Wilk test. Environmental variables, including water chemistry and hydrological data were standardised through linear transformation due to high variability within the variables analysed. Following this, analysis of variance was applied to test whether cladoceran abundance means differed between each season using Kruskal – Wallis tests and applying a significance of $p<0.05$. All exploratory data analysis was undertaken using PAST software (Hammer *et al.*, 2001). Shannon diversity (H) index and equitability (as a measure of evenness with which individuals are divided among the taxa present) based on the same index were also calculated (Hammer *et al*., 2006).

3.3 Results

3.3.1 Groundwater and slack climatological dynamics

C4 and ml2 slacks are topographically varied within, containing lower areas where water can persist (NE side of c4 and NW side of ml2) and higher patches where dryer conditions prevail when water table lowers (south side of both slacks). During the survey period of this study the hydrological seasons did not follow the ‘usual’ early spring-flooded, early summer-drying trend. The flood extent and mean depth for each survey is shown in Figure 3.4, which displays some evidence of water table lowering during the S16 survey, compared to the maximum extent of flooding. The winter conditions, however, show major contrasting differences; sampling corresponded to ‘fully flooded’, high water table conditions in W16, and the equivalent fortnight in W17 exhibited very reduced water levels for both sites.

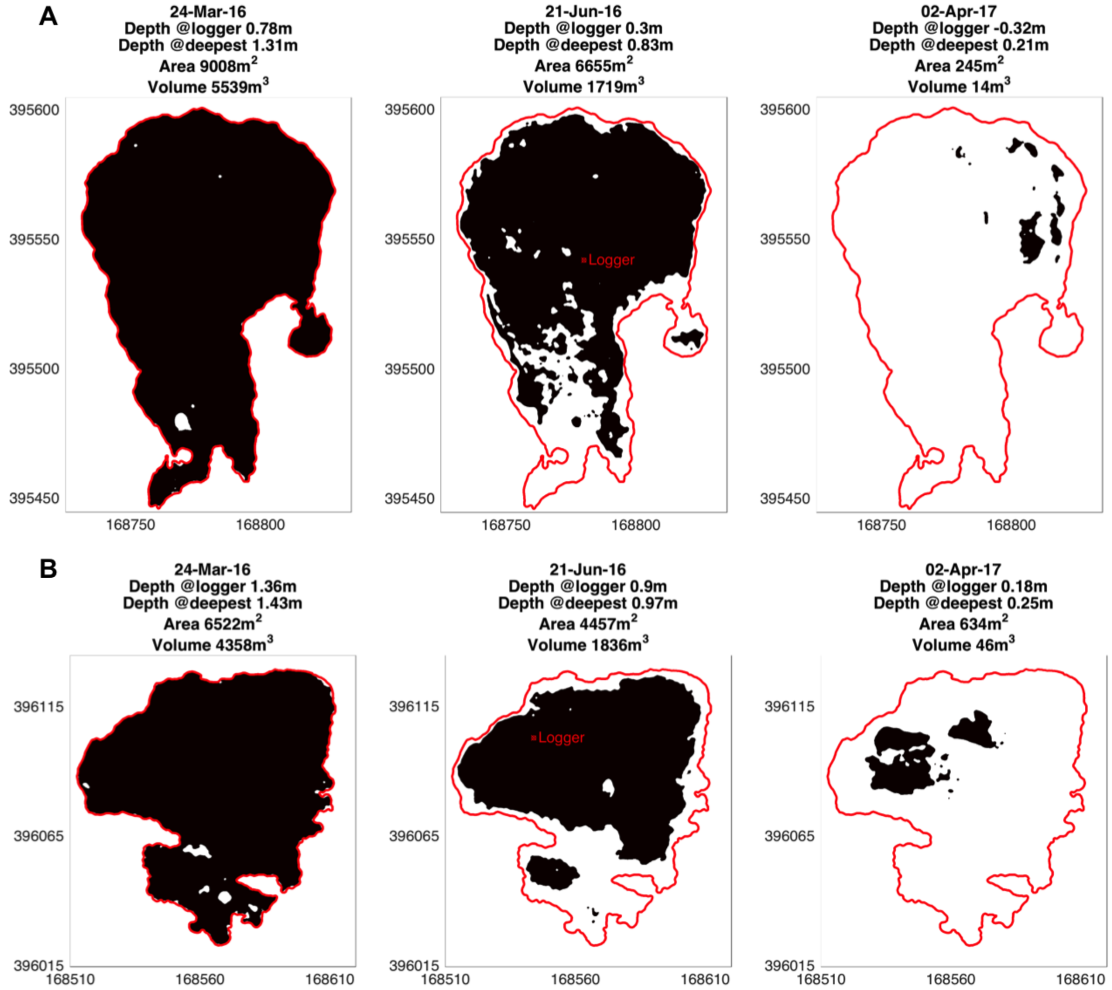


Figure 3.4 Average conditions of depth, area and volume of c4 (A) and ml2 (B) for the 28 days preceding each survey date. The outer line (red) shows the maximum recorded extent of the flooded area during the monitoring period.

The impacts seen on flooding extent of the slacks during W17 could be explained by a lack of rainfall preceding this period in addition to high evapotranspiration values. In fact monthly precipitation sums during the Oct 2016 - Sep 2017 were significantly lower than the year before or to date (Figure 3.5); furthermore, monthly evapotranspiration sums during 2016-17 were higher than the other years monitored.

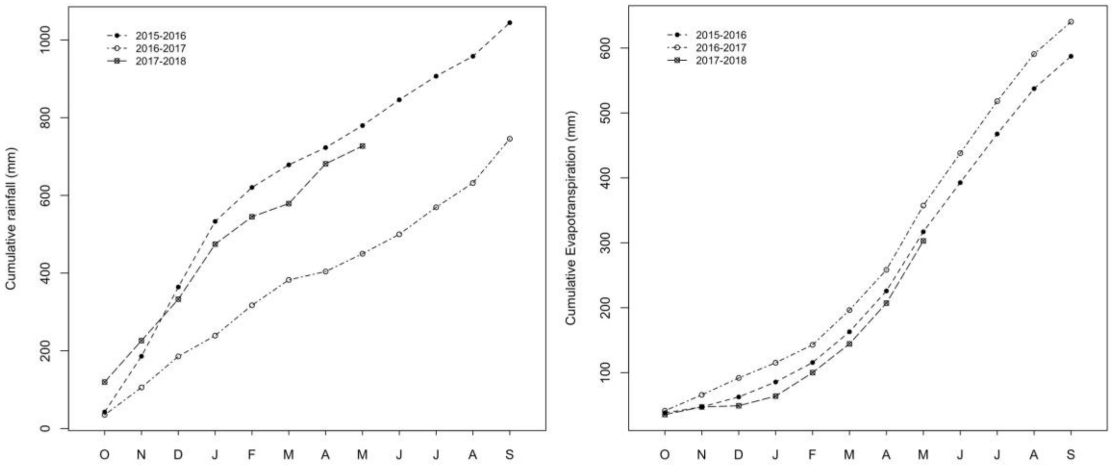


Figure 3.5 Cumulative rainfall and evapotranspiration showing the monthly average for each of the sampling years individually

Response of water temperature at both slacks is consistent with monthly and yearly oscillations for air temperature (Figure 3.6). At ml2 the increased water temperature in relation to c4 is due to the placement of the logger, measuring temperature sitting at the pond bed, whereas at c4, the logger is installed at a depth of 64cm below the bed of the slack, revealing less thermal amplitude. Nevertheless, the outside temperature is directly coordinated with water table temperature at seasonal and inter-annual periods, with average minimums of 5ºC at ml2 and 7ºC at c4 and maximums of 17ºC at ml2 and 14ºC at c4. Average air temperature ranges from 5ºC to 15ºC.

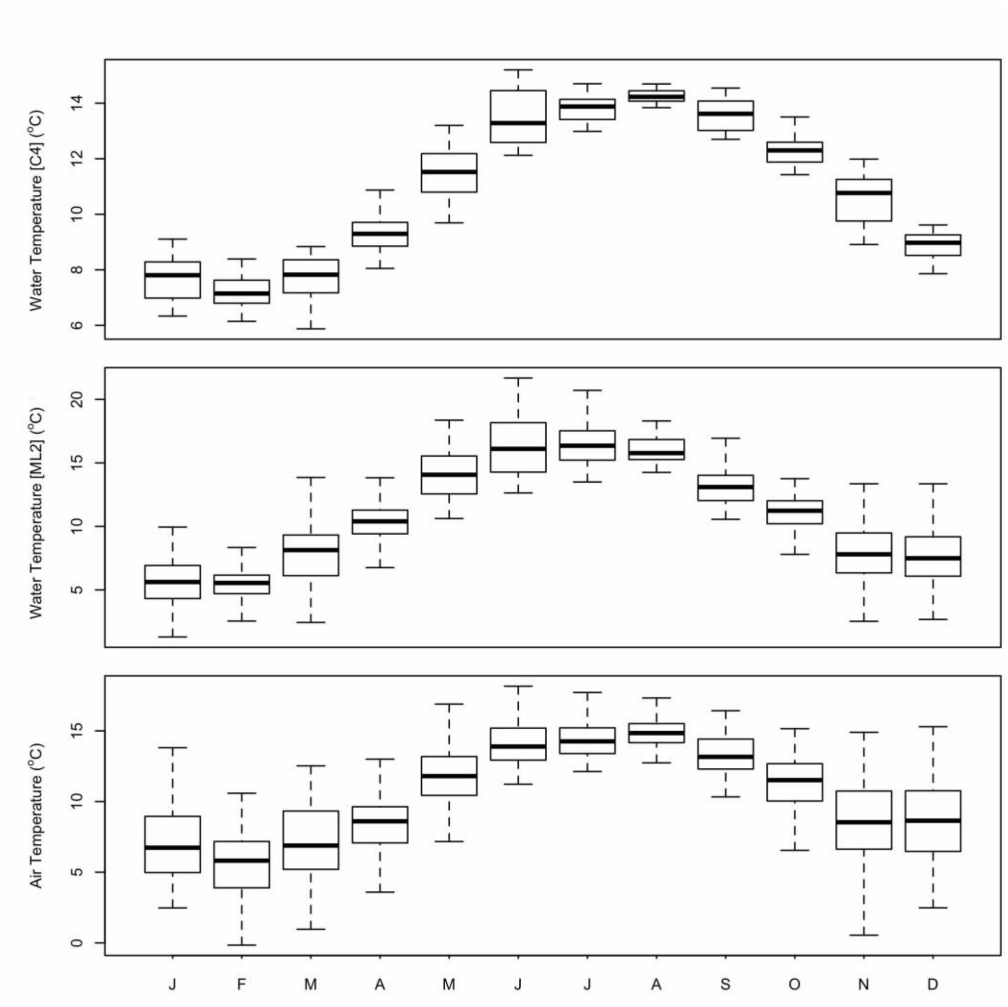


Figure 3.6 Monthly average temperatures recorded by the Sheskinmore Davis weather station (air temperature) and logger values for water temperature for c4 and ml2 slacks from September 2015 to May 2018. Note: logger at c4 is beneath the bed whereas ml2 logger is at the bed surface

3.3.2 Hydrology and seasonal flooding dynamics

Hydrological monitoring of the slacks showed a strongly seasonal regime that shifted between flooded and dry conditions over the course of the year (Figure 3.7 and Figure 3.8). The results also show that this water level regime is very variable (mean water table depth at the deepest part of the pond is 0.32cm and standard deviation 0.45cm for c4) and the system doesn’t completely dry every year. For ml2 however, as the logger dries it becomes impossible to provide a comparable measure as the average is using the ground (0) rather than a level at some depth beneath the ground, due to the placement of the logger at the pond bed. Still, maximum flooding for both slacks occur between February and April in each year, while lowest water levels experienced between September and December. The hydrological regime of each year is slightly skewed in that the time taken to reach maximum flooding is on average 2 months shorter than the time taken to reach minimum water levels from the winter maximum flooding.

Annual precipitation values change year to year, and there is notable variability in week to week and between seasons. Precipitation is greatest in the winter, with a distinct rise from a minimum around September (30-35mm month), peaking between December and February. Differences in monthly sums were considerable in two consecutive years (178mm in December 2015 compared to 80mm for the same month in 2016). Evapotranspiration has a stronger seasonal signature, with a relatively symmetrical rise to a summer high and fall to a winter low. Peak flooding of the slacks occurs in the period between peak precipitation and peak evapotranspiration, whilst the drier phases occur on the final decline in evapotranspiration and at the start of the rise in precipitation. The slacks receive water directly through precipitation, but also via the groundwater body held in the dunes surrounding the slacks. There is no evidence to suggest that this groundwater is supplied from beyond the adjacent dune aquifer, and is therefore all precipitation-fed**;** the timing of flooding and drying therefore reflects the interaction between direct rainfall and lateral groundwater. Given the vertical extent of the adjacent dunes though, it is likely that the dune aquifer presents a considerable supply of water through lateral groundwater flow. Connections between input and output of water in the slacks combine to drive a hydrology that responds to changeable weather, for example when the water table is kept high through seasons and evapotranspiration is minimised and vice versa, which leads to either sustained flooding through the year (e.g. autumn 2015 to autumn 2016) or a shorter hydroperiod elsewhere (e.g. W17).

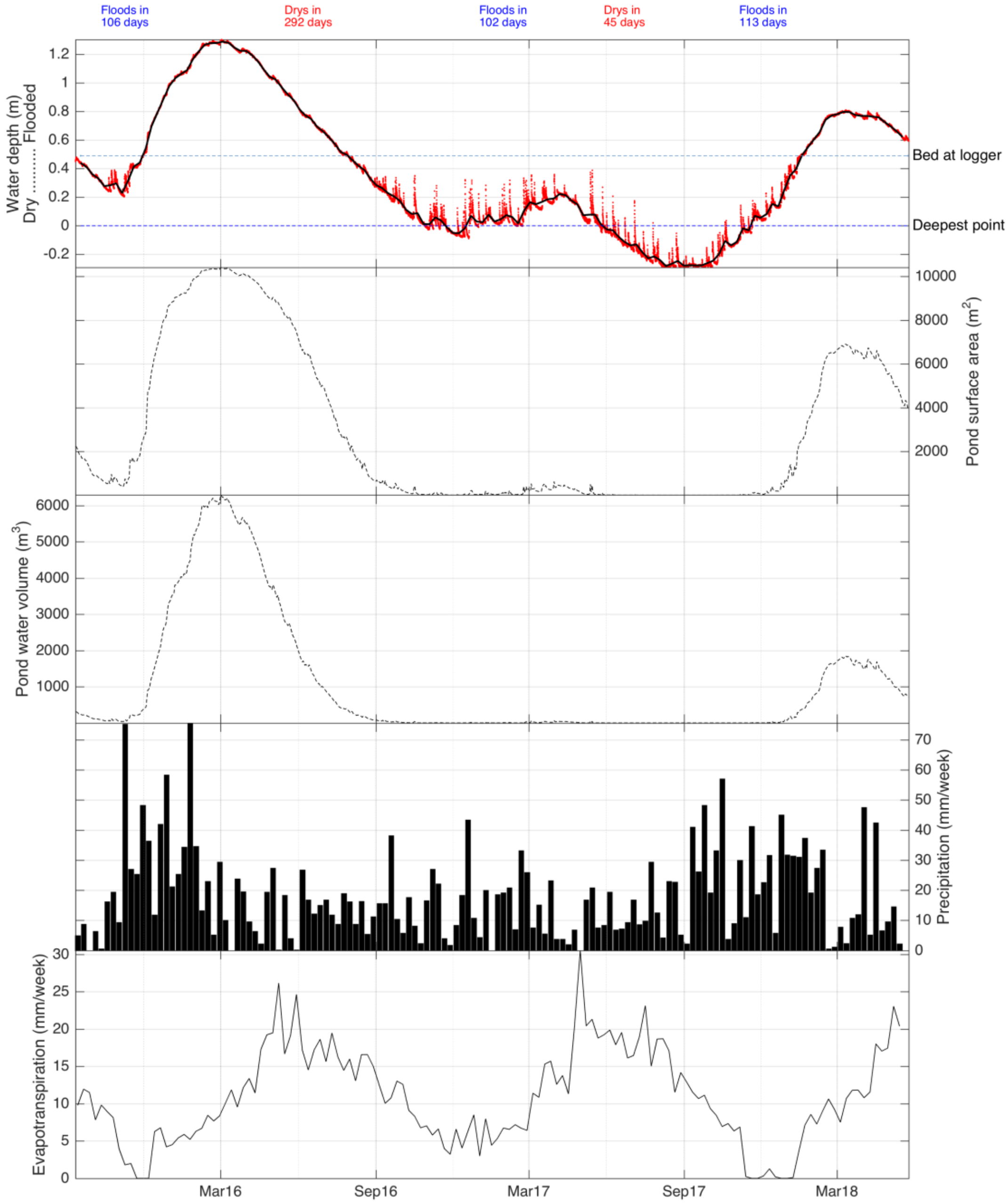


Figure 3.7 Time series of weekly water level (relative to the slack bed), slack surface area and water volume, precipitation and evapotranspiration recorded from October 2015 to May 2018 at c4.

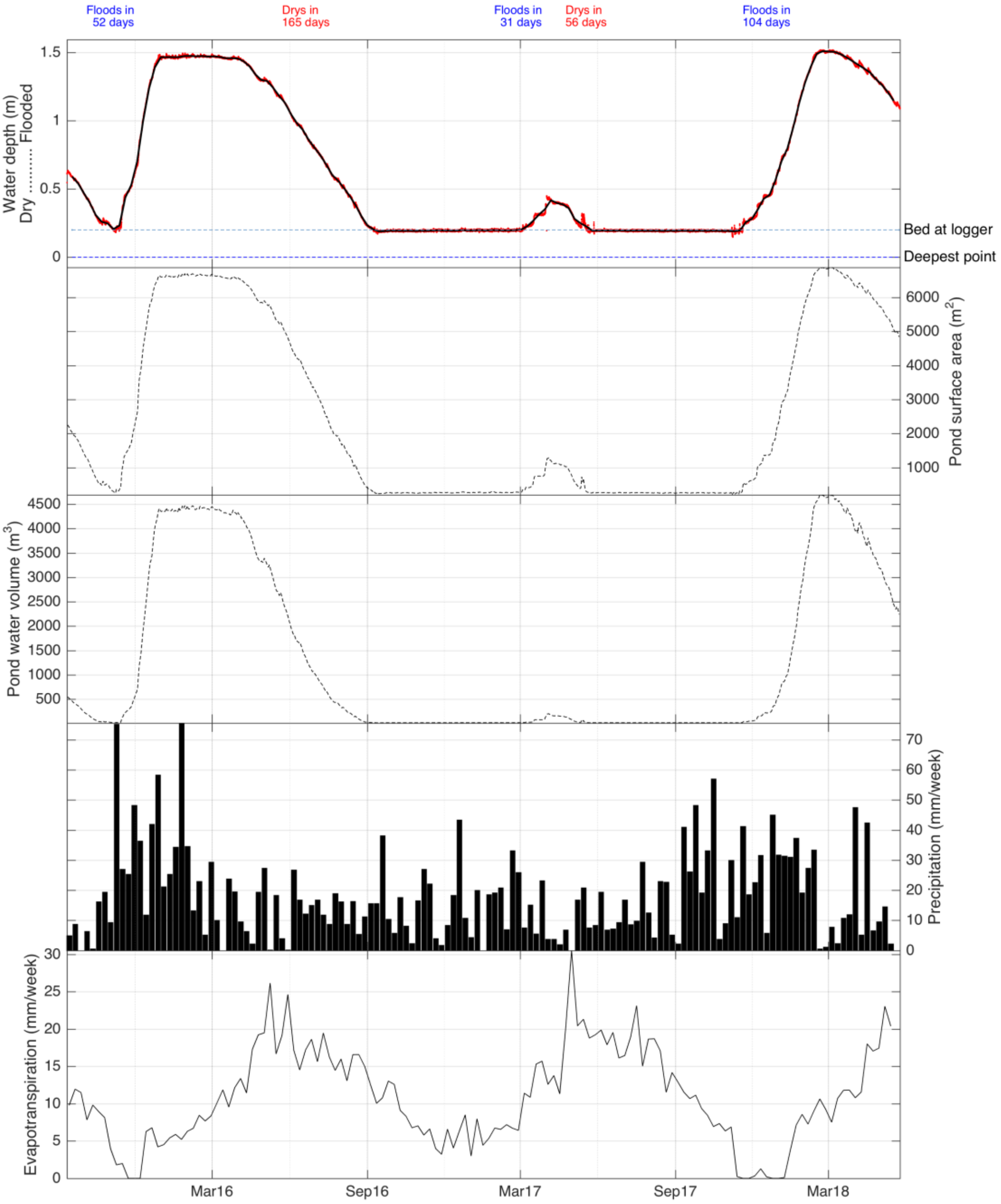


Figure 3.8 Time series of weekly water level (relative to the slack bed), slack surface area and water volume, precipitation and evapotranspiration recorded from October 2015 to May 2018 at ml2.

3.3.3 Seasonal variability in water chemistry

Average water chemistry values at both slacks were similar (Figure 3.9) but seasonal and inter-annual variability was different for the various water chemistry variables and between the slacks, with a stronger variation observed when the slacks were drying out – either from W16 to S16 or as seen in W17 when water table was at its lowest. Seasonal and inter-annual variation in water chemistry factors shows three types of variation: no variation through seasons nor years (I); seasonal variation, accounted for in terms of variability from W16 to S16 (and not comparative analysis from S15 to W16 or S16 to W17, as the lack of water in between makes it not suitable for seasonal comparison). (II); and inter-annual where, from one summer and/or winter to the next (III), water chemistry variables show fluctuations.

I. No variation – Conductivity showed no major changes through the sampling seasons at both slacks. Also c4 and ml2 are rather constant in terms of alkalinity (apart from the outlier value). In ml2 pH also showed low overall seasonal variability.

II. Seasonal variation – Changes from W16 to S16 are seen in water temperature, rising from an average of 10ºC to 16ºC in both slacks; nitrogen, decreasing at both slacks (from 40 to 20µg/L at c4, and from 25 to 15µg/L at ml2). Also SRP underwent a distinct rise during the same period at ml2 (jumping from 4 to 11µg/L) with chlorophyll *a* decreasing sharply from 6 to 1µg/L at c4.

III. Inter-annual variability – Comparing values from both summer samplings and both winter samplings, variables that show more inter-annual variability in summer are oxygen and pH at c4 and SRP at ml2. Winter variability is greater for chlorophyll *a* at c4 and for nitrates and temperature at both slacks. Total phosphorus also showed major changes in the W17 sampling at both slacks, where values rose below 50 to almost 100µg/L at c4 and from below 50 to over 200µg/L at ml2.

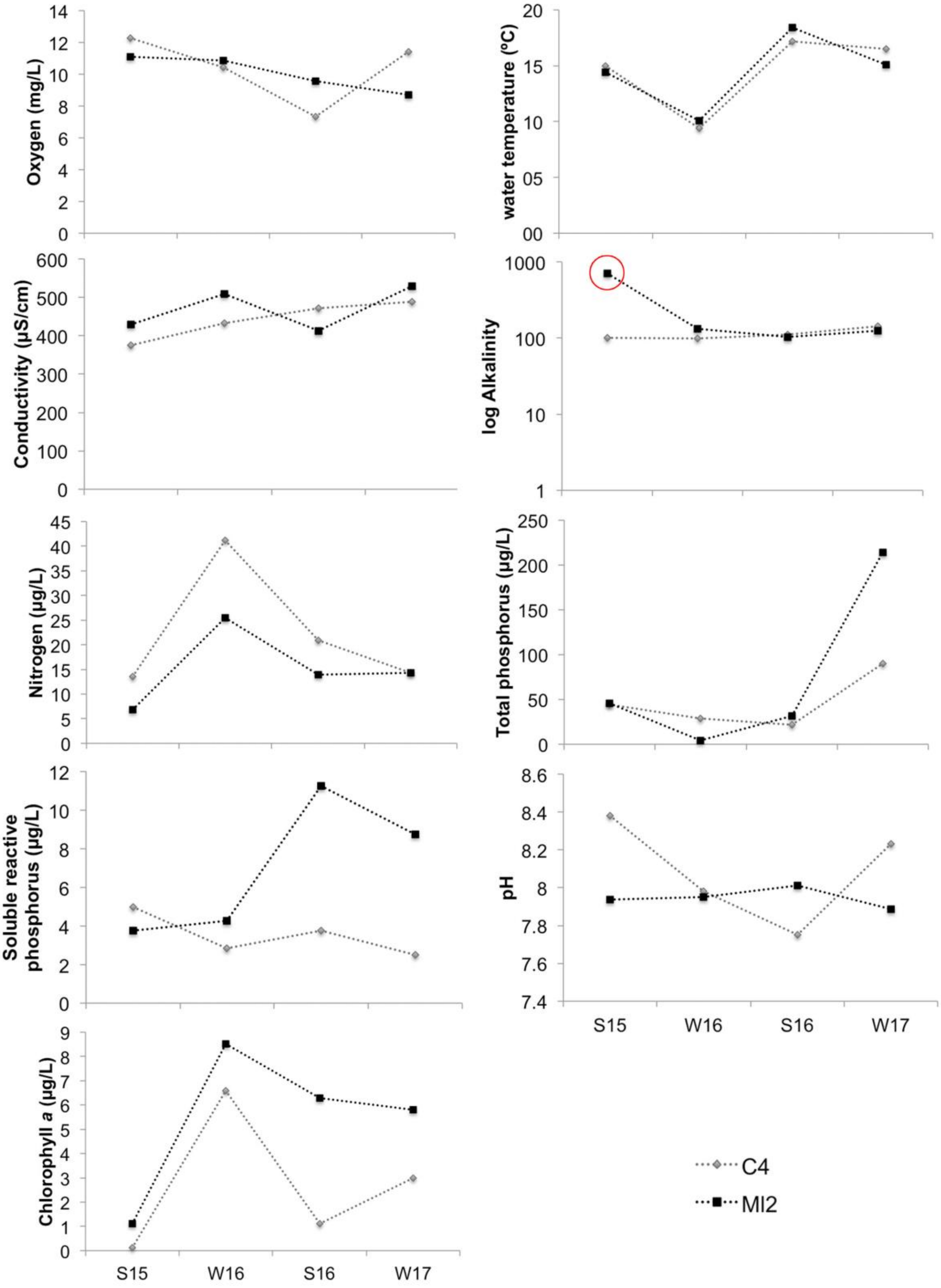


Figure 3.9 Seasonal variation of environmental water chemistry factors at c4 and ml2. Alkalinity S15 value for ml2 is identified out as an outlier

3.3.4 Cladoceran seasonal and inter-annual community structure

A total of sixteen cladoceran species were found at both sites, belonging to four families: Chydoridae, Bosminidae, Daphnidae and Macrothricidae. These were: *Alona costata, Alonella exigua, Alonella nana, Acroperus harpae, Alona affinis, Bosmina longirostris, Ceriodaphnia laticaudata, Ceriodaphnia quadrangula, Ceriodaphnia setosa, Chydorus sphaericus, Coronatella rectangula, Eurycercus lamellatus, Graptoleberis testudinaria, Macrothricidae sp, Simocephalus exspinosus* and *Simocephalus vetulus*. All species are widely distributed in Europe, mostly with a preference for littoral oligo to eutrophic habitats, rich in vegetation and with occurrence known for temporary waters (Alonso, 1991; Błędzki and Rybak, 2016). Some species are more frequent in shallow/small waterbodies (*A. costata*, *A. exigua*, *E. lamellatus*), others prefer sandy bottoms (*A. harpae*, *A. nana*) having both benthic and planktonic feeding habits (Davidson *et al.*, 2010), but the vast majority thrive among vegetation (especially species in the genera *Alona*, *Alonella*, *Ceriodaphnia*, *Coronatella* and *Graptoleberis*). *Chydorus sphaericus* and *Simocephalus* spp. are very common species with a high tolerance, able to adapt to a wide range of habitats, temperature and environmental conditions (Błędzki and Rybak, 2016).

Cladoceran abundance was significantly different between winter and summer samples (p<0.05) at both slacks (Table 3.1), and inter-annual variability is evident from S15 to S16 at C4, but not for Ml2 (Figure 3.10). The high no./L of *Alonella exigua* (189 no./L) could be influencing the values of the differences in abundance for Ml2, as visible on Figure 3.10, but standardization methods and even elimination of this outlier value still kept the statistical results unchanged. However, Ml2 showed differences in abundance, but non-significant (p=0.05) over two consecutive winter samplings. In this study, the lowest abundance of species was recorded for W17 with *A. costata*, *S. vetulus* and *G. testudinaria* (0.05 no./L), *Ceriodaphnia* spp. and *C. rectangula* (0.09 no./L.), *B. longirostris* (1.55 no./L) and *C. sphaericus* (2.73 no./L) present in C4 and only 5 species present in Ml2: *S. exspinosus* and *C. rectangula* (<0-04 no./L), *C. sphaericus* (0.08 no./L) and *C. quadrangula* and *A. exigua* with (0.7 no./L). *C. sphaericus* is a species capable of rapid population turnover and is considered a pioneer that can reach maturity in 3 days, explaining its potential rapid colonisation of ponds even when faced with environmental stress (Błędzki and Rybak, 2016). The conditions at Ml2 were such in W17 that even this species revealed the lowest abundance values recorded for all seasons at both slacks (0.08 no./L).

Table 3.1 Kruskal-Wallis *p*values for seasonal and inter-annual cladoceran abundance between both slacks (SS – summer 15 vs summer 16, WW – winter 2016 vs winter 2017, SW16 – winter and summer 2016)

	c4	ml2
SS	**0.023**	0.745
WW	0.488	0.05
WS16	**0.000**	**0.04**

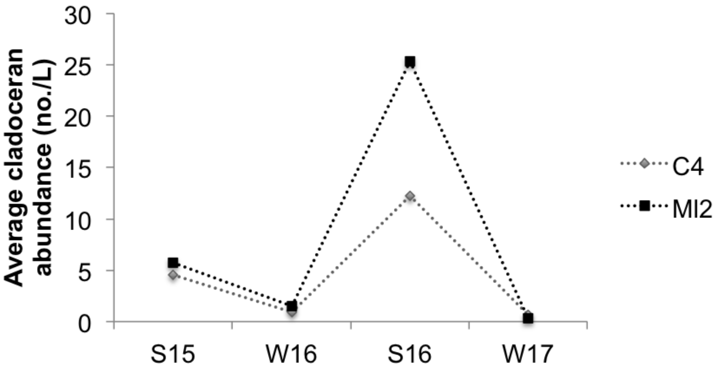


Figure 3.10 Seasonal cladoceran abundance for c4 and ml2

Highest diversity (Table 3.2) was attained in S16 in c4, when species such as *A. harpae* and *Ceriodaphnia* spp. appeared for the only time throughout the study. These species are well represented and widely distributed in Ireland and are often associated with dense aquatic vegetation such as stonewort (*Chara* sp.) and sandy sediment habitats (Duigan, 1992; Davidson *et al.*, 2010; Błędzki and Rybak, 2016). Ml2 showed a decrease in diversity in S16, although a new species occurred in this time interval, *G. testudinaria* (the only record for ml2), a species that was present in all 4 seasonal samplings at c4.

Other species were recurrently seasonal at c4. *A. affinis* was only found in summer samples. By contrast, the smaller sized *B. longirostris* was only found on winter samples, coincident with increased water volume, nitrogen and chlorophyll *a* values. No marked seasonal differences were seen for ml2 species. Table 3.3 shows the frequency of species that occurred at the four samplings for each slack.

Table 3.2 Seasonal changes in biodiversity indices based on Shannon abundance records for both slacks

			S15	W16	S16	W17
C4	cladoceran	diversity	0.875	0.418	2.102	0.968
		richness	8	8	16	7
		equitability	0.421	0.201	0.715	0.498
Ml2	cladoceran	diversity	1.397	1.447	0.953	1.055
		richness	5	8	10	5
		equitability	0.868	0.696	0.414	0.656

Table 3.3 Presence/absence of cladoceran species at C4 and Ml2 at each sampling season

	C4				ML2			
	S15	W16	S16	W16	S15	W16	S16	W16
no. Species	8	8	16	7	5	8	10	5
Alona costata		🟦	🟩	🟦		🟦	🟩	
Alonella exigua	🟩	🟦	🟩		🟩	🟦	🟩	🟦
Alonella nana		🟦	🟩		🟩	🟦	🟩	
Acroperus harpae			🟩					
Alona affinis	🟩		🟩			🟦	🟩	
Bosmina longirostris				🟦				
Ceriodaphnia laticaudata			🟩					
Ceriodaphnia quadrangula			🟩				🟩	🟦
Ceriodaphnia setosa			🟩					
Ceriodaphnia sp			🟩	🟦				
Chydorus sphaericus	🟩	🟦	🟩	🟦	🟩	🟦	🟩	🟦
Coronatella rectangula			🟩	🟦		🟦	🟩	🟦
Daphnia curvirostris	🟩							
Eurycercus lamellatus	🟩	🟦	🟩					
Graptoleberis testudinaria	🟩	🟦	🟩	🟦			🟩	
Macrothricidae			🟩					
Simocephalus exspinosus	🟩	🟦	🟩		🟩	🟦	🟩	🟦
Simocephalus vetulus	🟩	🟦	🟩	🟦	🟩	🟦	🟩	

3.3.4.1 Environmental controls on cladoceran seasonal abundance

Hydrological measurements and water chemistry factors combined were used to evaluate the influence of overall environmental controls on cladoceran compositional patterns using NMDS (Figure 3.11). Alkalinity exhibited an outlying value (Figure 3.9) and was therefore eliminated from ordination analysis. The first axis in the NMDS separates summer (top) from winter (bottom) samples, with winter 2017 orientated along the horizontal line of division. With a stress value of 0.08 generated by the algorithm, the multivariate distance between the samples is considered a good fit, indicating that the NMDS ordination is not arbitrary (Clarke, 1993; Letten, 2017). Seasonality produced slight shifts in community abundance values at both slacks, with major detachments visible for S16 at slack c4 and for W17 for both slacks. In terms of the hydrological driving of differentiation, external slack factors related to climate forcing (air temperature, evapotranspiration and rainfall) and slack inner hydrology (water volume, water depth, slack area and hydrology) primarily drive the separation between summer and winter, with higher air temperature and evapotranspiration values recorded for S16 at c4 (Figure 3.11 A). The presence of water in the slack promotes summer species, whereas the decrease in water capacity within the slack shapes winter species composition in accordance with higher air temperature and evapotranspiration in the summer. SRP and TP on the first axis (Figure 3.11 B) divide winter from summer community samples, with higher SRP and lower TP values in summer. Elevated conductivity appears to be associated with W17 communities.

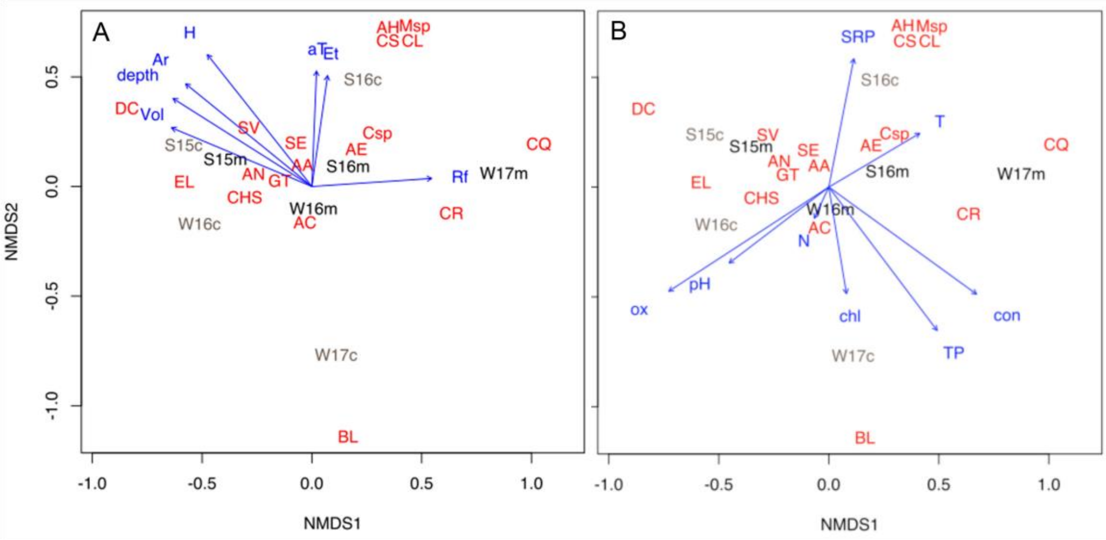


Figure 3.11 NMDS ordination plots combining both slacks cladoceran composition with species in red, seasons in black for ml2 and grey for c4 and environmental data as blue arrows. Plot A shows hydrological and climate parameters (Ar – slack area, Vol – slack volume, depth – water depth, H – hydroperiod (flood days) before sampling, aT – air temperature, Et – evapotranspiration, Rf – rainfall); Plot B with water chemistry parameters (ox – oxygen, pH – pH, SRP – soluble reactive phosphorus, N – nitrogen, chl – chlorophyll *a*, T – water temperature, con – conductivity, TP – total phosphorus)

Variables that were strongly correlated (r^2>0.6) with the 1st and 2nd NMDS axes (Table 3.4) were explored further (Figure 3.12) to ascertain more clearly how they affected species distributions across seasons at both slacks. In this analysis water chemistry appeared to be more of an influence on winter communities (especially oxygen, conductivity and total phosphorus) with hydrological parameters having more influence in the summer. Higher abundances of cladocerans as well as increased species richness were found in summer months associated with a greater amount of water in the slacks (area and depth) and an increased hydroperiod (number of days flooded) before sampling (H). Conversely, higher nutrient values (total phosphorus) and conductivity in winter were correlated with lower cladoceran abundances at both the c4 and ml2 slacks.

Table 3.4 NMDS axis scores and correlation and *pvalues* for significant environmental variables

	NMDS1	NMDS2	r^2	Pr(>r)
ox	-0.50620	-0.86242	0.74	0.104
con	0.80641	-0.59136	0.60	0.096
TP	0.69193	-0.72196	0.89	0.025
H	-0.62057	0.78415	0.85	0.054
Ar	-0.77539	0.63149	0.79	0.080
depth	-0.84448	0.53558	0.81	0.048

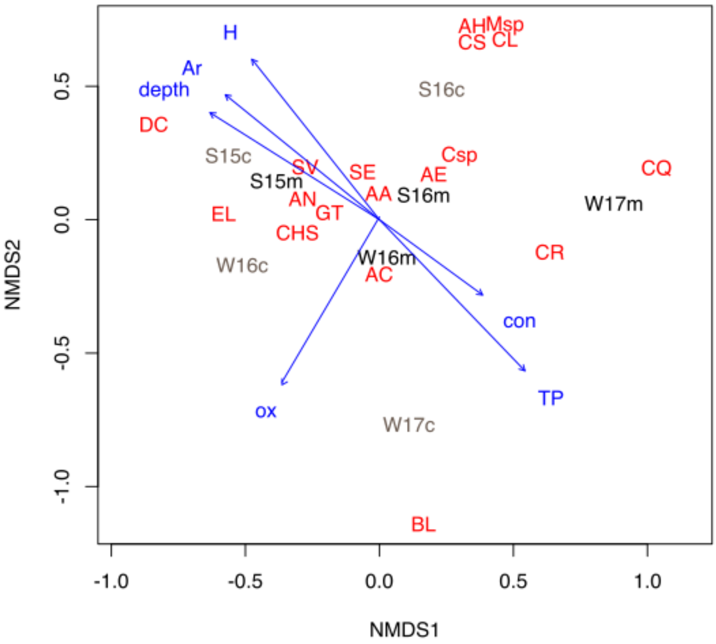


Figure 3.12 NMDS ordination plot of cladoceran abundance data for both slacks with species in red, including only strong relationship between environmental data (blue arrows) and axis. Seasons represented in black for MI2 and grey for C4. Species abbreviations: AH – *Acroperus harpae*, Msp – *Macrothricidae*, CS – *Ceriodaphnia setosa*, CL – *Ceriodaphnia laticaudata*, DC – *Daphnia curvirostris*, EL – *Eurycercus lamellatus*, SV – *Simocephallus vetulus*, AN – *Alonella nana*, GT – *Graptoleberis testudinaria*, CHS – *Chydorus sphaericus*, SE – *Simocephalus exspinosus*, AA – *Alona affinis*, AC – *Alona costata*, BL – *Bosmina longirostris*, AE – *Alonella exigua*, Csp – *Ceriodaphnia* sp, CR – *Coronatella rectangula*, CQ – *Ceriodaphnia quadrangula*

3.3.5 Vegetation

3.3.5.1 Life form composition

The total presence of species recorded at both slacks can be seen on Table 3.5. Plants were differentiated into terrestrial (fully terrestrial species that happened to be in water at time of sampling e.g. *Salix repens*), wet tolerant (species that are associated with wet zones or that have an early life stage in water, e.g *Juncus* spp. and *Myosotis laxa*); emergent (species that have their roots in water followed by an aerial structure, e.g *Eleocharis* palustris); and submerged species (living fully underwater, e.g. *Chara* spp.) (Figure 3.13).

Table 3.5 Presence/absence of plant species at c4 and ml2 at each sampling season

	c4				mL2			
	S15	W16	S16	W16	S15	W16	S16	W16
No. species	15	13	22	13	18	11	14	13
Caltha palustris			🟩	🟦				
Cardamine pratensis		🟦	🟩		🟩	🟦		
Carex nigra		🟦						
Carex sp	🟩		🟩	🟦	🟩	🟦	🟩	🟦
Chara sp	🟩		🟩				🟩	
Eleocharis palustris	🟩			🟦	🟩		🟩	🟦
Equisitum palustre			🟩		🟩		🟩	
Gallium palustre	🟩	🟦	🟩	🟦	🟩	🟦	🟩	🟦
Glyceria fluitans	🟩	🟦	🟩		🟩	🟦		🟦
Hippuris vulgaris								🟦
Hydrocotyle vulgaris	🟩	🟦	🟩	🟦	🟩	🟦	🟩	
Juncus articulatus	🟩	🟦	🟩		🟩		🟩	🟦
Juncus sp	🟩		🟩	🟦				
Leotodon taraxacoides					🟩	🟦		
Mentha aquatica	🟩	🟦	🟩	🟦	🟩	🟦	🟩	🟦

Myosotis laxa		🟦	🟩					
Potamogeton crispus			🟩					
Potamogeton natans	🟩	🟦	🟩					
Potentilla palustre	🟩		🟩					
Potentilla reptans					🟩	🟦		🟦
Ranunculus acris		🟦	🟩		🟩	🟦		
Ranunculus flammula	🟩	🟦	🟩	🟦	🟩	🟦	🟩	🟦
Roripa nasturtium-aquaticum				🟦				
Rumex crispus	🟩	🟦	🟩					
Salix repens	🟩		🟩	🟦	🟩		🟩	🟦
Shagnum sp	🟩	🟦	🟩	🟦	🟩	🟦	🟩	🟦
Shoenoplectus tabernaemontani				🟦				🟦
Shoenus nigricans					🟩		🟩	
Sparganium sp			🟩	🟦				🟦
Triglochin palustris			🟩				🟩	
Veronica scutellata					🟩		🟩	

The composition of vegetation life forms was similar for both slacks with wet tolerant species proportionally most important, likely due to the dominance of very shallow water. The percentage of emergent plant species showed high seasonal variability being higher in W17 at both slacks. Submerged species at c4 were more evident during the summer surveys, but were absent on the first two samplings (S15 and W16) at ml2. Terrestrial species were present at both slacks with higher proportions evident at ml2 in the first two samplings. Vegetation life-form proportions showed more inter-annual (S15-S16 and W16-W17) than seasonal (W16-S16) variability and this is in accordance to the hydrological regime. Over the 2-year sampling period, the hydrological regime of the slacks was also highly variable with no clear seasonal pattern.

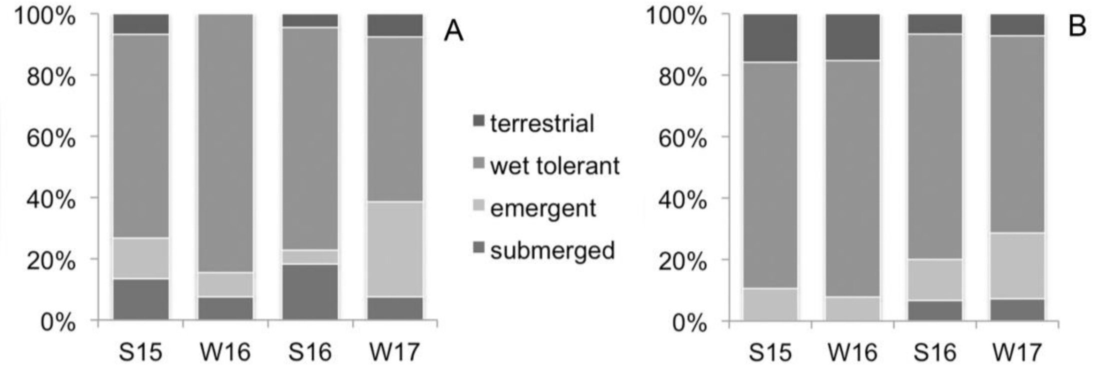


Figure 3.13 Frequency (%) of different vegetation life forms in c4 (A) and ml2 (B) over the four sampling seasons (S15 summer 2015; W16 winter 2016; S16 summer 2016; W17 winter 2017)

3.3.5.2 Patterns in vegetation

To understand how hydrology controls the aquatic vegetation of the slacks, spatial patterns and associations with topographic variation and flooding patches were explored. This analysis was made through interpretation of the seasonal vegetation abundance data, *in situ* observation, topographical surveying and aerial photographs. Both c4 and ml2 have complex and less defined margins to centre plant zonation (i.e. vegetation gradient according to species preferences of depth from margin to centre) as identified in the majority of seasonal waterbodies, where vegetation follows annual hydrological cycles of winter flooding and summer drainage (Grillas *et al.*, 2004; Curtis *et al.*, 2009). Still, a marginal outer layer of moss carpets combined with wet tolerant species was observed (*Sphagnum* spp. and *Hydrocotyle vulgaris* as an example) with occasional colonisation of terrestrial shrubs, mainly *Salix repens*. The inner sections are composed of elevated patches of *Juncus* spp surrounded by underwater and emergent growing species, such as *Gallium palustris*, *Carex nigra* and *Eleocharis palustris*, in the lower more inundated depression zones. Deeper patches are confined to the north-east of c4, but with several areas within the northern side of ml2. In these patches one can easily find the more aquatic species such as *Glyceria fluitans*, *Potamogeton* spp and *Veronica scutellata*, for example.

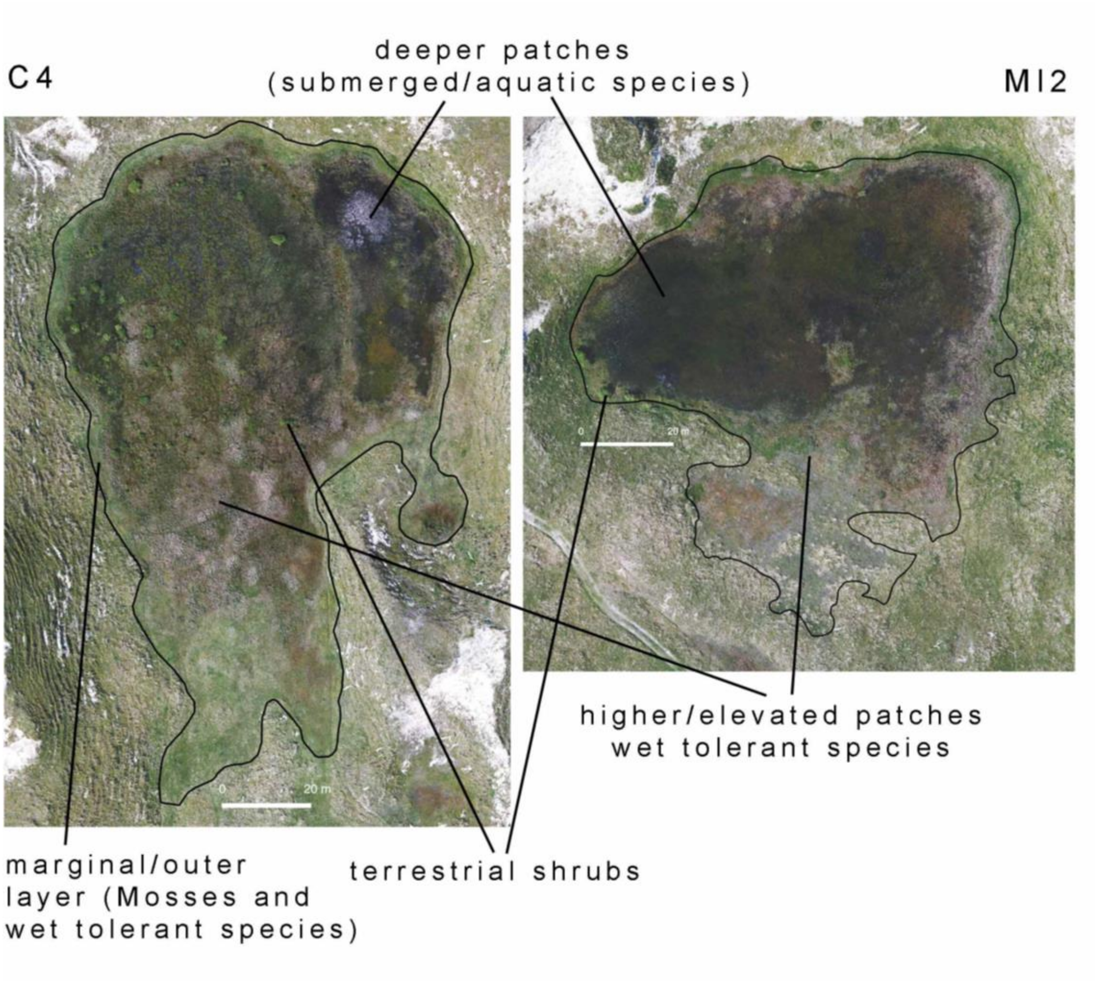


Figure 3.14 A schematic layout of the slacks topographical and hydrological variability

3.4 Discussion

3.4.1 The effects of climate on hydrological variability

The period of sampling and monitoring in this study covered two consecutive hydrological cycles (S15 through to W17), during which the slacks experienced two flooding events: expected S15 to W16 where water table rise followed a gradual increase from the autumn to spring; and unexpected S16 to W17 where low rainfall in autumn was insufficient to achieve high water levels in the slacks in the following hydrological cycle. Maximum temperatures during S16 were higher than average (26ºC) (Figure 3.15) and the minima were also high, but similar to the previous year (over 6ºC).

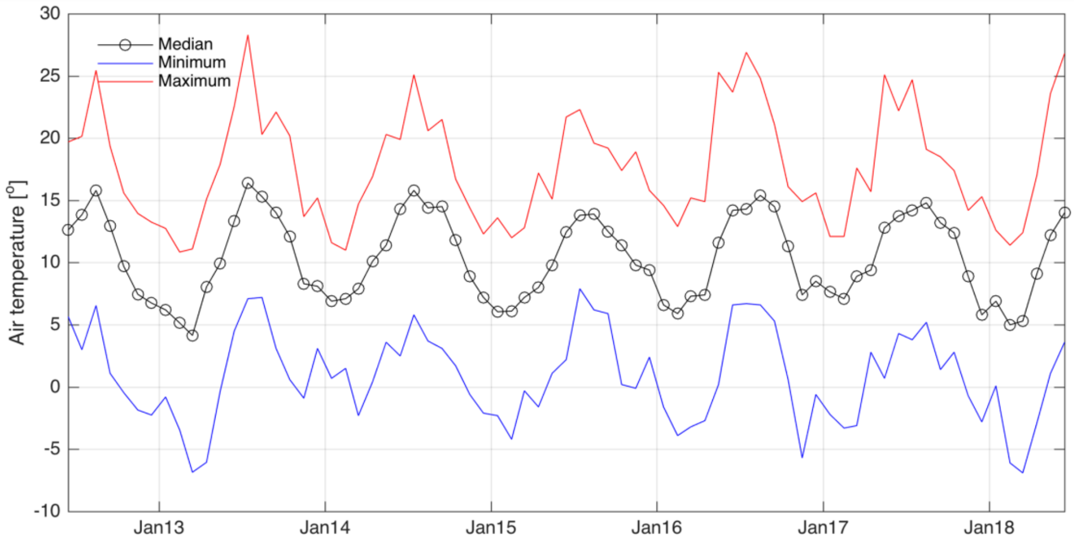


Figure 3.15 Air temperature recorded at Davis weather station from October 2012 up to May 2018

Rainfall through the following winter was lower than average (Figure 3.5). During W17, the area suffered a significant water table lowering affecting all dune wetlands at Sheskinmore Nature Reserve (Magee 2017; Burningham 2018). There is a strong seasonal hydrological regime in the dune slack systems dictated primarily by water inputs (precipitation) and outputs (evapotranspiration) that are directly related to climate and temperature.

The hydrological regime of the slacks at Sheskinmore largely follows a similar trend to other local dune slack systems, as for example at Magilligan in Northern Ireland (Robins and Wilson, 2017) where water table levels reach their maximum toward the end of winter, leading to a period of flooding across the slack floor. Within the context of the flooding and drying cycle, the hydroperiod variation within the slack is a consequence of the topographic layout of the slack bed. In larger slacks, as reported here, where subtle variations in topography are evident across a broad and relatively flat bed (relative to the surrounding dunes), this can lead to a distinct patchiness in flood frequency and water availability throughout the year (Figure 3.16). In both slacks, small isolated depressions allow water to persist for most of the year, even during the particularly dry period from 2016 to 2017.

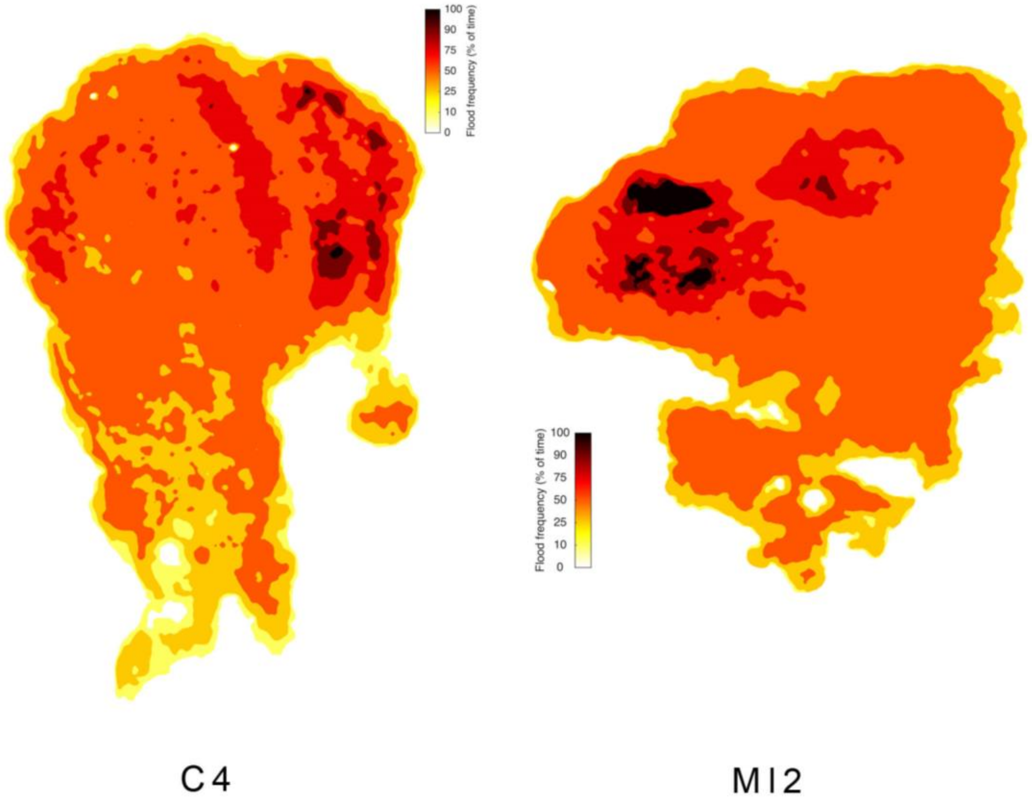


Figure 3.16 Estimates of spatial distribution in flood frequency based on the frequency distribution of water level records and the topography of the ponds. Note that the lack of below-bed measurement of water levels at ml2 means that the frequency distribution is biased to above-ground, flooding conditions.

The present 2-year hydrological study reveals a distinct shift in water balance at the slacks from one year to the other, especially from W16 to W17. A 6-year hydrological monitoring of c4 though, reveals a more balanced seasonal input (precipitation) and output (evapotranspiration) in the preceding years (Figure 3.17). Yet, results from the year 2012-2013 show low precipitation values, particularly in late winter 2013 (7.4 mm in February) paired with high peaks of evapotranspiration recorded in June and August of the same year, thus limiting the water availability at c4 in 2013. Similarly, Jeffries (2016) study showed strong relationship between rainfall events and wetness of dune slacks in the east of England, as well as dryness followed by severe drought in 2012-2013. This drought event was also felt in the Mediterranean (Trigo *et al.*, 2013), where impacts of lower precipitation had consequences on the following season hydrological cycle, i.e, from September to the following August. These results show that occasional inter-annual alterations in climatic conditions impact the hydrological dynamics at large scales as it did with slacks in Sheskinmore dune system, driven by decreases in precipitation and increases in evapotranspiration.

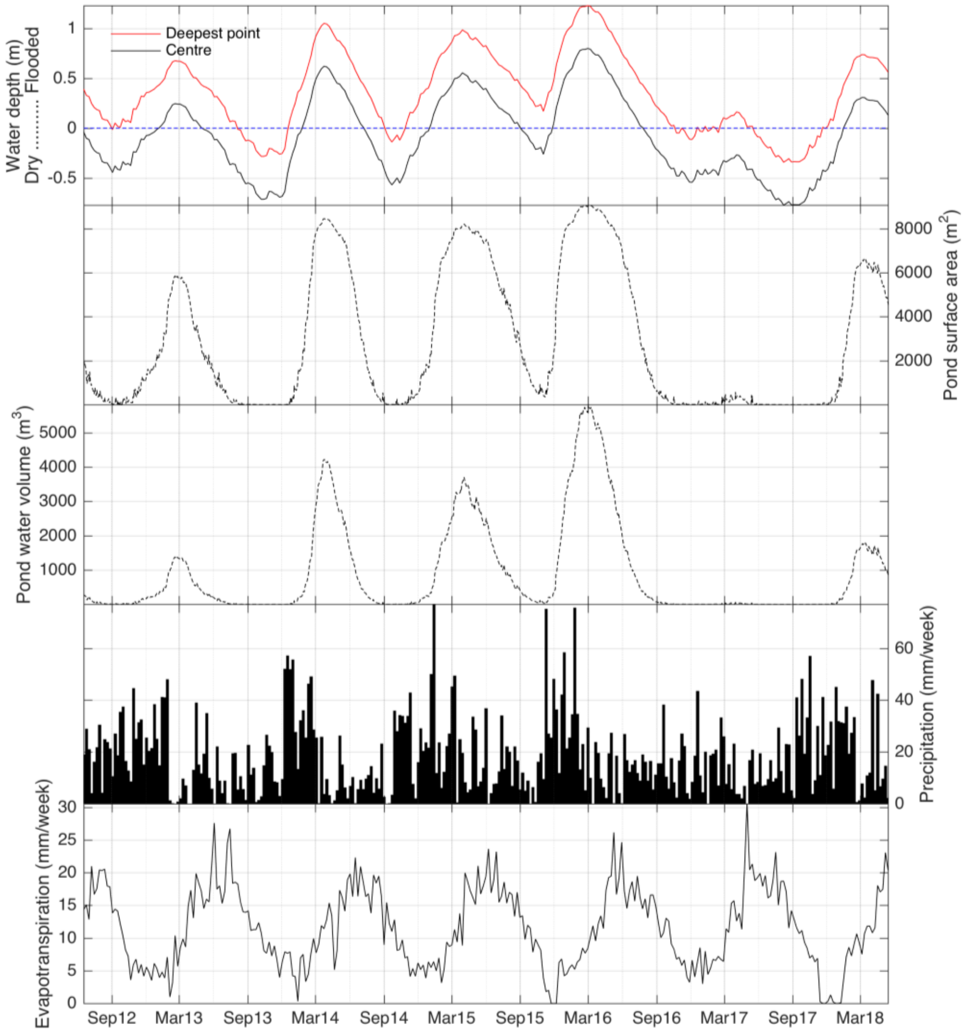


Figure 3.17 Time series of weekly water level (relative to the c4 slack bed), slack surface area and water volume, precipitation and evapotranspiration recorded from mid 2012 to mid 2018.

There is no doubt that inter-annual variability in weather conditions imparts a significant control on the hydrology and ecology of dune wetlands. These factors, in combination with the geomorphology of the slack depression influence both spatial and temporal ecological patterns within the dune slack by determining local microclimates (including temperature and humidity) and water availability (the extent of open, ponded water, saturated wetland, or dry patches with root access to water). Hydrological factors and processes not only define specific ecological niches across the slack i.e. the spatial patterns, they can also alter ecological development i.e. the temporal patterns. This implies that the ecology of the two slacks rely on ground water levels in turn influenced by precipitation.

3.4.2 Environmental variability

Over the 2-year period of hydrological and climate monitoring at Sheskinmore Nature Reserve, W17 was out of the envelope of variability expressed in preceding years. Fahd *et al*. (2007) suggested that the influence of severe drought in ponds may lead to a much more intense ecological change compared to dry periods before each annual flood. Drought here means that precipitation is decreased and not following seasonal patterns, whereas a dry phase occurs every year, as consequence of the lowering of the water table within the temporary waterbody. Droughts are often considered to impact summer conditions as observed by Jeffries (2016), but the monitoring of dune environments at Sheskinmore has shown that the impact of decreased precipitation can extend across all seasons, and in fact may be quite severe as seen for cladoceran seasonal species abundances for c4 and ml2 in W17, with the lowest values recorded.

The impact of water level lowering was also visible in terms of increased water chemistry values particularly for total phosphorus (increase from below 50µg/L up to 100µg/L at c4 and over 200µg/L at ml2) and soluble reactive phosphorus at ml2 only (changing from 4µg/L to 9µg/L) during the seasonal W16-S16 but also in W17 at both slacks. Although the values recorded were not alarming, the seasonal dynamics of the water phase of the slack does influence the fluctuation of these values. Rhymes *et al.* (2016) noted in a mesocosm experiment that a 10cm decrease in water table affected soil chemistry, by decreasing denitrification, with a 2-3 fold increase in soil nitrogen. High levels of nitrogen and phosphorus are considered a threat (as pollutants) on the quality standard of healthy dune slack environments in Ireland (NPWS, 2013). Elevated nitrogen inputs are linked to intensive grazing, agricultural intensification, groundwater pollution and anthropogenic pressure. On the other hand, although still low concentrations, winter values showed higher chlorophyll *a* at both slacks (from 7µg/L to 9µg/L at ml2 and from 3µg/L to 7µg/L at c4). Chlorophyll *a* is often used as a proxy for algal biomass, and an important feeding resource for cladocerans (Dallas and Drake, 2014), but also as a measure of the eutrophication potential (Vijverberg and Boersma, 1997). It is often related to TP levels as this latter variable is used as a measure of the productivity of shallow waterbodies (Søndergaard *et al.*, 2005), but in highly seasonal waterbodies however, such as the slacks studied, the impact on the chlorophyll *a* and TP is probably linked to the flooding/drying rhythms (Elkhiati *et al.*, 2013). The lowering of the water table at c4 and ml2 is then likely imposing shifts on the chemistry of the water at the slacks and therefore determining the species assemblages as a consequence, though local variations in water chemistry and hydrological regime are complex to assess, because it can vary considerably across a site and also between surface and groundwater (Jones *et al.*, 2006).

3.4.3 Seasonality differences in cladoceran abundance

Seasonal differences in crustacean abundance have been widely reported in the literature from tropical and Mediterranean regions (Mengestou and Fernando, 1991; Creek *et al.*, 2003; Miracle *et al.*, 2010) with generally higher abundances in spring/summer compared to autumn/winter. Temperate seasonal cladoceran variability in relation to climate and hydrology in slacks is not so well documented and this study adds to this knowledge for Irish dune slack habitats.

In this work, the cladoceran community was variable between years in terms of the summer community, but also varied seasonally. The overall cladoceran abundance ranged from 5 to 12 individuals/L in consecutive summer samplings S15 and S16 at c4 and from 6 to 25 individuals/L at ml2. By contrast in consecutive winter samplings (W16 and W17) cladoceran abundance changed from 0.9 to 0.7 individuals/L at c4 and 1.5 to 0.3 individuals/L at ml2. That abundance was higher in summer samples is in agreement with other similar studies, where winter/pioneer communities have lower diversity at the beginning of the wet season, compared to more established summer communities (Mengestou and Fernando, 1991; Antón-Pardo *et al.*, 2015). The results presented here suggest that cladocerans responds as much to inter-annual variability in hydro-climatic conditions as to seasons. This is primarily a product of a lowering of the water table in W17 and its consequences for pond area, depth, and water chemistry. But on the other hand, this does not explain why there is such a large difference between the two summers in terms of species richness and abundance, as hydrologically they were very similar. Fahd *et al.* (2007) verified that cumulative seasonal sampling revealed increased zooplankton richness over time, likely due to temporal and/or spatial changes in pond characteristics, followed by an unusual high rainfall event. What could be the case in the slacks studied, is that the conditions preceding the sampling in S16 influenced the appearance of new taxa (i.e., taxa not recorded in the previous 2 samples, e.g.: *A. harpae* and *Ceriodaphnia* spp at C4 and *G. testudinaria* at Ml2). At 20ºC cladocerans are able to reach sexual maturity in 5-10 days (Crosetti and Margaritora, 1987). Vandekerkhove et al. (2005) on the other hand, compared hatching timings at 15ºC for a set of temperate and Mediterranean cladoceran species and from 50% to over 70% of species hatched within 12 days. This means that cladocerans can rapidly reach high densities even when the wet phase is short. In addition, the slow rate at which cladoceran eggs evolve into juveniles in sexual reproduction, make cladocerans highly impacted by changes in environmental conditions such as temperature rising and water levels dropping (Jones & Gilbert 2016). These combined factors make cladocerans vulnerable and sensitive to the surrounding environment and therefore suitable for tracking short and long-term environmental changes (Davisdon *et al*. 2011; Błędzki & Rybak 2016).

The ecological preferences of the 'new' cladocera species that occurred in S16 are very similar, with most showing a preference for habitats rich in submerged and emergent aquatic vegetation (Fryer, 1968; Cazzanelli *et al.*, 2008; Błędzki and Rybak, 2016). Aquatic vegetation creates the habitat for a wide diversity of invertebrate species (Carpenter & Lodge 1986; Katende 2004; Ali et al. 2007; Peretyatko et al. 2009). To explore this relationship further, seasonal shifts and succession of plant species were analysed. Aquatic vegetation and cladoceran seasonal richness (Figure 3.18) shows a close correspondence at c4 throughout all sampling seasons, although at ml2 this relationship is only tight from W16 to S16. Inter-annual differences are more evident in the summer, with higher plant species richness in S16 for c4 and in S15 for ml2.

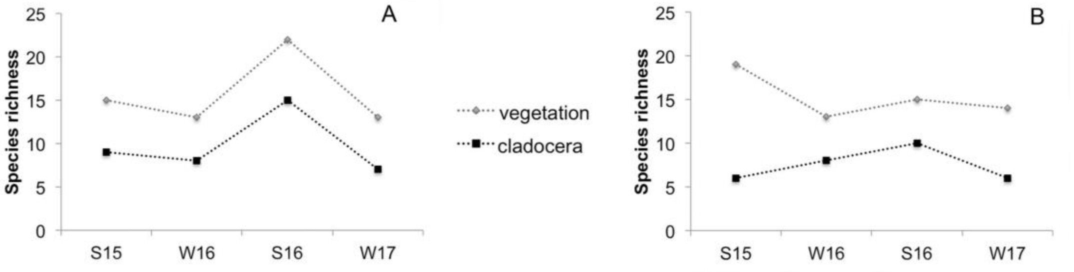


Figure 3.18 Cladocera and vegetation richness in relation to each sampling season at c4 (A) and ml2 (B).

In relation to plant frequency records (Figure 3.19), the presence of frequent (F), dominant (D) and abundant (A) plants at both slacks decrease steadily, with lower values recorded for W17, where neither D nor A species were present. What seems to occur is that the lower water level in W17 has extended the impacts to the loss of richness and a drop in the frequency records of plants and not so much that the presence of plant species is inducing this change in cladoceran values. The least frequent species (R) tend to increase from S15 to S16 at c4, but the occasional (O) are more frequent in W17, whereas at ml2, reach the lowest frequency in S16 and higher in W17. S16 has favoured the presence of a large number of concomitant plant species to occur, without any specific dominance.

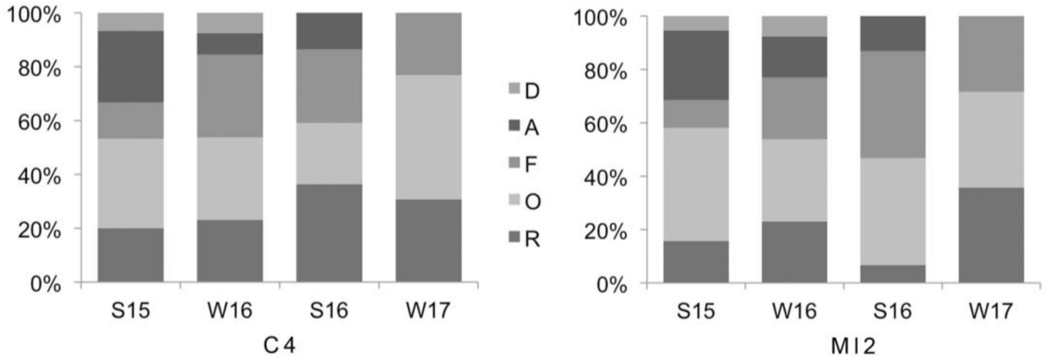


Figure 3.19 Seasonal frequency of the DAFOR scale for plants at c4 and ml2. D – dominant, A – abundant, F – frequent, O – occasional, R – rare

The lack of rainfall in the winter/spring W17 had a strong influence on the ecological functioning of the slacks within the dune system causing the disappearance of the winter flooding and leading to a period of drying earlier than usual within the hydrological year. Camacho et al. (2016) observed, that after strong rainfall in spring and abnormal rise of the water levels in Mediterranean temporary lakes, submerged macrophytes disappearance caused the system to enter heterotrophic conditions and biodiversity decreased. The same happened in the present study but with the abnormal lowering of the water levels instead. Less dominant species disappeared (e.g. *Gallium palustre*, *Glyceria fluitans*, *Sphagnum* sp.), giving way to emergent and wet tolerant species to occur more sparsely (e.g. *Juncus articulatus*, *Eleocharis palustris*), whereas submerged species were residual. The disappearance of submerged macrophytes leads to higher rates of respiration due to shading and increased algal blooms (Cazzanelli *et al*., 2008; Camacho *et al.*, 2016), that to certain extent would encourage growth and development of cladocerans with particular affinity for this type of food (e.g. *Chydorus sphaericus*, *Alonella exigua* and *Alonella nana*) (Barnett *et al.*, 2007; Błędzki and Rybak, 2016). However, results from this study showed considerable low biodiversity and abundance values for cladocerans in W17, even for the most resilient ones such as *C. sphaericus* in W17 and therefore, connections between aquatic plants and cladocerans are not so clear.

3.4.4 Vegetation hydro-patchiness

The topographic layout of the slacks seems to determine the distribution of different vegetation patches (Figure 3.20) and this is largely driven by i) inundation frequency (hydroperiod) or ii) water availability. Plant species in temporary waterbodies depend on soil type and humidity conditions, as well as water depth and topography (Ruiz, 2008). Temporary ponds comprise a wealth of plant species that can cope with varying degrees of complete submersion in water and species that can manage the drier periods and reduced access to, or availability of, water.

In the two slacks, wet tolerant species comprise a combination of reproductive strategies. Some competitive perennials have their budding parts either under the sediment or at the surface of the sediment, with the ability to regenerate every year (e.g. *Juncus articulatus*, *Carex nigra*). Fernnández-Aláez *et al.* (1999) found that an increase in helophyte (here broadly designated by wet tolerant species) richness in systems experiencing a greater variation in water levels reflected the topographic heterogeneity and shallowness of those Mediterranean waterbodies. Blackstock *et al.* (1993) analysed a seasonal inundated Welsh lake vegetation with resemblances to the Irish turloughs due to its geomorphological context. The study showed that species dominated different water depths with a very similar vegetation composition to the c4 and ml2 slack: punctuation of Salix bushes on the border of the pond, that would give way to wet tolerant and emergent species along the depth gradient (e.g. *Carex versicaria*, *Mentha aquatica* and *Gallium palustre*) of the wetland and on the shallow marginal side dominated by patches of moss carpet.

Even though the studied slacks have a wider array of wet to dry patches, they are considered ‘wet slacks’ following the Ranwell (1972) classification, where a water table is no less than 1m below surface. While located relatively close to the shoreline, both slacks are freshwater-fed, as evidenced by the presence of vegetation such as *Hydrocotyle vulgaris* and *Gallium palustre* (NPWS, 2013). The vegetation community also reflects the successional stages and flooding dynamics of the dune wet environments which show a great spatial and temporal variation according to the hydrological regime (Davy *et al.*, 2010).

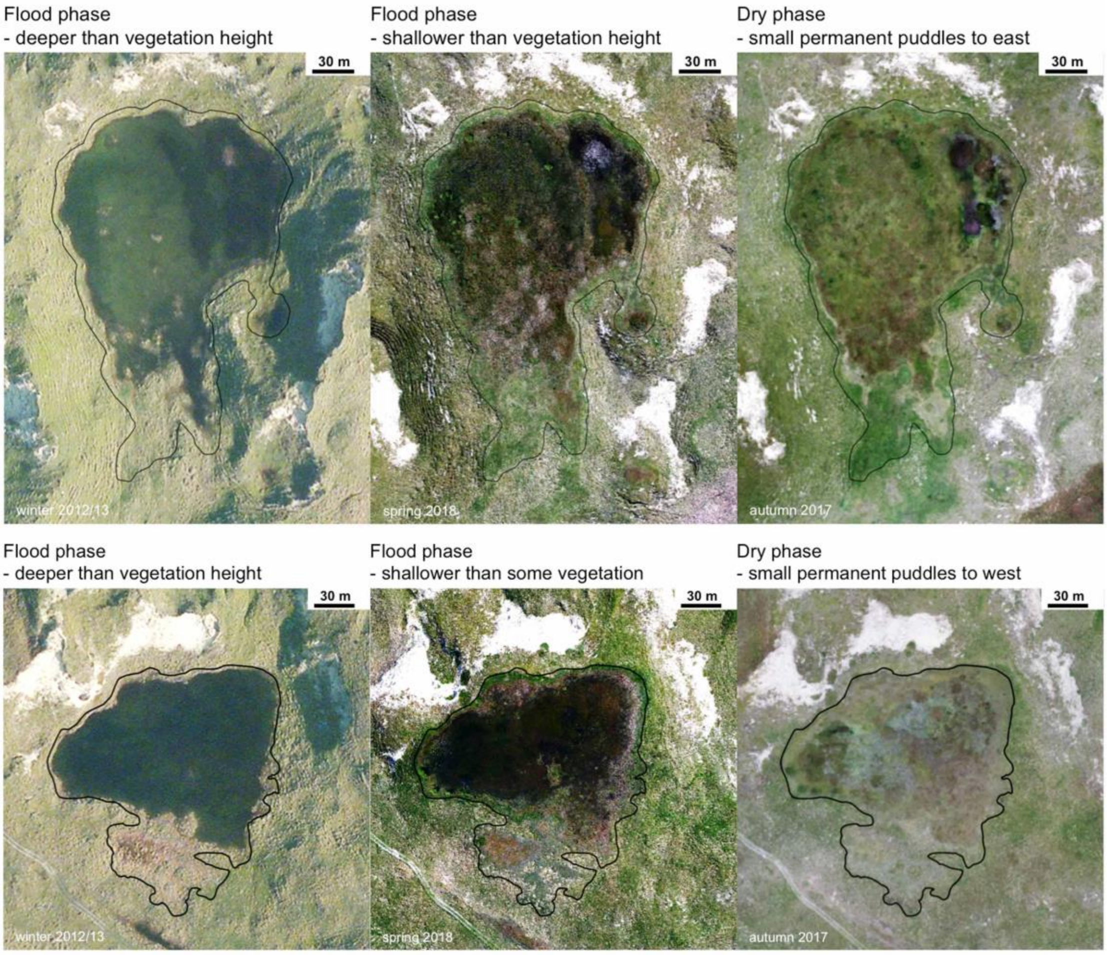


Figure 3.20 Aerial images showing the changes in flood extent relative to vegetation patches in c4 (top) and ml2 (bottom).

3.4.5 Climate change and future impacts on slack ecohydrology

Similarly to the studied slacks, Gardner (2016) showed that water levels at Sheskinmore Lough (a close large wetland located at the back of the dunes) were determined by precipitation and evapotranspiration, and based on climate change modelling, it was revealed that future ecohydrological dynamics in the wetland will likely be determined by changes in climate. Given the proximity of this Lough to the study sites, it is likely that these climatic influences will also impact the dune slacks at Sheskinmore. Human development has imposed its rules on the natural evolution and dynamic of coastal environments for the past millennia, however climate change is likely to become a stronger influential force of alteration on the coast for the next decades, with expected modifications on the natural patterns of precipitation and temperature (Jones *et al.*, 2011). At the Irish scale, the greatest threats that dune slacks face are related to changes to the natural dynamics of climate as well as hydrology, agriculture and recreation (Delaney *et al.*, 2013). All these factores have influenced the assessment of the overall conservation status of the HDS (2190) habitat as Unfavourable-Inadequate (declining), even where management is governed by strict regulations given that most of it is included within the Natura 2000 network (Houston, 2008; NPWS, 2013a).

Climate change in the 21st century is likely to cause impacts on hydrology with implications for biodiversity (Bates *et al.*, 2008), by reducing habitat suitability and threatening species with limited dispersal mobility (Hulme, 2005). Global precipitation and temperature trends show considerable variation from the past 100 years, with temperature increments of 0.5ºC to 2ºC expected by 2030 and between 2ºC and 3.5ºC by 2080 (Hulme *et al.*, 2002). In Europe, changes in precipitation intensity and frequency are expected for the future, with increased frequency in the north and decreased in the south, but subject to substantial seasonal, yearly and regional variations (Hulme *et al.*, 2002; Bates *et al.*, 2008). The UK Climate Impacts Programme (UKCIP) projects that future climate in UK and Ireland will be hotter and drier in the summer and warmer and wetter in winter (Hulme *et al.*, 2002). Based on the Irish Environmental Protection Agency report (McGrath *et al.*, 2005), climatic conditions in Ireland are due to be impacted as well, with mean monthly temperature increasing 1.25ºC and 1 to 5ºC over the next 40 years. Precipitation is predicted to decrease by around 10% in July in the south and in the north-west, and to rise by around 25% in winter with an increase in the frequency of extreme climatic events (strong winds and storms and predictions of over 20mm rainfall per day). Under these scenarios, Clarke (2010) suggests that changes in the balance caused by expected lower summer rainfall, higher evapotranspiration, together with higher winter rainfall amounts, will combine to reduce the annual recharge of coastal dune systems in NW England, with clear impacts on water dependent species. As a result, the increase in temperature and precipitation alterations, causing dune slacks to dry out or reduce significantly their hydroperiod, could result in a loss of many rare species (Jones *et al.*, 2011).

3.5 Conclusions

The dune slack systems at Sheskinmore show 2 years of inter-annual and intra-annual changes in water levels directly related to variations in rainfall. This study reveals that the effects of decreased precipitation during the autumn and winter was more severe than a summer drought, and its consequences extended across several of the following months. Water table level is the most important driver of the ecology of the studied slacks, with biological communities exhibiting increased inter-annual variability, as opposed to annual seasonal variation. Climate is undoubtedly an important influence on the optimal ecological functioning of dune slacks, and this chapter shows that inter-annual climate variability is capable of forcing more significant shifts in hydrology with substantial decline of species richness and abundance. Hydrology is imposing a strong control on cladocerans but also determining water chemistry fluctuation and vegetation patches colonisation. In this sense, vegetation was not directly related to cladoceran assemblages, but both biological groups responded similarly to water level fluctuations. As such, it is likely that weather changeability is the most important aspect of future climate change, but that there is a complex interaction between weather variability, regional weather and topography of waterbody. This study adds to examples of climate impacts on sensitive water dependant habitats and its communities and complements knowledge on Irish dune slack ecohydrological dynamics.

Chapter 4 - Spatial dynamics in dune ponds

4.1 Introduction

To understand the ecohydrolology of dune ponds, this chapter makes a broad scale analysis of controls on Cladocera and aquatic vegetation in a coastal dune system in west Donegal, northwest Ireland. The combination of environmental variation across the dune system, together with a complex topographical and hydrological context creates spatial heterogeneity in dune ecosystems more generally. Pond variation at local and regional scale is important due to the source of aquatic biodiversity that they provide, where high heterogeneity is key to high diversity at a landscape scale (Hassall, 2014; Hill *et al.*, 2017). The key aim here is to explore and characterise dune ponds across the landscape according to the variation in, and associations between, biological groups (aquatic plants and cladocerans).

The incorporation of spatial processes and environmental controls when assessing biodiversity of ponds in the landscape has been emphasised (Jeffries, 1998; Tolonen *et al.*, 2017) as this combination is essential for accurate assessment of pond status within the surrounding environment. Local factors such as water chemistry, climate, edaphic characteristics and hydrological dynamics of the ponds have been studied extensively and known to influence both fauna and flora community assemblage and dynamics in seasonal water bodies (Brooks and Hayashi, 2002; Sarma *et al.*, 2005; Rhazi *et al.*, 2006; Oertli, Céréghino, Biggs, *et al.*, 2009; Gascón *et al.*, 2012), but the incorporation of landscape features is not commonly appraised (Jeffries, 1998). Diversity and habitat heterogeneity are strongly affected by the surrounding landscape and contribute to maintaining species populations (Le Gall *et al.*, 2018). This chapter aims to explore if these assumptions are true for the species and ponds across the Magheramore dune system within Sheskinmore Nature Reserve by extending the landscape factors to intrinsic characteristics of the dune topography and the positioning of ponds within, so that the site's physical structure and the way this influences spatial dynamics in biological communities at a landscape scale is considered.

Following this, two hypotheses are tested: H1 Cladocera diversity is dictated by vegetation diversity and both are affected by environmental conditions; H2 Physical variability across the landscape is a key determinant of spatial variation in biological communities. To achieve this, the key research questions addressed in this chapter are:

- How does the environmental and spatial context of the pond within the dune landscape relate to the spatial patterns in ecology?
- Does the physical context (dune topography) impose controls on both cladoceran and vegetation communities across the dune system?
- Does high vegetation diversity correlate with high cladoceran diversity?

The Magheramore-Sheskinmore dune ponds were sampled and surveyed during one extended field campaign. June was selected for the survey as the most suitable season for capturing maximum range in both vegetation and cladoceran communities. Although late winter corresponds to the period of maximum flooding, the air and water temperature and overall climatic conditions during that time of the year compromises the development of biological communities, hence the decision to sample in summer when biological growth is at its maximum (Oliver, 2005; Sarma *et al.*, 2011). The challenge in the selection of the sampling period is to capture temporary pond habitats before the aquatic season ends and the dry period starts. Immediately before this stage, there is an increase in diversity and abundance, which together with environmental changes (temperature increase, water level decrease), leads to species maturation and adaptation to the new circumstances (Broeck, 2016). These features are visible through the development of resting eggs of cladocerans, and in the flowering of plants.

In June 2016, a total of 24 ponds were surveyed, comprising a variety of physical configurations and settings (Figure 4.1). To acknowledge the variability between ponds, the spatial monitoring included parameters that likely play a role in biodiversity and species composition such as: water chemistry (influencing overall biological health); aquatic vegetation (primary producers and species refuge and habitat structure); cladoceran community (representing the base of the food chain and key secondary producers); hydrology (defining the baseline reference on water level fluctuation); and topography (describing the local topographic setting and context relative to the predominant wind direction, neighbouring ponds and dunescape arrangement).

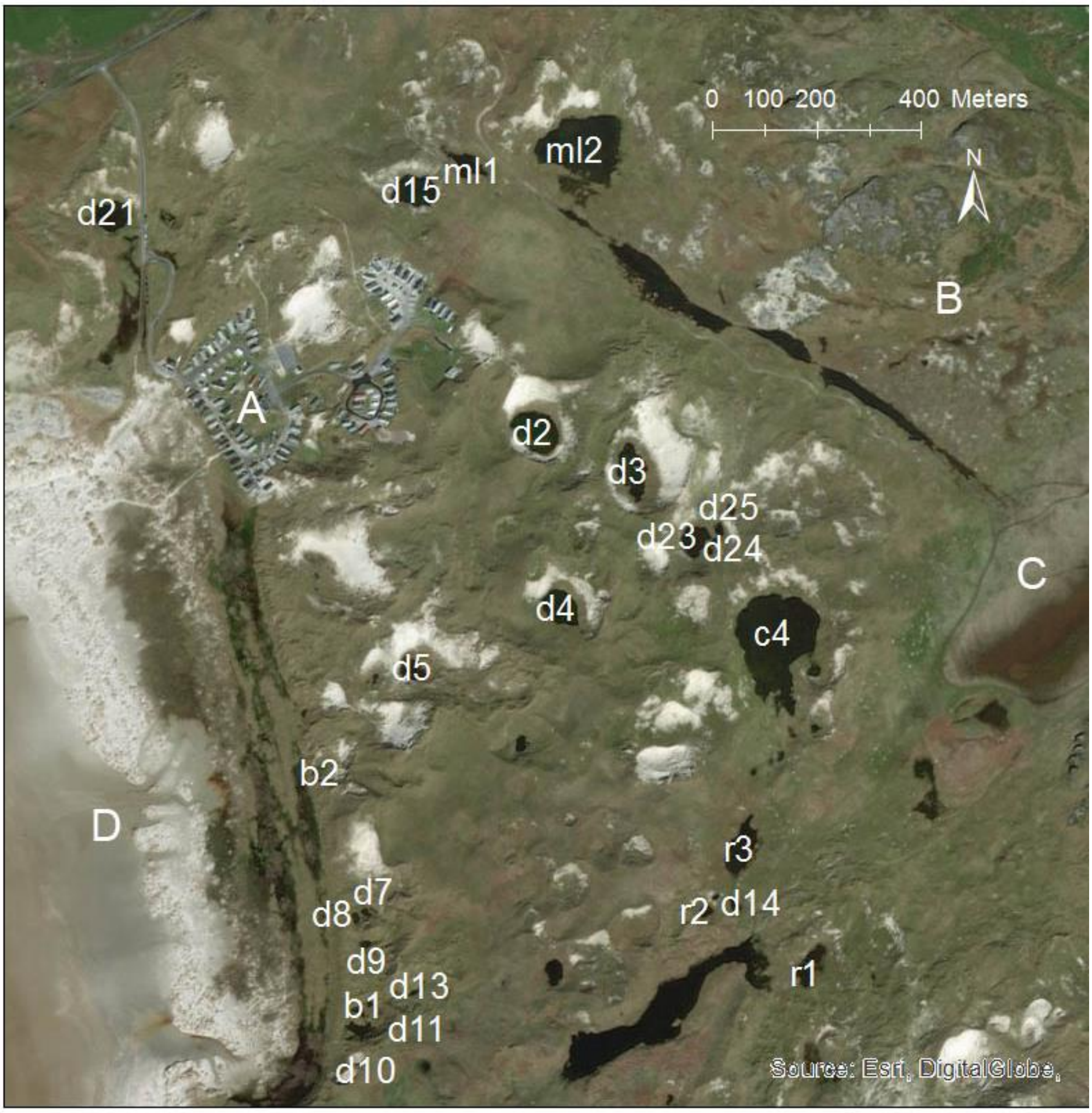


Figure 4.1 Sampling location and designated sites' codes at the Magheramore-Sheskinmore dunes, Ireland (Base Map: Bing imagery relating to winter 2011/2012 [2011-11-07 to 2012-03-28]). Sites of interest: A – Campsite, B - Mullyvea rocks, C - Sheskinmore wetland, D - Tramore Strand (beach)

4.2 Sampling and methodology

Water chemistry sampling included pH, oxygen, alkalinity, chlorophyll *a*, water temperature, conductivity, nitrates, soluble reactive phosphorus (SRP) and total phosphorus (TP). Cladoceran species were sampled by filtering pond water collected from the variety of microhabitats and depths present, and only vegetation that was growing in water was considered by walking in and around the pond to assess species abundances within the DAFOR scale. Topography and local geography was recorded with the use of a dGPS and aerial photography generated using an unmanned aerial system (UAS). To aid with visualisation of the landscape, spatial data layers were examined in ESRI ArcMap 10.1 (ESRI, 2015). In addition, Sentinel-2 imagery covering the site was explored visually to enable a qualitative assessment of pond hydrology. Launched in June 2015, the instrument captures four bands in the visible and near-infrared wavelengths at a resolution of 10m, with revisit timescales of less than a month (Walters and Scholes, 2017). Obtaining cloud-free imagery for northwest Ireland does reduce the temporal frequency of suitable images, but there is a sufficient number to capture the dune system during most seasons. Images were viewed as false-colour composites in order to maximise the visual clarity of wet versus dry areas, and for each pond location, an assessment of flood extent was undertaken. A description of the study area, and methodological approaches and techniques are explained in detail in Chapter 2 - .

4.3 Results

4.3.1 Environmental properties of dune ponds

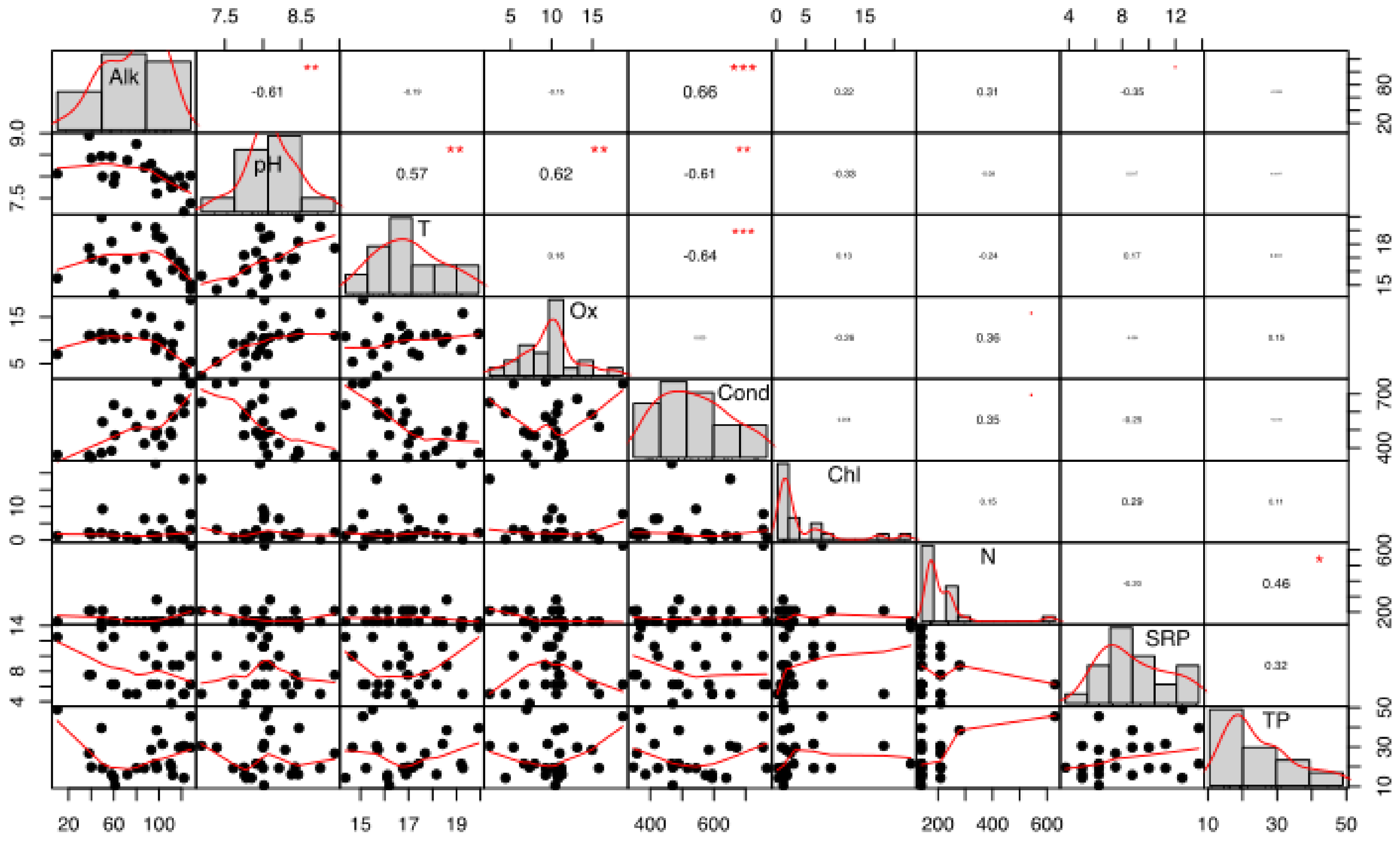
A summary of the mean chemical values is shown in Table 4.1. This includes the length of the measures taken from the sites and helps to retain maximum gradients in the data to help support which variables are used further in the multivariate analyses. In this case, pH and water temperature have very little deviation across samples, whereas conductivity, nitrates and alkalinity show more of the variability. Temperature measurements were taken at different times through the day and these could likely reflect some degree of solar heating and cooling. The gradient of variation (standard deviation of 1.5 ºC) is possibly reflecting this.

Table 4.1 Summary of water chemistry parameters: minimum, maximum, standard deviation and median values

Parameter	min	max	stdev	median
Alkalinity (µg/L)	10.00	128.00	32.92	95.00
Chlorophyll *a* (µg/L)	0.17	22.83	5.66	1.63
Conductivity (µS/cm)	347.00	767.00	131.01	503.50
Nitrates (µg/L)	139.40	628.90	10.24	139.40
Oxygen (mg/L)	2.41	18.72	3.59	10.53
pH	7.19	8.94	0.40	8.01
Soluble Reactive Phosphorus (µg/L)	3.75	13.75	3.02	7.50
Total Phosphorus (µg/L)	10.50	49.00	10.27	20.60
Water Temperature (ºC)	14.37	19.93	1.50	16.84

Spearman’s correlation coefficient between the different variables is shown in Table 4.2. Alkalinity is positively related to conductivity and negatively related to pH. pH is inversely correlated with alkalinity and conductivity but significantly related to temperature and oxygen. The temperature of the water is positively correlated with pH values, and negatively correlated with conductivity, denoting that higher water temperature in the ponds relates to more alkaline environments with less salt content. Chlorophyll *a* is not strongly correlated with any variable. Nitrate values are positively correlated with oxygen, conductivity and more strongly related to total phosphorus. But one high value is skewing the results here.

Table 4.2 Environmental variables correlation values (top triangle), histograms (diagonal) and scatterplots (lower triangle) with trendlines. red symbols (“*”, “**”, “*”, “.”, " “) are associated with significant p-values (0, 0.001, 0.01, 0.05, 0.1, 1). Abbreviations on the table correspond to (from left to right): Alk – alkalinity; pH – pH; T – water temperature; Ox – oxygen; Cond – conductivity; Chl – chlorophyll a; N – Nitrates NO_3^-; STP – soluble reactive phosphorus; TP – total phosphorus.**



4.3.1.1 Geospatial patterns in pond water chemistry

A pseudo-map approach was generated in R (R Core Team, 2014) to determine the relative positions of the sites so that the spatial organisation is accurate, at the same time that bring sites to a more condensed and readable form (Figure 4.2 and Figure 4.3). Spatial patterns are evident in some environmental characteristics, and not others. Higher alkalinity values are concentrated close to the shoreline in the south and south-west of the dunes. Similar to alkalinity, conductivity values show a southwest-northeast trend, with higher values concentrated on ponds further west, and lower values for those that are located inland. The overall pH values of the dune ponds are alkaline. The majority of the sites’ pH ranges between 7.5 and 8.5. Only r1 and b1 sites have slightly more neutral waters; d2 and d8 represent the more alkaline ponds from the dunes system. Most of the sites have very low chlorophyll *a* values, and sites r1 and r2 in the most southeast corner, have the highest concentration. Water temperature shows a north-south pattern, with middle range temperatures concentrated in the centre of the dune system. The majority of the coldest sites are located just under 100 metres form the vegetated backshore of Tramore Strand (west of the dunes), whereas the warmer temperature ones are on the middle to north side, although this could relate to the timing of the sampling as well, as mentioned before. Variability of nitrate (Figure 4.3) across the landscape is small, with low concentration on most of the sites. Site b2 had the highest value sampled, followed only by ml1. Although the north side of the dunes contains ponds with higher values of SRP, then the northeast southwest is generally middle ranged. Southeastern and central sites comprise the lowest values. Southern sites show lower oxygen values than ponds form those northward, and only three sites (b2, d8 and d11) have high values, all located on the west shoreline. Sites ml1, b2, d15 and d23 have the highest TP values. Lower values are more towards the south, although a strong pattern is not particularly clear. Middle range TP values, although also scattered, tend to congregate more on ponds closest to the beach backshore.

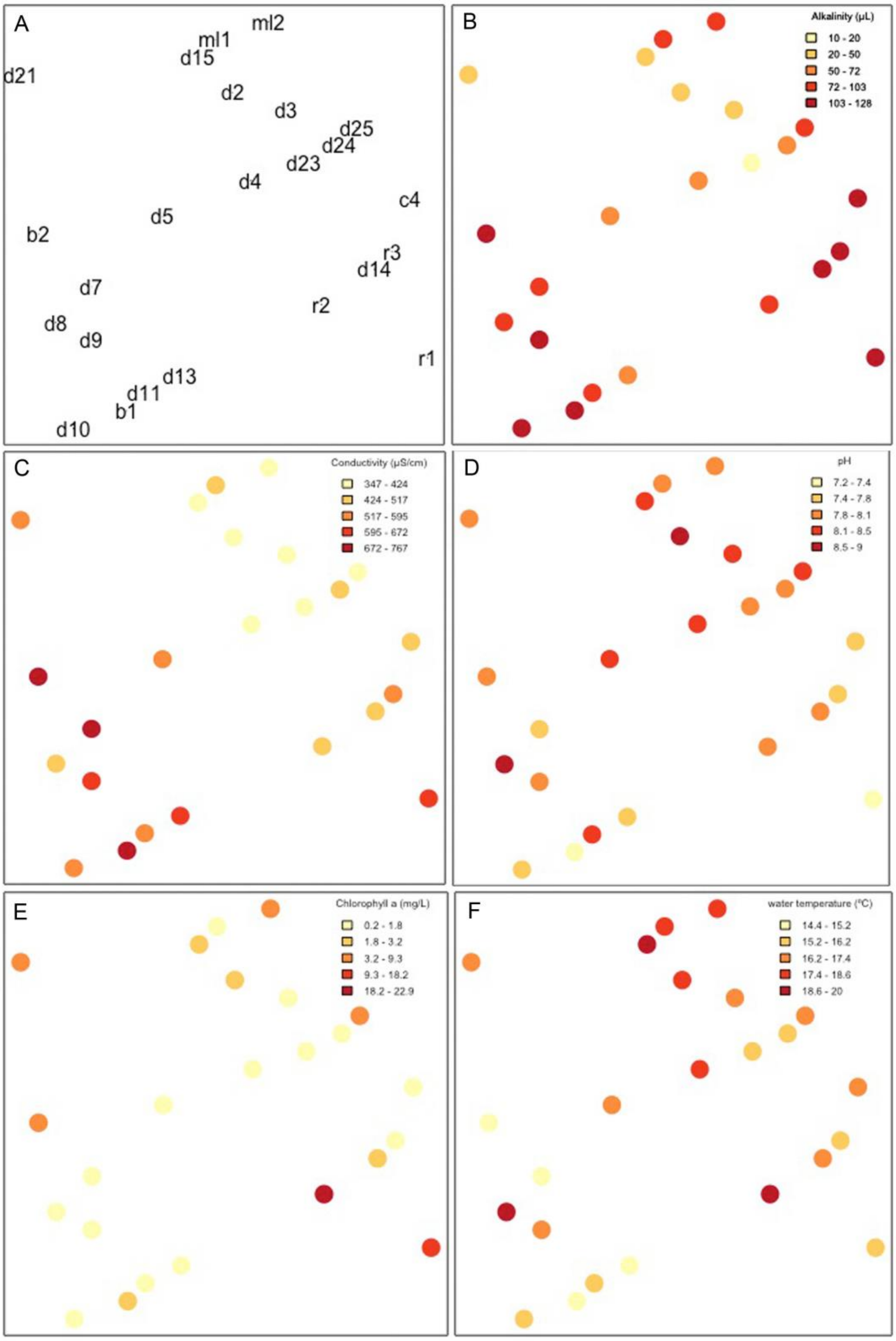


Figure 4.2 Spatial pattern in the Sheskinmore dune pond water chemistry: A – Site map, B – alkalinity, C – conductivity, D – pH, E – chlorophyll *a*, F – water temperature

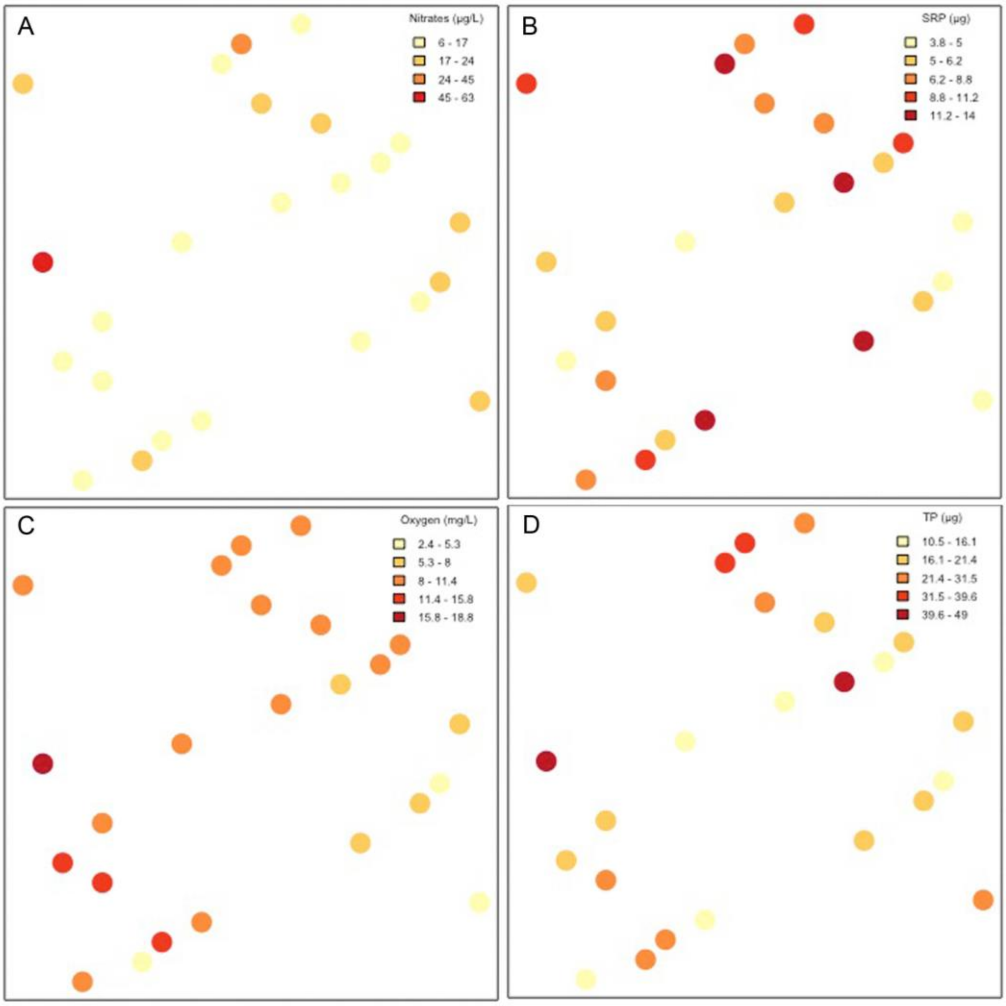


Figure 4.3 Spatial pattern in the Sheskinmore dune pond water chemistry: A – nitrates, B – soluble reactive phosphorus, C – oxygen, D – total phosphorus

Hierarchical clustering of sites using a Euclidean distance matrix for water chemistry data was undertaken to gain an understanding of groupings of sites with similar water conditions. The resulting dendrogram organises samples (ponds) based on their relative distances within this data space (Figure 4.4). Site b2 is identified through this analysis as being significantly distinct from the other ponds. Key characteristics of b2 that explain this are possibly linked to the high levels of TP, alkalinity, oxygen and the highest value of nitrates compared to all other sites. At smaller separation distances, sites b1 and d7, and to a lesser extent sites r1 and r3 are also highlighted as being distinct from the other sites. There is no clear spatial pattern to this organisation of ponds based on water chemistry, but like site b2, sites b1 and d7 comprise different chemical characteristics that include higher levels of conductivity and alkalinity, and the same range of values for oxygen, chlorophyll *a* and pH. Sites r1 and r3 share the same range of values for all variables apart from TP, pH, conductivity and chlorophyll *a*.

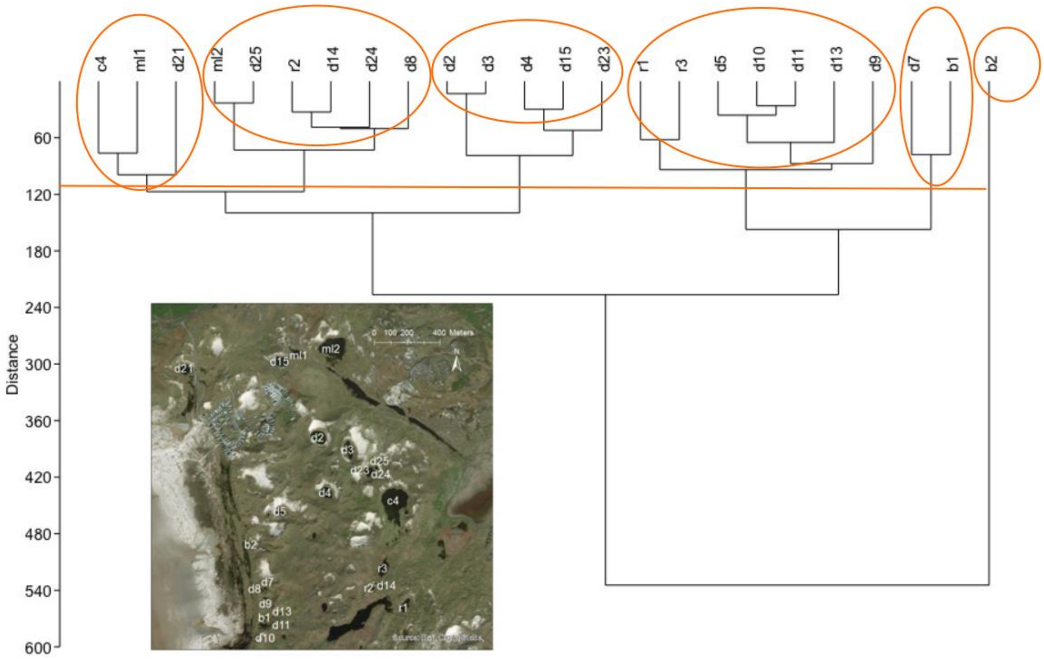


Figure 4.4 Hierarchy clustering of sites according to water chemistry variables with spatial map of Sheskinmore sites. Orange horizontal line shows the clustering threshold

To support the cluster analysis, ordination of the pond water chemistry was performed with PCA. Components 1 and 2 in this analysis explain 33% and 20% (eigenvalue = 2.97 and 1.79 respectively) of the total variance. The primary gradient is strongly explained by conductivity, alkalinity and pH, whereas nitrates, TP and oxygen are key variables aligned with the second component (Figure 4.5). Linked to the gradient defined by PC1, sites d2, d8 and d15 have higher pH, temperature and oxygen, with lower alkalinity and chlorophyll a, the exact opposite of sites b1 and r1. Site b2 distinguishes itself by having a higher measure of nitrates than any other site, and is a clear outlier driving the length of PC2.

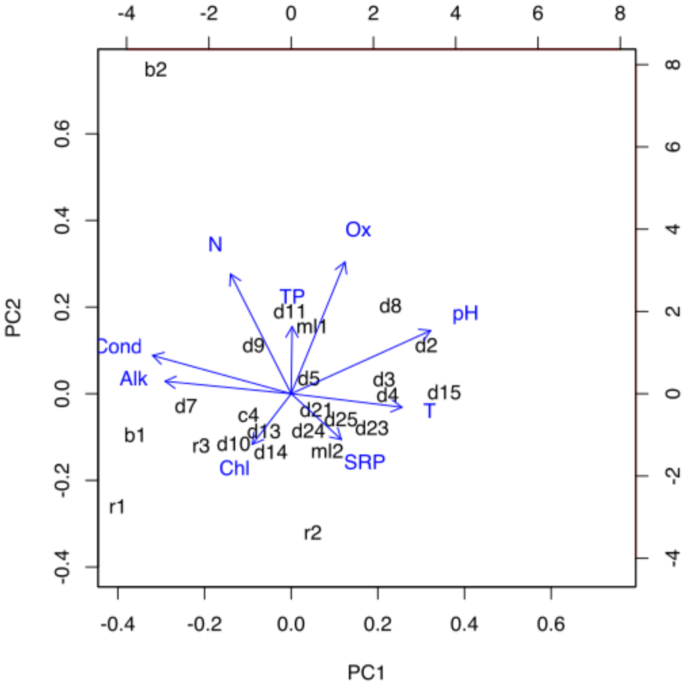


Figure 4.5 PCA ordination of sites according to water chemistry variables. Abbreviations: Cond – conductivity, Alk – alkalinity, N – nitrates, TP – total phosphorus, Ox – oxygen, pH – pH, T – water temperature, SRP – soluble reactive phosphorus, Chl – chlorophyll *a*

4.3.2 Hydrogeomorphology

The dune ponds across the Magheramore-Sheskinmore exist across a complex topography that has arisen through multiple phases of dune growth and destabilization (Burningham, 2008; Barrett-Mold and Burningham, 2010; Bolles, 2012). Here, the topographic positioning and spatial hydrological arrangement of the studied ponds is examined through a range of spatial data including satellite imagery, aerial imagery, dGPS data and UAS surveys from ongoing and other studies (Bolles 2012; Barrett Mold 2013; Gardner 2016), in addition to contributions from the park ranger and management board at the Reserve.

4.3.2.1 Inter-site distance matrix

Spatial trends in the region were studied based on distance matrix to understand how many ponds within a certain radius shared the same characteristics, and how elements are distributed across the landscape. This is only valid for assessing physical and chemical similarities, rather than understanding biological connections, as different modes of dispersal, in particularly for aquatic organisms, are passively disseminated between sites and dependent on other variables than just straight line distances (Leibowitz and Brooks, 2008; Horváth *et al.*, 2016). Topographic surveys using dGPS were undertaken to position the ponds spatially and vertically, and these data were used to create an inter-pond distance matrix for all pairs of sites. Cluster analysis was then used to form a hierarchical classification of sites in relation to their proximities and differences between pond clusters can be seen on Figure 4.6. Here, Euclidean distances created an hierarchical clustering, where longer lines correspond to longer distances across the dune ponds. A distance threshold of 200 metres was used to create different groups. There were 7 different clusters of sites, with 4 of them containing 3 to 7 sites. Two of the clusters comprised only 2 sites and one of them (d21) with a distance over 400 metres from all the others, being the most isolated site from the dune system studied.

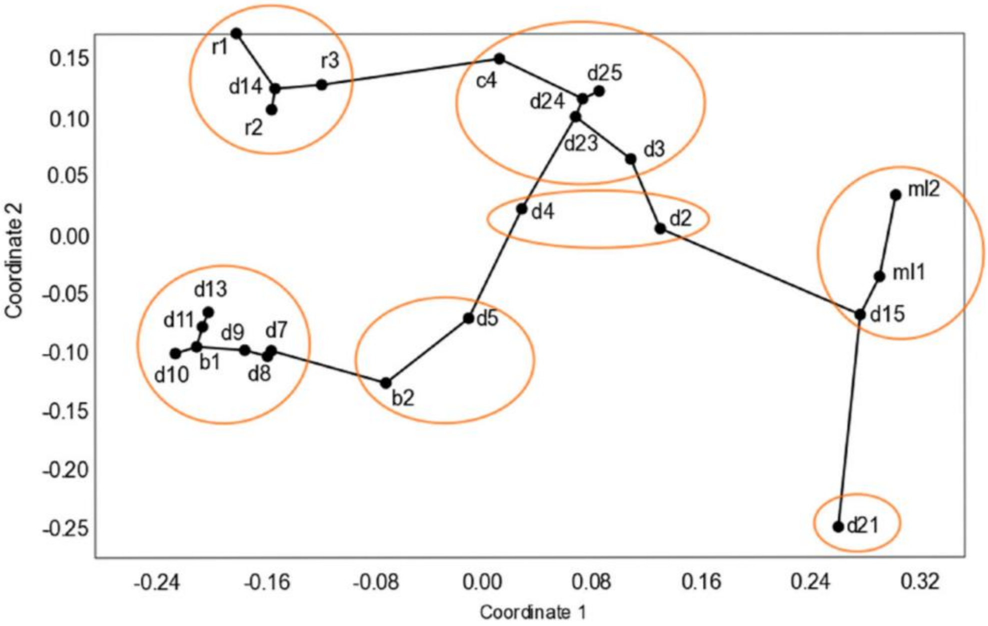


Figure 4.6 Spanning tree with clusters of sites less than 200m distance apart

4.3.2.2 Topographic framework

To understand the positioning of the different sampled sites in relation to mean sea level and relative vertical position within the dune landscape, the dGPS-derived elevations of the pond bed are presented in Figure 4.7. This illustrates the topographic differences of the ponds in the dune system. Ponds closer to the beach backshore generally have lower elevation, between 4 and 5 metres OD (e.g.: b2, d9 and d10), and those further inland are higher, from 7 to 8 metres OD (e.g.: c4, ml1 and ml2). Most of the sampled ponds lie within a range of 5-7 metres OD.

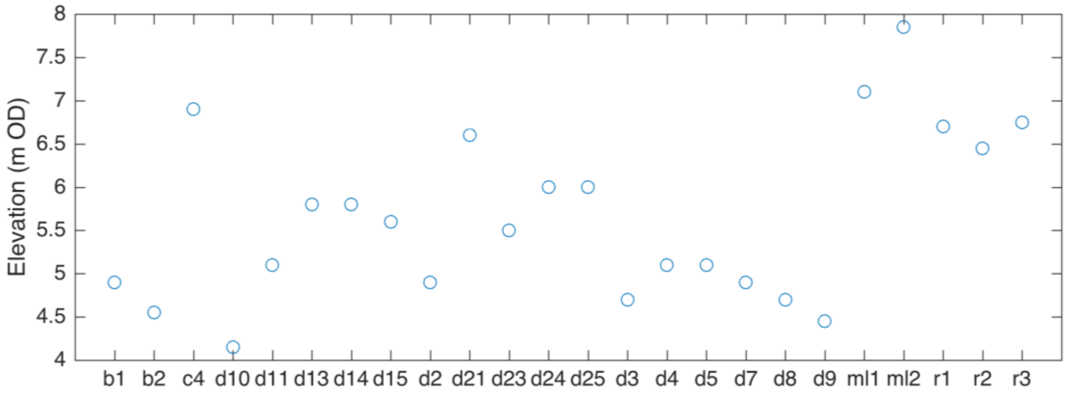


Figure 4.7 Pond bed elevation (metres) in relation to mean sea level (OD – ordnance datum)

4.3.2.3 Hydrological regime

Monitoring of all ponds in the Magheramore-Sheskinmore dune system was not possible, but the changing flood extent could be appreciated through the assessment of Sentinel-2 satellite imagery. For each pond, cloud-free imagery between August 2015 and February 2018 were reviewed through the Sentinel Hub Playground (available at https://apps.sentinel-hub.com/sentinel-playground), which allows the online visualisation of band composites and standard spectral indices such as NDVI. Remote sensing technologies enable earth observations of the habitat structure by a time-series analysis (part of the European Copernicus Programme and the NASA Landsat Data Continuity Mission) that enable monitoring of seasonal variations of habitat biophysical characteristics (Walters and Scholes, 2017). The degree of visible flooding was noted for each pond in each image, and these were classified on the basis of flooding continuity into 4 hydrological categories (**Table 4.3**). Satellite images reveal distinct fluctuation in water availability across the dune ponds, leading to a continuum between transient to permanent flooding. Ponds that easily dry out (category 1) correspond to the ones that were most frequently dry, with a short hydroperiod. Ponds in the category 4 are those that have sustained a permanent aquatic habitat all year round and from year to year. These tend to be ponds closer to the water table or the beach backshore. Hydrological category 2 refers to ponds that only dry in very dry years (here also classified as temporary I), and that in normal conditions keep patches or lower parts of the pond wet. Condition 3 corresponds to ponds that are predominantly wet but have registered a single drought event in the years studied (classified as temporary II); only ml1 and d4 show this characteristic.

Table 4.3 Spatial hydrological output based on Sentinel 2 satellite imagery (2015-2018)

hydrological category	description	sites
1	Easily dries out (ephemeral)	r1, r2, r3, d14, d24, d25
2	Occasional dries out (temporary I)	c4, ml2, d5, d21, d23
3	Frequently wet (temporary II)	ml1,d4
4	Permanently wet (permanent)	b1, b2, d2, d3, d7, d8, d9, d10, d11, d13, d15

4.3.2.4 Morphometrics of the pond depressions

The way that sites are interrelated according to their geometrical and spatial properties was examined by generating individual pond metrics for elevation, area, major axis length, circularity (Cox, 1927) and aspect ratio (ratio of minor to major axis lengths). The aspect ratio captures the extent to which the pond is equidimensional, where the index ranges between 0 (more elongated) and 1 (equidimensional). Circularity also ranges between 0 and 1, but relates more specifically to roundness, where a value of 1 is a true circle and a value closer to 0 is a more complex, angular shape necessitating a larger perimeter (Cox, 1927). To account for water context, the hydrological categories describing the hydrological regime in recent years, and also the water depth at the time of sampling, were included. Sites were grouped according to their physical characteristics (Figure 4.8) in a PCA ordination, where the first axis (PC1) explained 36.9% and the second axis (PC2) 31.3% (eigenvalues=2.58 and 2.19 respectively).

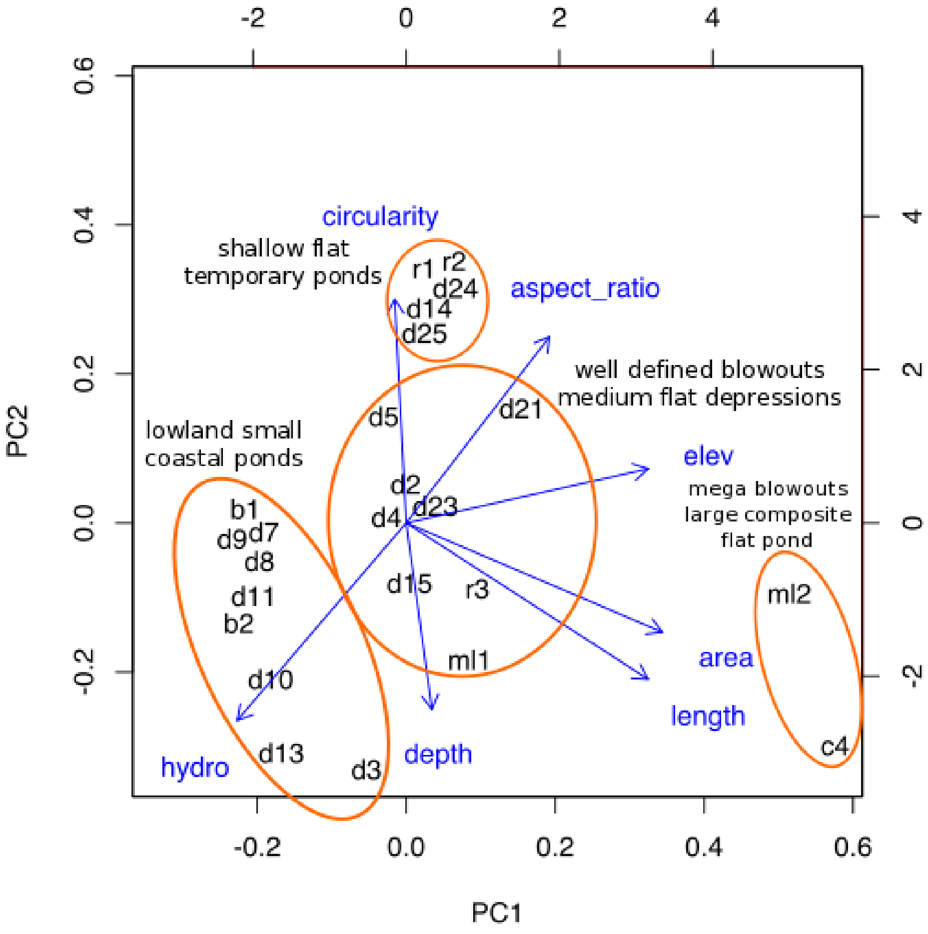


Figure 4.8 PCA ordination of sites according to topological variables. Abbreviations refer to: elev – elevation; hydro – hydrological category; depth – water depth, length – pond maximum length; area – pond area; circularity – roundness of pond; aspect_ratio – equidimensionality of pond shape

Ponds group themselves according to size (area and length) and hydrological categories, which constrain the first axis, and by shape (circularity) which determines the second axis groupings. The lower elevation, small coastal ponds are deeper, more elongated and have a more permanent water dynamic to them, contrasting with the shallow flatter, more circular and more seasonal ponds further inland. Ponds c4 and ml2 are distinctive from the others in terms of size, where they occupy large, extensively hollowed out blowout depressions, the largest blowouts in this dune system. These two sites have large flat areas where subtle topography has evolved through the development of small, within-blowout depositional features, and this has helped create a heterogeneous aquatic system with a complex microhabitat structure, as studied in more detail in Chapter 3 - .

4.3.3 Cladoceran composition

Individual rarefaction was used to compare taxonomical diversity in samples of different size to understand how many taxa might be expected in samples containing smaller total number of individuals (Hammer *et al.*, 2001). Pond samples that did not reach a plateau (Figure 4.9) suggests under estimation of cladoceran species richness. In the main, these were ponds that were shallow with limited water available (e.g.: r1, d14 from Figure 4.9a) or were slightly deeper with a large survey area (e.g.: d2 and ml2). Sites c4 and d8 for example, reached a plateau and have higher number of taxa, but are profoundly different ponds in terms of size (c4 large and d8 small). On the other hand, site d8 together with the other ponds shown in Figure 4.9c had the highest number of specimens, but still ml2 and d13 did not reach a plateau.

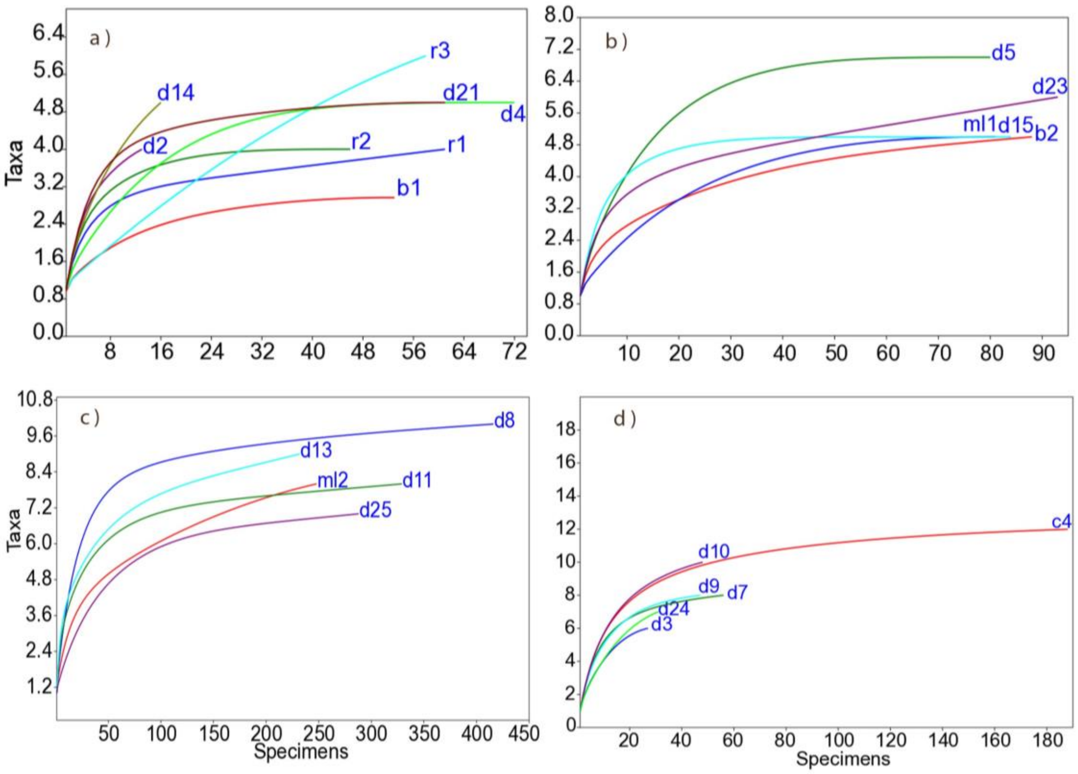


Figure 4.9 Different scale subsets of rarefaction curves for cladoceran specimens and taxa in each sampled site. Different scales for number of taxa and specimens in each subset a), b), c) and d)

Summer 2016 samples of the dune ponds included 30 different species and lifeforms, like male specimens or presence of ephippia (resting eggs) on the females. These help understand the dynamics of both habitat characteristics and population dynamics reflecting spatial and temporal processes. In total, 19 species were identified belonging to the 6 families Aloninae, Chydorinae, Bosminidae, Daphniidae, Eurycercidae and Macrothricidae. Of these, the 10 most common species were found in 58% of the sites (Figure 4.10) with *C. sphaericus*, *S. exospinosus* and *A. nana* occurring more frequently and found in 96% of the sites.

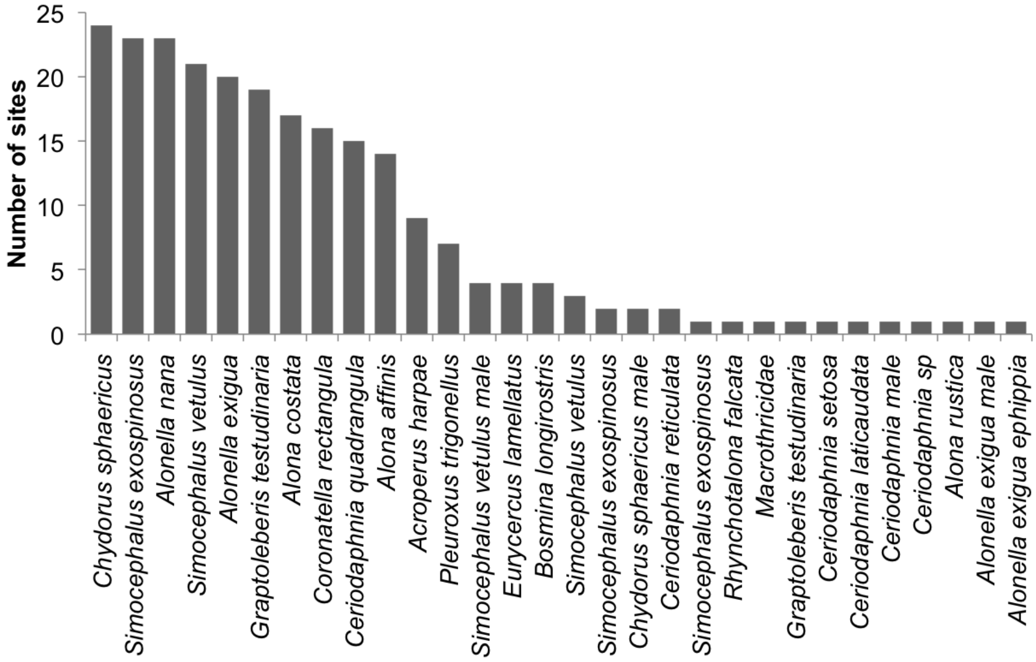


Figure 4.10 Total number of sites supporting individual cladoceran species

These last three species share some similarities and differences in terms of features and inherent characteristics (Table 4.4). *Chydorus sphaericus* is a pioneer species, very plastic, easily adapted to a wide range of environmental conditions as seen by the trophic range and hydrological tolerance. *Simocephalus exspinosus* is a very large sized species that tolerates slightly more eutrophic conditions than the other two; it is a filter-feeder unlike the other two which use a scraping method. *Alonella nana* is the smallest of the cladocerans and comes out as a species that thrives on sandy substrate waterbodies amongst vegetation and does not tolerate hydrological variability or eutrophic environments as the other two species do.

Table 4.4 Summary of main life history and features reviewed from the literature of the 3 most abundant species. Source: Lynch 1980; Dumont 1994; Błędzki & Rybak 2016; Rizo et al. 2017

species	zone	habitat	hydrology	feeding	body size	sexual period	trophic tolerance
Alonella nana	littoral	vegetation and sand	permanent	scraping algal particles from periphyton	<0.5 mm	autumn	oligo-meso trophic
Chydorus sphaericus	benthic and pelagic	vegetation	variable	scraping algal particles from periphyton	<0.5 mm	summer autumn	oligo-eutrophic
Simocephalus exspinosus	littoral	vegetation	variable	filtering from a stationary position	>2.5 mm	all year	meso-eutrophic

Species differentiation into males and ephippia features is represented in Figure 4.11. This representation helps to reveal which sites at the time of sampling had general different environmental characteristics that led to the appearance of asexual life forms – life-cycle phase in preparation for the next generation (Lynch, 1980; Caramujo *et al.*, 2013). These life form characteristics show a much lower incidence, appearing in only 6 sites, equivalent to 25% (specifically c4, d10, d11, d14, d25 and r1). Most notably, site d25 has the highest occurrence of cladoceran male and ephippia life forms presence.

Figure 4.11 Proportion of cladoceran male and ephippia presence across the sites

The spatial organisation of cladoceran families shows that Chydoridae, Daphnidae and Aloninae are the dominant families (Figure 4.12) and present at all sites apart from r1 (without Aloninae) and b1 (without Daphnidae). Macrothricidae was only found in pond c4 and Eurycercidae and Bosminidae are present in four ponds. In terms of diversity, site c4 contains all 6 families, being the more diverse, including a variety of species and adaptability. Sites b1 and r1 have the poorest representation, with only two families present. Site b1 includes small sized cladocerans (Aloninae and Chydoridae) only and r1 a combination of large and small sizes. Overall, in terms of both pond size and geographical location, there is little evidence of a distinct spatial pattern here.

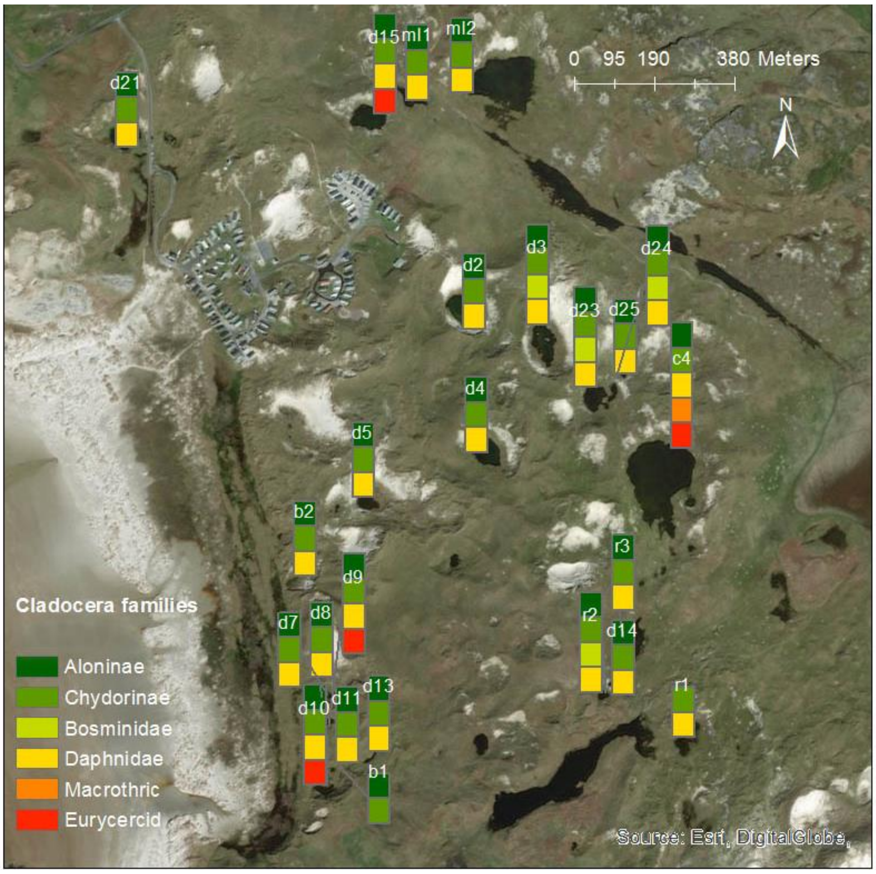


Figure 4.12 Presence of the six cladoceran families across sites

4.3.3.1 Cladoceran species assemblages

Twinspan (Hill, 1979) was used to examine the cladoceran species within the context of communities and sites; on the basis of the 30 taxa, the 24 sites could be divided into 6 main groups (Figure 4.13). Group 1 (n=4, eig=0.254) is indicated by *Bosmina longirostris*, a generally eutrophic species, represented here in the main by the shallow and rather exposed ponds that lack steeper walls. Group 2 (n=6, eig=0.221) has *Coronatella rectangula,* a fast swimmer that lives preferably amongst submerged vegetation (Viti *et al.*, 2013), as the indicator species. This group contains a range of large and shallow or small and deep ponds, with the majority of them concentrated on the west margin of the dunes, close to the beach backshore. Group 3, only contains two ponds (eig=0.217), and small individuals such as males and *Alonella nana*, known as the smallest of all cladocerans. Group 4 (n=4, eig=0.219) share *Eurycercus lamellatus* as indicator species and contain ponds that vary in size and depth.

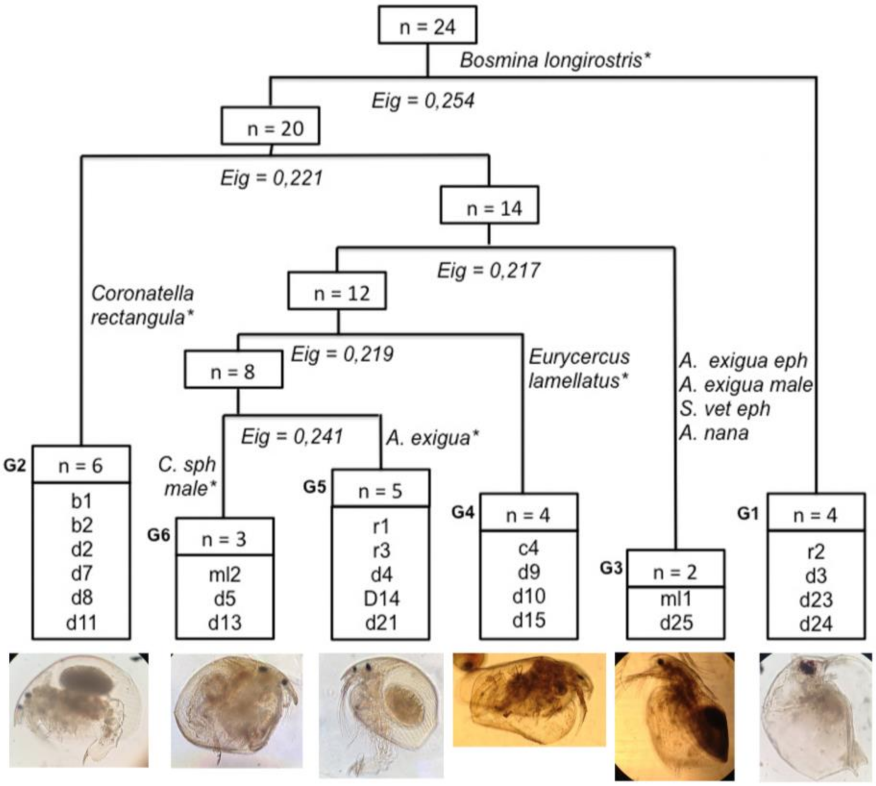


Figure 4.13 Twinspan dendrogram of cladoceran assemblages in June 2016, indicator species for each group (*) (*Simocephalus vetulus* ephippia for G3) and respective pictures and eigenvalues. Species abreviations: C. sph – *Chydorus sphaericus*; S. vet eph – *Simocephalus vetulus* ephippia; A. exigua eph – *Alonella exigua* ephippia. Note that species' pictures are in different scales

E. lamellatus is one of the largest (1.6-3.5 mm length) and lives preferably amongst *Chara* sp, feeding on bacteria rich environments, associated with vegetation detritus (Scourfield and Harding, 1941). Group 5 (n=5; eig=0.241) comprises a mixture of temporary/shallow and permanent/less shallow waterbodies, and has *Alonella exigua* as an indicator species, often associated with nympheids and other floating leaved plants (Dumont, 1994). The Group 6 (n=3, eig=0,241) indicator, *Chydorus sphaericus* male is the most common and widespread species, often considered a pioneer of establishment, tolerating a wide range of environmental changes and trophic conditions; this group contains ponds that vary in shape, size and depth (Scourfield and Harding, 1941; Błędzki and Rybak, 2016).

To explore the patterns of cladoceran assemblages, the spatial distribution of cladoceran communities based on the Twinspan groupings was plotted but shows no clearly defined pattern (Figure 4.14). Nevertheless, the largest group (2) is more concentrated toward the seaward margin, and groups 1 and 5 are primarily found toward the rear of the dune system. The other groups present themselves scattered throughout the landscape.

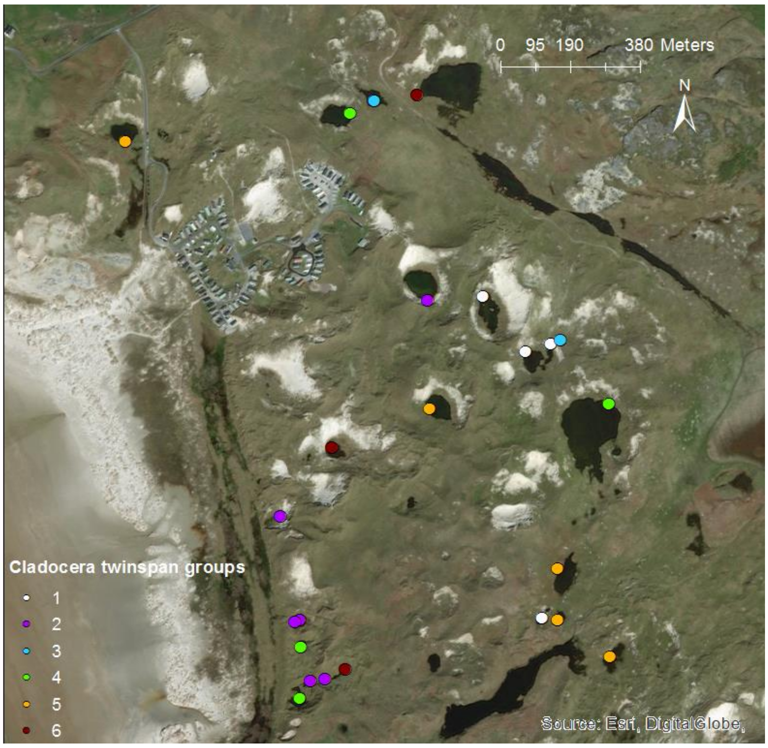


Figure 4.14 Spatial representation of the six cladoceran Twinspan groups

Cladoceran Twinspan groups were tested against all environmental controls (chemical and physical) to see whether there was any factor that would help explain variability within groups. The non-parametric Kruskal-Wallis test was used to determine significant variability between groups. Water chemistry variables did not have any expression in explaining variation within groups ($p>0.05$). Hydrological categories showed significant differences between groups ($p=0.01$) (Figure 4.15).

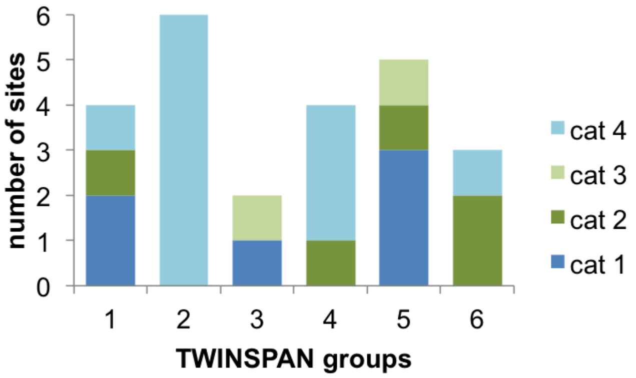


Figure 4.15 Hydrological categories frequency distribution within Cladocera Twinspan groups

Hydrologically, group 2 stands out as the only significantly different, with all sites belonging to cat 4 (permanently wet). Group 3, 4 and 6 range between two hydrological categories. G3 – ponds that easily dry, cat 1, or that are frequently wet, cat 3; and G4 and G6 share the same hydrological categories of ponds that occasionally dry, cat 2, or that are permanently wet, cat 4. Groups 1 and 5 have mixed hydrologies between their ponds, but G5 does not contain ponds that are permanently wet, cat 4.

4.3.4 Plant composition

4.3.4.1 Habitat characteristics

From the 50 plants listed (Figure 4.16), 47 were identified to species level. Nine plant specimens were recorded in 63% of the sites, and the three most pervasive species, *J. articulatus*, *R. flammula* and *H. vulgaris*, were present in over 88% of the sites. Nineteen species fit within the range of 21-54% of the total number of sites, and sixteen species were only present in less than 8% of the sites. Different life form categories of the plants were assessed and show that these sites comprise a broad suite of terrestrial and aquatic species (Figure 4.17).

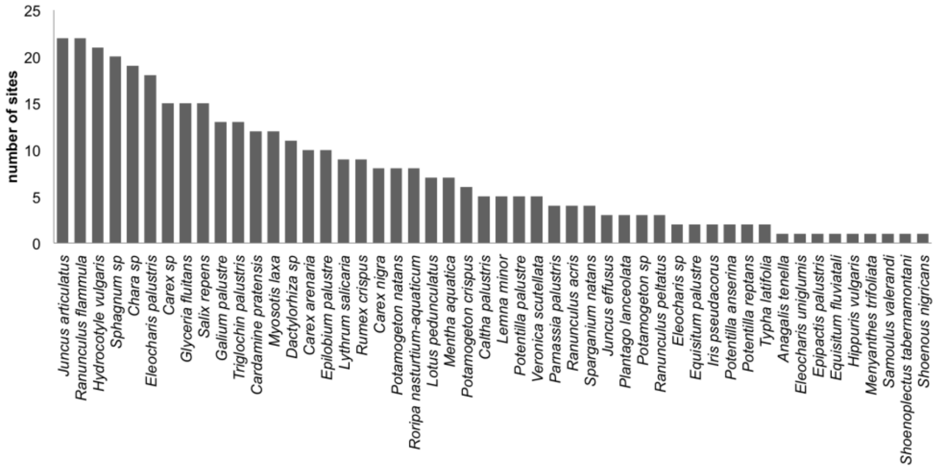


Figure 4.16 Total number of sites supporting individual plant species

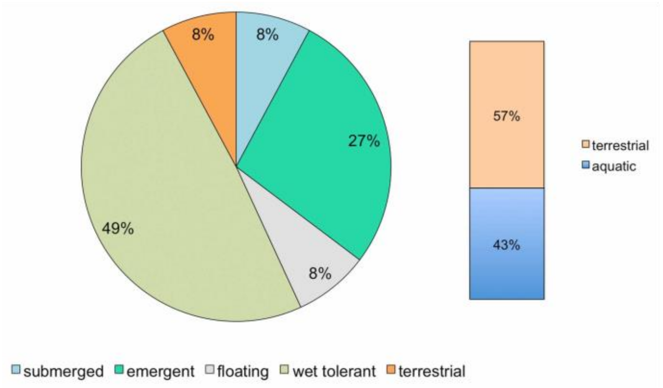


Figure 4.17 Frequency distribution of vegetation life forms in the sites sampled

The vast majority (49%) of vegetation species present in the ponds are wet tolerant, meaning that although not fully aquatic, they frequently occur along the margins or tolerate wet rhizomes (e.g. *Carex* spp, *Juncus* spp and many forms of *Ranunculus* spp). These are followed by emergent vegetation (27%), characterised by species that start their cycle in water, but emerge to reach maturity out of water (e.g.: *Eleocharis* spp, *Mentha aquatica*). Submerged species, that go through all life cycle in water (e.g.: *Chara* sp), floating species, that grow in water and reach maturity on the surface (eg: *Glyceria fluitans*, *Potamogeton* spp) and fully terrestrial species, the ones that have their life cycle in dry conditions (e.g.: some forms of *Potentilla* sp, *Salix repens*), each represent 8% of all the life form categories. In total, the sampled sites comprise 57% of non-aquatic species against 43% of fully aquatic vegetation.

Spatial visualisation of the vegetation life forms (Figure 4.18) shows that all the sites contain wet tolerant species, as the main composition of the plant community. Emergent species are absent from site r1 and very low represented in sites d15, d23-25. All of the sites have submerged species apart from b1. *Chara* sp used to be present at b1, but the resident population was lifeless at this time of sampling. Terrestrial species are the least represented of all life forms, but absent from d25, b2, d8, b1, d11, d13 and r1. Floating species although in low abundance are present in most of sites, and non-existent in ml2, d23, d25, r3, r2 and d14.

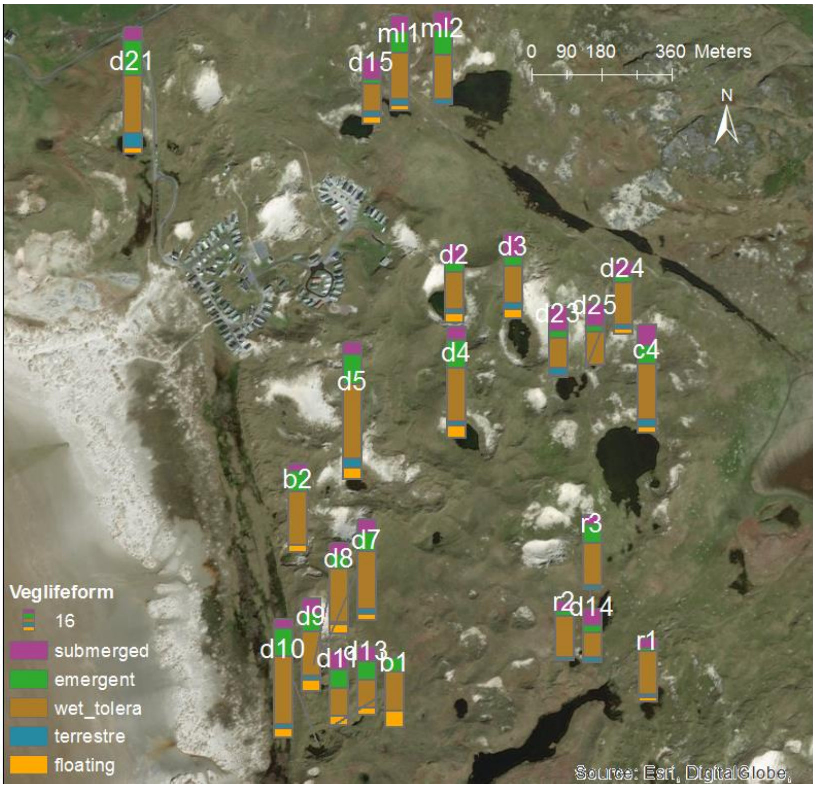


Figure 4.18 Spatial distribution of vegetation life forms across sites

4.3.4.2 Plant species assemblages

To understand the distribution of vegetation across sites, the vegetation data were analysed using Twinspan (Hill, 1979), organising the species into similar groups (Figure 4.19). From the 24 sites and 50 vegetation species, 6 groups are interpreted from the Twinspan divisions. Group 1 (division 2) contains 3 sites having *Rumex crispus* and *Dactylorhiza* sp as indicator species. These species fit within the ‘wet tolerant’ category, existing on the margins of the ponds. The ponds themselves are located relatively close to one another, in the central part of the inland dune system within deep sheltered blowouts, at the bottom of high dune walls. Group 2 (division=3) has both *Lythrum salicaria* and *Rorippa nasturtium-aquaticum* as indicator species in 5 sites. The first species commonly occurs along the margins of wet areas, whereas the second generally grows in fully aquatic environments. The sites range from small to medium in size, but all share deep topographic contexts, and apart from d4, they are all close to the shore. Group 3 (division=3) contains 5 ponds in the southwest corner of the site that lie closer to the beach backshore. The indicator species here is *Sphagnum* sp, which is a common feature of the slack dunescape at Magheramore-Sheskinmore, and is known to prefer acidic environments (Murphy 2002), usually dominating the pond bed area.

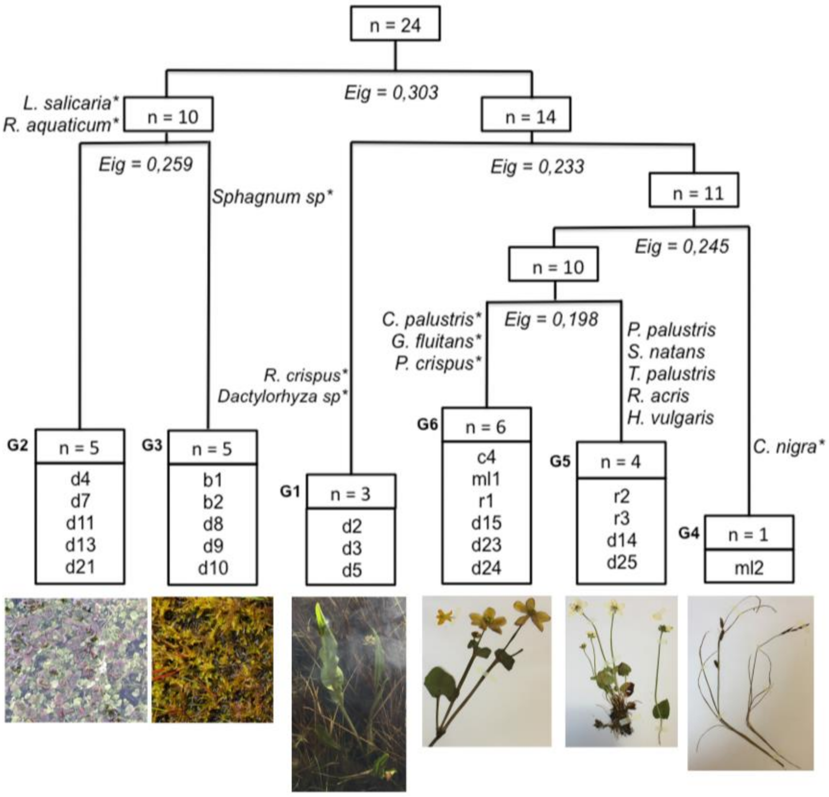


Figure 4.19 Twinspan dendrogram for the distribution of plants assembledges in June 2016, indicator species for each group (*) and eigenvalues. Species abreviations: L. salicaria – *Lythrum salicaria*; R. aquaticum – r*orippa nasturtium-aquaticum*; R. crispus – *Rumex crispus (Picture),* C. palustris – *Caltha palustris (Picture)*; G. fluitans – Glyceria fluitans; P. crispus – P*otamogeton crispus*; P. palustris – *Parnassia palustris (Picture)*; S. natans – *Sparganium natans*; T. palustris – *Triglochin palustris*; R. acris – *Ranunculus acris*; H. vulgaris – *Hydrocotyle vulgaris*; C. nigra – *Carex nigra (Picture)*.

Group 4 (division=4) is only represented by pond ml2 in the north of the site. The indicator species is *Carex nigra*, a species that tolerates wet periods, a feature that occurs in this large, topographically and hydrologically varied pond. Group 5 (division=9) is a collection of ponds with no indicator species that nonetheless share some similarities in having a dynamic hydrology, being dry during some part of the year. Group 6 (division=9) is the largest of the Twinspan groups. The indicator species are the emergent plant *Caltha palustris*, floating plant *Glyceria fluitans*, and the submerged *Potamogeton crispus*. This group comprised ponds with a range of sizes, shapes and geographical locations.

A spatial review of these Twinspan groupings is shown on Figure 4.20. Spatial configuration of the different vegetation groups reveals a slightly marked pattern. Groups 1 and 2 are more littoral and together with group 3 form assemblages of species that prefer more permanent waterbodies. Groups 5 and 6 are located further away from the beach backshore and 5 in particular, with a stronger component of more seasonal ponds. Comparable to the cladoceran spatial analysis, there is also a mixing of ponds with different physical characteristics that group these sites with similar vegetation communities.

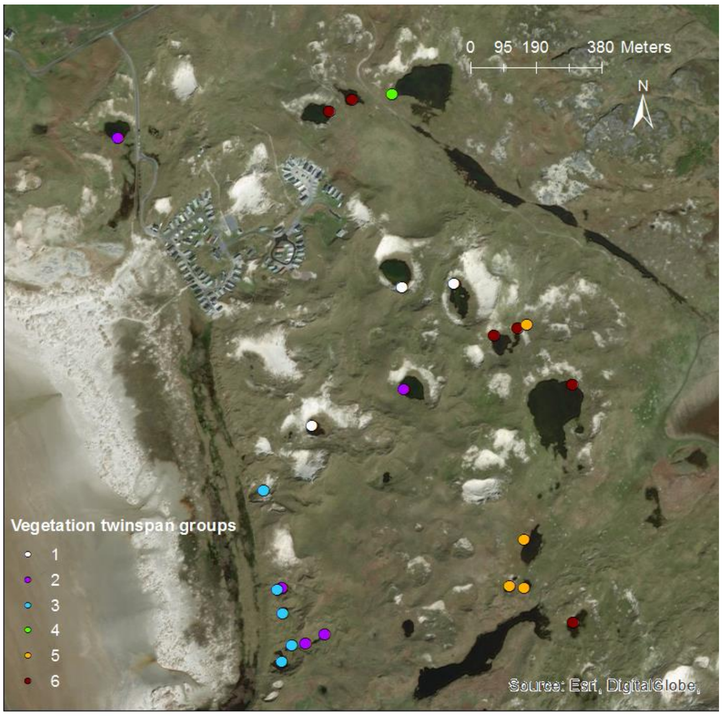


Figure 4.20 Spatial representation of the vegetation Twinspan groups

As with Cladocera groups, vegetation Twinspan groups were also tested against all environmental variables using Kruskal-Wallis test. Similar to the cladoceran results, water chemistry variables did not differ between groups ($p>0.05$) but hydrological categories significantly varied between groups ($p=0.008$). Figure 4.21 shows how these differences appear within the Twinspan groups.

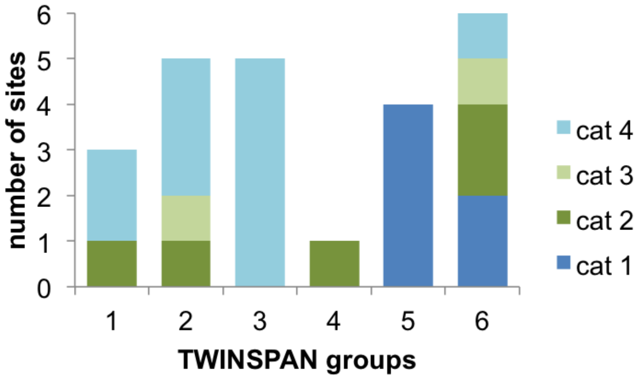


Figure 4.21 Hydrological categories frequency distribution within vegetation Twinspan groups

Hydrological categories also differentiated groups of vegetation, where groups 1, 2 and 3 are significantly different from groups 4 and 5. G4 represents one pond that occasionally dries (ml2) and G5 representing sites within category 1 (highly seasonal, easily dried ponds). G3 shows 5 ponds within the permanent hydrological category. G1 and G2 only represent ponds that have water throughout most of the time. G6 comprises all hydrological categories within.

4.3.5 Cladocera and plants

4.3.5.1 Diversity relationship

Pearson correlation revealed no significant correlation between cladoceran and plant diversity ($p=0,779$, $r^2=0,06$), for the nature of the association. Although positively linear, it is almost imperceptible (Figure 4.22).

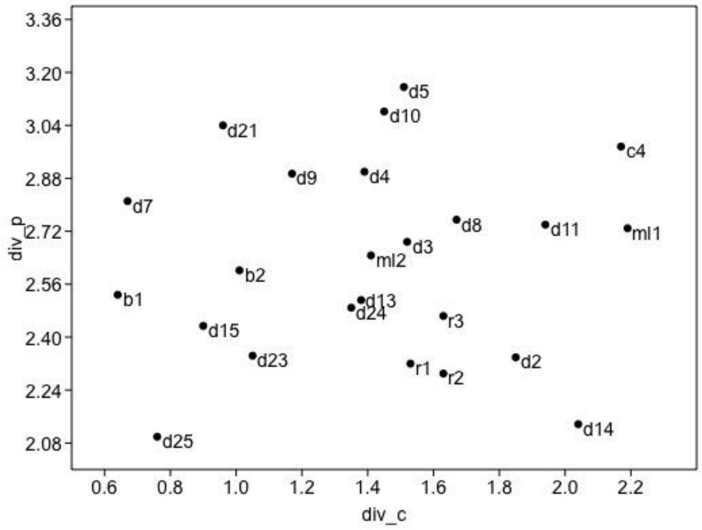


Figure 4.22 Relationship between cladoceran and plant diversity

4.3.5.2 Environmental variables influence on biological diversity

Contrary to Pearson correlation analysis, it is evident from both ordinations below (Figure 4.23 and Figure 4.24) that cladoceran and vegetation diversity respond in the same way to environmental factors. To understand how cladoceran and plant diversity performed in relation to water chemistry values, a PCA ordination was done (Figure 4.23). Axis 1 explained 25.35% and axis 2 explained 20.18% of variance (eigenvalues = 2.53 and 2.02 respectively). Conductivity was removed from the analysis as it was highly correlated with alkalinity. Both cladoceran and plant diversity responded similarly to water chemistry spatial variability. Diversity was higher in sites with high oxygen levels and low chlorophyll *a* values. Sites that contain higher biological diversity are c4, d5, d7 and d10 and lower diversity exists in r1 and r2. However, what is also visible is that high diversity relates to low chlorophyll a and low TP, which are negatively correlated

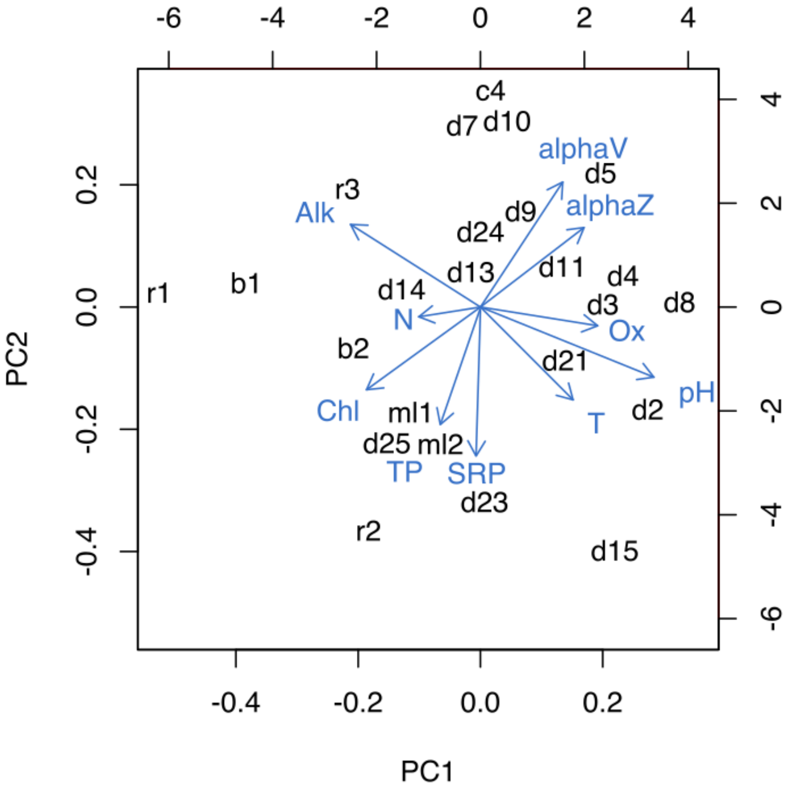


Figure 4.23 PCA ordination of cladoceran and plant diversity according to water chemistry. Abbreviations: Alk – alkalinity, alphaV – plant diversity, alphaZ – cladocera diversity, N – nitrates, Ox – oxygen, pH – pH, T – water temperature, SRP – soluble reactive phosphorus, TP – total phosphorus, Chl – chlorophyll *a*

Cladoceran and vegetation diversity was analysed in the context of site physical variables (Figure 4.24), and this explained more variability than water chemistry (axis 1 – 30.39%; axis 2 – 28.68%; eigenvalues = 2.74 and 2.58 respectively). Cladoceran and vegetation diversity increases in deeper ponds, but is also greater in ponds covering a larger area, those that are less uniformly circular, and those with a more permanent hydrological regime. Diversity is lower in more seasonal and smaller ponds.

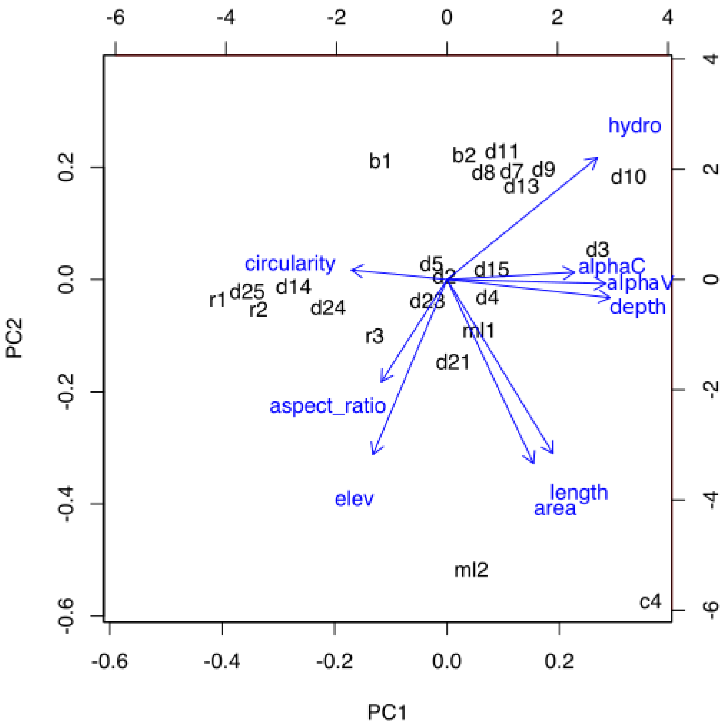


Figure 4.24 PCA ordination of cladoceran and plant diversity and influencing physical factors across sites. Abbreviations: hydro – hydrological category; circularity – roundness of pond; aspect_ratio – equidimensionality of pond shape; elev – elevation above sea level; alphaC – cladoceran diversity; alphaV – plant diversity; depth – water depth; length – pond maximum length; area – pond area

4.3.5.3 Biological assemblages

A tendency for certain species of cladocerans to be associated with different types of plant species according to their specific life forms (Figure 4.25) is highlighted. A full list of cladoceran species and codes used in the ordinations can be found in Appendix A. CCA ordination strength measured as the variance explained by plants life forms was 36,1% and eigenvalues for constrained axis was 0,3801 and 0,1526, for axis 1 and 2 respectively. On the first axis, submerged vegetation (e.g. *Chara* sp) is associated with the presence of *Alonella exigua* (Ae) in both male (Aem) and ephippia (Aee) forms, and sites ml1, ml2 and d25. Sites such as b1, b2, d2 and d8 are more associated with floating vegetation and cladoceran *Alona affinis* (Aa), *Glaptoleberis testudinaria* ephippia (Gle) and *Pleuroxus trigonellus* (Pt). Sites r2 and d23 are strongly associated with the presence of terrestrial vegetation (e.g. *Salix repens*) on the second axis as they stand are separated from the rest in relation to spatial organisation of cladoceran species abundances and plant life forms. These sites are also associated with male cladocerans and ephippians, such as *Simocephalus* spp (See, Sem) and *Chydorus* sp (Csm). Genus *Simocephalus* is considered a large cladoceran, but the presence of males (small ns) together with *Bosmina* sp (Bl), makes this set a rather small individual sized group, linked to ponds r2 and d23. Still on the first axis, but opposite to this, emergent species influence the Cladocera/sites combination here (e.g. *Juncus* spp).

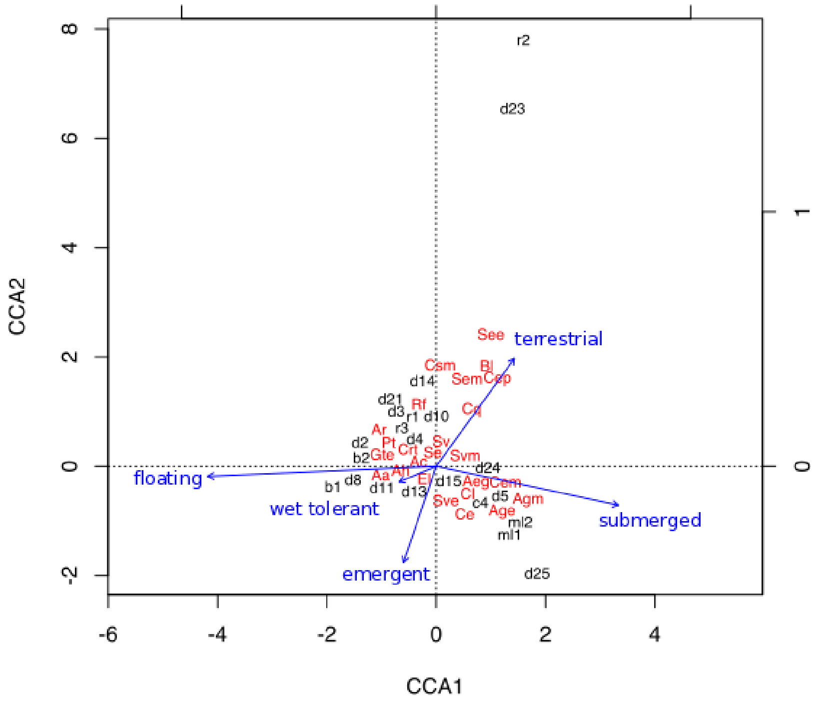


Figure 4.25 CCA ordination showing the relationship between cladocerans (red) and vegetation life forms frequency (blue) across sites (black). See appendix A for codes.

4.4 Discussion

The main aim of this chapter was to explore the roles of various pond water characteristics (water chemistry variables) and associated external factors (physical, geographical and hydrological) to understand the biological diversity (cladoceran and aquatic plant assemblages) of ponds across a dune landscape. The first question focused on how the environmental and spatial context of the pond within the dune system related to spatial patterns in ecology. Twinspan groupings for both cladocerans and plants revealed associations with pond elevation (measured relative to mean sea level) and hydrological regime. This divided ponds closer to the beach backshore from others further into the dune system, as well as differentiated groups belonging specifically to permanent versus temporary, seasonal flooding. The results demonstrated significant tolerance of both cladoceran and plants to a wide-ranging water chemistry (Figure 4.23), associated particularly with low chlorophyll *a* and TP. Water chemistry values are variable factors within seasons (Heegaard *et al.*, 2001) and these results may be the product of the small sample size, or small range of variation within the water chemistry, or even lack of variation within the ponds sampled. A broader study across the wider dune and lake environments of Sheskinmore (Gardner, 2016) presented water chemistry variables sharing the same range (example: pH, N, TP and SRP), which gives confidence that although a small sample size and an heterogeneous compilation of sites, values are comparable to related recent studies.

The second question explored whether the physical character of the dune system imposed controls on both cladoceran and plant communities. Physical variability however, showed more interesting results than water chemistry. Pond depth, hydrological regime and pond surface area were positively correlated with both cladoceran and vegetation diversity (Figure 4.24). Other studies have also found the importance of landscape features in explaining biological assemblages (Williams *et al.*, 2004) and in particular hydrology, in invertebrate (Waterkeyn *et al.*, 2008), zooplankton (Serrano and Fahd, 2005), amphibians (Babbitt and Tanner, 2000) and macrophytes (Rhazi *et al.*, 2009) differentiation, as well as size of waterbodies in embracing more species richness (Oertli *et al.*, 2002). These findings are also shared by Jeffries (1998), in reference to the fact that water chemistry alone is not sufficient in explaining spatial variation when studying an heterogeneous selection of sites.

The third question sought to establish whether high vegetation diversity correlated with high cladoceran diversity. This was found not to be true as diversity for both was almost unchangeable (Figure 4.22). However in this study, *Chara* sp and *Sphagnum* sp are shown to highly influence the patterns for cladoceran and vegetation associations. The presence of vegetation is known to influence zooplankton communities, for these provide shelter from predators, increase food availability and improve overall water quality. Studies have revealed that the microhabitat structure defined by the range in macrophyte species can promote multiple niches for different zooplankton genera (Choi *et al.*, 2014). In particular, small sized cladocerans (like *Aloninae* and *Chydoridae*) prefer free-floating vegetation, and large sized cladocerans (*Daphnidae*) prefer submerged vegetation (ibid). From all the different life forms, submerged vegetation is known to be a very important refuge for zooplankton (Bolduc *et al.*, 2016) as it shapes the community structure, biomass and functional diversity, by creating the perfect environment for hiding from predators, local source for food availability, shelter from currents and water flow and also improves water quality, especially for large slow moving cladocerans (Dodson *et al.*, 2009).

4.4.1 Environmental characterisation of the Magheramore-Sheskinmore dunes

4.4.1.1 Pond water chemistry variation

Water chemistry variables analysed, such as pH, conductivity, nutrient concentration and alkalinity, that are presumably important drivers of pond differentiation (Cottenie *et al.*, 2003), proved not to be so in this study in regards to promoting biological distinction. Water chemistry parameters showed some degree of variation across a relatively small area. Site b2 was the most distinct as it showed a separation from the other sites, mainly linked to high values of total phosphorus, oxygen, alkalinity and the highest value for nitrates. The b2 pond sits within a large and relatively inactive (mostly vegetated) blowout with a west-east structure (Figure 4.26A). It differentiates from the other ponds located close to the beach by being just metres from the embryonic dunes at the rear of Tramore Strand, with only a small dune barrier in between (Figure 4.26-B) where the seaward wall of the blowout has been eroded to just a short rim above the pond (c. 2m above the pond surface) whilst the landward walls rise to over 15m (Figure 4.26C).

Figure 4.26 Pond b2 and its framing on the landscape. A – general view (north); B – exposed seashore view (west); C – tall sand dune facing wall (east)

All the other dune ponds are more sheltered, either being further away from the backshore and/or contained within a more intact depression structure. A possible explanation for high levels of nitrates here may be due to its proximity to the strand, as in early stages of dune formation, along the high tide mark, there are deposits of drift material that contain nitratesous organic matter enabling then the settling of pioneer vegetation species and the beginning of the embryonic dune stage formation (Delaney *et al.* 2013). However, this explanation is ruled out as the strand and low dune plain have been advancing for at least 20 years (Barrett-Mold and Burningham, 2010). Another source is through wet deposition of nitrates dissolved in rainwater, as emphasised by APIS report (2016). This report points out the UK west coast as one of the risk areas of nitrogen pollution via wet deposition, because the coast receives the highest rainfall and those coastal sites that experience sea fogs contain higher pollutant concentrations. Chemistry measurements from other water sources such as rainfall, groundwater and brackish waters (from the marshland at the beach strandline) were not taken, as neither was the ionic composition of the pond water, implying that accurate determination of the sources of the chemistry variability would be a good addition to understand the environmental diversity at the dunes and how water is moving in and out of the ponds. Sites b1 and r1 have low pH, temperature and oxygen levels, but are high in alkalinity and chlorophyll *a*. Chlorophyll *a* is a biological parameter related to primary production, responding directly to the chemistry of the water (Heegaard *et al.* 2001) and in this case, related to alkalinity levels too. pH levels across the dunes were within the alkaline range, reflecting the generalized calcareous sand that forms the dune system.

Spatial variability in water chemistry can be quite complex in dune slacks and changeable between pond water and groundwater (Jones *et al.* 2006). When comparing both water chemistry and physical variability across sites, hydrological categories were revealed to be the most important drivers of spatial differentiation in ecology. The physical ordination showed a stronger response (PCA1=37%, PC2=31%), compared to the former (PCA1= 33%, PCA2=20%).

4.4.1.2 Physical variation

Topological, structural and physical results show a separation of the dune system into two main sets: one with ponds located closer to the beach, lower elevation, deeper and more permanent; and the other comprising a set of ponds located higher in the dune scape (relative to mean sea level), further away from the shore, where fluctuations in hydrology lead to a more varied regime between temporary and permanent. These findings were shared by the separate analysis performed either on physical structure of sites only, and biological groupings generated by Twinspan. Results illustrate that the water dynamic in the ponds follows a continuum of temporariness. Ponds' unique features seem to be derived by the underlying geomorphological bed, positioning in relation to mean sea level and the hydrological dynamics. Based on this, different permanence stages occur at different sites: from permanent (Figure 4.27 A and B) to seasonal (Figure 4.27 C and D) and two temporary categories in between (Figure 4.27 E and F – hydrological category 2; and G and H – category 3). The physical structure of the Magheramore-Sheskinmore dune system exerts a strong geomorphological framework on dune pond systems that imposes controls on the local environment, hydrological behaviour and biological composition. These qualities generate a complex structure that characterises the pond landscape with an inherited heterogeneity. This chapter only looked at variability at one point in time over the landscape, but the incorporation of temporal satellite imagery data was relevant as a mean for understanding spatial organisation, presence and movement of water in the dune system. The hydrological dynamic in waterbodies is a complex multidimensional variable (Brendonck *et al.*, 2017) that should only be estimated based on long-term observations for reliable information. Water movement and availability in the ponds is changeable and a clear hydroregime denomination is not easily defined.

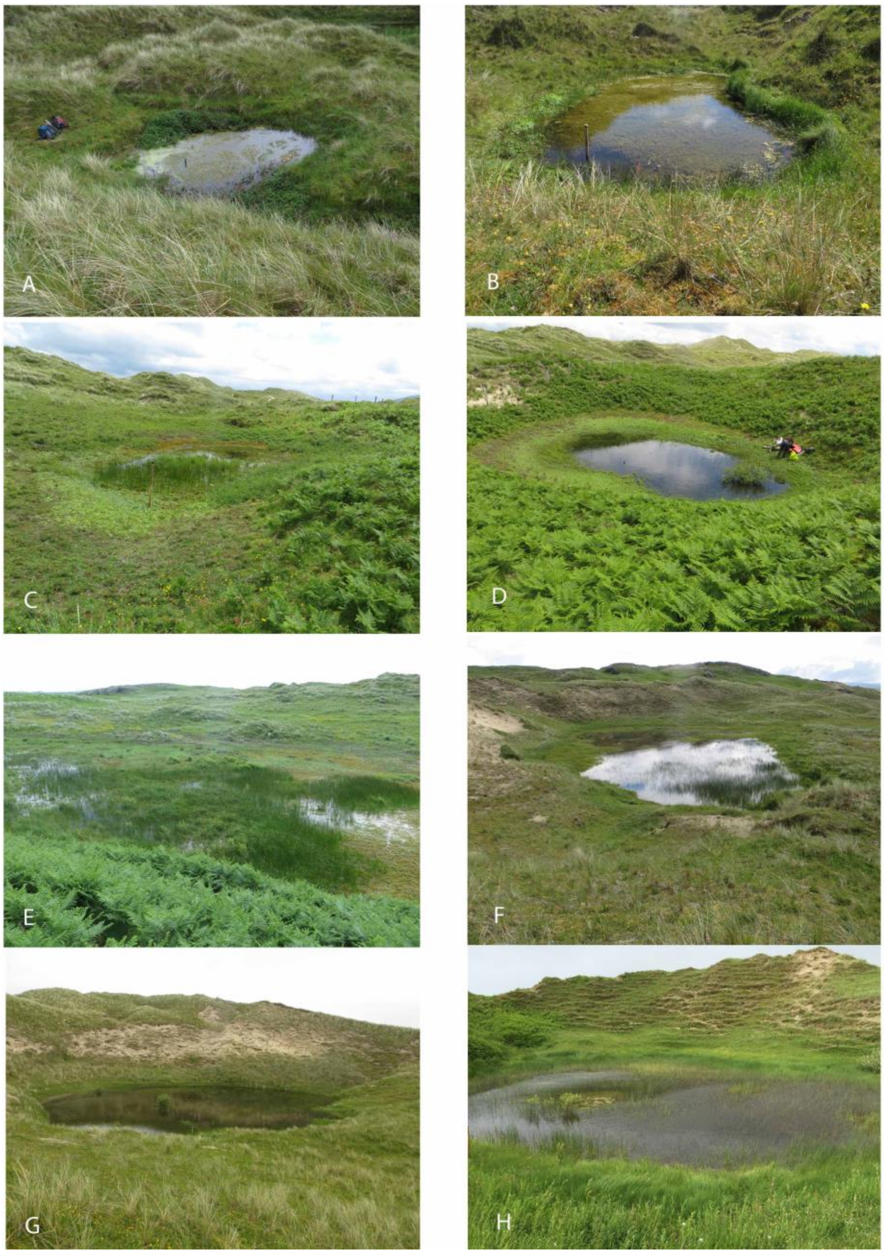


Figure 4.27 Groupings of sites defined by physical characteristics: A (b1) and B (d8) – permanent coastal ponds; C (d14) and D (r2) – shallow, highly seasonal ponds; E (c4) and F (ml2) – large flat blowout pond-wetland systems; G (d15) and H (d21) – ponds within well defined blowout depressions

4.4.2 Patterns in cladoceran and plant communities

In terms of cladoceran diversity, site c4 contains all families recorded across the dune pond system at Magheramore-Sheskinmore, including a variety of species and adaptability. This site was also notable due to its' size (represented by area and length in Figure 4.8), being the largest pond in the dune system (major length 383m; area 6617m^2); ml2 was similarly large (major length 293m; area 4762m^2) relative to the other ponds. The blowouts that these ponds exist within comprise steep and tall rims, and a much lower, broad and relatively flat central area. But subtle variations in topography of these broad beds have created patches and microhabitats of vegetation that seem to be associated with localised variability in water permanence across the surface bed (see also Chapter 3 -). This heterogeneity in local geomorphology, and the relatively large accommodation space are likely important characteristics for promoting high variety within this site, since the array of food source, shelter and physical differentiation gives space to species with different requirements and ecological performances to co-exist (Oertli *et al.*, 2002). Schuler *et al.* (2017) showed that habitat heterogeneity and size revealed increased zooplankton richness in larger sites. On the other hand, ponds b1 (14m^2) and r1 (67m^2) are comparatively smaller, with reduced amount of topographical variability. Site b1 includes the small sized cladocerans and r1 a combination of large and small sizes. Abundance of large cladocerans is important as it is indicative of the trophic state of freshwater habitats (Haberman and Haldna, 2014). Larger body size cladocerans may be related to increased food availability (Dodson *et al.* 2009). Presence of large species is also a sign of an absence of predators (Lynch, 1980). Hence, the size and general physical heterogeneity spectrum of the ponds captured within Sheskinmore dune scape is likely influencing the amount of species variability and assemblages seen.

Males and ephippia on the other hand, corresponded to 25% of the total species present. Their appearance in sites d25, d14 and r1 could be linked to the fact that these are very shallow, temporary waterbodies that by the sampling time (summer) were already transitioning to their dry phase. From these, d25 showed the highest abundance of cladoceran sexual features. Cladocerans undergo a cyclical parthenogenesis (asexual reproduction) several times during favourable environmental conditions, producing a population relying totally on female clones. When conditions change (e.g. temperature, desiccation), a life cycle delay happens, or diapause, where the development is stopped (Hand *et al.*, 2016), and a shift to sexual reproduction occurs to breed males and haploid eggs, ready to be fertilised (Lynch, 1980). Ephippia are thus fertilised eggs from sexual reproduction, and these are prepared to undertake resting stages, surviving under severe conditions, until favourable conditions return and enable the hatching and restoration of the community (De Meester *et al.* 1999). In the present work, the extent to which the presence of sexual life forms of cladocerans in some ponds reflect the physical and spatial variability within the landscape, either connected to pond type or even because the pond is entering the desiccation phase, is difficult to assess. Establishing a pattern for the diapausing mechanism is not easy, as the responses vary according to different environmental stimuli such as photoperiod, temperature, desiccation, all predictive of the forthcoming environmental change (Hand *et al.*, 2016; Paes *et al.*, 2016). The data in the present study only relate to one point in time, so this means that there is the potential for these diapause stages to be present in all the ponds later in the season when they start to become drier. Still, at the same point in time, ponds d25, d14 and r1 were going through an advanced stage of desiccation and physical factors like small size and circularity were common features of these ponds (Figure 4.24). On the other hand, factors to do with water chemistry were not so relevant (Figure 4.23).

Most of the vegetation present was wet tolerant, meaning that species can exist in both dry and wet environments. The presence of fully terrestrial species in the samples implies that at the time of the sampling, they were in water, as only species that had their roots in water were surveyed. The fluctuation of water input through precipitation, and water output through evapotranspiration can happen within a short amount of time and species need to be able to adapt and survive in these circumstances. Even though these are species that like to be close to water, they do not survive in a fully wet environment, which suggests the rapid variation in water levels of the ponds. The water flow dynamic in temporary systems leads to phases of rapid change reflecting the direct availability for vegetation, and the results show precisely the adaptability of the vegetation species to those shifts in the habitat conditions. Dune slack vegetation deals with a dynamic water regime that in some way impacts the water chemistry and availability of certain nutrients. Wet tolerant species found on the sites, like *S. nigricans*, *Juncus sp* and *Carex sp*, have developed an internal spongy tissue, named aerenchyma, that promotes the oxygenation from the shoot (emerged, in contact with air) to the root (submerged and in an anoxia stage), hence their ability to survive and even thrive under waterlogged conditions (Grootjans *et al.* 1998). The water depth that these ponds reach enables them to contain fully aquatic species, within the categories of submerged and floating, whereas the shallowness of others as well as the areas around the margins, only 27% of the total species were found to thrive in the present study. Nicolet *et al.* (2004), also found that emergent species were the most common from temporary habitats in England compared to fully aquatic plants.

Overall the relationship between communities across the dunescape at Magheramore-Sheskinmore is not easily explained. On the contrary, this apparent disconnection is possibly happening due to a combination of specific conditions that ponds embrace here, including local and external factors not measured in this study. One of these factors is the presence of predators (Zokan and Drake, 2015). Species like frogs, toads and newts were frequently spotted on the sites, which are known to feed upon smaller invertebrates (Lynch, 1980). Another approach for explaining spatial organisation of communities is through the way that species recolonize other water bodies. This variable was not specifically measured in this study but nonetheless, its influence may be at work. Spatial dispersal of zooplankton and vegetation is passive through reproductive propagules or resting stages (Leibowitz and Brooks, 2008). Dispersal is measured as wind, water and animal vectors and these ultimately depend on the hydrological phase of the water body (Brendonck *et al.* 2017). Wind dispersal acts when ponds are dry and the egg/seed bank gets exposed to erosion. As the overall weather conditions in west Donegal coast, the exposure to northwest prevailing winds and more often than not, gale force winds (Mckenzie & Cooper 2001; Horváth *et al.* 2016), this method of dispersal of species may be predominant at the Magheramore-Sheskinmore dunes. Mckenzie and Cooper (2001) explain how dune formation in west Donegal follows the prevailing wind direction, however, in the Tramore Strand (where Sheskinmore Nature Reserve is placed) the landscape is very much exposed and dunes are formed by complex multimodal wind regimes, coming from various directions, and that a particular distribution pattern of the sand here is not easily defined. Dispersal via water connections occurs when water levels are high and enable connectivity between waterbodies. This is unlikely the case of the dune ponds at Magheramore-Sheskinmore, as the distance between sites does not enable water connectivity like in other systems such as floodplains (Leibowitz & Brooks 2008; Obolewski *et al.* 2016). However, the presence of water increases the animal vector dispersal of zooplankton. Sheskinmore is known to be a reference location for bird watching as it is a feeding ground for many migratory bird species and permanent residence to others (NPWS, 2015). Adding to this, other animals prosper on the dune system and rely on their waterbodies as a source of freshwater; these include foxes, rabbits and the occasional cattle, that possibly act as vectors of dispersal as well.

The biodiversity that ponds encourage in the landscape makes them a habitat of conservation importance. Relatively small water bodies, such as temporary ponds, are good for large scale surveys, because they increase the understanding of the underlying ecological community structure and biodiversity patterns at a landscape scale (De Meester *et al.* 2005). Species distribution is the result of a combination of various factors, either external such as environmental characteristics or internal, linked to biotic interactions or population dynamics (Legendre and Legendre, 1998). This study showed in fact that the dune pond landscape at Magheramore-Sheskinmore is quite heterogeneous and for this reason it is able to hold, within a relatively short space, a multifaceted pond structure, in both physical and biological terms that clarifies the niche richness and complexity of the site. These are extremely important features that promote increased biodiversity and species richness, as sites with a variety of sizes promote ecological variability in terms of a wide range of freshwater species (Oertli *et al.*, 2002). Spatial configuration and structure of environmental conditions is the base for the habitat heterogeneity model (also called niche diversity) (Massicotte *et al.*, 2014), that in itself suggests an increase in species richness and functional diversity by means of expansion of the array of habitats with different resources that favour distinctive species adapted to diverse environments in space and time. However, spatial heterogeneity is not always easy to describe, because it is a mixture of environmental factors and ecological community processes (Borcard & Legendre 2002; Jeffries 2008; Schuler *et al.* 2017). This is enhanced particularly due to the varied communities present and the complex ecosystem interrelations in process. Spatial surveys are extremely important in an early survey stage because they can provide answers on the spatial arrangement of the site and species, as well as help understand which factors may be influencing their distribution and/or abundance (Borcard and Legendre, 2002). Large scale biological surveys not only assess the biodiversity and the significance of those species on the dynamics of a region, but also gather important information that can be used for implementing adequate conservation programs (Li *et al.* 2006; Jeffries 2008; Hill *et al.* 2018).

The current research provides the first ever spatial assessment of cladoceran communities in context of vegetation and environmental variables in dune slacks of the Irish coast. Dune slack Irish freshwater micro faunal investigation has not been previously recorded. Studies focusing on Irish zooplankton communities reflect matters of the marine coast and Irish Sea or large Loughs (Pociecha and McCarthy, 2011), and this sort of knowledge on coastal freshwater systems, such as dune slacks, is lacking. Furthermore, works that embrace local small-scale variables (environment) with spatial processes as potential drivers of community structure and functional diversity of zooplankton are still uncommon (Massicotte *et al.*, 2014). This study tried to bring together an holistic approach, combining factors that may be influencing the dune pond landscape organisation, and extended the already well explored dune slack vegetation community dynamics to include a study of freshwater Cladocera.

4.5 Conclusions

When comparing both water chemistry and physical variability across sites, hydrological categories were revealed to be the most important drivers of spatial differentiation in ecology. Geographical, topological, structural and physical positioning within the dunes separates ponds closer to the beach backshore from the ones further into the dune system, and this differentiated groups of ponds belonging to different hydrological categories, illustrating that the water dynamic in the dune ponds follows a continuum of temporariness.

Pond depth, hydrological regime and pond surface area were positively correlated with both cladoceran and vegetation diversity. Cladoceran diversity was linked to vegetation diversity and both were shaped by environmental conditions, but by spatial and physical factors in particular, in which larger ponds contained higher diversity, whereas smaller and particularly dryer ponds contained different cladoceran assemblages and life stages. Furthermore, the physical variability across the landscape was revealed to be a major factor for sustaining the biodiversity of coastal ponds at Sheskinmore Nature Reserve. Still, the relationship between communities across the dunescape is not easily explained. The apparent disconnection is possibly due to a combination of specific conditions that ponds embrace, including local and external factors not measured in this study. These are a combination of pond water chemistry, geomorphological setting, proximity to the water table and vulnerability to climatic changes. Nevertheless, the acknowledgment and incorporation of cladoceran diversity and hydrological regime adds an explanatory component to the overall spatial organisation of Magheramore-Sheskinmore ponds landscape and what is known so far on the coastal dunes of Ireland.

Natural drivers determine cladoceran and vegetation community structure (species richness and composition) and spatial dynamics in this low impacted ecosystem, however physical variability is the most important factor in explaining variability, and therefore should be taken into consideration when assessing ecological dynamics in extensive semi-natural freshwater dune landscapes, and should also be included in the monitoring strategies for conservation management.

Chapter 5 - Cladoceran communities of Mediterranean Ponds – Hydrogeomorphological controls

5.1 Introduction

Mediterranean temporary ponds (MTP) are seasonal waterbodies highly threatened by anthropogenic pressure (Zacharias *et al.*, 2007; Ruiz, 2008; Rhazi *et al.*, 2012). Although with a sometimes short behaviour, the flood timing allows specialised animal and plant species to thrive in this habitat. MTP occur mostly within the Mediterranean area and are considered a priority habitat according to the Natura 2000 network of the European Union (Natura code 3170, EC Habitats Directive (92/43/CEE)). In Portugal MTP exist in range of geographic and physical contexts, from coastal to inland, including natural and agricultural areas (Fonseca and Monteiro, 2011; Pinto-Cruz *et al.*, 2011), and within different underlying sediment topologies and geomorphologies (Machado et al. 1999; Pinto-Cruz et al. 2009). MTP in southwest Portugal embrace a variety of waterbodies conferring a complex structure that enhances biodiversity. Ponds here are still threatened by human pressure, often used as wasteland deposits and are subject to morphological manipulation of the pond structure for agricultural and grazing purposes, sediment extraction and *Eucalyptus* sp. plantation (Machado et al. 1999 (a); Pinto-Cruz 2010; LIFE Charcos 2013; Ferreira & Beja 2013).

The studied habitats have been monitored in terms of their flora and fauna diversity and dynamics via the EU 'LIFE Charcos' Project (LIFE12NAT/PT/997). The LIFE Charcos project aims to protect MTPs in southwest Portugal (a Site of Communitarian Importance (SIC) under Natura 2000), by reversing a serious decline in pond numbers (52% in the past 10 years) and promoting their conservation. However, the incorporation of microcrustaceans in the bioassessment and overall contribution to biodiversity and conservation practices, in terms of regional and hydrogeomorphological changeability, has not yet been studied, hence the work presented herein. Understanding the underlying hydrogeomorphology of temporary ponds is a challenge that only a few studies have attempted (Sun *et al.*, 2006; Leibowitz and Brooks, 2008).

Pond hydrology is determined by the external (weather related factors such as temperature and precipitation) and internal characteristics (especially pond physical structure, local shading and topographic and geological setting) of individual ponds

(Sidle and Onda, 2004; Leibowitz and Brooks, 2008). Water input to ponds from precipitation is either direct into the pond basin, or indirect via surface runoff (especially where there is a connection to streams and ditches or where the pond lies at the base of a slope) or through a rise in local groundwater levels (Grillas *et al.*, 2004; Sun *et al.*, 2006). The interaction between groundwater and pond water is influenced by the geomorphology of the terrain and groundwater level position, as well as soil characteristics and lithology beneath the pond bed (Sidle and Onda, 2004; Leibowitz and Brooks, 2008). The main loss of water is through evapotranspiration (caused by temperature and respiration) directly from the pond surface in contact with air and vegetation within the pond and/or in the surrounding catchment area (Grillas *et al.*, 2004; Zacharias and Zamparas, 2010). The topography of the terrain (especially as it relates to moisture gradients) and composition of the substrate influences the depth and water-level dynamics of a pond (Fernnández-Aláez *et al.*, 1999), given that it provides insight on water flow directions (Sun *et al.*, 2006) The structure and composition of the sediment affects water permeability that in turn controls the input and output of groundwater and surface water. For this reason, the pond depression and catchment characteristics are fundamental factors affecting the hydrological regime of temporary ponds (Leibowitz and Brooks, 2008). Pond hydrology affects cladoceran diversity both directly via the availability of water (water depth and permanence) and indirectly by influencing the spatial distribution and abundance of aquatic vegetation that cladocerans utilise as refuge and feeding habitat. In temporary ponds the zones that act as borderlines between wet and dry areas create complex structures, generating varied conditions for a wide range of species. These damp areas are often a mixture of mud, sand, bare soil and vegetation, where semi-terrestrial species like beetles, snails and spiders live and aquatic invertebrates breed (Biggs *et al.*, 1994). Sediment structure is crucial for biodiversity and it is crucial to understanding how water moves through its layers.

Although some studies acknowledge the link between hydrology of temporary ponds to sediment and geomorphological contexts (Ruiz, 2008; Stelzenmüller and Martìn, 2009; Díaz-Paniagua *et al.*, 2010), only few studies explore this relationship with biological communities (Zunzunegui *et al.*, 1998; Muñoz-Reinoso, 2001; Amami *et al.*, 2013), and only one study up to date considers its connection with cladocerans (Espinar and Serrano, 2009).

This chapter examines cladoceran composition and diversity in ponds in two different regions (separated by c. 80km) on a north-south coastal gradient in southwest Portugal (Figure 5.1 and Figure 5.2). At the Malhão (MAL) sand dune system 5 ponds were sampled and monitored (Figure 5.1), while in the Vila do Bispo (VB) a further 6 ponds were investigated (Figure 5.2). Initially two more ponds in MAL were included in the study but during the sampling campaigns, absence of water kept those ponds dried throughout, disabling the ability of sampling. Ponds were selected to maximise the hydrological gradient, based on variability of size, shape, vegetation cover and surrounding marginal characteristics, as well as distribution across the landscape.

The ecological dynamic processes amongst Mediterranean ponds on a north south latitudinal gradient in SW Portugal, could suggest a change in biological community composition and biodiversity. This is primarily because different geomorphological contexts may influence the way water moves below the ground, which then impacts vegetation and consequently a myriad of aquatic dependent life forms, amongst them, microcrustaceans. Mediterranean ponds are considered habitats with high and unique vegetation richness, but little is known about how cladoceran communities vary along hydrogeomorphological gradients at a regional scale. Added information on the regional diversity of cladocerans according to hydrogeomorphological differences of Mediterranean ponds will upgrade the information available and help to support the development of appropriate conservation and management strategies.

Sites were surveyed and monitored to capture the temporal characteristics of the hydrological regime within the context of spatial variations in geomorphology, over 4 sampling campaigns covering early summer and late winter in the years of 2015, 2016 and 2017.

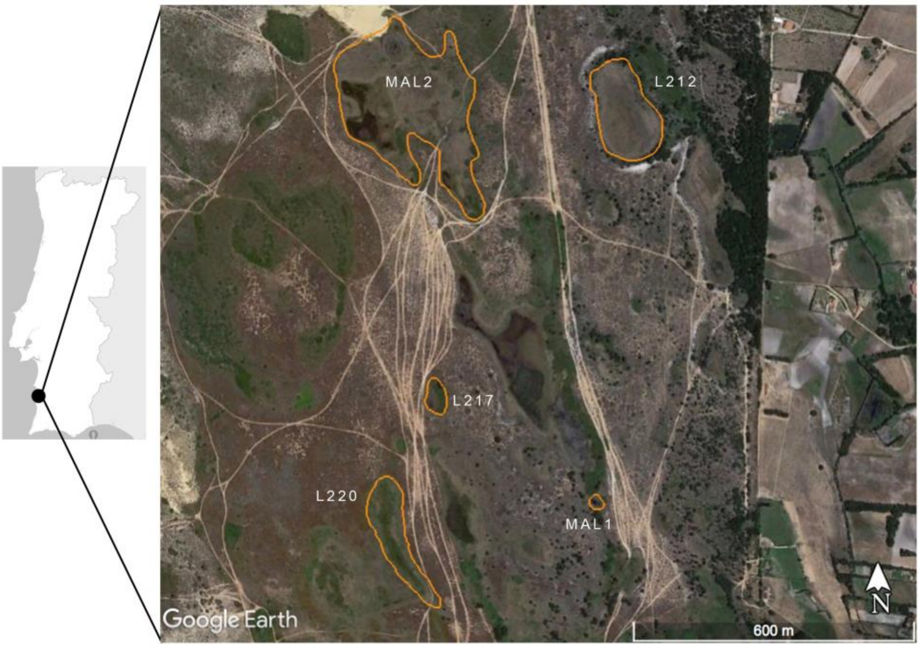


Figure 5.1 Map of the studied ponds at MAL region noting individual sites sampled

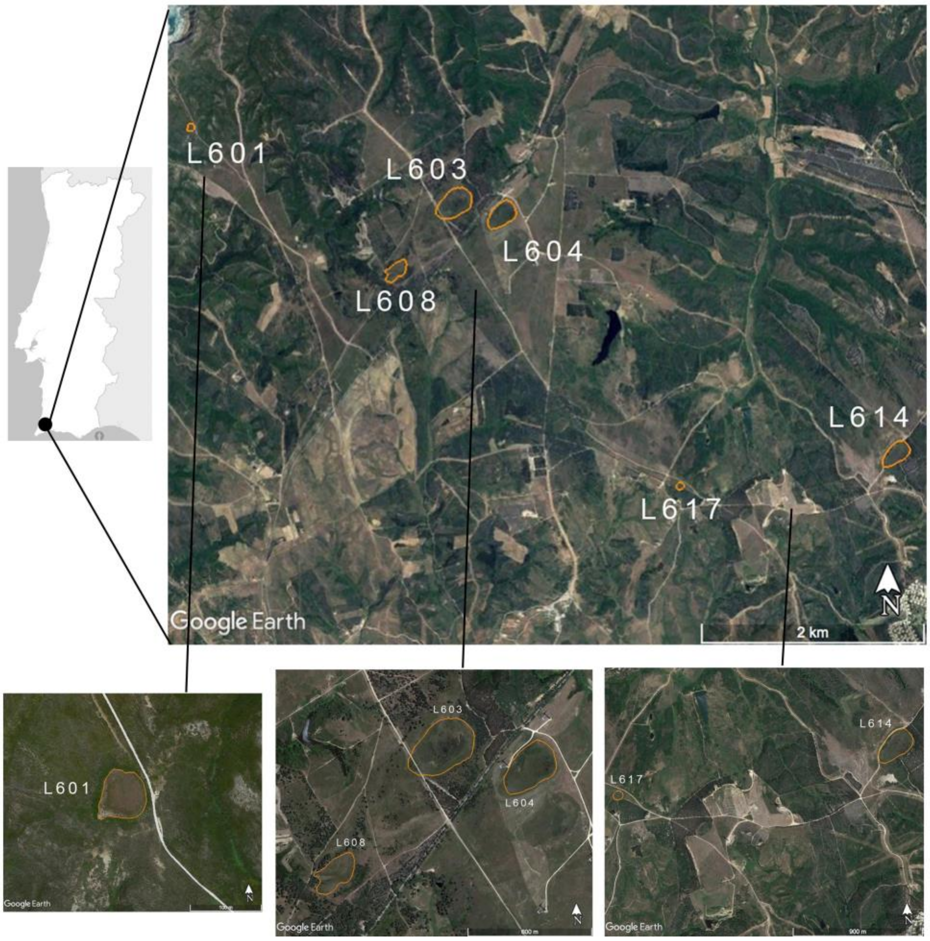


Figure 5.2 Map of the studied ponds at VB region noting individual sites sampled

Despite the fact that all the studied ponds in MAL and VB have characteristics that define them as temporary ponds, structural differences are likely constraining the role that hydrological regime plays in these two systems. This is because the eolian system of the two geomorphologic units are differentiated into what looks like mobile dunes (MAL) and stabilized sands (VB), and as referred, the extent to which these geomorphological differences have an effect on cladoceran composition has never been analysed.

The two groups of ponds were compared in terms of their species composition (cladoceran and vegetation) as well as environmental context. The main intent of this chapter is to characterise the hydrogeomorphological and ecological differences between the two regions. Hypothesising that species diversity significantly differs in ponds with distinctive hydrogeomorphologies, the chapter explores how the hydrological regime of the pond in relation to its setting determines the nature of cladoceran communities. The key research questions addressed here are:

- Do cladoceran communities show a regional differentiation within Mediterranean temporary ponds?
- Is hydrogeomorphology the central factor in maintaining high species diversity in ponds at a regional scale?
- Does hydroperiod influence cladoceran and plant communities?

5.1.1 Regional context

The Portuguese southwestern coastal plateau extends for over 150km north - south, and up to 20km from the coast to inland. The lithology of this region dates from the marine Carboniferous era. It is formed of schist and greywacke, on top of which a height of up to 20 metres of sand composite rests (Alves, 1998). The regions of MAL and VB experience a Mediterranean climate with an Atlantic influence. Both sites have ponds across a range of sizes and shapes, but they share habitats classified as Mediterranean Temporary Ponds (Habitat 3170*), and are also considered Important Areas for Ponds (IAP), based on their biodiversity value and pond density (Ewald *et al.*, 2010). At both regions grazing is practised extensively and evidence of cattle and wild boar trampling and disturbance on the pond beds is frequently seen.

The MAL ponds are located on a coastal dune system around 65m above sea level. The region is dominated by Pleistocene aeolianites (lithified dune sediments) which are variably weathered and eroded, creating a contemporary mature dune system elevated and not directly connected to the coastal beach systems (Alves, 1998). This dune complex contains a series of temporary ponds with a somewhat homogenous sediment core profile, mainly consisting of sands. Around 15km inland from MAL are the Cercal hills (max 372m above sea level) which influence the dune system chemically (via mineral run-off), but also affect local climate as well (Alves, 1998). Ponds here occur within sand dune depressions, where marked topographic lows bring the ground level closer to the water table, enabling the existence of a stable, though seasonal, freshwater habitat (Figure 5.3).

The VB ponds are located at the southwest tip of Portugal, around 120m above sea level. These ponds hold water for a few months in the year due to the presence of a clay impermeable layer (Machado *et al.* 1999). Above this, the more permeable sediment is dominated by a sandy composite, but it has a much thinner structure than MAL. In this system, very close to the pond bed (sometimes within 50cm depth), underlying layers of schist and greywacke present an impermeable layer. Rainwater gets retained within depressions in the landscape, thereby creating ponds that dry during the summer and autumn (Machado *et al.*, 1999; Fonseca and Monteiro, 2011). In this landscape ponds are most easily discerned in terms of vegetation differentiations rather than marked topographic depressions, resembling the ‘daya’ ponds in Morocco (Amami *et al.*, 2013). Water is generally retained by means of a subtle topographic depression with marginal “walls” of terrestrial/bushy vegetation (Figure 5.4). More often than not, these ponds remain unnoticed to the untrained eye especially when dry, as the topographic barrier is not markedly distinct.

Figure 5.3 Malhão dunes and ponds at the top (Mal2) and bottom (Mal1) showing the morphological and topographical aspects of the region

Figure 5.4 Vila do Bispo ponds showing the different morphological and topographical aspects of the region. From top left to bottom right: L604, L608, L614, L603

5.2 Sampling and analytical approach

Sediment and topographic data collection and sampling was undertaken in late summer (October 2015) when the ponds had all dried out. This enabled a full survey of the basin and catchment of the ponds and an increased likelihood of installing piezometers at deeper levels below the dry sediment bed. Sediment data from core profiles was also collected in all ponds during this time, using an auger that was twisted all the way through the sediment until it reached the water table. Sediment samples were collected at various depths, depending on the proximity of the pond bed to the water table or accessibility. Only at site L617 (VB) the task of sediment extraction was not possible until reaching the water table depth due to the very hard conditions of the dry clay layers. Also, site L603 (VB) sits on top of a schist layer that occurs at 50cm from the pond bed. For this reason, although data was collected from all layers of the sediment profile for all sites, analyses were focused on the top 40cm to allow a comparative analysis. Each layer of sediment was separated into small bags with notes taken of different sediment textures and colours. This enabled a stratigraphic characterisation of the sediment beneath the pond surface.

Loss on ignition (LOI) was used to estimate the % organic matter (OM) and carbonate content (CO_3) of each layer of the sediment cores as described by Heiri *et al*. (2001). Sediment samples were first oven-dried at 105ºC to constant weight for 12 hours. This eliminated all water content. After this, organic matter was combusted to ash at a temperature of 550°C for 2 hours. The difference between weights gives the percentage organic content (OM) of the sediment. Lastly, percentage carbonates was derived from mass loss in the sample when heated at 950ºC for a further 4 hours. The difference between weight lost at 550ºC and at 950°C was multiplied by 1.36 (difference between molecular weights of CO_2 and CO_3) to derive the carbonate content which was then expressed as a percentage of dry weight (Heiri *et al.*, 2001). LOI was performed on samples to guarantee that carbonate sedimentary shells and organic material would be excluded from the grain analysis such that it did not compromise the minerogenic component of the sedimentological analyses. Grain size analysis was calculated using a Malvern Mastersizer 2000 particle size analyzer (Rawle, 2003). This method uses a laser diffraction system that measures the angular variation in scattered light when the beam passes through particles in a sample (Horiba, 2013). A small amount of sample was pre-wetted with a few drops of water that acted as a dispersant before being transferred into the dispersion unit. Clay sediments create stiff aggregates that are not easily dispersed in natural conditions (Gackstetter, 2007). In these cases a few drops of Calgon© (sodium hexametaphosphate) was added to increase the dispersal of the particles. Samples were then left overnight to give sufficient time for sediment disaggregation to take place. After this procedure some samples were put through a period of sonication to disperse the remaining agglomerates into their primary particle size (Gackstetter, 2007). The programme Gradistat (Blott and Pye, 2001) was used to calculate grain size statistics for each sample, and to organise the data into grain size categories (% sand, % mud and % silt). Sand was measured as the percentage of sediment grains with size varying between 63μm and 2mm; mud as % grains ranging between 3.9 and 63μm size; and silt as grain sizes <3.9μm. All measurements were replicated 3 times for each sample and an average from the first 40cm of sediment below pond bed was calculated and used thereafter.

Munsell soil colour charts were used to classify each sediment layer according to a defined colour. A small sample of the sediment was taken and laid out to dry on top of a white piece of paper. After it had dried out completely the colour was then compared with the Munsell chart and the best match was noted. This was implemented to all layers from all the ponds. The results of this analysis can be found in Appendix B.

Cladoceran data were collected as explained in Chapter 2 - . For comparisons of cladoceran abundance values data were log and square root transformed when required. One-way ANOVA or Kruskal-Wallis tests (depending on the normality of data) were used to test the null hypothesis that the MAL and VB data sets had the same mean. These analyses were undertaken using the PAST software package (Hammer *et al.*, 2001), and a p-value of 0.05 was used as the threshold of significance (i.e. $p<0.05$). The Bray-Curtis dissimilarity index was used to compare cladoceran and vegetation communities between sites and regions, using R software (R Core Team, 2014). Correlations between variables were performed using a Pearson correlation index when data were normally distributed and the Spearman's correlation test when they were not.

Owing to the nature of the ponds and climatic circumstances, absence of water limited the data collection and thus not all sites were sampled in all the four seasons. Still, hydrological regime was monitored in 6 of the 11 ponds (see Table 5.1), following the methodology described in Chapter 2 - . Weather data were obtained from Instituto Português do Mar e da Atmosfera (IPMA 2015) as explained also in Chapter 2. A summary of the sampled sites and of sampling occasions and missed samplings is given in Table 5.1.

Table 5.1 Site sampling in different seasons. Blank spaces correspond to absence of data. Hydrology was monitored at ponds specified in bold, indicated by the presence of a logger

region	pond	Latitude (N)	Longitude (W)	May 15	Feb 16	May 16	Feb 17	logger
Malhão	L212	37°45'20.9"	8°47' 31.75"		x			
	L217	37°45'20.8"	8°47'32.7"		x	x	x	
	L220	37°44'54.1"	8°47'52.6"		x		x	x
	Mal1	37°44'54.1"	8°47'34.04"	x	x	x	x	x
	Mal2	37°45'19.0"	8°47'53.6"	x	x	x	x	x
Vila do Bispo	L601	37°7'43.8"	8° 55' 9.39"		x		x	
	L603	37°07'18.7"	8°53'35.2"	x	x	x	x	x
	L604	37°7'16.5"	8°53'19.68"	x	x	x	x	
	L608	37°7'1.2"	8°53'57.52"	x	x	x	x	
	L614	37°06'02.9"	8°50'58.8"	x	x	x	x	x
	L617	37°05'58.4"	8°52'12.5"		x		x	x

5.3 Results

5.3.1 Regional geomorphology

5.3.1.1 Pond bed stratigraphy and sedimentology

Understanding the sediment structure of substrate underlying the pond in terms of its composition enables the characterisation and differentiation of ponds locally in terms of soil types and sediment sources. Grain size analysis was performed to understand the context and texture of the underlying sediment according to its mean grain size, and eventually to appreciate if the sediment is more aggregated or loose to allow the passage of water. This provided information on variation in percentage of sand, mud and silt of sediment for each site. Results from the grain size analysis give an idea on variation in sediment size and composition between layers of each individual pond for the total depth collected at both regions MAL (Figure 5.5) and VB (Figure 5.6). All ponds from both regions contain around 40% of sand throughout the sediment layers, with no apparent distinction between MAL and VB, meaning that these are pure end members of sand sediment constitution (>63µm). L212 represents an exception in the MAL region, with higher %mud (from 3,9µm to 63µm) and %silt (below 3,9µm) particularly on the first 35cm of sediment layers. Still, the stratigraphy from all the other ponds at MAL is composed of coarser sediments. VB ponds L601, L617 and the first 10cm of L608 are slightly more variable, with up to 10% of mud in their composition. The rest of the layers fall within the sand with percentages varying from around 10% to over 60%. In summary, despite some nuances, the sediment structure of both regions is similar.

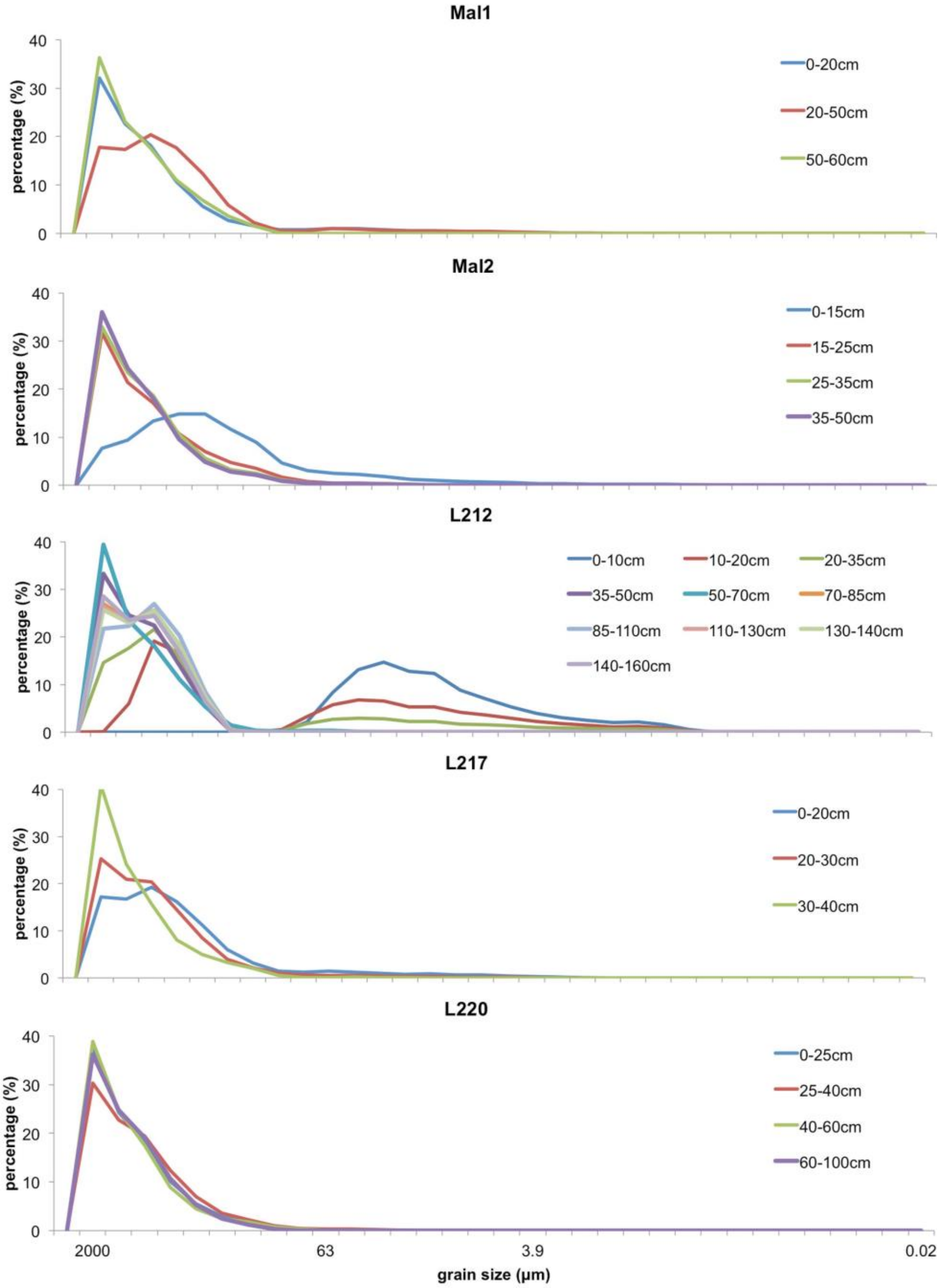


Figure 5.5 Grain size stratigraphy for each MAL pond along depth

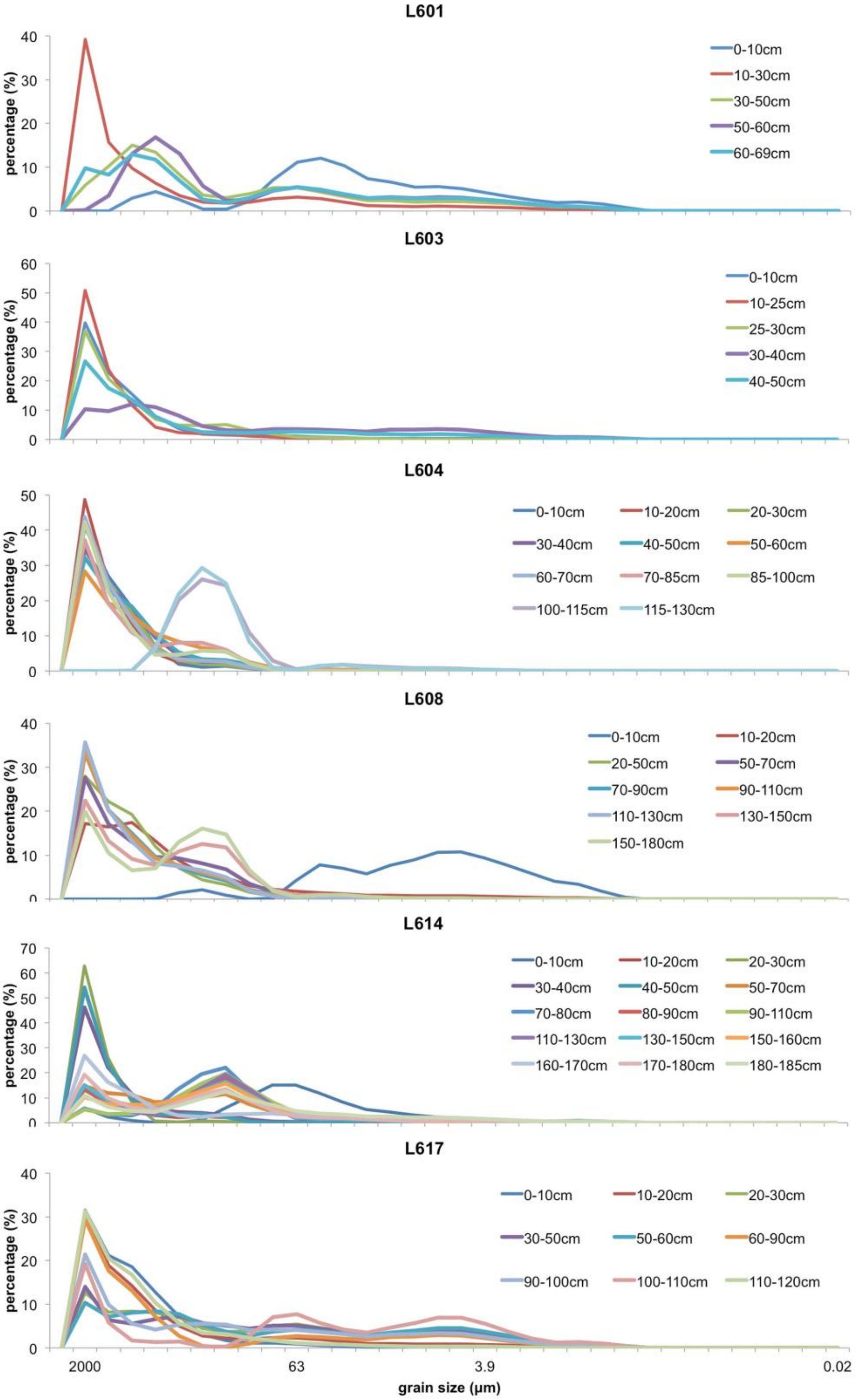


Figure 5.6 Grain size stratigraphy for each VB pond along depth

After the visualisation of the stratigraphy for all the ponds at both regions it is pertinent to understand if these differ between regions. As mentioned before, to allow for a coherent comparison of the sites, a choice was made to analyse only the first 40 cm of sediment layers. Figure 5.7 gives a comparison of sand, mud and silt sediment percentages between regions at 40cm depth. The MAL ponds have sediments composed of a high percentage of sand, whereas the VB ponds contain also a high percentage of sand, but finer grains are also present. Mud and silt were present at lower percentages in both regions but were slightly more elevated in the VB ponds. Kruskal-Wallis and ANOVA tests were performed (data non-normal even after transformation for %sand) and results turned out to be non-significant in terms of differences between the MAL and VB regions for all of %sand (*P*=0.516), %mud (*P*=0.632) and %silt (*P*=0.332). This means that, although underlying geological setting of both pond regions is different, (consolidated sand versus schist), the structure of the top sediment is fairly similar.

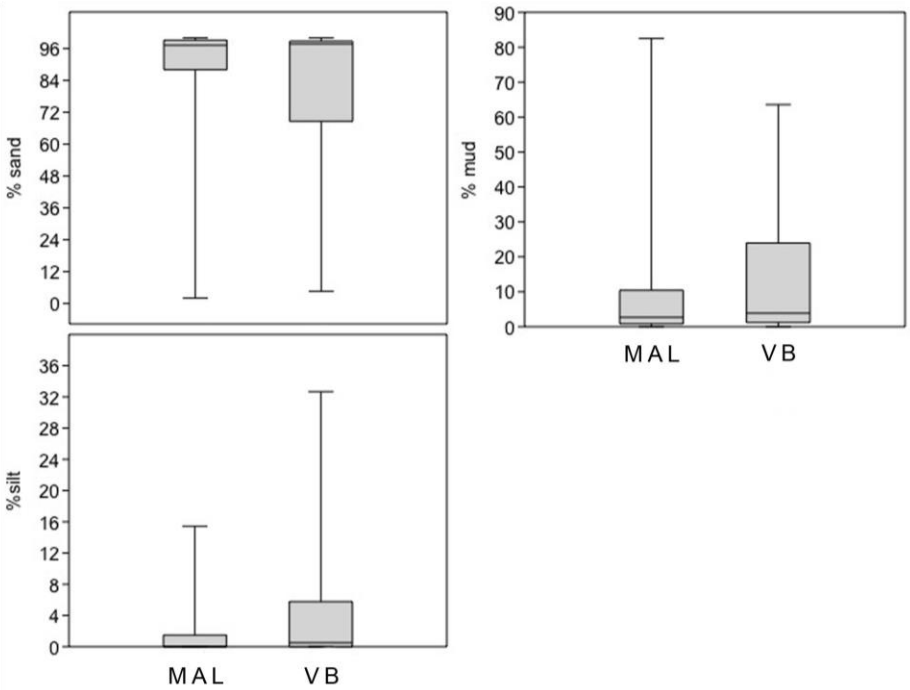


Figure 5.7 Average %sand, %mud and %silt content of the uppermost 40 cm of sediment for both the MAL and VB pond sites

5.3.1.2 Sediment composition

The organic content (OM) of soils reflects the accumulation of organic material over time and is directly linked to the presence and productivity of aquatic vegetation (Davy *et al.*, 2006) and partly by invertebrate feeding (Woods, 1999; Błędzki and Rybak, 2016). In temporary ponds however, OM is oxidised every time the pond dries, leading longer periods of permanence for temporary ponds compared to permanent waterbodies (Biggs *et al.*, 1994). Carbonate content (CC), measured as CO_3, is usually defined as promoting sand buffering capacity (capacity to buffer the sediment pH change) and is derived from the erosion of calcareous rocks and/or shell debris. In older dunes, carbonates are progressively dissolved and leached away (Davy *et al.*, 2006) and these processes ultimately determine the hydrochemistry of dune ponds, and influence aquatic vegetation development.

Percentage of OM and CC of the uppermost 40 cm of pond bed sediment were generated from the LOI analysis (Figure 5.8). Results show that, overall, mean values differ between both regions, with MAL having lower OM and CC than VB. Organics are low, but values are again overall very similar between the ponds and pond regions. When comparing the MAL and VB ponds, %OM is lower in the former (<4%), whereas in VB ponds it is slightly higher. This means that the accumulation of organic material, although low, is greater in the VB ponds. This may possibly be due to the porous nature of the sandy substrate that promotes the wash away of OM with infiltration. Carbonates are also very low (<1.7%) suggesting that the sands are leached and/or little new carbonate material is being introduced to the ponds, but values differ slightly between the MAL and VB ponds. In the MAL ponds, values persist below 0.3% in the majority of sites. Although dune sands with shell material in their composition are expected to have high CC (Davy 2006), this is not the case in the MAL dunes. By contrast the VB ponds contain a slightly higher %CC (1-1.8%).

Generally, in both the MAL and VB ponds low values of both OM and CC imply dominance by subsurface drainage, whereas in the VB ponds, higher OM and CC values may suggest that the opposite is true, perhaps due to an underlying impermeable layer. Kruskall-Wallis tests (data non-normal even after transformation) revealed significant differences for %OM (p=0.04) and %CC (p=0,00) between MAL and VB.

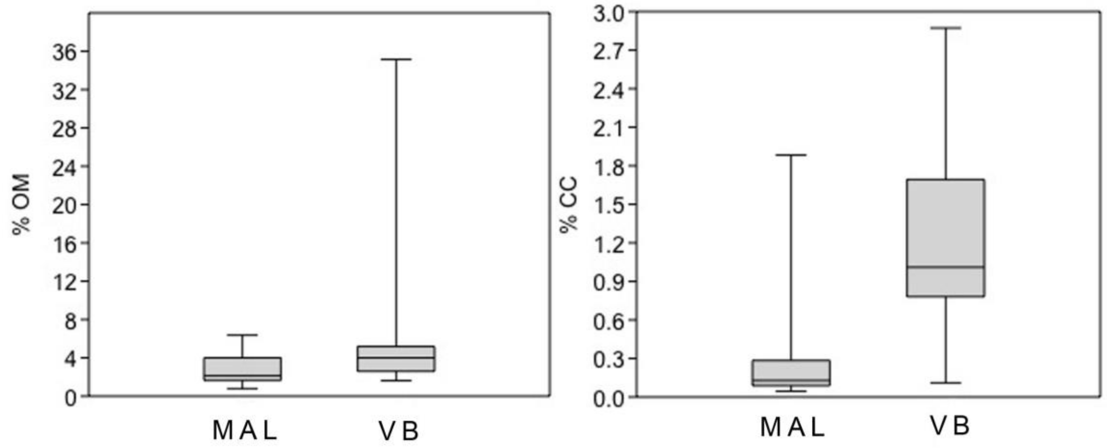


Figure 5.8 The % organic matter (OM) and carbonate content (CC) of the uppermost 40 cm of pond bed sediment in the MAL and VB ponds

5.3.2 Pond's hydrological cycle

5.3.2.1 Climatic framework

Data from the local weather institute, IPMA (2015), recorded average temperatures of 20.3ºC in the summer and 10.6ºC in winter over the 30 years from 1971 to 2001 in the Alentejo district, local to the MAL dunes. In the Algarve district, where VB stands, the average for the same period was slightly higher than MAL at 21.3ºC in summer and 11ºC in winter. A very similar annual precipitation total of 395mm was recorded for Alentejo (min 239mm, max 624.2mm) compared to 394mm for the Algarve (min = 208.3mm and max = 656.8mm) for the same period. Precipitation occurs mostly from October to March in both regions, and is generally low (<40mm/month) or absent in the summer months. To more accurately analyse values during the hydrological monitoring undertaken for this study, climate data from a weather station in Sines was used to directly compare with MAL region data (located 28km away). Data was also provided by IPMA for VB from the Aljezur weather station (circa 33km distance away) and a locally installed barometric logger that captured hourly climatic information. Figure 5.9, Figure 5.10 and Figure 5.11 show monthly records from 2010-2017 for both MAL and VB regions.

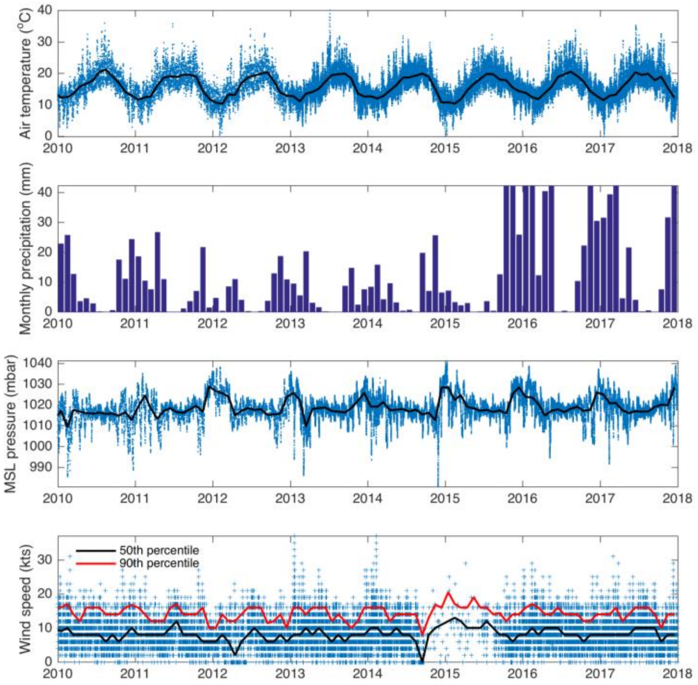


Figure 5.9 Review of weather data from Sines (2010-2017 inclusive) in the MAL region

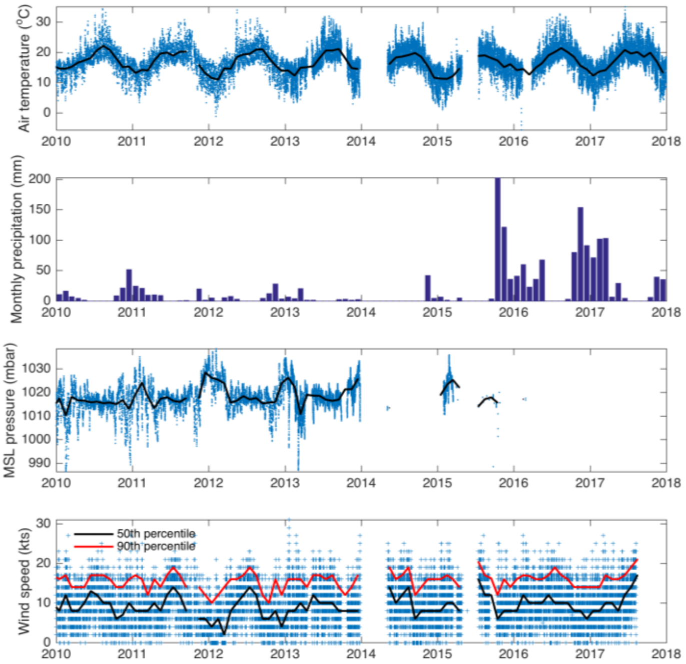


Figure 5.10 Review of weather data from Aljezur (2010-2017 inclusive) in the VB region. Blanks correspond to missing data

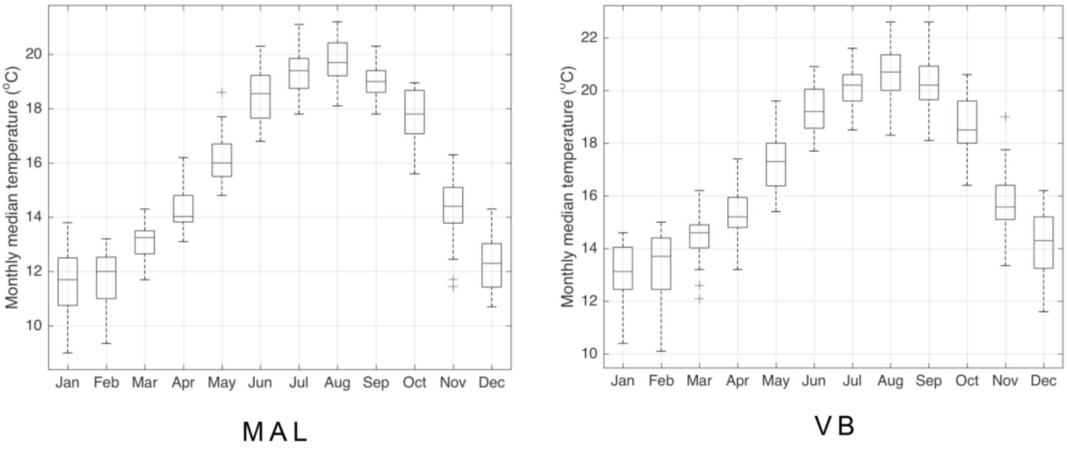


Figure 5.11 Summary of monthly temperatures (based on weather records from 2010-2017) in MAL and VB regions. Note different scales

The hydrological year begins with the first rains in autumn (September) that last until late spring (the start of May in 2016, and June in 2017), with some intermittent rainfall periods in between (Figure 5.12). From May to September, it was mainly dry in the two study regions, but with a spell of rain in mid June 2016 in the MAL region (Figure 5.12 A). The temperature range is similar between both regions with lowest values around February 2016 (around 5ºC) and January 2017 (circa 0ºC), and highest values in mid summer for both locations.

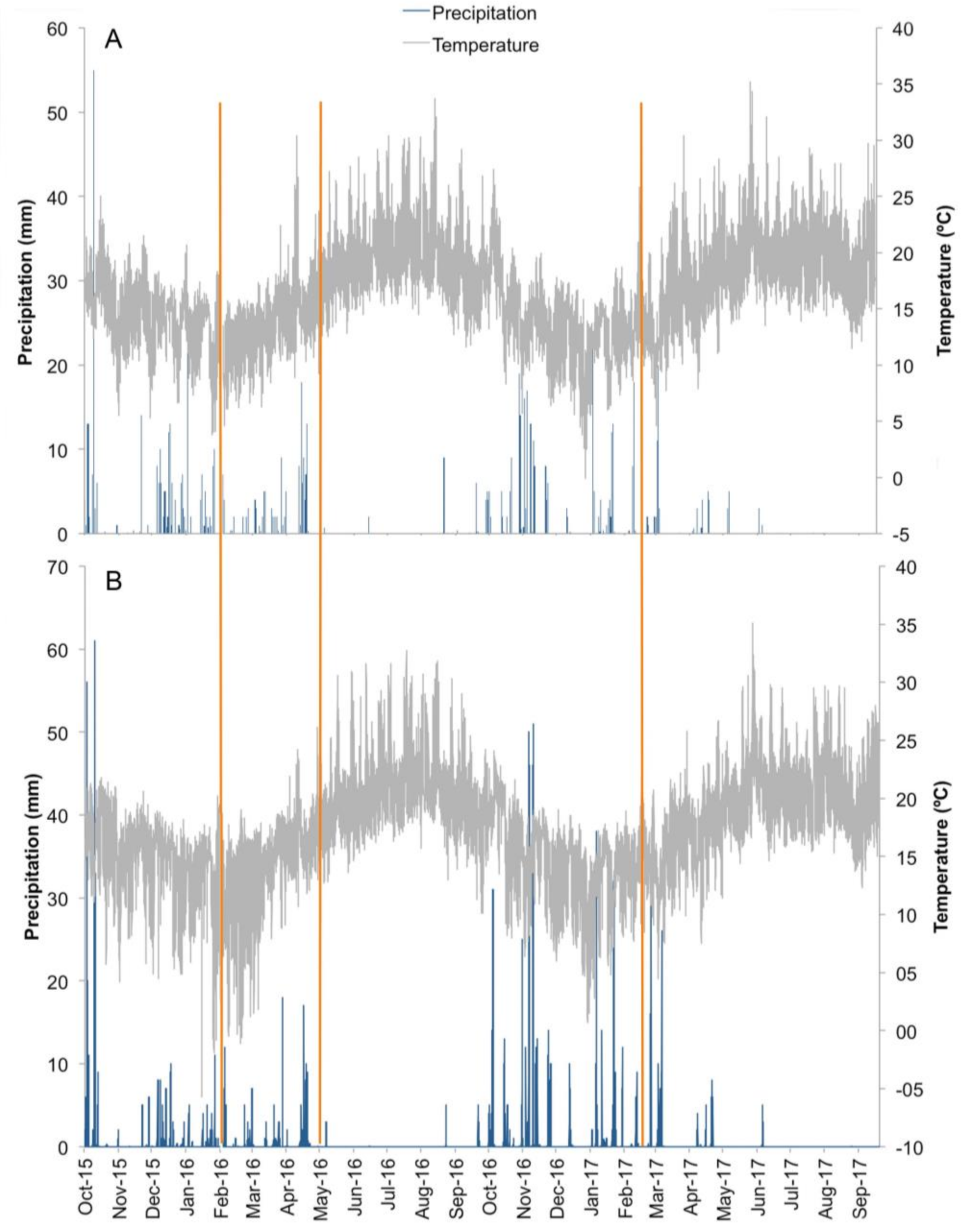


Figure 5.12 Hourly temperature and precipitation values for MAL (A) and VB (B) from Sines and Aljezur weather stations, respectively. Vertical orange lines correspond to the periods of data collection

5.3.2.2 Hydrological regime

The duration of the water cycle was measured in 6 ponds (represented in bold on Table 5.1). Water availability before each sampling was accounted for (Table 5.2) and differences between both regions are visible, but not significant (ANOVA *P*>0.05). MAL ponds had less days of water whereas VB had more before each sampling. For the 2 years of hydrological monitoring, the total number of days of inundation for each site shows that VB sites L603 and L614 had the longest hydroperiod and L617 the shortest of all sites in the two regions. In comparison the MAL ponds had values between those from VB, with L220 having the longest hydroperiod. The sum of total days of inundation throughout the 2-year study was 813 for MAL against 961 for VB. Ponds L220 and L617 were dry during summer 2016 sampling. Rainfall on average was slightly increased in the VB region (Figure 5.12), which might explain lower water availability in the MAL ponds prior to each sampling. Some variation is evident for hydroperiod between and within the two study regions exists, however other factors influencing the output of water in the system (such as groundwater flow or evapotranspiration) may also help to explain this.

Table 5.2 Number of days of inundation before each sampling season plus total number of days of inundation for the period of October 2015 to September 2017 in the MAL and VB hydrological monitored ponds

		W16	S16	W17	Total
MAL	mal1	32	121	9	248
	mal2	98	186	6	257
	L220	39	dry	23	308
VB	L603	120	217	77	421
	L614	121	217	64	413
	L617	29	dry	16	127

Analysis of the hydrological regime of each pond shows that the change in water level through the seasons is quite consistent within a region (Figure 5.13). All sites display distinctly seasonal hydrological regimes with maximum water depths occurring from autumn to late spring and lower values related to the drying out period in the summer/autumn months. In both regions, there is a strong alignment between water level behaviour in the ponds, meaning that the ponds experience flooding and drying at similar times. The flooded period is marked by a number of small-scale water level variations that occasionally lead to local drying. In the first flood season, MAL2 remains flooded throughout, but both MAL1 and L220 experience the occasional, short-lived drying. During the second flood season, the average level of all these ponds is reduced, but notably more so in MAL2 which only floods for very short periods. Water levels in MAL1 and L220 fluctuate considerably, but these do flood more frequently than MAL2. Both hydrological seasons in VB follow the same pattern, with ponds L603 and L614 flooding throughout the season and pond L617 only responding to increased water input of water from October 2015 and March 2017, coincident with rainfall peaks (Figure 5.13). The response and ability of this latter pond to flood is decreased compared to ponds L603 and L614.

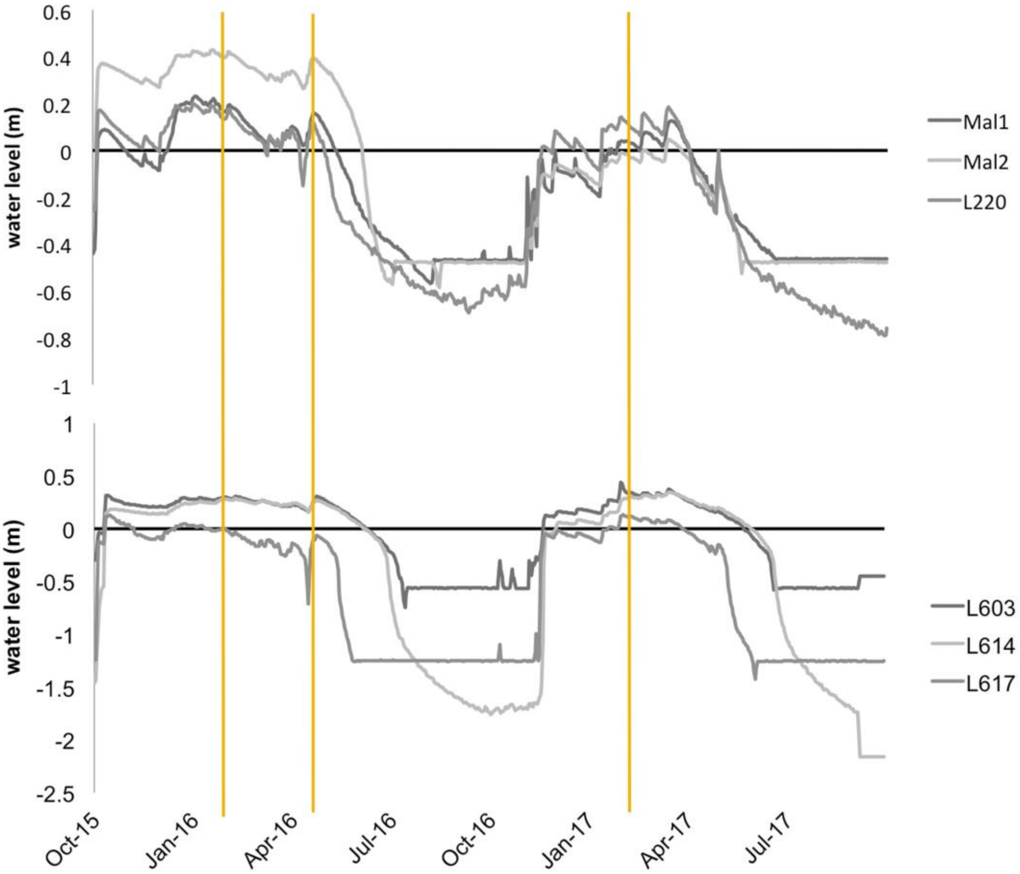


Figure 5.13 MAL (top) and VB (bottom) hydrological regime showing daily water levels for the period of the study in the hydrologically monitored ponds. Black horizontal line reflects the bottom of the pond bed and vertical orange lines to the periods of data collection

5.3.2.3 Drying and flooding behaviour

Considering how these ponds each respond to climatic events, namely rainfall and air temperature, the next Figures focus on some of the detail of the different hydrologies on the winter/summer drying phase (Figure 5.14 and Figure 5.15) and on the autumn/winter flooding phase (Figure 5.16 and Figure 5.17). There is a clear response from all ponds to increasing temperature records throughout the winter/summer phase at both MAL and VB regions, with striking high responses to sudden rises in temperature, as seen in May 2016. This resulted in a particular effect on the sudden drop of water levels across all ponds, though with greater impact on pond L220 at MAL and L617 at VB, dropping from 20 to 40cm below pond bed (Figure 5.14 and Figure 5.15). Levels are recovered afterwards with rainfall events.

During the flooding phase, here covered from October to January of the following year (Figure 5.16 and Figure 5.17), the response of pond's water level variability is more related to rainfall events rather than temperature. Mal1 and L220 in MAL region (Figure 5.16) and L603 and L617 in VB region (Figure 5.17) have very rapid responses even to short-lived rainfall events, whereas ponds Mal2 in MAL and L614 in VB have slower responses. Temperature also has some effects on the water level variability but with a much lower expression than in the winter/summer.

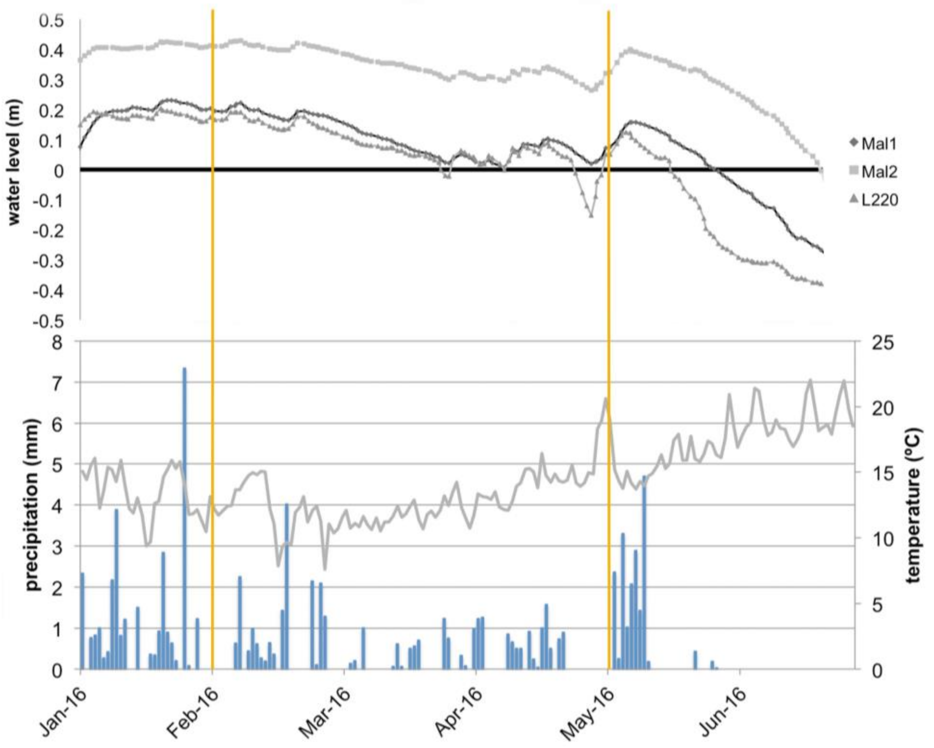


Figure 5.14 MAL hydrological and climatic regimes showing daily levels for winter and summer phases in the Mal1, Mal2 and L220 study ponds. Black horizontal line

reflects the bottom of the pond bed and vertical orange lines to the periods of data collection

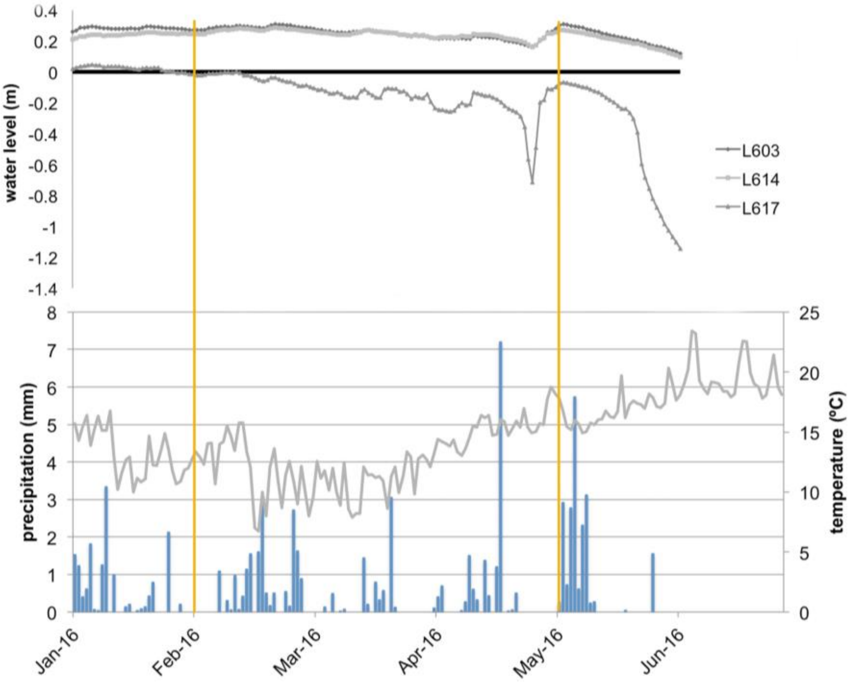


Figure 5.15 VB hydrological and climatic regimes showing daily levels for winter and summer phases in the L603, L614 and L617 study ponds. Black horizontal line reflects the bottom of the pond bed and vertical orange lines to the periods of data collection

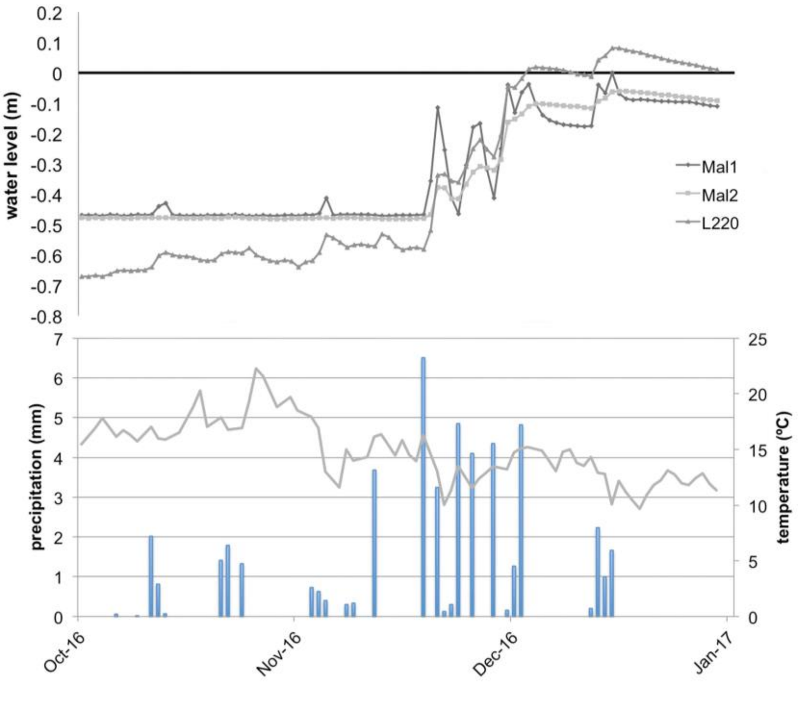


Figure 5.16 MAL hydrological and climatic regimes showing daily levels for autumn and winter in the Mal1, Mal2 and L220 study ponds. Black horizontal line reflects the bottom of the pond bed

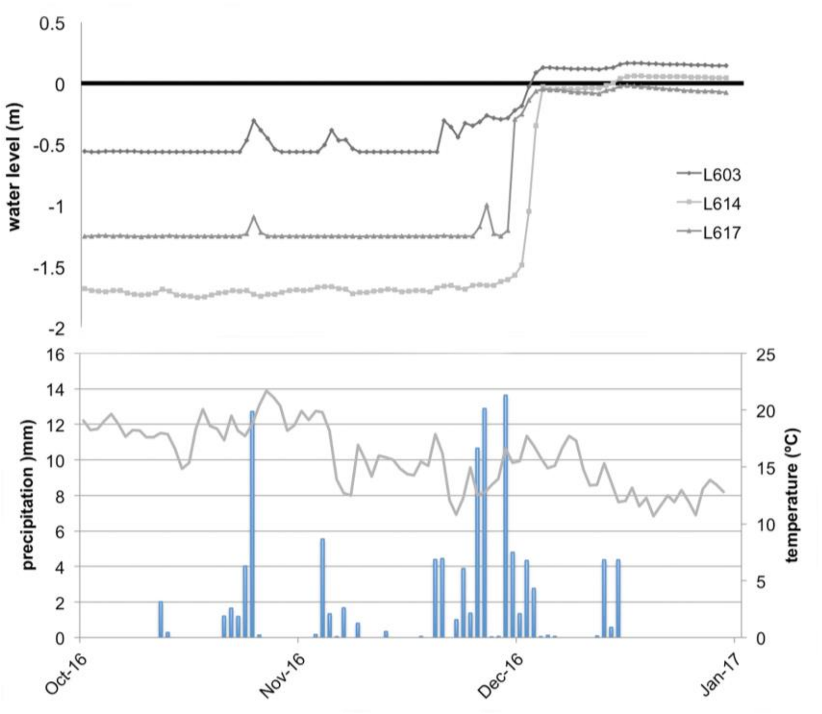


Figure 5.17 VB hydrological and climatic regimes showing daily levels for autumn and winter in the L603, L614 and L617 study ponds. Black horizontal line reflects the bottom of the pond bed

5.3.3 Environmental variability

Comparison of water chemistry and environmental data from both sampling seasons (summer and winter) revealed a significant difference for most of the environmental factors studied and for that reason, a separate analysis according to each season was undertaken. A summary of the results is given in Table 5.3. All ponds at both regions are mildly acidic (pH<7), with low alkalinity and conductivity values. The nutrient concentration in the water (TP, SRP and nitrates) reveals low numbers, though TP shows slightly higher values for MAL sites in winter. For the summer samples, there are no marked differences in terms of water chemistry and environmental factors in both regions. The difference between ponds in the summer is only significant for oxygen, the VB ponds with higher values than the MAL ponds. For the winter samples, 5 out of 11 variables showed significant differences between the regions. These were chlorophyll *a*, pH, oxygen, alkalinity and conductivity. The total number of sites sampled in each of the two seasons differs as in winter there were more ponds with water. In winter season chlorophyll *a*, alkalinity and conductivity are significantly higher in the MAL ponds, whereas pH and oxygen are higher in VB ponds.

The total of 34 water samples (20 from VB and 14 from MAL ponds), combining the 4 seasons, were compared between both regions. Only pH, oxygen, conductivity and SRP showed significant differences ($p<0.05$). From these differences, MAL exhibits lower values than VB for all significant variables, apart from conductivity (Figure 5.18). The results suggest increased nutrients at MAL. On the other hand, at VB, ponds reflect a more pristine nature and perhaps this is revealed in the increased oxygen levels (more photosynthetic activity) and slightly more alkaline pH.

Table 5.3 Means, standard errors (s.e.) and significance of differences between regions for environmental and water chemistry variables (A. summer sampling (May 2015-2016); and B. winter sampling (Feb 2016-2017)), *P* values correspond to ANOVA and Kruskal-Wallis (1) tests; *significant results.

	A. Summer			B. Winter		
	MAL	VB	*p* value	MAL	VB	*p* value
number of sites	5	8		9	12	
	mean (s.e.)	mean (s.e.)		mean (s.e.)	mean (s.e.)	
water depth at sampling (cm)	38.2 (4.45)	37.13 (7.36)	0.917 ns	38.67 (4.43)	50.63 (5.78)	0.138 ns
water temperature (ºC) [1]	21.50 (1.28)	22.77 (0.89)	0.379 ns	15.08 (0.75)	14.96 (0.43)	0.884 ns
chlorophyll *a* (µg/L)	4.43 (2.03)	7.96 (5.22)	0.979 ns	13.19 (5.49)	2.85 (0.45)	0.002**
pH	6.38 (0.10)	6.72 (0.18)	0.204 ns	6.11 (0.16)	6.81 (0.07)	0.000***
total phosphorus (µg/L)	69.82 (24.33)	67 (15.10)	0.887 ns	141.51 (45.79)	89.3 (27.82)	0.217 ns
soluble reactive phosphorus (µg/L)	11.99 (2.64)	10.62 (1.92)	0.675ns	16.90 (5.54)	14.92 (2.32)	0.995 ns
Nitrate (NO_{3-}) (µg/L)	39.78 (8.04)	23.24 (1.87)	0.03 ns	60.61 (10.84)	42.67 (5.47)	0.128 ns
oxygen (mg/L)	4.49 (1.17)	9.12 (0.44)	0.001**	5.93 (1.15)	10.10 (0.55)	0.002**
alkalinity (µg $CaCO_3$/L)	36.8 (17.81)	30 (10.54)	0.652 ns	52.78 (26.94)	14 (3.93)	0.000***
conductivity (µS/cm)[1]	1128 (297. 81)	775.25 (166.56)	0.285 ns	1192.33 (186.01)	411 (105.46)	0.001**

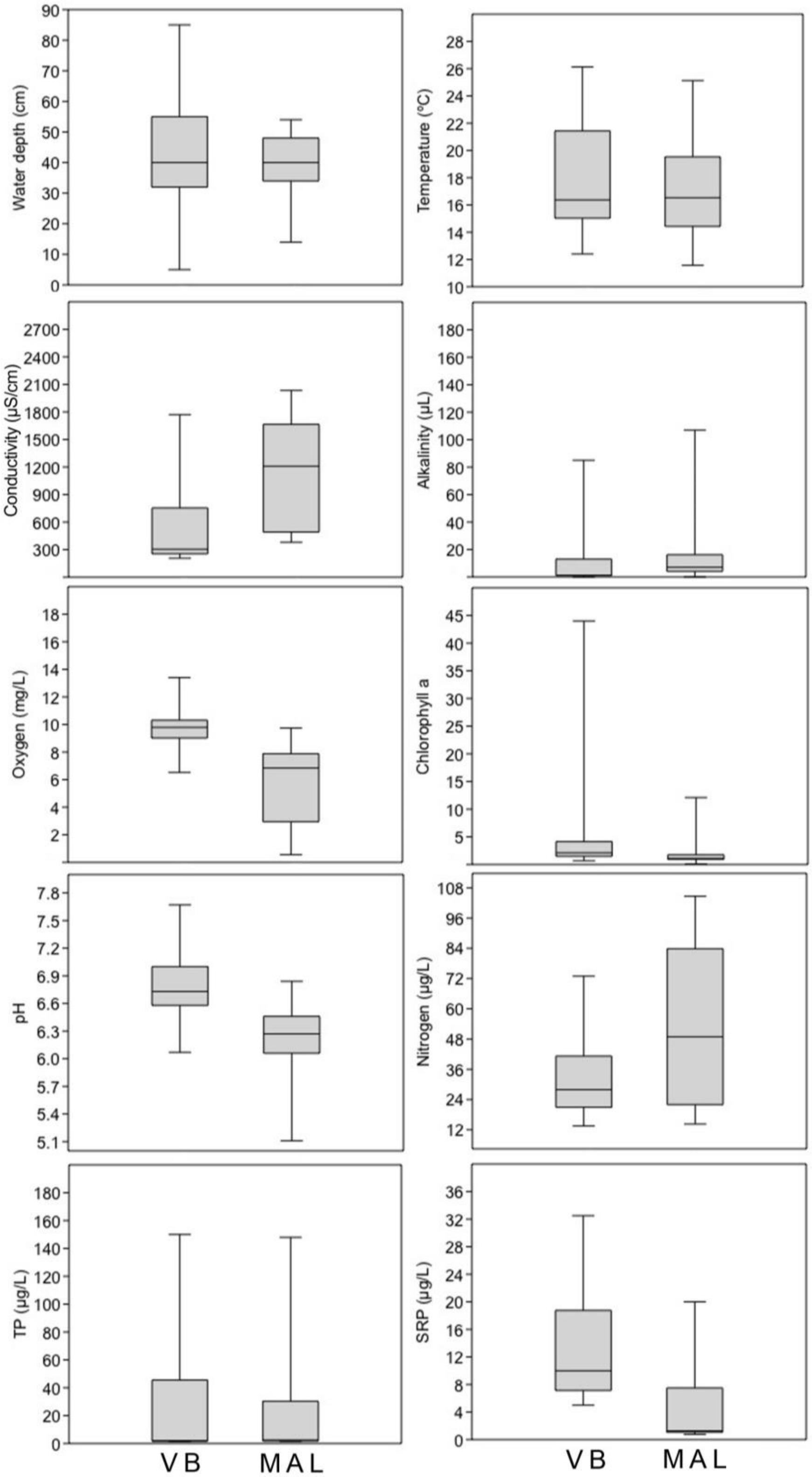


Figure 5.18 Boxplots of the distribution of the main environmental and water chemistry data with respect to VB and MAL regions

Non-Metric Multidimensional Scaling (NMDS) analysis (Kruskal, 1964) was used to discriminate between sites and to identify regional trends in the environmental data, using the “vegan” and “picante” packages in R software (R Core Team, 2014). Confidence ellipses were used to identify and represent each region (Figure 5.19). The relationship between each environmental variable and the sampled sites was tested and the most important variables were identified by their correlation with NMDS axes 1 and 2 (Table 5.4). Variables that correlate with axis 1 and 2 are hydrology (measured as days with water before the sampling date) and pH, respectively. Hydrology (H) demonstrates a strong correlation with axis 1 denoting extended days with water in some VB ponds prior sampling, compared to MAL. pH on the other hand, although slightly acidic as mentioned before, is higher in VB region and lower in MAL, strongly dictating the NMDS organisation along the 2nd axis.

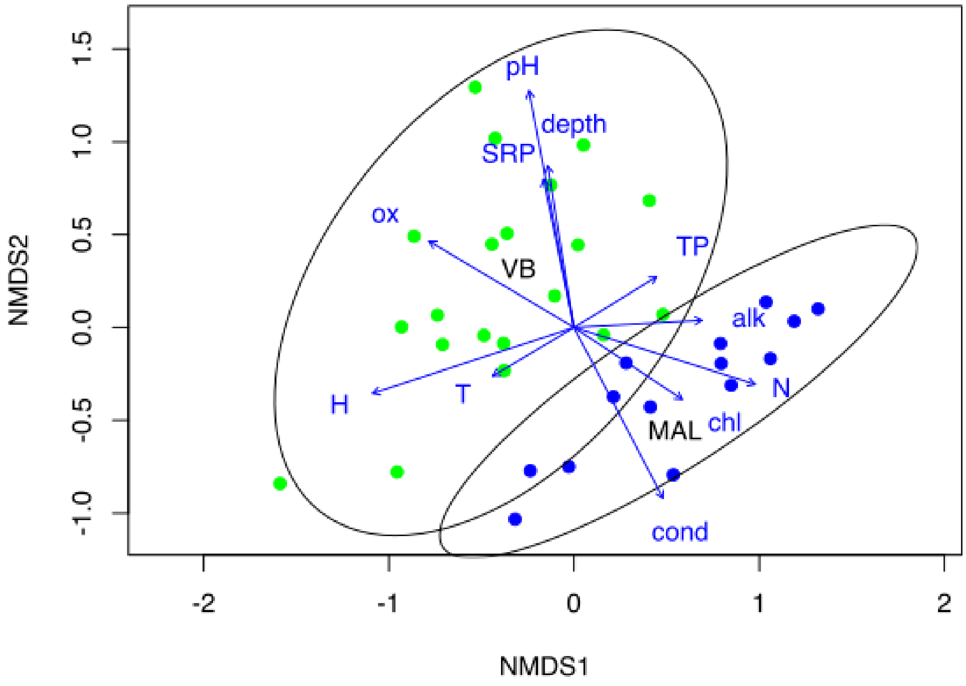


Figure 5.19 NMDS plot showing environmental variables in relation to VB and MAL regions. Abbreviations in blue: pH – pH; depth – water depth, SRP – soluble reactive phosphorus; ox – oxygen; TP – total phosphorus; alk – alkanlinity; N – nitrates; chl – chlorophyll *a*; T – water temperature; H – flooding (days) before sampling; cond - conductivity

Stress value for the NMDS was less than 0.2, in conformity with the actual multivariate distance between samples (Letten, 2017). Data from the two different regions are well separated in the NMDS, but they also overlap for both axes. The key drivers of separation in the plot are conductivity and chlorophyll *a* (both higher at the MAL sites), and oxygen which is higher at VB. pH, water depth, and SRP are also important in distinguishing the VB sites from MAL where values for these variables are all lower.

Table 5.4 *P*-values for Pearson correlation between environmental variables and NMDS axis 1 and 2. Bold values show significant correlations (*P*<0,05)

Variable	Axis1	Axis2	r2	Pr(>r)
Water depth	-0.16040	0.98705	0.2520	0.131
Chlorophyll *a*	0.83356	-0.55242	0.1594	0.346
alkalinity	0.99847	0.05523	0.1556	0.357
pH	-0.18767	0.98223	**0.5474**	0.003 **
Temperature	-0.85909	-0.51182	0.0847	0.559
Oxygen	-0.86007	0.51017	0.2694	0.130
conductivity	0.46363	-0.88603	0.3498	0.075 .
Nitrogen	0.95449	-0.29825	0.3416	0.071 .
Sluble reactive phosphorus	-0.20223	0.97934	0.2163	0.199
Total phosphorus	0.85319	0.52161	0.0889	0.584
Hydrology (flooding days prior sampling)	-0.95071	-0.31008	**0.4249**	0.032 *

5.3.4 Species assemblages

5.3.4.1 Cladoceran species

From the 11 ponds, a total of 23 species of cladocerans were found in the sampling. From these 23 species of cladocerans, the presence of males and females with ephippia were also separately noted (Table 5.5), but subsequent analysis integrated the specific sexual life-forms into the main represented species.

Table 5.5 Cumulative counts of cladoceran taxa and codes including different morphologies (male and female with ephippia) present in the studied ponds for the

summer and winter seasons in both MAL and VB. Bold show the most frequent and underlined the least frequently found species

Cladocera	code	summer MAL (n=5)	 VB (n=4)	winter MAL (n=5)	 VB (n=6)
Alona affinis (Leyding, 1860)	Aa	1		2	1
Alona affinis ephippia	Aae	1			
Alona rustica (Scott, 1895)	Ar			2	
Alonella excisa (Fischer, 1854)	Ae	3	3	6	4
Alonella excisa ephippia	Aee	1			
Alonella excisa male	Aem	2			
Bosmina longirostris (Müller, 1776)	Bl			6	
Ceriodaphnia dubia (Richard, 1894)	Cd				3
Ceriodaphnia laticaudata (Müller, 1867)	Cl	1	2		1
Ceriodaphnia male	Cm			1	
Ceriodaphnia quadrangula (Müller, 1785)	Cq	3	2	4	1
Ceriodaphnia quadrangula ephippia	Cqe			1	
Ceriodaphnia reticulata (Jurine, 1820)	Cr	3	3		4
Ceriodaphnia reticulata ephippia	Cre				1
Ceriodaphnia sp	Csp	2	2	2	3
Chydorus sphaericus (Müller, 1776)	Cs	3	1	6	5
Chydorus sphaericus ephippia	Cse		2		1
Chydorus sphaericus male	Csm	3	2	2	1
Coronatella rectangula (Sars, 1862)	Crc	4	2	5	1
Coronatella rectangula ephippia	Crce	1	1		
Coronatella rectangula male	Crcm	1			
Daphnia hispanica (Glagolev & Alonso, 1989)	Dh			1	
Daphnia hispanica male	Dhm			2	
Diaphanosoma brachyura (Liévin, 1848)	Db	4	1	1	
Diaphanosoma brachyura ephippia	Dbe	1			
Diaphanosoma brachyura male	Dbm	1			
*Dunhevedia crassa (*King, 1853)	Dc	3		4	
Dunhevedia crassa ephippia	Dce	2			
Ephemeroporus margalefi (Alonso, 1987)	Em	4		5	
Ephemeroporus margalefi ephippia	Eme	2			
Ephemeroporus margalefi male	Emm	2			
Karualona iberica (Alonso e Pretus, 1989)	Ki			1	
Leydigia iberica (Kotov & Alonso, 2010)	Li			1	
Macrothrix hirsuticornis (Norman & Brady, 1867)	Mh			2	
Megafenestra aurita (Fischer, 1849)	Ma			1	
Moina micrura (Kurz, 1875)	Mm	3			
Moina micrura ephippia	Mme	1			
Moina micrura male	Mmm	1			
Scapholebris rammneri (Dumont & Pensaert, 1983)	Sr	3	2	2	
Simocephalus exspinosus (De Geer, 1778)	Se	4	3	6	4
Simocephalus exspinosus ephippia	See	1			
Simocephalus vetulus (Müller, 1776)	Sv	2		6	1

Simocephalus vetulus ephippia	Sve	1

The most frequent cladoceran species (found in over 55% of the sites and represented in bold in Table 5.5) are *Simocephalus exspinosus*, *Alonella excisa*, *Chydorus sphaericus*, *Coronatella rectangula*, *Ceriodaphnia reticulata* and *Ceriodaphnia quadrangula*; these species are all quite common and widespread in Europe. They belong to the daphniid and chydorid families, both of which are diverse with species adapted to a range of trophic (oligotrophic to eutrophic), hydrological (seen in both permanent and temporary ponds) and habitat conditions (Alonso, 1991; Barnett *et al.*, 2007; Błędzki and Rybak, 2016). Species found at only one site in only one sampling (corresponding to 5% of the total, underlined in Table 5.5) were *Daphnia hispanica*, *Karualona iberica*, *Leydigia iberica* and *Megafenestra aurita*, only found in the VB ponds. The first three species are endemic and have a very limited distribution in the Iberian Peninsula (Alonso, 1991), whereas *Megafenestra aurita*, although widespread in Europe is for the first time recorded in the study ponds. Błędzki & Rybak (2016) show an up to date record of all cladoceran species found up to 2015. The limited records from Portugal are demonstrated here, not as the absence of a certain species taxa, but as the absence of local studies of this kind.

5.3.4.2 Plant species

A total of 49 species of plants were identified in the ponds surveyed in southwest Portugal. This included all species that occurred in water at the time of sampling, and for this reason some terrestrial and semi-aquatic species are also included. Four plants were found in over 50% of the samplings, these being *Agrostis stolonifera*, *Baldelia ranunculoides*, *Galium palustre* and *Juncus heterophyllus* (in bold on Table 5.6). These are all species that occur mostly in the vicinity of water bodies and are commonly widespread and cosmopolitan. Similarly to Cladocera, some plant species were only recorded once during the samplings (underlined on Table 7), these were *Cladium mariscus* and *Juncus conglomeratus*. Given that these are wet tolerant plants, it is likely that the results reveal the absence of water around the plant by the time of sampling, hence not accounted for (as is the case of *C. mariscus* – whose presence is marked by a large perennial agglomerate at pond Mal2), or in the case of the latter species, its presence may have remained unnoticed due to the undergrowth or lack of distinguishing features such as flowers, at the time of sampling.

Table 5.6 Cumulative counts of plant taxa and codes present in the studied ponds for the two seasons in both MAL and VB. Bold show the most frequent and underlined the least frequently found species

Vegetation	code	summer MAL (n=5)	summer VB (n=4)	winter MAL (n=5)	winter VB (n=6)
Agrostis stolonifera (L.)	agr_stol	4	5	4	7
Anagallis tenella (L.)	ana_ten	2			
Antinoria agrostidea (DC.)	ati_agr			1	3
Apium nodiflorum (L.)	api_nod			5	4
Baldellia ranunculoides (L.)	bal_ran	3	8	4	8
Bolboschoenus maritimus (L.)	bol_mar	2	2	4	8
Callitriche brutia	cal_bru				2
Carum verticillatum (L.)	car_ver			8	8
Cladium mariscus (L.)	cla_mar	1			
Cotula coronopifolia (L.)	cot_cor		3	3	1
Cynodon dactylon (L.)	cyn_dac			4	4
Cyperus longus (L.)	cyp_lon	1			3
Dittrichia viscosa (L.)	dit_vis		2	4	5
Eleocharis palustris (L.)	ele_pal	1	6	3	4
Eleocharis sp. undiff.	ele_sp		5	2	1
Eryngium corniculatum (Lam.)	ery_cur		7		1
Galium palustre (L.)	gal_pal	1	1	4	12
Glyceria declinata (Bréb.)	gli_dec	2	3	3	
Holcus lanatus (L.)	hol_lan			6	5
Hyacinthoides vicentina subs transtagana (Franco & Rocha Afonso)	hya_vic			1	8
Hydrocotyle vulgaris (L.)	hyd_vul	3		1	2
Hypochaeris radicata (L.)	hyp_rad			2	
Illecebrum verticilatum (L.)	ill_ver	1	6	1	5
Isoetes setaceum (Lam.)	iso_set	2	3	1	1
Isoetes vellatum	iso_vel			3	4
Isolepis fluitans (L.)	iso_flu		2	3	9
Isolepis sp. undiff.	iso_sp			2	3
Juncus conglomeratus (L.)	jun_con			1	
Juncus effusus	jun_eff		1	1	
Juncus heterophyllus (Dufour)	jun_het	5	6	3	3
Juncus maritimus (Lam.)	jun_mar		1	3	2
Juncus sp. undiff.	jun_sp	2	4	1	7
Leontodon taraxacoides (Vill.)	leo_tar			2	
Leucojum autumnale (L.)	leu_aut				2
Littorella uniflora (L.)	lit_uni			2	1
Lobelia urens (L.)	lob_ure				3
Lotus hispidus (Willk.)	lot_his		1		1
Lythrum junceum (Banks & Sol.)	lyt_jun	1	1		2

Mentha pulegium (L.)	men_pul			2	1
Myosotis laxa (C.F.Schultz)	myo_lax		5		2
Myriophyllum alterniflorum (DC.)	myr_alt	1	4		4
Panicum repens (L.)	pan_rep	3		3	7
Paspalum paspalodes (L.)	pas_pas	2	7	1	
Phalaris coerulescens (Desf.)	pha_coe			1	
Pulicaria paludosa (Link)	pul_pal				3
Ranunculus ophioglossifolius (Vill.)	ran_oph				3
Ranunculus peltatus (Schrank)	ran_pel	2	6		3
Ranunculus sp. undiff.	ran_sp	1	2	3	5
Samolus valerandi (L.)	sam_val	1		3	1
Schoenoplectus lacustris (L.)	scho_lac		1	1	
Silene laeta (Aiton)	sil_lae	1	1		

5.3.5 Community characteristics

Cladoceran and vegetation biodiversity, calculated here using the Shannon diversity index H, is lower for MAL compared to VB, and this is corroborated by species richness estimates (Figure 5.20). When abundance is considered for cladocerans, however, there is a higher number of individuals per litre in the MAL ponds. The significance of variation between regions amongst the different biota revealed that, for cladocerans, abundance (ANOVA F=7,03; *p*=0,002) and richness (ANOVA F=15,91; *p*=0,000) differed significantly between regions, whereas diversity did not (ANOVA F=3,37; *p*=0,08). Plant biodiversity indices varied significantly between regions for diversity (K-wallis H(chi^{2})=9,59; *p*=0,002) and richness (K-wallis H(chi^{2})=8,537; *p*=0,003). In summary, the VB ponds contain a higher number of cladoceran and plant species per pond compared to MAL, however, the number of cladocerans per litre in the MAL sites is larger. Although cladoceran diversity indices are not markedly different between regions, plant diversity indices are significantly different.

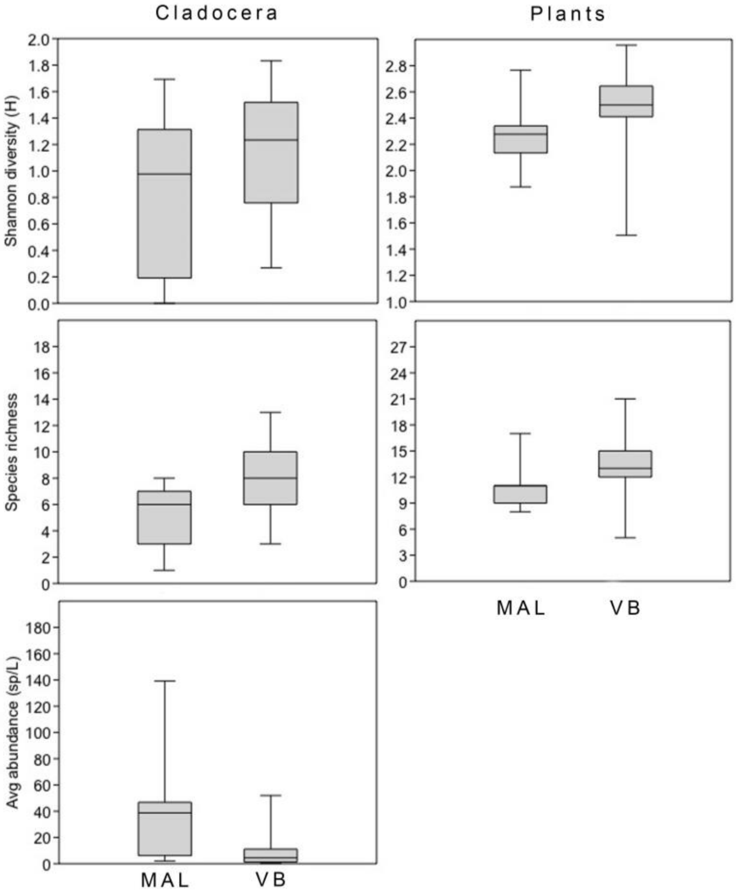


Figure 5.20 Boxplots of diversity, species richness and abundance indices for cladoceran and vegetation between both MAL and VB regions

A Spearman's rank correlation was undertaken to understand which environmental factors were correlated with the differences seen in abundance, richness and diversity values for both biota (Table 5.7). Cladoceran diversity and vegetation richness were the only parameters not significantly related to any environmental variable. Variables that explain cladoceran abundance are pH, oxygen, hydrology (as flooding in days before sampling) and nitrate $NO3^{-}$. Cladoceran richness is related to depth, chlorophyll *a*, pH and conductivity. pH is also a strong factor explaining regional differentiation between the VB and MAL sites and again shows a strong correlation with the cladoceran diversity indices. Vegetation diversity was highly correlated with pH, oxygen, and conductivity. Water chemistry is clearly strongly influencing vegetation, whereas the influence of water depth and hydrology together with pond water chemistry determine cladoceran community structure.

Table 5.7 Spearman’s rank correlation coefficient (R_s) and pvalues (in bold) between environmental factors and cladoceran and vegetation biological indices for all seasons and ponds. Bold values show significance ($p<0.05$). Abbreviations stand for: depth – water depth; chl – chlorophyll *a*; alk – alkanlinity; pH – pH; T – water temperature; ox – oxygen; cond – conductivity; N – nitrates; SRP – soluble reactive phosphorus; TP – total phosphorus; H – flooding (days) before sampling

	Cladocera						Vegetation			
Var	Abundance		Richness		Diversity		Richness		Diversity	
	p	***Rs***	***p***	***Rs***	***p***	***Rs***	***p***	***Rs***	***p***	***Rs***
depth	0,060	0,326	**0,003**	-0,492	0,083	0,301	0,286	0,188	0,070	0,316
chl	0,151	-0,252	**0,005**	0,466	**0,045**	-0,347	0,505	0,118	0,581	-0,096
alk	0,661	-0,078	0,608	0,091	0,627	0,086	0,128	-0,266	0,421	-0,140
pH	**0,025**	0,383	**0,022**	-0,390	0,208	0,221	0,547	-0,107	**0,025**	0,390
T	0,347	0,166	0,517	0,115	0,284	0,189	0,252	0,202	0,107	-0,281
ox	**0,002**	0,517	0,072	-0,312	0,138	0,260	0,413	0,145	**0,001**	0,565
cond	0,827	-0,039	**0,019**	0,400	0,774	0,051	0,064	0,321	**0,029**	-0,379
N	**0,013**	-0,420	0,691	0,071	0,264	-0,197	0,424	0,142	0,752	-0,055
SRP	0,217	0,217	0,533	-0,111	0,919	0,018	0,060	0,326	0,141	0,256
TP	0,318	-0,177	0,870	0,029	0,240	-0,207	0,155	0,249	0,702	-0,067
H	**0,016**	0,589	0,927	0,025	**0,030**	0,542	0,091	0,437	0,642	0,120

5.3.6 Cladoceran and vegetation regional community distinctiveness

TWINSPAN (Hill, 1979) was used to characterise, separately, the cladoceran and vegetation communities across the regions. Applied to the cladoceran species, the first division of the TWINSPAN analysis divided communities into the two regions (Figure 5.21). The indicator species at this division were *Chydorus sphaericus* for the MAL sites and *Ephemeroporus margalefi*, *Diaphanosoma brachyura* and *Simocephalus vetulus* for the VB sites (not shown on the figure as only indicator species for each individual group are represented where they exist). Subsequent divisions from this point generated four groupings covering VB sites of which groups 1, 3 and 7 comprise VB sites only, and group 6, the largest group, contains 1 site from the MAL region. Groups 2 and 4 contain only MAL ponds and group 5, similar to 6, contains one site from VB amongst the remaining 6 from MAL.

Group 1, generated at division 2, contains 3 different samples from one site (L608), with the indicator species *Moina micrura*. This species has been found to be a late coloniser of ponds, with an ability to thrive in eutrophic conditions, appearing only in the last phases of the water cycle, before pond drying (Crosetti and Margaritora, 1987; Błędzki and Rybak, 2016). Pond L608 is the only VB pond with highly turbid water and one of the reasons for this is the presence of large branchiops that disturb the pond bed sediments. It is therefore consistent that G1 contains samples from summer and one from a dry winter (2017) since *M. micrura* presence is indicative of a site with these features. Division 5 separated groups 3 from 6 and 7, capturing the summer and winter sampling seasons. G3 contains VB sites from summer samplings with the indicator species being *Diaphanosoma brachyura* (widely distributed and cosmopolitan species, adapted to low resource habitats - Błędzki & Rybak 2016) whereas groups 6 and 7 contain only winter samples from VB. This last group has *Alonella excisa* as an indicator, a species that is widely distributed in Europe, with a preference for submerged vegetation, living mainly at the bottom of slightly eutrophic, shallow waterbodies (Alonso, 1991; Duigan, 1992).

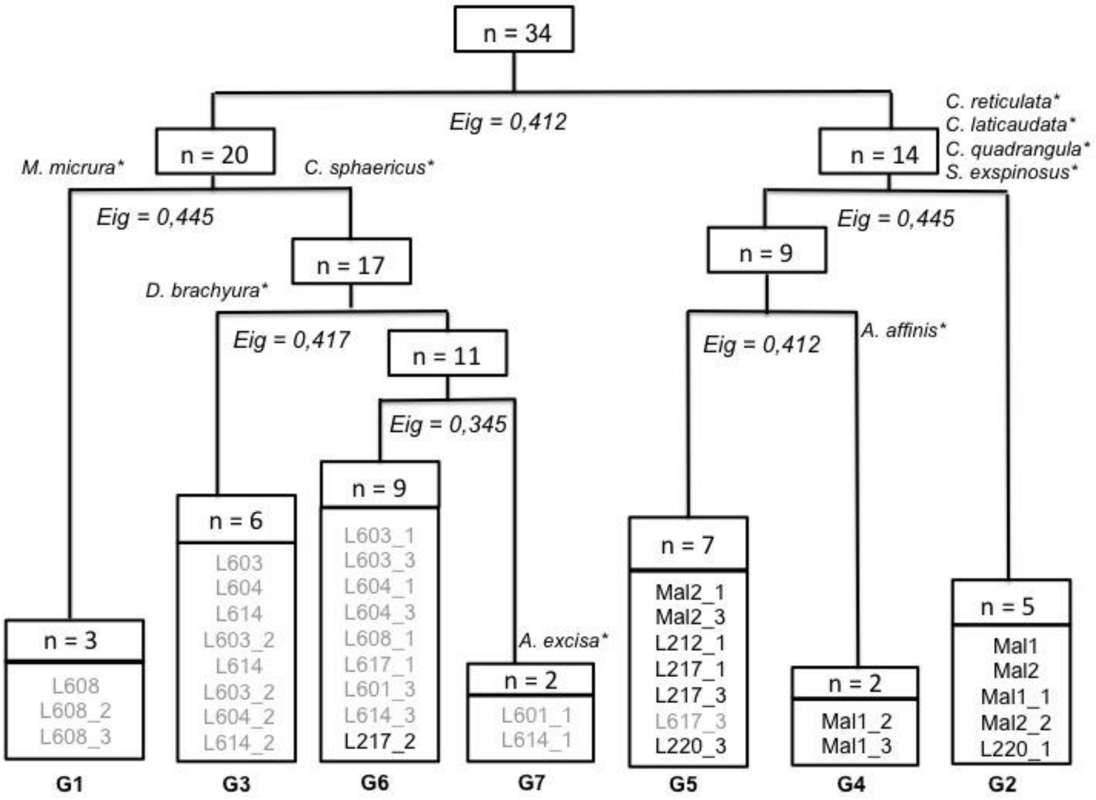


Figure 5.21 Cladocera TWINSPAN dendogram showing eigenvalues and indicator species (*). Black sites correspond to MAL, grey sites correspond to VB and the number in front relate to the sampling seasons: nothing (S15), _1 (W16), _2 (S16) and _3 (W17)

From the MAL-dominated groups, only G5 is separated according to seasonality, whereas the other two correspond more to samples from consecutive samplings. This means that groups 2 and 4, even after the drought period, are characterised by the same species community: G2 has three *Ceriodaphnia* species and *Simocephalus exspinosus* as indicators, all large sized species from the daphnid group, adapted to a range of trophic environments found mostly amongst aquatic vegetation (Błędzki and Rybak, 2016). G4 has *Alona affinis* as an indicator, also a large species from the chydoridae group, which although widely distributed in Europe, is more commonly found in cold waters, also amongst vegetation (Duigan, 1992; Błędzki and Rybak, 2016). Cladocera community cluster division by the TWINSPAN reveals distinct communities in both regions, with preference for more specialised and medium to smaller sized species in VB ponds, as opposed to more ubiquitous and medium to larger sized species assemblages in the MAL ponds.

The TWINSPAN cluster organisation of the vegetation communities showed clear separation of sites between regions and within this, between sampling seasons as well (Figure 5.22). Groups 1, 4 and 5 comprise sites exclusively from the MAL region, whilst groups 2, 6 and 7 belong entirely to VB. Group 3 is the only end group with a mixture of sites from VB and MAL, as well as seasons (samples from summer 2016 and winter 2017). The first division of the TWINSPAN plainly separates winter samples from the rest (Groups 1 and 2 on division 2). Group 5 (division 7) also also separates a site from the same sampling period (site L220_1). *Apium nodiflorum* marks this in both divisions as being the indicator species. This is a fully aquatic species with a preference for sandy substrates (Bracamonte *et al.*, 2014). Here it indicates ponds in the MAL region (G1 and G5) and in wintertime when water levels are high and there is a tendency for a well developed aquatic vegetation.

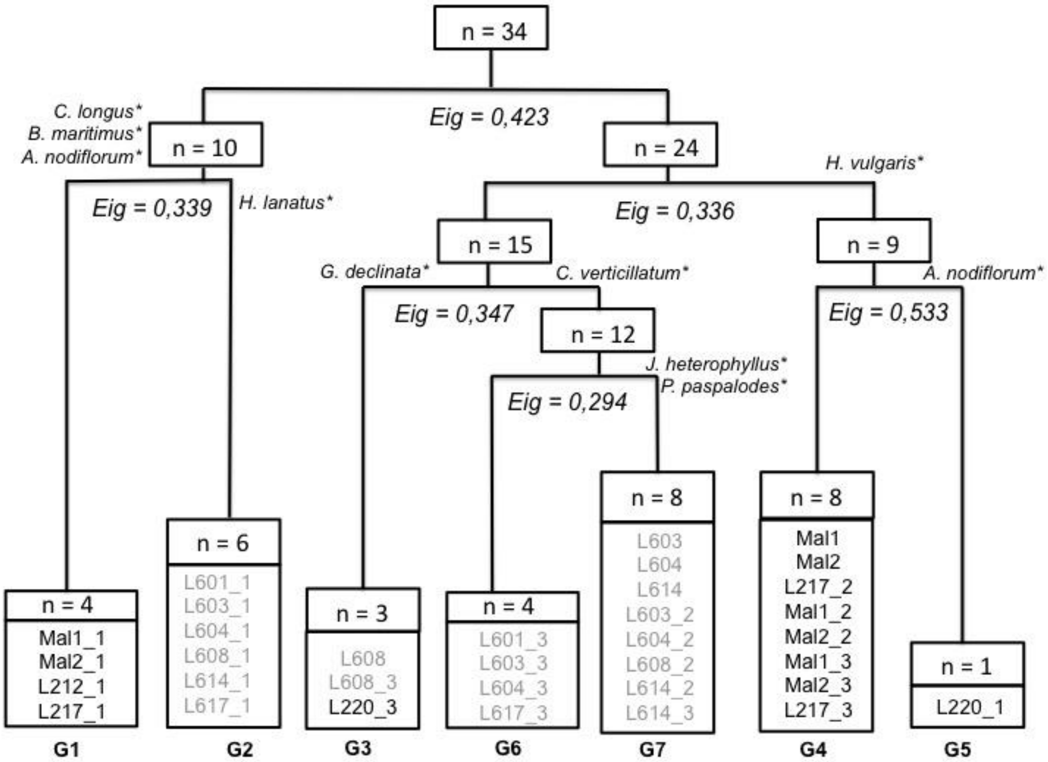


Figure 5.22 Vegetation TWINSPAN dendogram showing eigenvalues and indicator species (*). Black sites correspond to MAL, grey sites correspond to VB and the number in front relate to the sampling seasons: nothing (S15), _1 (W16), _2 (S16) and _3 (W17)

Groups 2 and 6 are VB sites sampled in winter having *Holcus lanatus* and *Carum verticillatum*, respectively as indicator species. *H. lanatus* is not considered an aquatic species but is a grass commonly seen close to ponds as an opportunistic terrestrial that colonises temporary ponds in dry years (Grillas *et al.*, 2004). *C. verticillatum* is an Atlantic species found in open areas on top of sandy sediments of seasonal waterbodies where organic matter accumulates (Bracamonte *et al.*, 2014). Group 7 represents VB sites sampled in the summer and the indicator species of this environment are *Juncus heterophyllus* and *Paspalum paspalodes*. The former species is a terrestrial taxa with an aquatic form, that thrives and develops under water and flowers above it (Bracamonte *et al.*, 2014). The latter species is not considered a macrophyte and is not native to Europe, but is a grass present in Mediterranean habitats found amongst other temporary pond vegetation species (Alfonso *et al.*, 2016). Group 3 is indicated by *Glyceria declinata*, an aquatic species commonly found in the Iberian peninsula in Mediterranean temporary ponds (Bracamonte *et al.*, 2014; Flora-On, 2018). Group 4 is characterised by three MAL sites sampled in various seasons and has *Hydrocotyle vulgaris* differentiating this at division 3. This is a common and widespread species in Europe that prefers humid areas, occurring mainly on the margins of wet habitats (Bracamonte *et al.*, 2014; Flora-On, 2018), and sometimes associated with peaty - more acidic - soils (Landsdown, 2014). In general, the main vegetation communities are separated according to sampling season and region, with a combination of aquatic and wet tolerant species found in the same vegetation community, indicating an inundation gradient where annual species occur more in winter and perennials (helophytes) in the summer.

5.3.7 Associations between vegetation and Cladocera

Pearson correlation between species richness of Cladocera and vegetation at each site showed a positive and significant relationship (R=0.518 *P*=0.002 – Figure 5.23).

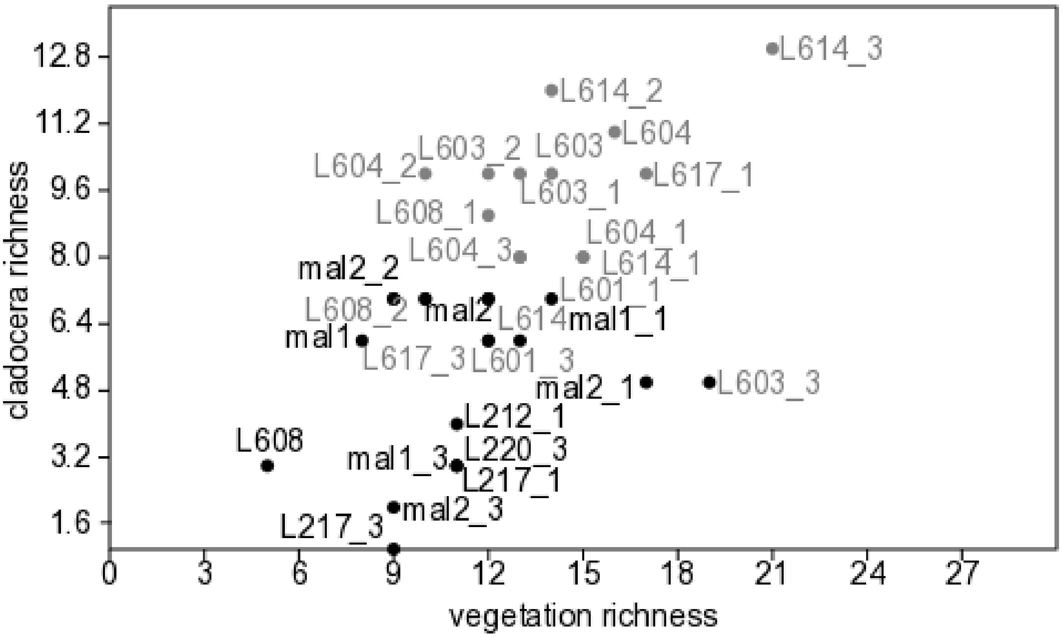


Figure 5.23 Correlation between Cladocera and vegetation species richness. Black sites correspond to MAL, grey sites correspond to VB and the number in front relate to the sampling seasons: nothing (S15), _1 (W16), _2 (S16) and _3 (W17)

As mentioned before, NMDS was applied to cladoceran species abundance data according to all sampled ponds in all seasons. Influences of cladoceran species and plant species on the assemblage distribution of the sites were analysed in two different plots separately. Stress value for each NMDS was less than 0.2, in conformity with the actual multivariate distance between samples (Letten, 2017). Pearson correlation coefficient strength (|R|>0.5) was used to test which species were more correlated with the first and second NMDS axes - 6 cladoceran and 11 plant species (Figure 5.24 and Figure 5.25). Cladocera species associated with the VB ponds were *Simocephalus vetulus*, *Ephemeroporus margalefi*, *Alonella excisa* and *Moina micrura*, whereas *Simocephalus exspinosus* and *Chydorus sphaericus* characterised the MAL ponds. This finding is in agreement with the TWINSPAN results. *C. sphaericus* is highly correlated with axis 1, and separates MAL and VB ponds. *M. micrura* correlates more with axis 2 and the VB ponds.

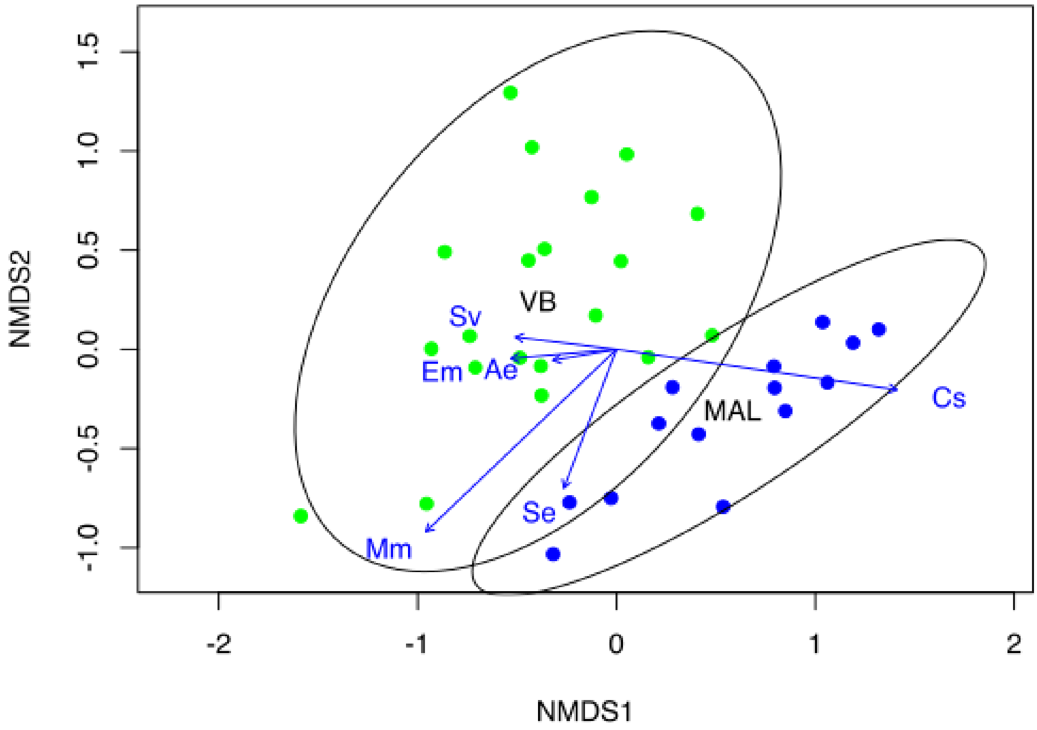


Figure 5.24 NMDS of Cladocera species in the VB ponds (green) and MAL ponds (blue). Species indicated by vectors are: Sv- *Simocephalus vetulus*; Em – *Ephemeroporus margalefi*; Ae – *Alonella excisa*; Mm – *Moina micrura*; Se – *Simocephalus exspinosus* and Cs – *Chydorus sphaericus*

Both species are adapted to living in hydrologically unstable regimes and tolerate warm temperatures, however, there are specific traits that differentiate these two species from one another (Scourfield and Harding, 1941; Alonso, 1996; Barnett *et al.*, 2007; Błędzki and Rybak, 2016). In particular body size is larger in *M. micrura* (0-5-1.2mm compared to 0-33-0-49mm in *C. sphaericus*) and filtration and feeding is also distinct:

M. micrura filters water from a stationary position, whereas *C. sphaericus* scrapes algal particles from periphyton. Further, the habitat preference of *M. micrura* is in the littoral of turbid/clay waters, whereas *C. sphaericus* prefers to live in the benthic/pelagic area amongst vegetation.

In terms of vegetation, NMDS shows similar results to the TWINSPAN analysis. *Apium nodiflorum*, *Glyceria declinata* and *Agrostis stolonifera* are very much representatives of MAL flora, whereas *Eryngium cornicolatum* and *Baldelia ranunculoides* are more commonly found in the VB ponds. MAL ponds are largely dominated by the competitive perennial *A. stolonifera* which reduces the light availability in the pond, able to penetrate only in the centre, and this is where annual species like *A. nodiflorum* and *G. declinata* tend to occur. In contrast, VB ponds have less vegetation density and light penetration enables a wider range of aquatics to occur.

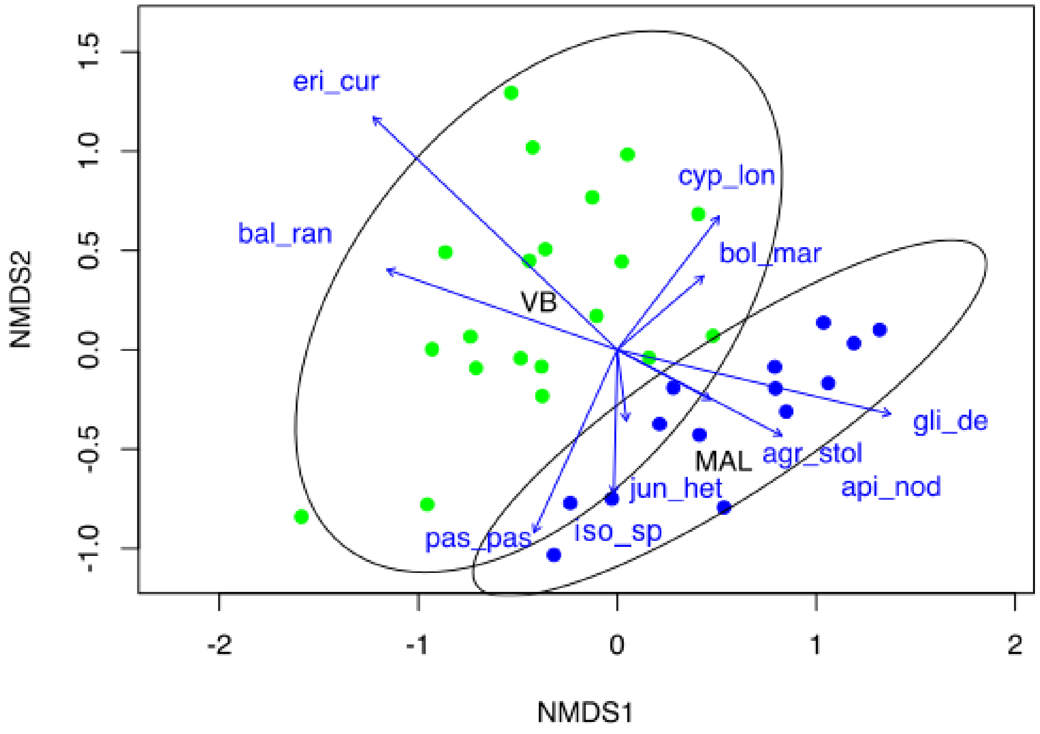


Figure 5.25 NMDS of plant species in the VB ponds (green) and MAL ponds (blue). Species indicated by vectors are: eri_cur - *Eryngium corniculatum*; bal_ran - *Baldelia ranunculoides*; cyp_lon - *Cyperus longus*; bol_mar - *Bolboschoenus maritimus*; jun_het - *Juncus heterophylus*; agr_stol - *Agrostis stolonifera*; gli_de - *Glyceria declinata*; api_nod - *Apium nodiflorum*; iso_sp – *Isolepis sp*; pas_pas - *Paspalum paspalodes*

5.3.8 Hydroperiod and species turnover

Analysis of the variation in species composition relative to hydroperiod was undertaken for the 6 sites where hydrological measures were collected. Correlation coefficients between hydroperiod (measured as flooding days before sampling) and cladoceran species abundance, diversity (Shannon indices) and richness and vegetation species diversity (Shannon indices) and richness values are summarised in Table 5.8. For all biodiversity indices measured, only cladoceran diversity exhibited a significant correlation with hydrology in the MAL region (*p*=0.005). Species richness and abundance for cladocerans were not influenced by the number of days before sampling. The vegetation community shows no correlation with hydrology measures in either of regions.

Table 5.8 Results of a Pearson correlation analysis between hydrology and biodiversity indices for cladoceran and vegetation in VB and MAL regions. Bold score represents significant results

		hydro vs cladocera		**hydro vs vegetation**	
	indices	**R**	***p* value**	**R**	***p* value**
VB	Richness	0,66	0.543	0,12	0.921
	Abundance	0,79	0.424	NA	NA
	Diversity	0,64	0.555	0,07	0.956
MAL	Richness	-0,64	0.558	-0,21	0.868
	Abundance	-0,10	0.936	NA	NA
	Diversity	**-1,00**	**0.005**	-0,30	0.806

5.4 Discussion

5.4.1 Portuguese Mediterranean ponds

Since the classification of MTP as a priority habitat for conservation (EU Habitats Directive 92/43/CEE), there has been an increased interest and attention to this habitat in Portugal (Fonseca *et al.*, 2008). Studies to date have focused on gathering information on the status and threats to Portuguese temporary ponds (Beja and

Alcazar, 2003; Farinha *et al.*, 2007; Presado *et al.*, 2010; Fonseca and Monteiro, 2011), with special attention often paid to large branchiopods (Machado et al. 1999 (a); Machado et al. 1999 (b); Fonseca et al. 2008; Korn et al. 2010), vegetation (Pinto-Cruz *et al.*, 2009, 2011; Pinto-Cruz, 2010) and amphibians (Ferreira and Beja, 2013). Few zooplankton and hydrological studies have thus far been undertaken in the regions considered in the present study. So far, only one study is known for microcrustaceans in southwest Portugal (Caramujo and Boavida, 2010) with hydrological study limited to a single investigation of temporary pond groundwater hydrochemistry balance (Salvador *et al.*, 2017). Most of the other research has been focused on contributions to support and guide management strategies and conservation plans, and this has been primarily driven by increasingly intensive agricultural practices and consequent degradation of MTP habitat (Farinha *et al.*, 2007). Other Mediterranean countries like Spain (Serrano et al. 2008; Quintana et al. 2018), France (e.g. Grillas et al. 2004), Italy (including both the mainland and islands - Crosetti & Margaritora 1987; Bagella & Caria 2012), Morocco (Laila Rhazi *et al.*, 2001; Broeck, 2016) have undertaken comparatively more research on temporary ponds across many fronts for several years. Developments within the subject in Portugal still remain limited and incomplete. Therefore, the rationale for ecohydrological research on Portuguese MTP is clear, given a general paucity of research to date.

The main focus of this chapter was in analysing pond hydrogeomorphology in two regions (MAL and VB) to try to understand how the hydrology of the pond in relation to its setting determined the nature of cladoceran communities. The two regions are acknowledged to be different, primarily in terms of their visual attributes: the mobile sand dune landscape of MAL against the more compact wide flatter depressions of the landscape at VB. Both pond districts can be classified as MTPs. However, the MAL ponds also resemble and behave similarly to humid dune slacks. Indeed these ponds may actually sit somewhere between these two pond types (Carla Pinto-Cruz *per. obs.*). The vegetation assemblages, size and hydrological regime of the ponds found in both the MAL dune system and VB have led to their classification as MTPs (Pinto-Cruz, 2010; LIFE Charcos, 2013, 2015).

5.4.2 Effect of sediment on hydrological variability

Sediment composition of the pond bed and below impacts the behaviour of water within the pond, particularly in terms of controlling water retention and permeability (Alves, 1998), as finer sediment has an increased ability to retain large quantities of water, as well as holding the majority of the nutrients and OM. In the MAL ponds, low values of both OM and CC could imply subsurface drainage, whereas more elevated values in the VB ponds, suggest more productivity in terms of OM and CC. Therefore, even with the same level of subsurface drainage, the base levels differ between regions. Salvador *et al.* (2017) tried to understand how water was moving within the pond systems of SW Portugal and found a north-south contrast in groundwater chemistry, suggesting different pond water/groundwater interactions at a regional scale, however the core reasons for this remained unexplained by Salvador study. This present work also shows that differences in CC and OM between regions do exist, but the causes for this remain difficult to assess.

Data from the top 40 cm of the sediment profile of the ponds revealed a similar structure in terms of sediment texture and granulometry between regions. An earlier study performed on the top 20 cm of some MAL ponds revealed that the sediment was characterised by a lower water retention capacity and low acidity (Alves, 1998). These results are also confirmed in the present study where coarser sand grains dominate, with no significant difference between MAL and VB regions. The pond L212 however, showed itself to be an outlier, with more fine sediment (mud and silt) and less sand than the other MAL sites. Ponds L603, L604 and L614 in VB are also comprised notably of sand. Machado *et al.* (1999) points out the ponds' characteristics where large branchiops occur in the SW Portugal, and defines most pond substrates in the VB region to comprise both sand and clay, apart from L614, which is sandy. The variability in substrate sedimentary characteristics between sites is greater than between regions. Alves (1998) showed fine and medium size sand grains with values ranging from 50-90% sand on the top sediment layer of MAL ponds. Mal2 showed a lower proportion (50%) of coarse and a higher proportion of fine sand (44%) compared to sites sampled by Alves (1998) study. Alves (1998) also noted that finer grain sizes increased towards the northwest side of the dune system and therefore values for Mal2 were different from the other MAL sites.

Water level loggers showed different timings of flooding and drying between the ponds, demonstrating water-holding differences between them, with direct influence of rainfall and temperature. The relationship between hydrology and geomorphology across temporal and spatial scales is pertinent to improve conservation and management practices (Sidle and Onda, 2004). In particular, the relationship of surface and groundwater in different geomorphic settings has only started to emerge in the past decade (ibid). Possible reasons that could explain the strong response of sudden drop in water levels in the summer, when temperatures increase, as opposed to a more slow response from other ponds, could be related to the proximity of some ponds to planted forests for forestry production (eucalyptus and pine trees). This occurs around all three ponds in VB, but not so much in MAL. One other explanation might relate to the density of vegetation within the pond itself, as ponds L220 and L617 have scarcely any bare sediment, with a lot of vegetation colonising the pond bed area, hence having higher influence on the usage of water for transpiration. This is also different between summer (drying) and winter (flooding) as the water table is kept at lower levels and either the lack of viable vegetation to exist or the depth at which the water is in relation to plants roots, makes it less visible to impact temperature rises and water level drops in the winter times at both regions. It seems likely that those ponds whose levels drop faster than others may not have any other source of water beyond the local water table (ponds L220 and L617), whereas ponds that tail off more slowly are likely to have some sort of spring fed water source or alternatively a sediment texture/composition that holds water in the pond basin (Mal1, Mal2, L603 and L614). This suggests that, even within the same region, hydrogeomorphological variability exists between individual ponds. According to the data analysed in this study it still remains difficult to explain how water is moving and how does it differ from pond to pond even within the same region.

The exact processes that explain these differential hydrological dynamics remain to be fully understood, mainly because the results do not follow an expected difference in underlying sediments between both regions. Pinto-Cruz *et al.* (2009) showed the influence of clay in determining the distribution of plant communities according to different temporary ponds of the SW coast of Portugal, but results of the present work do not show a clear indicator for this type of region or ponds differentiation. This work analysed sediment layers that are in direct contact with vegetation roots, but how the water moves in and out of the ponds remains poorly understood. According to Grillas (2004) geomorphology is the second most important factor that determines the distribution of temporary ponds, just after the climatic influences, as it determines the shape of the terrain in which water retention is able to occur to form a pond. Hence the inter - relation of geomorphology and hydrology in MTP needs more investigation to understand water movement in and out of the pond and also laterally on the local groundwater level in relation to sediment and bed rock, as the hydrology is inevitably influencing species dynamics within the pond ecosystem.

5.4.3 Hydroperiod as a driver of environmental variability

Significant differences between the regions for environmental variables were evident for winter, coincident with maximum flooding, as opposed to only oxygen levels in summer samples, when ponds are heading towards the desiccation phase. The small number of sites in the summer sample period may also influence the lack of significance. When flooding is at maximum and water levels are at their highest, dilution promotes decreases in nutrient concentration; when water levels start to drop, the process is reversed and nutrient concentrations increase (Florencio *et al.*, 2014). The range of pH and conductivity values has been found to be similar to other studies of the MAL ponds (e.g. Pinilla 2014; Tornero *et al.* 2016). High oxygen levels, pH, low conductivity and reduced nutrient content characterised the VB ponds and these tend to be more oligotrophic, whereas the MAL ponds had the appearance of slightly dystrophic habitats, with lower pH and brown acidic water. However this classification is also based on high nutrient values (Wetzel, 2001) and MAL ponds revealed low phosphorus and nitrogen content – hence not dystrophic. Even though grazing at both regions exist by both domestic cattle and wild boar, there seems to be more impact seen on the MAL ponds, in terms of pond bed trampling. This is verified because MAL ponds are located close together, whereas VB ponds are scattered along a wider landscape. In VB only ponds L603 and L608 had evidence of cattle whereas the other ponds were more visited by wild boar. 23,2% of the 237 ponds along the Portuguese SW coastal plateau are regularly grazed by livestock (Beja and Alcazar, 2003). MAL ponds may differ in terms of their water acidity, higher conductivity and lower oxygen because of the effects of grazing, particularly with the increased concentration of nutrients, when water levels in the pond are low.

Diversity for cladocerans showed significant correlation with hydrology in the MAL region. The fact that hydroperiod in the MAL ponds in 2017 was much lower in comparison with the same time in the previous year may be an explanation for the different diversity values seen. In the MAL region, during the first half of the year in 2017, species diversity for Mal1 and Mal2 remained very low in comparison with the same time interval in 2016. As a consequence, pond Mal2 was only able to hold water for few occasions in the 2016/2017 hydrological year when cladoceran abundance was very high. It may be so that with the decrease in the volume of water, the water column contains more density of cladoceran as species tend to aggregate where water still exists, hence a possible explanation for the high abundance (number of species per Litre) found.

Although there was no significant difference between regions in terms of the different flooding days before sampling for each pond, this variable together with pH, was important in explained the ordination patterns for the sites (Figure 5.19). This perhaps suggests that hydrological differentiation is not so markedly evident between regions, but more related to intrinsic specificities of the sites.

The largely weak contribution of the selected variables as possible explanatories of regional differentiation amongst Mediterranean temporary ponds in the SW Portuguese coast is likely indicating the apparent randomness in this habitat. What seems to happen is that ponds here act not so much as a collective species pool, but more like islands that individually contain their own hydrogeomorphological characteristics. Still, sediment composition and water chemistry were the best predictors of regional species composition and diversity patterns. Water chemistry revealed that in MAL, there were higher values linked to increased nutrients (higher conductivity and lower pH), likely related to the extensive grazing that is practised in the area, and these factors lead to higher cladoceran abundance, composed of large sized cosmopolitan species. VB ponds on the other hand, reflected a more pristine nature (higher pH and oxygen levels), with higher cladoceran richness, represented by more medium to smaller sized species and increased hydroperiod.

5.4.4 Local and regional differentiation of species biodiversity

The surveyed ponds in the present work reveal a rich and diverse community of cladocerans, comparable to studies elsewhere in MTPs. Cumulative results over the 2 year samplings showed a total of 23 cladoceran species in both regions, with 19 species found in VB and 12 found in MAL over the 2016 (February and May) samplings alone. The only comparative record for Portuguese quantitative data was during a fortnighlty sampling study performed over two years (Caramujo and Boavida, 2010), and this revealed the presence of 10 cladoceran species in the MAL region. Plants in the studied ponds also revealed a diversified community with a cumulative number of 47 identified to species level, 38 species from MAL and 40 from VB. This number is however lower than others recorded from MTP’s in SW Portugal, where large scale studies have revealed over 120 species in just one type of temporary pond (Pinto-Cruz *et al.*, 2009). Still, the present values have enabled the characterisation and representativeness of plant species in the studied ponds to be established and their relationship to hydrological factors and seasonal variability of cladocerans.

Comparison of the pond regions revealed distinct assembladges of both cladoceran and vegetation communities. Cladocera showed variability related to abundance according to specific water chemistry variables, such as chlorophyll *a*, pH, oxygen, conductivity and nitrogen, but also to water depth and hydrology. Vegetation showed direct associations with water chemistry including alkalinity, pH, oxygen and conductivity. Regional differentiation was marked by lower pH and less flooding days before sampling, which led to higher cladoceran abundance in the MAL ponds, and higher cladoceran diversity and richness in the VB ponds that had a higher number of days with water before each sampling, hence increased hydroperiod. Crustacean richness was found to increase with shorter hydroperiods in Sahuquillo & Miracle (2013) study. Although the MAL region had a shorter hydroperiod, this study showed an increase in cladoceran abundance for MAL sites when water levels were low. The shortening of water availability in MAL could have reduced the scope of species that were able to exist in that time, due to hatch time limitations. But for those species that were able to get into the water column reproductive rates could have been increased, thus allowing to attain higher densities, in less water volume. A study by Sahuquillo & Miracle (2010) showed that in a drier year, rarer crustaceans were not found, but the number of smaller short-lived species (rotifers) increased. This is likely to be the case in MAL ponds.

5.4.5 Biological uniqueness in MTP

Cladoceran community cluster division using TWINSPAN revealed distinct communities in both regions, with preference for more specialised and medium to smaller sized species in the VB ponds, as opposed to more ubiquitous and medium to larger sized species assemblages in the MAL ponds. Some species in the VB ponds have been recorded for the first time in the SW study region. Included in these are three Iberian relics with a very limited distribution that are rarely reported in the literature. *Karualona iberica* is a species of Gondwanan origin, reported in Europe only from Portugal and Spain, but having a distribution that extends to north Africa and southeast Asia (Błędzki and Rybak, 2016). *Leydigia iberica* is a species that up until very recently had only been found in one location in Seville, southern Spain, in a temporary clay, turbid pond with limited vegetation (Alonso, 1991; Błędzki and Rybak, 2016). *Daphnia hispanica* is an Iberian endemic with a confirmed presence in MAL ponds from the Caramujo & Boavida (2010) study. All these endemic species were found only in VB ponds, with no records over this study in the MAL region. MTP are in fact an important habitat and refuge for rare species (Machado *et al.*, 1999; Sahuquillo and Miracle, 2010) as it is also confirmed for cladoceran in southwest Portugal. Large branchiopod crustaceans have been found before in some ponds of VB region, with special importance given to the rare species *Chirocephalus diaphanus*, *Cyzicus grubei* and *Triops cancriformis mauritanicus* (Machado et al. 1999). In addition, the occurrence of endemic species in VB ponds (*Leydigia iberica*, *Karualona iberica* and *Daphnia hispanica*) highlights the importance of the ecological conditions in these systems.

The main vegetation communities were separated according to region and to sampling season – winter annuals and summer perennials. In the high water level phase (winter), more aquatic species were expected to be present. Instead, a mixture of aquatic and wet tolerant species represented the vegetation communities in these ponds. For instance, *C. longus*, *B. maritimus* and *A. nodiflorum* are indicators of winter MAL group 1. Here, combinations of fully aquatic (*A. nodiflorum*) with wetland (*C. longus*) species seem to occur. Similarly, *J. heterophyllus* (aquatic) and *P. paspalodes* (wetland) indicate group 7, a group with VB sites sampled in the summer. Although, the species found in the ponds in both regions are commonly found in other MTPs, these ponds reveal a range of conditions that enable the existence at the same time and in the same season, of different vegetation physiological characteristics. Díaz-Paniagua *et al.* (2010) found a more consistent and distinct seasonal differentiation between vegetation in Doñana National Park temporary ponds. Although not accounted for in this study, both the VB and MAL regions contain sites with variable sizes and areas and this may explain the inconsistency and mixture of aquatic and semi-aquatic vegetation species found in both regions, as it is the case for other taxon variability at MAL (Tornero *et al.*, 2016). Another possible reason for the lack of plants' seasonal differentiation could be the shallow nature of these ponds that provide the necessary habitat for more wet tolerant species to co-exist with fully aquatic species, independent of season. In addition to this, the survey approach in this study did not differentiate between margin and central pond samples, but combined all species that had their roots in water, which may in itself have led to the present results. In an agricultural landscape of Norway, Edvardsen & Økland (2006) found that pond area and margin were the best predictors of terrestrial and semi-terrestrial vegetation and that this heterogeneity increased the variability and frequency of less common species.

Some of the more emblematic species present in this study include: *Littorella uniflora*, an abundant species in northern Europe but rare in Portugal (Flora-On, 2018); *Isoetes setaceum*, indicator of MTP ponds; *Juncus heterophyllus*, classified as near-threatened by IUCN red list (de Bélair and Grillas, 2010), due to its short limited range (distributed only in south-western Europe and northwest Africa) and scattered population; *Illecebrum verticilatum*, common in central Europe but rare in the south and Mediterranean areas (Castroviejo, 2012). In addition to this, *Hyacinthoides vicentina* subs *transtagana* is a Portuguese endemic (Castroviejo, 2012; Flora-On, 2018), also found in the VB region (already mentioned previously as not being aquatic, but thriving in the vicinity of water). This is a species with a very limited distribution (SW Portugal), and its presence in the area is an indication of the uniqueness of the region in terms of the wider faunal and floral diversity. Hydrological seasonality and hydroperiod length of the studied sites is an essential component to the maintenance of high biodiversity value of these ponds, seen both locally and regionally, which sustain a high level of specialised and unique species of microcrustaceans and plants with global conservation importance.

In response to the research questions set in the beginning of this chapter, the answers are:

- There is a distinction between regions as well as seasons, with VB ponds revealing the presence of more rare endemics, seen in higher diversity and richness, as opposed to high abundance in MAL ponds.
- Hydrology impacts and shapes cladoceran diversity with increased hydroperiod, but geomorphological influence is complex and not directly determining species diversity by the elements analysed in this study, as it does not show regional differences in sedimentology.
- Results show that hydroperiod influences both cladoceran and plant communities in terms of local organisation and that cladoceran and plants follow similar richness and regional patterns.

5.5 Conclusions

The hypothesis set to be tested in this chapter (H:' species diversity significantly differs in ponds with distinctive hydrogeomorphologies') fails as regional differentiation was not found in terms of hydrogeomorphological characteristics. However, cladoceran communities did show a regional distinction between the ponds, connected to water chemistry, sediment composition and hydrology. The variability of the hydroregime and sediment structure is most relevant at the local, rather than the regional scale, but more research is needed to understand the role of local and regional differences in shaping cladoceran and plant assemblages in southwest Portugal MTPs. The present study makes a significant contribution to biodiversity knowledge for MAL and VB region, adding a substantial cladoceran species list that further reinforces the highl and unique biological value of SW Portuguese MTPs and in turn the need for long-term monitoring and conservation of these ponds.

Chapter 6 - Discussion

This thesis examines the ecology and habitat preferences of Cladocera in coastal temporary ponds in Western Europe: at sites from the northwest (NW Ireland) to the southwest (SW Portugal). The study described and unravelled ecohydrological processes influencing coastal ponds with varying water level regimes at a range of temporal and spatial scales. It explored local (C4 and Ml2), spatial (all ponds at Sheskinmore, Ireland) and regional (Malhão and Vila do Bispo, Portugal) arrangements of sites that varied in terms of inherent physical and climatic characteristics with a common goal, namely understanding the seasonal hydrological balance of input, permanence and output of water within the waterbodies' catchment and the influence of this dynamic on aquatic ecology.

Results have shown the inter-connection between climate, hydrology and consequent biological assemblages, with the overall ecosystem responding to a complex combination of the factors analysed. The variability in climate, specifically in the year 2016-2017, with lower precipitation and higher temperatures in Ireland and Portugal added an unanticipated twist on 'common' seasonal hydrological events, with interesting consequences for species assemblages. In addition to this, the individual characteristics of the ponds and heterogeneity of the sites within the landscape adds to the complexity of the coastal pond environment.

This work shows that the conservation of temporary coastal systems needs to take into account hydrological, environmental and geomorphological factors, and biological surveys need to be expanded to more taxonomic groups, including other trophic levels, to better comprehend the controls on ecosystem complexity and biodiversity. Cladocerans were the main biological groups studied to take into account external climatic forces, intrinsic pond geomorphological variability and pond morphology as well as landscape dynamics, providing a suite of scientific evidences on the present status and future threats to the ecohydrological balance of temporary pond systems.

6.1 Cladocera in temporary ponds – an overview

The climate of northwest Ireland and southwest Portugal has some important contrasts, most notably in terms of precipitation and temperature. Ireland is generally wetter and colder than Portugal, but both coasts experience strong westerly winds (sometimes gale force). Importantly, at both locations, weather conditions throughout the monitoring period (summer 2015 to winter 2017) incorporated a significant dry phase where rainfall events were less frequent in the second sampling year (2016-2017), creating an extended drier period that lasted until the following year.

The c4 and ml2 slacks (Chapter 3) comprise the two largest waterbodies within the Sheskinmore (NW Ireland) dune slack system but nevertheless are representative of the Humid Dune Slack (HDS) ecology, with fluctuating water regimes that are dependant on groundwater as their water source (Grootjans *et al.*, 1998). Their dependence on rainfall, that directly fills the slacks with water and indirectly via rising of the groundwater levels or feeding from adjacent dunes, highlights a vulnerability in terms of their capacity to retain water and maintain biodiversity when rainfall is low. This issue was evidenced by increased inter-annual variability of cladocerans as opposed to intra-annual seasonal variation. Summer 2016 was characterised by higher diversity for C4 and winter 2017 for a drop in biodiversity at both slacks. Cladocera and plant species followed the variation subtleties of the water regime within the slacks, but plants also revealed a complex relationship with the different topographic patches of water permanence across the slacks' surface.

Chapter 4 focusing on the Sheskinmore system evaluated local small-scale environmental variability and spatial processes as drivers of cladoceran assemblages. Results show that, within a rather small area, where ponds are separated by a few meters and up to 2km apart (the longest distance between 2 ponds), cladoceran composition and physical variability differs, mainly according to hydrological categories, which are then linked to the proximity of each site to the water table. Geographically, the chapter indicates a separation of ponds between those located closer to the Tramore strand, and those more distant from this part of the dune system. Surprisingly though, water chemistry was not so relevant to species composition variation in space. The survey at the Sheskinmore dune ponds provides the first ever record of the spatial arrangement of the ponds, cladocerans and plants, and recognition of the variables that are influencing their distribution.

When looking at the ecohydrology of temporary ponds at a regional scale (Chapter 5), sediment composition and water chemistry were better predictors of the regional differentiation of cladoceran composition, rather than geomorphological differentiation between regions. The main differentiations relied on the fact that the dune ponds at Malhão (MAL) were characterised by shorter hydroperiod, lower %OM and %CC, increased conductivity and lower pH, leading to higher cladoceran abundance. By contrast, the Vila do Bispo plateau (VB) ponds had a longer hydroperiod, higher %OM and %CC, reflecting higher pH and oxygen levels and associated increased cladoceram species richness. Results show that, even though these ponds are classified as the same natural habitat – MTP – when comparing different regions, cladoceran communities are shaped by a combination of factors and explanations for this vary according to individual pond characteristics and collective differences between the MAL/VB groups of ponds. Thus, distinctive regional characteristics promote different habitats, in the same way as the large c4 and ml2 slacks do individually, and the Sheskinmore pondscape does collectively.

The research presented here improves understanding of the subtleties and sensitivity of the ecology of temporary ponds to factors like hydrological dynamics and climatic shifts and the way these influences operate on ponds' inner ecological organisation. Furthermore, this study shows that some of the unique species recorded require particular attention in terms of local land management and conservation strategies if they are to be preserved, independent of scale. Studies encompassing larger scale monitoring demonstrate the improvement of regional biodiversity (Beja and Alcazar, 2003; Barrett-Mold and Burningham, 2010; Sayer *et al.*, 2012; Hill *et al.*, 2017) that a diversified pondscape can provide and therefore, it is important to note that biodiversity is enhanced when the hydrological variability and heterogeneity within an individual pond and at a landscape scale is maintained and considered in management plans.

6.2 Temperate and Mediterranean temporary ponds: similarities and contrasts

The anthropogenic pressures and climatic threats that seasonal freshwater ponds face are transversal to latitudes. The sites investigated here are within protected areas for conservation and nature reserves – Sheskimore Nature Reserve, Ireland and Parque Natural do Sudoeste Alentejano e da Costa Vicentina, Portugal - and site-specific conservation measures are not sufficient to stop and eliminate the impacts caused to the ecosystem by these threats. Climate change is also a major cause for concern, where variability on the annual rainfall amount has proved to affect these sites profoundly, limiting water availability and therefore diminishing species abundance and diversity values drastically.

The research focused on two EU Habitat Directive classified site types, namely Humid Dune Slacks and Mediterranean Temporary Ponds; a classification based on vegetation composition. However, it is important to acknowledge the similarities between the Irish and Portuguese sites despite very different physical and climatic settings. Indeed, a myriad of cladoceran species were living and using these seasonal waterbodies in the same way, independent of their bio geographical adaptations and, 11 out of the 33 cladoceran species identified in this work were found in both countries (Table 6.1), equivalent to 33% of the total species recorded.

Table 6.1 Inventory of the cladoceran species sampled in this study

	Ireland	Portugal	Ire + Por
N=33	N=9	N=12	N=11
Acroperus harpae	🟩		
Alona affinis			🟦
Alona costata	🟩		
Alona rustica			🟦
Alonella excisa		🟩	
Alonella exigua	🟩		
Alonella nana	🟩		
Bosmina longirostris			🟦
Ceriodaphnia laticaudata			🟦
Ceriodaphnia quadrangula			🟦
Ceriodaphnia reticulata			🟦
Ceriodaphnia setosa			🟦
Chydorus sphaericus			🟦
Coronatella rectangula			🟦
Daphnia curvirostris	⬛		
Daphnia hispanica		🟩	
Diaphanosoma brachiura		🟩	
Dunhevedia crassa		🟩	
Ephemeroporus epiaphantoii		🟩	
Ephemeroporus margalefi		🟩	
Eurycercus lamellatus	🟩		
Graptolebris testudinaria	🟩		
Karualona iberica		🟩	
Leydigia iberica		🟩	
Macrothricidae sp	🟩		
Macrothrix hirsuticornis		🟩	
Megafenestra aurita		🟩	
Moina micrura		🟩	
Pleuroxus trigonellus	🟩		
Rhynchotalona falcata	🟩		
Scapholeberis rammneri		🟩	
Simocephalus exspinosos			🟦
Simocephalus vetulus			🟦

Acropercus harpae for instance is a species that tolerates cold, with preference for sandy bottoms and submerged vegetation (e.g. *Chara* spp and *Potamogeton* spp), but does not tolerate hydrological changes and eutrophication (López-Blanco *et al.* 2011; Błędzki and Rybak, 2016), hence the appearance in the Irish sites and not in the Portuguese ones. *Alonella exigua*, *A. excisa* and *A. nana* are very similar in terms of physiognomic features and yet, *A. exigua* and *A. nana* were only recorded in the Irish sites, whereas *A. excisa* was found in the Portuguese sites. A summary of the main distinctive features of these three species is summarised in Table 6.2. A. *excisa* is more adapted to both temporary and permanent waterbodies, living in the bottom and able to tolerate slightly eutrophic conditions, whereas *A. exigua* and *A. nana* are more adapted to permanent water conditions, and exist in waters with slightly higher calcium content. The trophic tolerance and ability to sustain hydroperiod variations may be some of the reasons why these species were unique to either of the countries (more seasonal ponds in Portugal, as opposed to more permanent waterbodies found in Ireland), but also the specific conditions of the sites determine the abiotic conditions too, given that in Błędzki and Rybak (2016) these three species are defined as widely distributed in Europe.

Table 6.2 Summary of main features reviewed from the literature for the *Alonella* species. Source: Lynch, 1980; Dumont, 1994; López-Blanco *et al.*, 2011; Błędzki and Rybak, 2016 and Rizo *et al.*, 2017

	habitat	**position in the water**	**plant preference**	**hydroperiod**	**feeding**
A. excisa	littoral, between plants	bottom	*Chara* spp., *Sphagnum* spp.	temporary and perman	scraping algae and detritous
A. exigua	between plants	nympheids	submerged floating	permanent	scraping bacteria and detritous
A. nana	between plants	sandy littoral	*Sphagnum* spp.	permanent	very fine bacteria or detritous

	body size (mm)	**swim (body length/sec)**	**trophic preference**	**pH**	**Ca2+ (mg/dm)**
A. excisa	0.29-0.43	18.9	slightly eutrophic	>3.8	<1.4
A. exigua	0.30-0.40	47		3.8-9	higher
A. nana	0.20-0.28	7.6	oligo-mesotrophic	>3.2	<2.2

Bosmina longirostris, *Chydorus sphaericus* and *Coronatella rectangula* are species that are tolerant to high trophic conditions, with greater plasticity and resilience (López-Blanco *et al.*, 2011), and for these reasons are more likely to exist at both regions. *Diapanasoma brachiura* and *Moina micrura* for example, were only sampled from the Portuguese sites and possible reasons for this rely on the fact that these species are more adapted to meso-eutrophic conditions, shallow and turbid and oligohaline (low salinity) conditions. However, *D. brachiura* has been reported from both countries, *M. micrura* has not been recorded for Ireland, as it is more typically found in shallow lake habitats (Błędzki and Rybak, 2016), hence another reason for appearing more on the Portuguese sites.

6.3 Defining temporary ponds

6.3.1 The definition challenge

Humid dune slacks are defined as "(…) damp or wet hollows left between dunes where the groundwater reaches or approaches the surface of the sand. Their most distinguishing feature is a seasonally fluctuating water table which usually reaches a maximum in winter and spring and drops in summer" (Houston, 2008). NPWS (2013) also defines HDS as being "(…) characterised by the occurrence of a water table (…). In winter, where there is relatively high rainfall and low evaporation, the water table normally rises above the soil surface and inundation occurs. In spring and during the major part of the summer, the water level drops, but the top layer of the soil remains damp". Ponds on the other hand, as mentioned before, have many different definitions based on their size, depth, water supply, use, location, geomorphological characteristics, etc. European definitions for a pond vary from Biggs *et al.* (2005) who limit the pond to a size range (>1m^2 and <2ha.), independent of its origin, water permanence or depth; Oertli *et al.*, (2000) who add in a maximum depth of 8 m, in which plants are still able to occur; and EPCN (2008), that broadly defines a pond as "a temporary or permanent standing waterbody between 1m^2 and 5 hectares in surface area".

Based on these definitions, and given that some of the ponds occurring at Sheskinmore are permanently wet, and therefore do not follow the seasonal filling and drying out fluctuations in the water level (apart from c4 and ml2, which clearly fall into the category of slacks), the more generic term 'pond' was therefore used in the thesis. In SW Portugal, ponds have been previously assessed as MTP's, but the dune ponds at MAL do have similar functioning and structure to dune slacks in terms of the water table dependence, where pond geomorphological structure and formation is apparently similar. The MTP habitat classification relies not only on the inundation zone, but also on the spatial vegetation gradient from the centre (wet) to the periphery (dry). A dune slack is associated with coastal dune depressions where the water table is near the surface (Semeniuk and Semeniuk, 2011) and this is the case at both MAL and ponds studied in Ireland. In Portugal, Humid Dune slack vegetation communities are associated with two habitat types (3130 - oligotrophic to mesotrophic standing waters with a vegetation of *Littorelletae uniflorae* and/or *Isoeto-Nanojuncetae*; and 3170* MTP) (Houston, 2008), so the MAL dune system may in fact contain sites within both classifications in terms of wetland vegetation. However, this work has focused only on the inundated zones of the ponds and therefore similarities between MAL ponds and dune slacks at Sheskinmore might have been enhanced.

Throughout the work, the lines that defined and classified ponds and slacks' systems as separate were not easy to see and often similarities were more common than disparities. When referring to c4 and ml2 in this thesis they were mentioned as slacks, however, in the entire Sheskinmore dune, waterbodies were referred to as ponds. Even here, when relating to an individual water body, it is easier to put it in context of the wider nomenclature and ecological classification – such as slack or pond – based on its vegetation assemblage. But on the wider landscape, the hydrological component defines all these systems (slacks and ponds) as temporary/seasonal waterbodies, so the classical pond versus slack definition gets difficult to determine when referring to the main principles of filling up and drying out of dune freshwater bodies. A more generalised way of looking at it is by considering the definition of wetland. Even so, the precise definition is not easily agreed given the international range of different nomenclatures as well as types of wetlands. In general terms, a wetland encompasses both land and water environments, as they can be seasonal (such as temporary ponds), but nevertheless, water is the primary factor controlling the environment and associated habitats (Silva et al., 2007). Although useful to determine scientific definitions for different habitats, finding a common ground for naming such a wide range of similar and yet inherently different temporary ponds is clearly associated with challenges.

6.3.2 Continuum of temporariness

Despite the geographical and climatic differences in this study, western European coastal areas provide a continuum of environments for temporary ponds. This is seen under a suite of hydrological variability mixed with geomorphological, climatic and coastal settings, which progressively shape the different environments in which species can exist. In essence, littoral environments contain in themselves a multitude of features that enable temporary freshwater systems to occur and contribute to the local, regional and coastal biodiversity. This study showed there is a continuum of water permanence within individual sites (seen on the different patches of water availability at C4 and Ml2) and also at the landscape scale (Sheskinmore dune system containing a variety of water permanence ponds) and lastly at the regional scale, with MAL and VB demonstrating, not only the adaptability of species that are able to co-exist in these systems according to different water dynamics, but also the implications that this continuum has for promoting the complexity, heterogeneity and diversity of these freshwater systems. What connects all of this is the fact that there is a variable water level regime, which in a sense cuts the relation to only temporary ponds and embraces different water level waterbodies, since some of them throughout this work have shifted their hydrological categorisation and were not so temporary or permanent as previously thought. Furthermore, all of the studied systems contain species able to cope with this variability and have built up resilience to inter-annual climatic unpredictability.

Defining hydrological categories across systems experiencing different water permanence is common practice, though the degree of temporariness to permanence, is something that is not replicated from place to place. The hydroperiod categories differ according to the region sampled, often linked to the relationship between climate and local geomorphology. Beja and Alcazar (2003), for instance, have defined hydrological categories according to drying scores for a SW Portuguese pond study. However, the most common hydrological classifications vary in regards to days/weeks/months of the flooding duration (Babbitt and Tanner, 2000; Díaz-Paniagua *et al.*, 2010), or categories/classes based on the hydroperiod length (Florencio *et al.*, 2013; María Sahuquillo and Miracle, 2013; Alfonso *et al.*, 2016), analogous to the one adopted in this study (see Chapter 4 -). Even after categorising the inundation period of temporary ponds, the inter-annual variability conditioned by rainfall timing and events induces variation within the same waterbody, where ponds can be classified as having longer hydroperiods in one year and shorter in the next. As such, a single classification of hydro categories of water permanence in temporary ponds becomes problematic (Díaz-Paniagua *et al.*, 2010). There is a need for more flexibility when determining an hydrological category, but is also necessary to consider the ecological meaningfulness of the category too, in terms of the species presence and tolerance.

Temporary ponds are faced with a continuum of hydrological regimes, primarily due to the lack of consistency of water input (precipitation, groundwater, other), which then influences the environmental variability within biotic and abiotic factors that ultimately determine species composition. Therefore an attempt to put ponds into categories, permanent – ephemeral and categories in between is a little simplistic and ultimately a classification for temporariness that also incorporates controls on the ecology of temporary ponds is needed. Ultimately, monitoring ponds through different hydrological years is needed to classify them properly. In addition, the balance of precipitation and evapotranspiration sometimes change the direction of the groundwater flow, dependant on sediment stratigraphy of the wetland-upland continuum (Sun *et al.*, 2006). The fact that winter 2017 was a dryer period has had a prolonged effect on the recovery of the groundwater levels up to its regular state at Sheskinmore, despite the plentiful rain in the following year (Figure 6.1). It seems likely that it will take some time for the water levels across the dunes to rise up enough to supply all the ponds and it demonstrates the role of the wider dunescape in supplying groundwater to the ponds in this area.

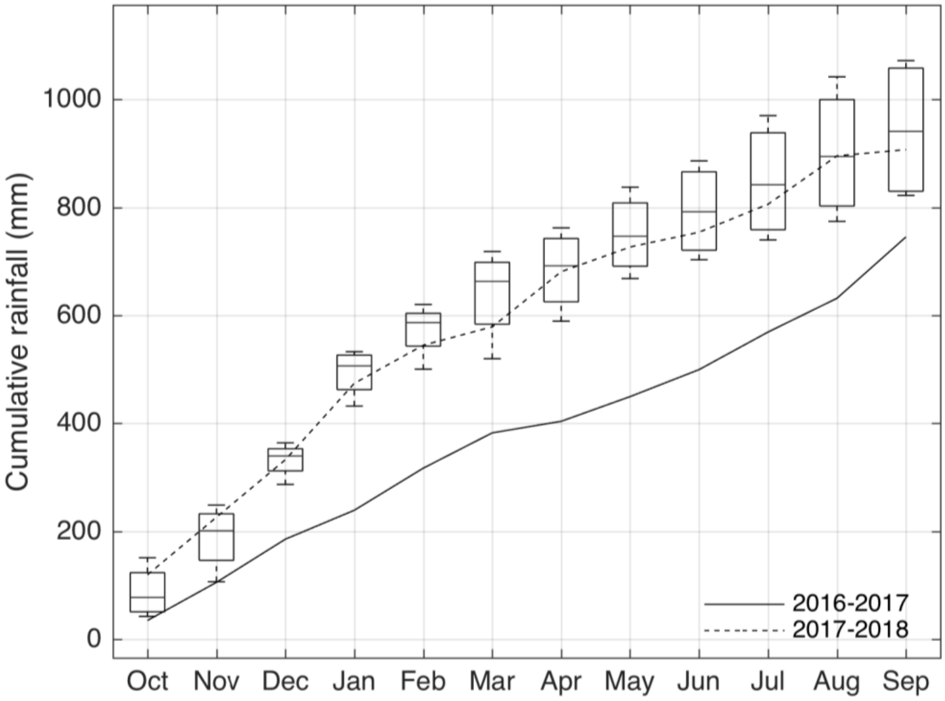


Figure 6.1 Cumulative rain boxplot taken from Davis weather station, Sheskinmore

In previous years, the amount of water that could be held in the sand of the dry dunes has proved to be enough to supply the ponds and slacks throughout the year. So, when the wider body of water gets depleted, as it occurred in 2016-2017, the ponds show signals of taking longer to respond to new rainfall as the dunes soak up a lot of water too. This is showing the influence of different climate changes and human interventions on the vulnerability and function of these ponds and that different pond/slack systems along the ‘continuum of temporariness’ will respond differently to these events in space and time.

6.4 Conservation of freshwater habitats in Europe

The preservation of biodiversity is a topic of great importance in the global ecological debate, given that it is greatly threatened by human pressure, due to either local interference (intensification of agriculture, touristic development, increased pollution, mentioning a few) or widely visible effects of climate change (temperature rise and modifications on the precipitation regime and overall water cycle). At a worldwide scale, the alarming evidence of the increased rates of degradation of temporary freshwater systems is widely recognised (Deil, 2005; Dudgeon, 2010). In Europe, the classification of different types of waterbodies by the Habitats Directive (Directive 92/43/EEC) has recognised the particular threats and needs for conservation within them, which has led to a shift in attention towards their protection (management and conservation) and for the designation of priority habitat status. Since this time, there have been just a few initiatives that have focused on ponds and temporary ponds. The European Pond Conservation Network (EPCN), which was formed in 2004 (Oertli *et al.*, 2009), has increased networking amongst researchers, promoting public awareness as well as the conservation of ponds and freshwater bodies, by bringing together the latest research developments through the organisation of conferences and workshops (EPCN, 2008). This organisation has also implemented the Important Areas for Ponds (IAP) concept which seeks to recognise and inform on ecologically important and unique national and international pond regions of freshwater biodiversity importance (Keeble *et al.*, 2009; Ewald *et al.*, 2010), to then assist with the development of strategies for pond monitoring, protection, management and conservation. Over 250 areas across Europe, Northern Africa and Middle East have been identified as IAP’s. In Portugal, Malhão was assessed as an IAP based on pond density (8 ponds per km^2). Vila do Bispo region contains 2 IAP’s, VB and Sagres (where some of the ponds studied in this work are also placed), based on the priority habitats surrounding the ponds. In the UK and Northern Ireland a number of designated sites have been classified (Keeble *et al.*, 2009; Keeble *et al.*, 2009a), though this classification has not yet been extended to the Republic of Ireland. Although not specifically for ponds, a review of habitats and species in Ireland (Lucey and Doris, 2001) has identified 400 Special Areas for Conservation (SAC’s), also known as NATURA 2000 sites, containing flora and fauna of European importance for conservation. Sheskinmore Nature Reserve is one of them, though the assessment report was labelled incomplete and insufficient in 2001.

Dune slack habitat assessment was reviewed in a report by NPWS (2013) for Ireland. Rather sadly, this review evaluated the overall conservation of Irish dune slacks as ‘inadequate’ with future prospects of decline. This was mainly due to the fact that dune systems in Ireland are often preferred sites for recreation, with development of golf courses, beach houses, caravan parks, agricultural intensification and sometimes sand quarrying (Lucey and Doris, 2001). Equally in Portugal, human pressure is also quite severe at the studied sites, with factors linked to recreation, intensive agriculture, habitat degradation and lack of awareness of these habitats prevailing (Beja and Alcazar, 2003; Farinha *et al.*, 2007; Presado *et al.*, 2010). So at present, the academic world, local environmental agencies and authorities, as well as Natural Park rangers are entities that need to be aware of the needs, sensitivity, threats and mechanisms around the optimal functioning of temporary ponds, within the context of HDS and MTP. The challenge though is transmitting this knowledge to the stakeholders and community.

The EU LIFE Charcos Project (LIFE12 NAT/PT/000997) is a clear success case in Portugal, which has enhanced the connection between people and ponds via educational activities and engagement with local authorities and landowners. The project has achieved in the restoration of degraded ponds, and by creating interpretation centres that helped to bring awareness of the impacts and needs of these systems to the wider community. Similarly in the UK, a number of activities have been developed and applied through the years involving thousands of volunteers with the purpose of protecting, researching and conserving species and ponds. The Freshwater Habitats Trust ‘People, Ponds and Water’ initiative has been aimed at monitoring, managing and protecting ponds in England and Wales (Ewald *et al.*, 2018). Also the ‘Norfolk Ponds Project’ (NPP), has been directed at reversing the decline in Norfolk (eastern England) farmland ponds, by restoring and managing neglected, threatened and overgrown ponds (available at http://www.norfolkfwag.co.uk/). Similar to People Ponds and Water the NPP has also sought to engage farmers, landowners and the public with ponds and their problems and ecological potential. People Ponds and Water and the NPP are two good examples of projects that have initiated pond-engaging activities with volunteers, stakeholders and landowners, to improve wildlife and conservation value. In the Republic of Ireland these sort of focused activities are not present, apart from the RSPB ‘Pond dipping’ activity in Northern Ireland (RSPB, 2018), with an educational perspective on the biological richness of life in ponds. Given the current declining status of dune slack habitats, as well as the variety of unique and rare temporary water systems in Ireland, like the turloughs (priority habitat in Annex I of the EU Habitats Directive (EEC, 1992)) (Skeffington *et al.*, 2006), the need to engage local public, authorities and government has never been so pertinent and more activities that involve and link academic research and science with the general public are highly encouraged.

6.4.1 Zooplankton (Cladocera) and water quality status

In Europe, alongside the Habitats Directive implementation, the Water Framework Directive (WFD) has also been in place since 2000, and European countries have since been developing monitoring programs, species inventories and bioassessment techniques in a wide range of areas. The WFD defines ecological status of aquatic systems based on their quality, structure and functioning, measured by a set of biological quality elements (BQE) (European Commission, 2000). However, while plants are included in the WFD it does not acknowledge zooplankton as an essential BQE, only taking into account phytoplankton, aquatic plants, macroinvertebrates and fish (WISE, 2009). The lack of fish in temporary ponds only emphasises the importance of including zooplankton in ecological assessments of water quality status. Nonetheless, this fact has not prevented the freshwater pond community from generating proposals for research, management and protocols that include zooplankton in pond conservation and appraisals and assessments (Boix *et al.*, 2012; Gilbert *et al.*, 2014; Van den Broeck *et al.*, 2015).

The absence of zooplankton in WFD assessments of ecological quality has received much criticism from the freshwater research community (Davidson *et al.*, 2011; Eggermont and Martens, 2011; Jeppesen *et al.*, 2011; García-Chicote *et al.*, 2018). Zooplankton are an integral component of food webs, as they represent a link between primary producers and predators, but in addition to their importance in contemporary ecological status assessment, they are excellent tracers of past environmental conditions, providing valuable information on the interactions between benthic and pelagic community changes through time as a response to stressors such as eutrophication (Davidson *et al.*, 2007, 2011; Jeppesen *et al.*, 2011). However, when considering hydrological shifts, cladoceran community changes have also proved to be powerful tools for assessing not only the past history of a waterbody (López-Blanco *et al.*, 2011), but also inter-annual variation on shorter contemporary timescales as shown in the present study.

6.4.2 Main challenges and future work recommendations

A waterbody is an ecosystem in itself, with a myriad of factors acting in the overall structure of its being. Therefore studies that are limited to a few variables (either biological, chemical or physical) will inevitably miss the complete picture. Plus constraints of sampling (in space and time) often limit the degree to which species are recorded, such that the whole biodiversity is rarely fully characterised.

This study focused on cladocerans due to their adaptation to the temporary habitat in terms of life-cycle abilities, but also because they are good indicators of the environmental conditions, responding rapidly to changes, as already mentioned. However, extending the scope of study to other communities, such as other zooplankton groups (copepods and rotifers), known to vary in environmental tolerances and dispersal characteristics could be very fruitful (Dallas and Drake, 2014). Phytoplankton and macroinvertebrates, as well as amphibians and reptiles would certainly expand knowledge regarding habitat dynamics, species and trophic interactions and top-down versus bottom-up relations and dispersal patterns. In addition, paired studies of plant and cladoceran interactions would provide insight into the way that cladoceran assemblages in these temporary ponds behave, affording a stronger link in relation to the inherent topographical patchiness of seasonally fed waterbodies. Thus the understanding of the inherent biological and ecological features of the studied ponds in Ireland and in Portugal is still in its early stages and more knowledge is required to fully comprehend the complexity of these systems.

Hydrological monitoring is not easily accessible and the extent to which it is possible to investigate the details of the hydrodynamics of ponds in the long-term is restricted by budgets and proportional to the size of the survey area. Some studies refer to the need for multiple hydrological monitoring points to correct the single-point measurements, to better represent the cross-section average, as well as better understand the movements between groundwater and local pond water flow (Wagner *et al.*, 2006), but given the nature of the study and its budget and time restriction, this was not possible here. In addition, there is also the risk of destruction when the loggers are in place, as was the case in similar studies (Dr. José Paulo Monteiro *pers. obs*), where an hydrological monitoring station at Vila do Bispo was vandalised by people, and also as happened in this study, where cattle at Sheskinmore accidently broke and destroyed one of the installed loggers. All these variables make this component difficult to study, especially when large and rather remote areas are concerned.

Another suggestion for further work is to look at deeper layers of sediment beneath the ponds, both in terms of investigating past history and environmental evolution of the sites in terms of the biological communities from the eggbank (Brendonck et al., 2017), but also to understand the sediment texture and geomorphology of the site in more detail. In this study, the sediment structure revealed its differences in attained depth and colour, showing that at the time of sampling, the length and amount of the sediment samples collected was proportional to the depth at which the water table was located. However, in adjacent ponds the water table was maintained at different depths. The geomorphology of the underlying pond sediment bed in both regions studied here presented different structures and sedimentological textures, and this variation is also evident, not only between regions (MAL and VB, Portugal), but also within each layer of the individual pond sites. A more systematic study of pond sediment stratigraphies would provide some basis to understanding the subsurface sediment layering role on lateral groundwater flow direction, with comprehension of groundwater and surface water interactions (Sun *et al.*, 2006).

Probably the biggest challenge of all facing temporary ponds is future climatic changes. With forecasts pointing out alterations in precipitation, whether it is decreased in Southern Europe or increased but seasonally altered in Northern Europe, it may be that the hydroperiod in already short-lived ponds, will decrease (in the south) and significantly alter seasonally (in the north), and a wide range of species from microcrustaceans, to plants, to amphibians, will suffer the consequences of this, via the inability to complete their life-cycle. These changes will likely give rise to increases in more opportunistic widespread species, namely those able to colonise new sites and live under different circumstances. Inevitably these species will outcompete specialised species (like the Iberian endemics found in the Portuguese ponds) and impoverish the diversity pool of these systems. To minimise the likely impacts of this scenario, a few suggestions are proposed here:

- Intensify the multidisciplinary study of temporary ponds within different biological groups as well as investigate the physical and morphological individualities of ponds in addition to the landscape in which they occur;
- Manage disturbed ponds and eventually create new ones where necessary, to favour the widespread distribution of species and maintain high biodiversity standards;
- Move species to new ponds where the impacts of climate change may be more severe and build an egg and seedbank storage to enable this reposition in the future;

- Identify relict individual sites (with presence of endemics and/or rare species across biological taxa) as well as enlarge the IAP areas to more locations and countries;
- Restrict access to some of the ponds or locations of groups of ponds so as to not alter their natural state;
- Improve networking and public engagement by raising awareness for policy makers, NGO's and landowners to ultimately create appropriate legislation.

6.5 Conclusion

This thesis represents the first attempt to characterise and compare coastal ponds in NW Ireland and SW Portugal in terms of their cladoceran assemblages and hydrological variability. The present work has improved current knowledge on the ecohydrology of coastal temporary waterbodies, which is limited in Portugal and non-existent in Ireland. This PhD addressed some of the gaps currently present in the temporary pond literature by providing the following knowledge:

1. Hydroperiod is a key factor regulating temporary pond function by having a major effect on biological communities, particularly amongst Cladocera and plants in NW Ireland and SW Portuguese ponds;
2. There is more inter annual (yearly) variation of biological communities in temporary ponds compared to intra-annual (between seasons);
3. Sediment properties and surrounding environment (geomorphology, topography and climate) are linked to hydrological regime;
4. Ecohydrological processes are an essential component for the conservation status and management of temporary ponds;
5. Interaction between weather variability, local hydroregime and topography of waterbodies, impacting species in space and time is complex.

Accurate management policies need widely known conservation practices. Moreover, local targeted needs within the specificities of each ecological framework, of which different temporary ponds exist, require to be taken in consideration as well. Currently, the uniqueness and biological richness of temporary ponds is well documented with a wide range of geographical and typological locations and habitats studied. Yet, the challenge remains in integrating a multidisciplinary ecological approach to the study of these systems, in particular improving understanding of biological communities, especially microcrustaceans, with support from hydrological, climatic and geomorphological integration and interpretation.

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Appendix

Appendix A Cladocera species and life forms' names and codes

Species	code	Species	code
Acroperus harpae	Ah	*Chydorus sphaericus*	Csp
Alona affinis	Aa	*Chydorus sphaericus* male	Csm
Alona costata	Ac	*Coronatella rectangula*	Crt
Alona rustica	Ar	*Eurycercus lamellatus*	El
Alonella exigua	Aeg	*Graptoleberis testudinaria*	Gt
Alonella exigua ephippia	Age	*Graptolebris testudinaria* ephipia	Gte
Alonella exigua male	Agm	*Macrothricidae*	Msp
Alonella nana	An	*Pleuroxus trigonellus*	Pt
Bosmina longirostris	Bl	*Rhynchotalona falcata*	Rf
Ceriodaphnia sp	Ce	*Simocephalus exspinosus*	Se
Ceriodaphnia setosa	Cs	*Simocephalus exspinosu*s ephippia	See
Ceriodaphnia laticaudata	Cl	*Simocephalus exspinosus* male	Sem
Ceriodaphnia quadrangula	Cq	*Simocephalus vetulus*	Sv
Ceriodaphnia reticulata ephipia	Cep	*Simocephalus vetulus* ephippia	Sve
Ceriodaphnia male	Cem	*Simocephalus vetulus* male	Svm

Appendix B Colour profile from pond sediment cores from MAL (left) and VB (right). Colour codes and names are given as standard munsell color chart units

MAL				VB							
Pond	**depth (cm)**	**code**	**colour**	**Pond**	**depth (cm)**	**code**	**colour**	**Pond**	**depth (cm)**	**code**	**colour**
MAL1	0-20	4/1 10YR	dark gray	L601	0-10	6/3 10YR	pale brown	L614	0-10	3/2 10YR	very dark greyish brown
	20-50	5/1 10YR	gray		10-30cm	6/3 10YR	pale brown		10-20cm	4/4 10YR	dark yellowish brown
	50-60	5/2 10YR	grayish brown		30-50	4/3 2.5Y	olive brown		20-30	4/4 10YR	dark yellowish brown
MAL2	0-15	3/1 10YR	very dark gray		50-60	6/3 2.5Y	light yellowish brown		30-40	5/6 10YR	yellowish brown
	15-25	5/2 2.5Y	grayish brown		60-69	6/3 2.5Y	light yellowish brown		40-50	5/6 10YR	yellowish brown
	25-35	4/2 2.5Y	dark greyish brown	L603	0-10	5/2 10YR	grayish brown		50-70	7/4 2.5Y	pale yellow
	35-50	4/1 10YR	dark gray		10-25cm	6/2 2.5Y	light brownish gray		70-80	7/4 2.5Y	pale yellow
L212	0-10	6/1 10YR	gray		25-30	6/2 2.5Y	light brownish gray		80-90	6/4 2.5Y	light yellowish brown
	10-20cm	6/1 10YR	gray		30-40	8/1 5Y	white		90-110	5/6 10YR	yellowish brown
	20-35	8/1 7.5YR	white		40-50	7/1 5Y	light gray		110-130	4/4 2.5YR	olive brown
	35-50	6/2 2.5Y	light brownish gray	L604	0-10	4/3 2.5Y	olive brown		130-150	5/4 2.5Y	light olive brown
	50-70	5/5 10YR	yellowish brown		10-20cm	5/2 2.5Y	grayish brown		150-160	5/5 10YR	yellowish brown
	70-85	7/1 10YR	light gray		20-30	4/4 10YR	dark yellowish brown		160-170	3/1 2.5Y	very dark gray
	85-110	6/3 10YR	pale brown		30-40	4/1 5Y	dark gray		170-180	4/2 2.5Y	dark greyish brown
	110-130	5/2 2.5Y	grayish brown		40-50	5/2 2.5Y	grayish brown		180-185	4/1 5Y	dark gray
	130-140	5/3 10YR	brown		50-60	6/2 2.5Y	light brownish gray	L617	0-10	4/2 10YR	dark greyish brown
	140-160	5/3 10YR	brown		60-70	6/3 5Y	pale olive		10-20cm	5/2 10YR	grayish brown
L217	0-20	5/2 2.5Y	grayish brown		70-85	6/4 5Y	pale olive		20-30	7/3 2.5Y	pale yellow
	20-30	5/3 2.5Y	light olive brown		85-100	8/3 5Y	pale yellow		30-50	8/2 2.5Y	pale yellow

(Appendix B cont)

MAL				VB							
Pond	**depth (cm)**	**code**	**color**	**Pond**	**depth (cm)**	**code**	**color**	**Pond**	**depth (cm)**	**code**	**color**
L217	30-40	5/2 10YR	grayish brown	L604	100-115	7/4 5Y	pale yellow	L617	50-60	7/4 10YR	very pale brown
L220	0-25	4/2 10YR	dark greyish brown		115-130	7/4 5Y	pale yellow		60-90	8/2 10YR	very pale brown
	25-40	3/1 5Y	very dark gray	L608	0-10	5/2 2.5Y	grayish brown		90-100	8/1 10YR	white
	40-60	4/1 5Y	dark gray		10-20cm	5/2 2.5Y	grayish brown		100-110	8/3 5YR	Pink
	60-100	4/2 2.5Y	dark greyish brown		20-50	6/2 2.5Y	light brownish gray		110-120	8/2 7.5YR	pinkish white
	110-117	3/1 5Y	very dark gray		50-70	4/2 10YR	dark greyish brown				
					70-90	4/2 10YR	dark greyish brown				
					90-110	5/1 5Y	gray				
					110-130	7/1 5Y	light gray				
					130-150	6/2 2.5Y	light brownish gray				
					150-180	8/3 5Y	pale yellow				