Effects of habitat management and restoration on freshwater ecosystem population dynamics.

The research presented in this thesis was carried out at the department of Environmental and Landscape Sciences (DISAT), Università di Milano-Bicocca, Milano, Italy.

Cover image: an adult of *Aeshna cyanea* that is drying the wings in the sun, after leaving its exuvia (Photo by Sergio Canobbio).

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Effects of habitat management and restoration on freshwater ecosystem population dynamics.

TUTOR: Dott.sa Valeria Mezzanotte
DOTTORANDO: Dott.sa Laura Sartori

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Chapter 1

General introduction

1.1 Wetlands and biodiversity

Freshwater ecosystems, and especially wetlands, are generally acknowledged to be habitats of high biodiversity and they have been valuable as sources, sinks and transformers of a multitude of chemical, biological and genetic materials (Mitsch and Gosselink 2007). Although wetlands have significant ecological functions and recognized social and economic uses, they are threatened by a number of anthropogenic pressure sources, the most important of which include increased nutrient loading, contamination, acid rain and invasion of exotic species (Brönmark and Hansson 2002). They are also under threat in many parts of the world due to land conversion to agricultural and residential uses (Daniels and Cumming 2008). Climate change may increase the impacts of these threats through reductions in rainfall and increased temperature, decreasing flow and altering timing and variability of flow regimes (Kingsford 2011).

While freshwater communities may be highly diverse and ⁄or heterogeneous under pristine conditions, homogenization can occur both within and among these communities due to human activities, including hydrological modification, chemical pollution, landscape fragmentation and exotic species introductions (Rahel 2002). Disturbance is regarded by many freshwater ecologists as playing a central role in determining the structure of communities (e.g., Resh et al. 1988, Lake 1990, Fisher and Grimm 1991, Poff 1992, Giller 1996).
There is a need to deepen the effects of environmental degradation and specific environmental stressors (including habitat modification) on wetland biodiversity, wetland processes and homogenization of a variety of taxonomic groups, in order to protect and restore valuable

wetland habitats. Macroinvertebrates are directly affected by the physical and chemical integrity of their surrounding environment, including water quality (EPA 2002), and respond to a complex of localised environmental factors in some sort of hierarchical arrangement (Winterbourn 1981). They are known to influence the rates of nutrient cycling and decomposition, have a central position in wetland food webs, and integrate environmental impacts and changes through time (Scatolini and Zedler 1996, Sharitz and Batzer 1999, Brady et al. 2002, Stanczak and Keiper 2004). Therefore, they are potentially extremely useful as indicators of restoration success (Campbell et al. 2002).
Some studies (e.g., Heino 2000, Sudduth and Meyer 2006, Walsh et al 2005 a,b) suggested that water body size, habitat heterogeneity and hydromorphology can positively affect biota in both impaired and restored freshwater environments. For these reasons the management of these peculiarities have become increasingly important in order to protect and increase wetlands biodiversity.

1.2 Artificial and constructed wetlands as restoration measure

Artificial ponds are valuable to society, since they are often created for purposes such as water supply, floodwater retention, recreation and education, or wildlife management and research (Oertli et al. 2005). They are also often the results of mitigative measures to compensate for habitat destruction and the subsequent loss of species (National Research Council 1992). For these reasons, the introduction of artificial wetland ecosystems can create new ecological resources, especially in territories where water is scarce. However, it can be useful to understand the ecological function of these habitats, especially at the invertebrate community level (e.g., Gee et al., 1997; Herrmann et al. 2000, Ruhí et al. 2009). Research on constructed wetlands has most frequently addressed their efficacy in pollutant removal and flood mitigation, with less attention paid to the functional aspects of the constructed wetlands ecosystem (Mitsch et al. 1998, Spieles et al. 2006).

In this sense, a deeper knowledge of the biodiversity hosted in these environments is needed to evaluate if newly created ponds are appropriate management tools for biological conservation (Ruhì et al. 2009). Although biodiversity analysis have often been based only on species richness, it is important to consider, among others, aspects concerning taxonomic relatedness (Warwick and Clarke 1995).
Local factors, such as fish stocking, pond use, egg banks, water regime, hydroperiod length or habitat heterogeneity, can relevantly influence the community structure (e.g., Schneider and Frost 1996, Della Bella et al. 2005, Gascòn et al. 2005). Even in highly interconnected ponds, local environmental constraints can be strong enough to prevail over regional homogenizing forces and structure local communities (Cottenie et al. 2003). For these reasons it is important to understand the effective way to manage and restore natural and artificial wetland ecosystems, in order to protect or even increase the local biodiversity.

1.3 Aims of the thesis

This project has evaluated the effectiveness of the interventions provided by Parco Pineta di Appiano Gentile e Tradate (a regional park in Lombardy, North-Western Italy) in freshwater ecosystem management (wetlands and streams restoration, introduction of constructed wetlands, biological communities management), considering their effect on population dynamics. It has been contextualized in a broader view concerning the study of the complex relationships between biodiversity, environmental variables of different aquatic ecosystems, and human presence (considered both as perturbing agent and restoration promoter).

Specifically, the study has included:

- General overview of taxonomic and functional biodiversity of aquatic and riparian ecosystems of Parco Pineta;
- Definition of the environmental characteristics of the ecosystems (with particular reference to water quality,

hydrology and morphology) and the alterations bearing down on them;
- Analysis of population dynamics (especially at macroinvertebrate community level) for conservation aims;
- Evaluation of the ecological status and effectiveness of restoration interventions;
- Determination of further possible actions for the restoration of compromises and/or disappearing ecosystems.

The general overview of taxonomic and functional biodiversity of aquatic and riparian ecosystems has been obtained by periodic sampling; the applied experimental design has also produced a faunal biodiversity checklist of aquatic ecosystems in the park. In particular, the study has focused on macroinvertebrate communities, carried out by qualitative periodic sampling in the different aquatic ecosystem microhabitats.
The definition of the environmental characteristics of aquatic ecosystems and alterations has occurred through the determination of water physico-chemical properties and the measurement of hydromorphological parameters. Finally, the project has evaluated biotic interactions between populations to check the overall metapopulation framework and the population dynamics. The results could be useful to plan future interventions for conservation management.

1.4 Outline of the thesis

In this work wetlands with different features and origin have been analysed from various points of view, trying to define which variables were more important in influencing the local and regional biodiversity hosted in these ecosystems.

In **chapter 2** a constructed wetland system has been considered focusing on its primary treatment aim. Its efficiency in pollutants and microbial removal was analyzed. The removal capacity of each treatment stage have been evaluated and compared, in particular considering the high efficiency in disinfection processes. Seasonal influence on treatment capacity was also evaluated.

In **chapter 3** constructed wetlands have been analysed as possible ecosystems and compared with some artificial pools and natural ponds spread within the Park territory, in order to evaluate if artificial interventions could be considered as restoration measure. The analysis has been based on the macroinvertebrate community present in the different category of ecosystems. The results showed in chapter 3, permit to considered all the considered wetlands as part of the local ecological network.

In **chapter 4** the hydrological characteristics, the morphology and the distinctive environmental features of each considered wetland have been described, in order to evaluate which were the most important variables that could influence the macroinvertebrate community assemblages and the biodiversity level. Area and habitat heterogeneity resulted to be the main characteristic that could really influence the community variability and then they have been considered through a single index, proposed to describe the wetland ecosystems.

A different perspective have been adopted in **chapter 5,** where the macroinvertebrate community have been considered as metapopulation. Dispersal mode and species traits were taken into account to define the connection between the single community of each wetland. Macroinvertebrate community were described as composed by four life-strategy groups, that showed different preferences between the various wetland category. The differences observed between artificial, natural and constructed wetlands resulted to be influenced also by season.

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Chapter 2

Evaluation of the performance of a constructed wetland system for the treatment of domestic wastewater in a natural protected area

Abstract

A constructed wetland (CW) system composed of a subsurface flow wetland (SSF), a surface flow wetland (SF) and a facultative pond was studied from July 2008 until May 2012. It was created to treat the domestic sewage produced by a hamlet of 150 inhabitants. Monthly physicochemical and microbiological analysis were carried out in order to evaluate the removal efficiency of each stage of the process and of the total treatment system. Pair-wise Student's *t*-tests showed that the mean removal of each considered parameter was significantly different (α=0.05) between the various treatment phases. Two-way ANOVA (analysis of variance) and Tukey's HSD (honestly significant difference) tests were used to find significant differences between wetland types and seasons in the removal efficiency of the considered water quality parameters. Significant differences in percent removal efficiency between the treatment phases were observed for total phosphorus (TP), total nitrogen (TN), ammonia nitrogen (NH_4^+-N) and organic load (expressed as COD, chemical oxygen demand). In general, the wastewater treatment was carried by the SSF phase mainly, both in summer and in winter. *Escherichia coli* (*E. coli*) removal ranged from 98% in winter up to >99% in summer, that corresponded approximately to 2-3 orders of magnitude. Disinfection was not influenced by the season, but only by the treatment phase. Probably filtration and adsorption to macrophyte roots and substrates were the main *E. coli* removal mechanisms.

Keywords: constructed wetland, wastewater treatment, microorganism removal, *Escherichia coli*.

Submitted manuscript.

2.1 Introduction

Constructed wetland (CW) systems have been widely used over the last 25 years as an alternative to conventional systems for the wastewater treatment of small communities due to their minimum power consumption and low maintenance costs (Mara et al. 1992, Brix, 1994, Vymazal 2002, Bécares 2006, Puigagut et al. 2007). CW sewage treatment systems, based on the natural wetland's distinctive features, have been developed for large-scale application in Europe and in the United States. These treatment techniques are described as environmental friendly and sustainable (Cooper 2010), being build with low investment, low cost, less energy-intensity and essential ecological functions in comparison to conventional sewage treatment systems (Sun et al. 2007, Chen et al. 2008). The interest towards CWs is also related to the ecological role they can play, providing habitat for different species. CWs show high efficiency in removing of nitrogen, phosphorus (e.g., IWA 2000, US EPA 2000, Donga et al. 2007, Hafner et al. 2006, Lin et al. 2008, Yang et al. 2008) and also provide suitable conditions for pathogen removal. Several studies have shown that CW systems allow a substantial improvement of microbiological quality of wastewater, similar to that obtained by conventional technologies (Green et al. 1997, Hill and Sobsey 2001).
Processes for bacterial removal in pond systems include physical and chemical factors such as solar irradiation (UV light) and temperature (Curtis and Mara 1992, Davies-Colley et al. 1999, Zdragas et al. 2002), filtration (Arias et al. 2003), adsorption and subsequent sedimentation (Davies and Bavor 2000) and biological factors such as predation and competition, attack by lytic bacteria and bacteriophages (viruses), natural death and decomposition. Even hydrophytes, that are an indispensable component in CWs, play an important role in antibacterial activities and, producing allelochemicals, affect the growth of other living beings, including bacteria and viruses (Zhang et al. 2009).
Wastewater disinfection has become increasingly important considering the necessity of reclamation for water-reuse, particularly

in continents and countries where water is a scarce resource (Asano 1998, Anderson et al. 2001, US EPA 2004). In order to carry out a sustainable resource management, wastewater should not be purified simply to be discharged directly into rivers and oceans, as the input of treated effluents often contributes to maintain environmental flows (Greenway 2005). CW systems can also be useful for water-reuse purposes, enhancing natural disinfection processes.
The aim of this study was to evaluate the efficiency of a combined CW (subsurface flow wetland, free surface flow wetland and facultative pond) in the removal of nutrients, COD and of the faecal indicator *E. coli.*

2.2 Materials and methods

2.2.1 Study site

The study was performed in a CW system placed in Venegono Inferiore, a small village in province of Varese (Lombardy, North-Western Italy). This CW has been created to treat the sewage produced by a hamlet of 150 inhabitants. It is a mixed system continuously fed with domestic raw wastewater that are treated after a pre-processing Imhoff tank at a flow rate of 37.5 m^3/day with an organic concentration of 186.7 g BOD_5/m^3 (measured at the oulet of pre-processing Imhoff tank, responsible for a 30% removal f the raw sewage BOD_5).
The whole system (Fig. 2.1) consists of a subsurface flow wetland (SSF), cropped with *Phragmites australis*, followed by a free surface wetland (SF) cropped with *Thypha latifolia* and by a final facultative oval-shaped pond (200 m^2, 0.5 m depth of water), the last being built with the main purpose of being colonized by the local biological communities (especially invertebrates and amphibians). The first SSF wetland has 513 m^2 area, 0.85 m mean depth and 3.7 days hydraulic retention time (HRT). The surface organic load at the inlet is 13.7 g BOD_5/m^2*day (equal to a volumetric load of 16.06 g

BOD/m^3*day) . The SF wetland area is 300 m^2, its depth is 0.7 m and HRT is 5.6 days. SF and the final facultative pond have been spontaneously colonized by *Lemna sp.* entirely covering the free water surfaces since the system started to work.

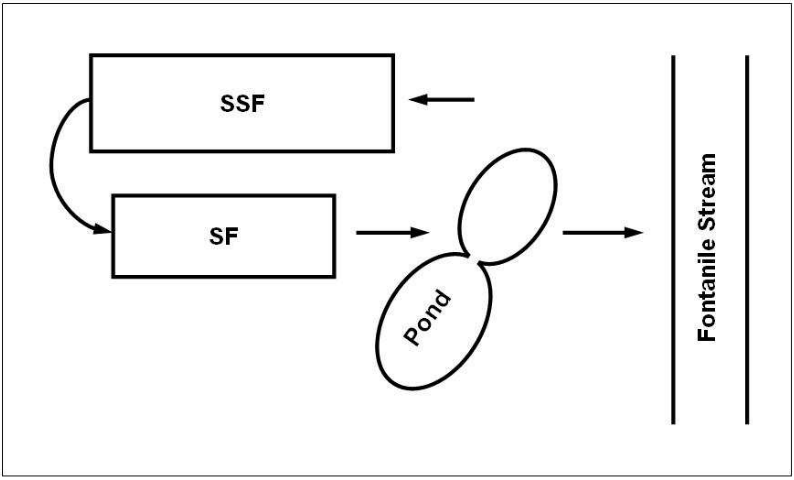


Fig. 2.1. Scheme of the studied CW system.

2.2.2 Sample collection and analyses

Wastewater samples were collected monthly from July 2008 until May 2012 at the inlet and at the outlet from SSF and SF wetlands in sterile plastic containers (500 ml for physicochemical parameters and microbiological counts) and transported to the laboratory for analysis. All samples were kept refrigerated until the microbiological analyses, which were done within the following 24 hours, according to Standard Methods (APHA, AWWA, WEF, 1998). Some water parameters (temperature, dissolved oxygen, oxygen saturation and conductivity) were measured in situ, using a Hach-Lange probe with a LDO oxygen sensor. The other considered chemical parameters were pH, COD, total phosphorus, total nitrogen and ammonia nitrogen.

E. coli were counted by the membrane filtration method according to Standard Methods for the Examination of Water and Wastewater (APHA, AWWA, WEF, 1998), using 0.45 μm pore-size cellulose nitrate filters (Sartorius Stedim Biotech) and selective agar (Chromogenic *E. coli* agar - EC X-GLUC agar, Biolife).

2.2.3 Data analyses

Statistical analyses were carried out using XLSTAT 7. Pair-wise Student's t-tests were used to check if the percent removal of each considered parameter was significantly different between the two treatment steps. Two way analysis of variance (ANOVA) was used to check the influence of treatment type and seasonal variation (and the interactions between treatment and season) in the removal of nutrients and faecal bacteria. Subsequent pair-wise comparisons were performed using Tukey's HSD (honestly significant difference) post hoc tests.

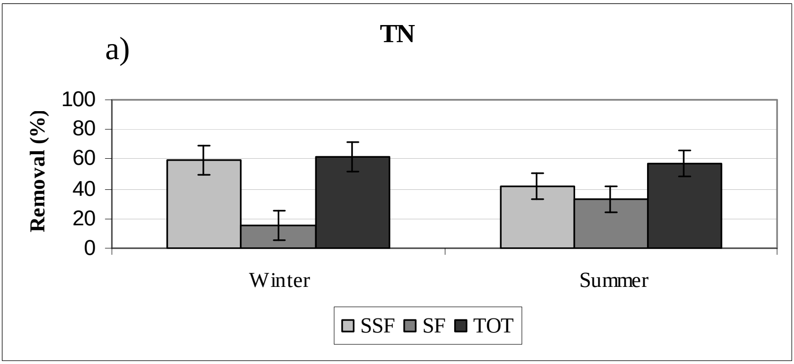
2.3 Results and discussion

A summary of the average concentrations of the considered parameters both in the influent and final effluent is presented in Table 2.1, that reported also the average percent removal in the different wetland treatment phases. For *E. coli* the mean count was $2.30*10^5$ CFU/100 ml in the influent and $1.10*10^3$ CFU/100 ml in the final effluent, with a removal of about 2 orders of magnitude. The overall bacterial removal in the system was on average higher than 99% and was mainly due to SSF. As to the other considered water quality parameters, the lower percent removal (51.71%) was observed for NH_4^+-N. Removal efficiency was slightly higher for TP and TN (55.01% and 59.36% respectively). A higher percent removal was measured for COD, whose value decreased by about 75%. As observed for *E. coli*, the most important role in removing the inlet pollutants was played by SSF rather than SF.

Table 2.1. Influent and final effluent average concentrations and microbial counts (mean and st. dev. in parentheses) and % removal efficiency at each stage of the CW system.

	Influent	Removal (%)			Final effluent
		SSF	SF	Cumulative treatment system	
TP (mg/l)	4.856 (2.133)	41.85	28.19	55.01	2.189 (1.970)
TN (mg/l)	40.950 (20.162)	49.95	24.57	59.36	16.267 (12.820)
NH_4^+-N (mg/l)	33.010 (17.094)	42.74	25.00	51.71	14.022 (11.421)
COD (mg/l)	160.43 (111.50)	70.97	11.19	75.24	34.16 (26.47)
E. coli (CFU/100ml)	$2.30*10^5$ ($1.37*10^5$)	97.10	88.28	99.71	$1.10*10^3$ ($1.70*10^3$)

For each parameter seasonal means and seasonal removal rates were calculated, considering a growing season (called “summer”) and a quiescence season (called “winter”). TN removal (Fig. 2.2 a) was different through seasons both in the SSF and in SF processes, even though the total system efficiency did not show relevant seasonal differences (62.00% in winter, 57.20% in summer). The same trend was observable even for NH_4^+-N (Fig. 2.2 b). For both these parameters, SSF showed a higher efficiency in winter (59.41% for TN and 48.10% for NH_4^+-N) than in summer (41.55% and 37.97% respectively), while the SF process seemed to be more efficient in summer (32.92% and 32.52% respectively) than in winter (15.17% and 16.55% respectively). TP removal (Fig. 2.2 c) was better in summer for each single phase of the process (45.89% in SSF and 33.70% in SF) and even for the total treatment, that in the growing season removed on average 61.20% of the inflow concentration. The removal efficiency was higher for COD (Fig. 2.2 d) than the other parameters both in winter and in summer in each treatment stage and in the total CW system. Winter mean removal of the total treatment system were 82.93%, while in summer it decreased at 65.13%. *E. coli* percent removal carried out by the CW system was always very high being 98.42% in winter and 99.67% in summer. Fig. 2.3 represents the removal expressed in $\log(N/N_0)$. It is possible to observe that the total mean log removal was over than 3 orders of magnitude in summer (-3.56) and over than 2 orders of magnitude in winter (-2.67). Even in this case the higher removal rate was performed by the SSF treatment (- 3.35 in summer, -2.40 in winter) rather than SF (-0.21 in summer, -0.27 in winter).



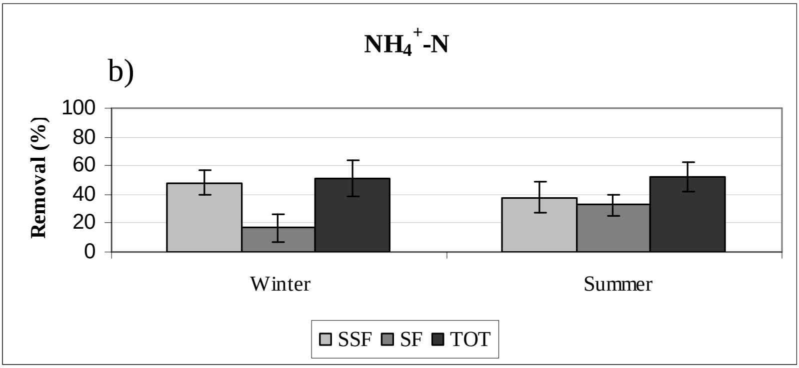
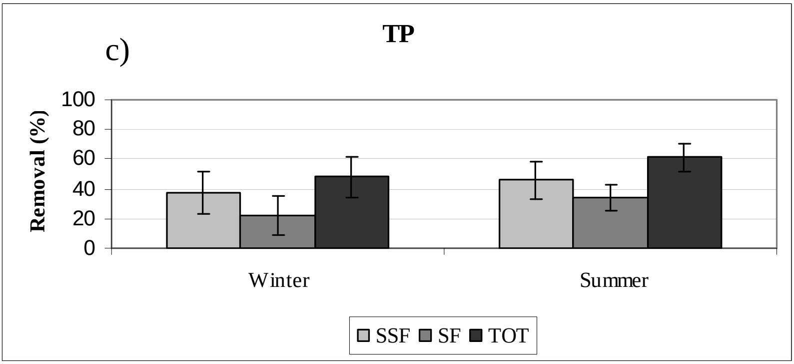


Fig. 2.2 a,b. Seasonal % removal (mean ± st. error) performed by the SSF, the SF and the total treatment system.



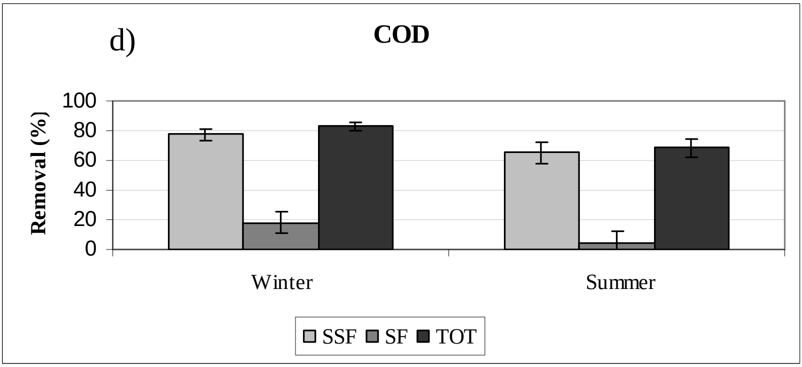


Fig. 2.2 c,d. Seasonal % removal (mean ± st. error) performed by the SSF, the SF and the total treatment system.

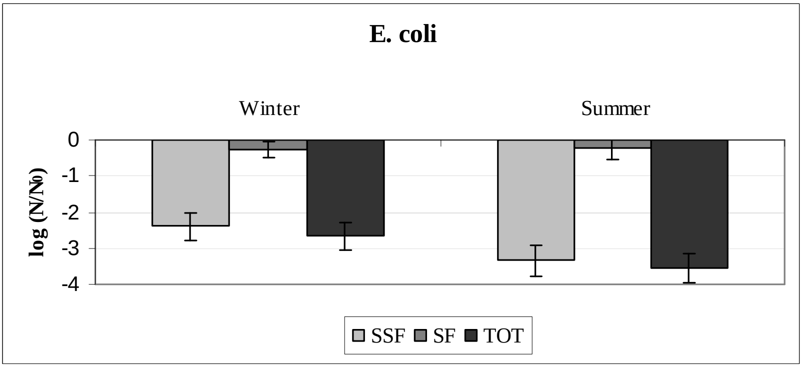


Fig. 2.3. Seasonal log removal (mean ± st. error) performed by the SSF, the SF and the total treatment for *E. coli.*

Pair-wise Student's *t*-tests were used to check if the removal of each considered parameter was always significantly different (α=0.05) between the various treatment phases. The concentrations decreased significantly at each phase of the treatment process and out of the whole CW system. *P*-values of the tests are reported in Table 2.2. The only not significant difference was found for *E. coli* between the outlets from SSF and the SF out.

Table 2.2. Comparison of the concentrations measured in and out of SSF, out of SF and out of total treatment (Pair-wise Student's *t*-test *p*-values, α=0.05).

	TP	TN	NH_4^+-N	COD	E. coli
in - out SSF	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
in – out SF	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
out SSF – out SF	**0.003**	**<0.0001**	**<0.0001**	**0.013**	0.152

The removal of nutrients, COD and microorganisms were analyzed as a function of CW treatment and season, as underlined by two-way ANOVA (Table 2.3). The model did not resulted significant for TP removal (p=0.303), which seemed to be independent from treatment and season. For the other considered parameters the model resulted significant (p=0.006 for TN) or even highly significant for treatment (p<0.0001 for COD, p<0.0001 for *E. coli*) but not for season, except for COD (p<0.0001 for treatment and p=0.011 for season). TN removal resulted to be significantly different between SSF and SF phase (treatment p=0.001), but not influenced by the season. The same statistical difference was found for NH_4^+-N, whose removal resulted significantly influenced by the treatment phase (treatment p=0.035). The *E. coli* log removal resulted to be significantly different along the treatment process (p<0.0001) but not influenced by the season.

Table 2.3 – Significant differences in the removal efficiency between treatments and seasons for each parameters (Two-way ANOVA, $p<0.05$).

Metrics		**DF**	**Sum of squares**	**Mean of squares**	**F value**	***p* value**
TP	Model	5	7741.952	1548.390	1.247	0.303
n = 51	Residuals	45	55883.507	1241.856	-	-
	Total	50	63625.459	-	-	-
	Season	1	1579.302	1579.302	1.272	0.265
	Treatment	2	6077.399	3038.700	2.447	0.098
	Season*Treatment	2	45.927	22.964	0.018	0.982
TN	Model	5	13802.903	2760.581	3.832	**0.006**
n = 51	Residuals	45	32420.105	720.447	-	-
	Total	50	46223.008	-	-	-
	Season	1	36.363	36.363	0.050	0.823
	Treatment	2	11507.819	5753.909	7.987	**0.001**
	Season*Treatment	2	2752.832	1376.416	1.911	0.160

Table 2.3 (continued) – Significant differences in the removal efficiency between treatments and seasons for each parameters (Two-way ANOVA, p<0.05).

Metrics		**DF**	**Sum of squares**	**Mean of squares**	**F value**	***p* value**
NH_4^+-N	Model	5	7799.125	1559.825	1.733	0.147
n = 51	Residuals	45	40509.307	900.207	-	-
	Total	50	48308.432	-	-	-
	Season	1	64.396	64.396	0.072	0.790
	Treatment	2	6517.478	3258.739	3.620	**0.035**
	Season*Treatment	2	1454.995	727.497	0.808	0.452
COD	Model	5	45921.805	9184.361	27.798	**<0.0001**
n = 51	Residuals	45	14867.819	330.396	-	-
	Total	50	60789.624	-	-	-
	Season	1	2308.584	2308.584	6.987	**0.011**
	Treatment	2	43456.452	21728.226	65.764	**<0.0001**
	Season*Treatment	2	9.502	4.751	0.014	0.986
E. coli (log N/N_0)	Model	5	95.413	19.083	16.734	**<0.0001**
n = 51	Residuals	45	51.315	1.140	-	-
	Total	50	146.728	-	-	-
	Season	1	4.495	4.495	3.941	0.053
	Treatment	2	86.158	43.079	37.778	**<0.0001**
	Season*Treatment	2	2.674	1.337	1.172	0.319

Tukey's HSD tests underlined that for each parameter the significant differences (p<0.05) in removal were mainly between SF and SSF treatments. These results confirmed that the more efficient treatment process in the studied CW system was carried out by the SSF wetland rather than SF wetland. For TN Tukey's test showed significant difference between SSF-SF (p=0.022) and between SF-total treatment (p=0.001). The same differences were found for bacterial log removal, that also resulted to be significantly different between SSF and SF (p<0.0001) and between SF and total treatment (p<0.0001). NH_4^+-N removal resulted significantly different between SF and total treatment (p=0.033). COD was the only parameter whose removal resulted significant differences even among seasons (p=0.011), other than between treatment phases (SSF-SF, p<0.001; SF-TOT, p<0.0001).
Table 2.4 shows the mean seasonal microbial removal rates (log removal*day^{-1}, log removal*m^{-2} day^{-1}) in the different wetlands of the system, considering their area and their HRT (expressed in days). The SSF wetland was the most effective in the removal of *E. coli*, both in winter and in summer.

Table 2.4. Mean microbial removal rates (st. dev. in parentheses) in winter and in summer.

		SSF	SF	TOT
Log (N/N_0)/d	Winter	-0,65 (0.29)	-0,05 (0.12)	-0,29 (0.12)
	Summer	-0,90 (0.34)	-0,04 (0.17)	-0,38 (0.13)
Log(N/N_0)/(d*m^2)	Winter	-1,27*10^{-3} (5.69*10^{-4})	-1,59*10^{-4} (4.01*10^{-4})	-3,53*10^{-4} (1.42*10^{-4})
	Summer	-1,76*10^{-3} (6.71*10^{-4})	-1,27*10^{-4} (5.72*10^{-4})	-4,71*10^{-4} (1.59*10^{-4})

Different processes may be involved in the removal of contaminants and microorganisms in a natural wastewater treatment system. In particular bacterial fate is influenced by the type of wetland and associated factors that cause their removal or inactivation. In this study the removal efficiency of a combined CW system for contaminants and *E. coli* were evaluated. Wetland system effectively removed *E. coli* with mean reductions ranging from 98% in winter to >99% in summer. These value are within the ranges reported in literature for CW treating domestic wastewater (Soto et al. 1999, Greenway 2005, Reinoso et al. 2008), showing that *E. coli* were between the most efficiently removed microorganisms. The type of wetland significantly influenced the removal efficiency, both for pollutants and microorganisms during the study period. The SSF phase was the most efficient, obtaining the highest removal for all the considered parameters (Table 2.1).
Many researches have assessed that several processes could contribute to bacterial removal in CW systems. In SF wetlands and ponds, adsorption on settleable solids and further sedimentation (Grimason et al. 1996) and solar irradiation (Curtis et al. 1992, Davies-Colley et al. 1997) are considered to be the main bacterial removal mechanisms, in addition to predation by antagonistic organisms (Manage et al. 2002), physicochemical conditions (Araki et al. 2000) and toxins excreted by certain algae (Oufdou et al. 2001). In SSF wetlands filtration and adsorption to macrophyte roots and substrates (Williams et al. 1995, Gerba et al. 1999, Sleytr et al. 2007) were shown to be the main removal mechanisms. It has been assessed that the presence of macrophytes has a lethal effect on faecal indicators, such as *E. coli* (Gersberg et al. 1989). In fact, it is well known that some macrophytes, including *P. australis*, produce root exudates which are toxic to a range of bacteria, including *E. coli* (Ottová et al. 1997). In addition to this direct effect, the enhanced development in the rhizosphere of population of bacteria with antibiotic properties (e.g. *Pseudomonas* spp.) may also contribute to *E. coli* removal (Ottová et al. 1997). Also, it is possible that fungi may carry out a similar role (Decamp and Warren 2000). Adsorption and inactivation can be considered the primary factors controlling

virus attenuation within submerged flow treatment systems (Vega et al. 2003). These processes were probably predominant in the studied CW system producing bacterial indicators removal or inactivation.
With regards to seasonal differences, some authors have observed higher removal efficiencies during the hot season (El Hamouri et al. 1994, Karathanasis et al. 2003), although others have not found seasonal changes (García et al. 2006, Reinoso et al. 2008, Abreu-Acosta and Vera 2011). In fact, Hatano et al. (1993) demonstrated that the effect of temperature and seasonal variation of microorganism removal may vary with different pathogen species and type of wetland vegetation.
In this study no seasonal differences were observed for *E. coli* removal, although they were observed for the other considered contaminants. In particular, COD percent removal resulted to be more efficient in winter rather than in summer.

2.4 Conclusions

COD and nutrient removal carried out by the cumulative treatment system in the study period was >50% for NH_4^+-N, >55% for TP and TN and >75% for COD. The main treatment process was performed by the SSF, in winter as in summer. For the considered chemical parameters, the studied CW system seemed to be even more efficient than similar CW composed by SSF treatment phase (e.g. Mezzanotte et al. 2012).
E. coli were efficiently removed in the studied CW system, with no differences between seasons. The mean removal was up to 2 orders of magnitude, that corresponded to >99% of the inlet concentration, and allowed to respect the Italian standards for discharge. The main bacterial removal was carried out by the SSF wetland, which was also responsible for the most part of the treatment process for the other analysed water quality parameters. COD was the only parameter whose removal resulted significantly different among seasons besides between treatment phases.

Filtration and adsorption to macrophyte roots and substrates were probably the main removal mechanisms for the considered faecal indicator, as shown by the absence of differences related to temperature variations. The scarce contribution of SF wetland in the total treatment process may be linked to the presence of *Lemna sp.*, that at the end of its seasonal life cycle decomposed in the basin. In addition, it did non permit the disinfection process carried out by solar radiation, covering all the free water surface. The better removal of nutrients in SF in summer can depend on *Lemna* uptake, which, of course, did not affect COD and *E.coli.* At the same time, at the end of summer the accumulated *Lemna* biomass provided a sort of internal COD load, so that COD removal, if referred, as usual, to COD input, appeared lower, even if bacterial activity was probably comparable in the two seasons. These are common problems which need to be taken into account in surface flow CW which should be carefully managed in order to prevent the accumulation of *Lemna* biomass and to stimulate macrophyte colonization (with consequent shading effect).

In the analyzed situation, the considered natural treatment system is effective but is chiefly due to the first step (i.e. Sub-surface flow system). The overall efficiency could be improved by the above mentioned measures. The improvement of microbiological quality of wastewater is similar to that obtained by conventional technologies, reducing the impacts due to traditional treatments.

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Chapter 3

Comparison of macroinvertebrate assemblages and biodiversity levels in constructed wetlands and natural ponds.

Abstract

Constructed wetlands are valuable options because of their role in water supply, floodwater retention, nutrient loading and water treatment capability, at the same time allowing the restoration of lost habitats and helping biodiversity conservation. There is still little knowledge of the biodiversity that can develop in these artificial environments, especially at the invertebrate community level. The macroinvertebrate assemblages, the water chemistry and the environmental characteristics of natural and artificial pools and constructed wetlands in Pineta Park (Northern Italy) were studied in order to evaluate the effects of local factors on the macroinvertebrate communities. Natural ponds were considered as reference sites. Statistical analyses (Principal Components Analysis, Canonical Correspondence Analysis, Analysis of Variance) were carried out on the whole dataset. PCA showed that ponds were divided into clusters, depending on their morphology and their water quality and independently from their artificial or natural origin. The overall biodiversity level (Taxa Richness, Shannon and Pielou Indices) of natural ponds and constructed wetlands was similar, while the composition of the communities varied. CCA highlighted the differences in the composition among the various kinds of ecosystem and pointed out the relationships between macroinvertebrates and environmental variables.

Overall, constructed wetlands showed the potential to be valuable elements of the ecological network, right from the start of their insertion in the environment, due to fast colonizing invertebrates. However, long term assessment is needed to understand if the community composition can become comparable to other kinds of wetland.

Key words: natural and constructed wetlands, freshwater macroinvertebrates, habitat heterogeneity, diversity level, community composition.

Submitted manuscript.

3.1 Introduction

Although wetlands are habitats of high biodiversity and have significant ecological functions and recognized social and economic uses, they are threatened by a number of anthropogenic pressure sources, the most important of which include increased nutrient loading, contamination, acid rain and invasion of exotic species (Brönmark and Hansson 2002). They are also under threat in many parts of the world due to the conversion of natural land to agricultural and residential uses (Daniels and Cumming 2008). Climate change might increase impacts of these threats by reducing rainfall and increasing temperature, decreasing flow and altering timing and variability of flow regimes (Kingsford 2011).

Recognition of ecological services provided by wetlands has stimulated renewed efforts to protect, manage and construct them (Mitsch et al. 1998, Zedler 2006). Artificial ponds are valuable to society, since they are often created for purposes such as water supply, floodwater retention, recreation and education, or wildlife management and research (Oertli et al. 2005). Often, they have an important role to compensate for habitat destruction and the subsequent loss of species (National Research Council 1992). For these reasons, the introduction of artificial wetland ecosystems can create new ecological resources, especially where freshwater environments are reducing. These interventions can have different aims; they can be useful as wastewater treatments or can be inserted as naturalistic elements in the local ecological network.

There is still little knowledge on the processes taking place in these artificial environments and on the ecological function of these habitats, especially at the invertebrate community level (Gee et al. 1997, Hermann et al. 2000, Ruhí et al. 2009). Research on constructed wetlands has most frequently been addressed to the evaluation of their efficacy in pollution removal and flood mitigation, with less attention to their role as ecosystems (Mitsch et al. 1998, Spieles et al. 2006). Only a few studies (e.g. Spieles and Mitsch 2000, Fairchild et al. 2000, Balcombe et al. 2005, Becerra-

Jurado et al. 2009) have analysed the macroinvertebrate communities of constructed wetlands and the driving environmental factors that influence them. Thus, a deeper knowledge of the biodiversity hosted in these environments is needed to evaluate if artificially created ponds and constructed wetlands are also appropriate restoration tools for biological conservation (Ruhí et al. 2009).
Macroinvertebrates are directly affected by the physical and chemical integrity of the surrounding environment, including water quality (EPA 2002). They are known to influence the rates of nutrient cycling and decomposition, to have a central position in the wetland food webs and to integrate environmental impacts and changes through time (Scatolini and Zedler 1996, Sharitz and Batzer 1999, Brady et al. 2002, Stanczak and Keiper 2004). Therefore, they are potentially extremely useful as indicators of restoration success (Campbell et al. 2002).
We examined the macroinvertebrate communities and their relationships with water quality and habitat heterogeneity in eight ponds in a natural park in Italy. We chose four artificial ponds created for various purposes and four natural ecosystems that have been considered as reference sites. The aims of the work have been (1) to improve the understanding of factors affecting the invertebrate community structure in artificial ponds and constructed wetlands, (2) to assess the role of the selected ponds and wetlands in improving biodiversity and (3) to compare the biodiversity level among artificial ecosystems and reference sites.

3.2 Methods

3.2.1 Sampling Sites

Eight wetlands were selected within Pineta Park, a natural park in Northern Italy, 35 km northwest of Milan (Fig.3.1). They lie in a hilly wooded and agricultural area, consisting of clay terraces originating from Pleistocene erosion, which allow water accumulations. The whole park, enclosed by an urbanized area, is placed in the wider territorial area of the Alps piedmont hills and covers 4860 ha.

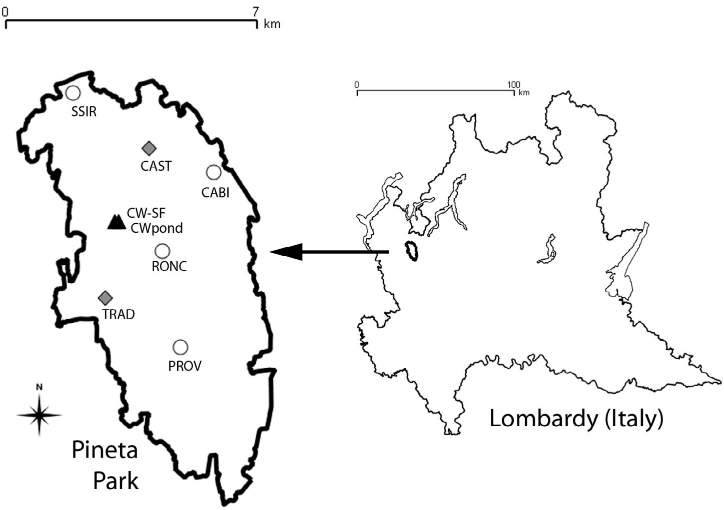

Fig. 3.1. Study area with the location of the eight considered wetlands (white dots = natural ponds; black triangles = constructed wetlands; grey diamonds = artificial pools).

On average, the maximum summer temperatures (July) are approximately 22.0°C and the minimum winter temperatures (January) are 1.6°C; annual temperature variation is around 20.3°C. During the year rainfall is about 1400 – 1500 mm, with a primary maximum in spring and a secondary in autumn. The climate can be defined as mildly continental.

The wetlands considered for this study have been classified as natural (NAT), artificial (PARK) and constructed (CW). Their areas range between 50 m^2 and 1500 m^2. NAT ponds are the larger, and originated partly by the erosion of running water, and partly by the human extraction activities carried out in the past centuries. They are characterized by the availability of numerous microhabitats, due to the banks morphology, the variable depth (>1 m) that change gradually and the presence of typical wetlands vegetation (different macrophytes and riparian grasses). The studied NAT ponds are four: Cà Bianca (CABI), San Siro (SSIR), Proverbio (PROV), and Roncamocc (RONC). PARK pools have been introduced by the Park management for naturalistic purposes, mainly to provide habitat and recovery for amphibian species. They are oval-shaped, 50 m^2 surface and 1 m depth. The considered PARK pools are two, Tradate (TRAD) and Castelnuovo Bozzente (CAST). The two CW pools are part of a constructed wetland for the treatment of wastewater produced by a hamlet placed in a wooded area, and consist in a surface flow pool (CW-SF) and in a following smaller pond (CWpond). They are 306 and 105 m^2 respectively and both reach a maximum depth of 0.6 m.

3.2.2 Habitat characteristics

Within each pond some environmental characteristics have been considered to describe the whole ecosystem. Particularly, we observed the presence of submergent, emergent and floating macrophytes. We estimated the presence of the various kind of macrophytes as significant if they covered the wetland surface for at least 20%. We also evaluated the presence of riparian vegetation in a 1 m buffer from the water edge and of wood structures such as floating and immersed trunks, roots and living trees.

The physical and morphological habitat differentiation has also been observed, considering the presence of gradual bank slopes, bends in the pond perimeter, variable depths, permanent shady or sunny areas given by the canopy coverage. These characteristics of the water bodies have been considered as presence/absence, and then the sum of the presences has been counted, thus originating a score between 0 and 5. This score has been accounted to be indicative of “low” morphological differentiation if included in the 0-2 range, or indicative of “high” morphological differentiation if included in the 3-5 range (Table 3.1).

Table 3.1. Environmental variables presence/absence and morphological diversity in each pond. CW= Constructed wetlands, NAT = natural ponds, PARK= artificial pools.

	CW		PARK		NAT			
	CW pond	**CW-SF**	**CAST**	**TRAD**	**CABI**	**PROV**	**RONC**	**SSIR**
Submergent macrophytes	X	X	✓	✓	X	✓	X	✓
Emergent macrophytes	✓	✓	✓	✓	✓	✓	✓	✓
Floating macrophytes	✓	✓	✓	X	✓	✓	X	✓
Riparian grass	✓	X	✓	✓	✓	X	✓	✓
Woody structures	X	X	X	X	✓	✓	✓	✓
High morphological diversity	✓	X	X	X	X	✓	X	✓

3.2.3 Sampling for water quality analyses

Physico-chemical and microbiological parameters have been analysed in samples collected at the same time as macroinvertebrate qualitative samplings. Some water parameters (temperature, dissolved oxygen, oxygen saturation and conductivity) were measured in situ, using a Hach-Lange probe with a LDO oxygen sensor. For the other parameters (pH, COD, total phosphorus, total nitrogen, ammonia nitrogen, *Escherichia coli),* water samples have been collected in bottles and analysed in laboratory within the following 24 hours, according to Standard Methods (APHA, AWWA, WEF, 1998).

3.2.4 Macroinvertebrate sampling

Macroinvertebrates were sampled seasonally between June 2008 and August 2009 by qualitative seasonal samplings. Within each pond samples have been taken with a 500 µm mesh size net (0.05 m^2), with sweeps in rapid sequence (two replicates for the smallest ponds; four replicates for the bigger ponds, as suggested in Gascón et al. 2008) from every different identified habitat, considering vegetation species (submergent, emergent and floating macrophytes; riparian grass), bottom characteristics, solar exposition and coverage, banks morphology and wood structures presence.

Sampled macroinvertebrates were preserved in 90% ethanol before being sorted, counted, identified at the lowest taxonomical level possible (usually species or genus; family for Diptera and Oligochaeta) and then conserved in 4% formaldehyde. Taxa richness, Shannon Index and Pielou Index were evaluated using the same taxonomical level in all the water bodies, for the purpose of comparison. The overall methodology was followed for both artificial and natural wetlands.

3.2.5 Data analysis

Data analyses have been carried out using XLSTAT 7 (Principal Component Analysis - PCA - and Analysis of Variance - ANOVA) and CANOCO 4.5 (Canonical Correspondence analysis - CCA) software. PCA was performed to evaluate the relationships and the relative importance of each environmental and water quality variable in this study.

To evaluate any significant difference in community assemblages and biodiversity, ANOVA one-way model was used with the three groups of sites as treatments. Thus, we considered the groups of sites (NAT, PARK and CW) as predictor variables (treatments) and the biodiversity indices as response variables. A posteriori pair-wise comparisons between treatments were carried out using Tukey's HSD test.

A CCA was performed to evaluate the relationships between macroinvertebrate communities and site environmental characteristics. Standardization of environmental variables was automatically performed by the software (CANOCO 4.5; ter Braak and Smilauer 1998). A preliminary Detrended Correspondance Analysis (DCA), performed on the invertebrate community data, showed a gradient length >3 SD, indicating a unimodal response and, thus, justifying the use of CCA. Only the environmental variables significantly related (Monte Carlo permutation test, $P < 0.05$) to macroinvertebrate distribution were retained. Variables showing strong multicollinearity (Variance Inflation Factors > 20) were also excluded from the analysis (see results).

3.3 Results and discussion

Morphological diversity of ponds is shown in Table 3.1. SSIR and PROV resulted to have the highest values for morphological microhabitat diversity and macrophyte species, while CW-SF was the least differentiated for morphology and vegetation. Even if CAST e CABI had a low level of morphological differentiation, they were characterized by the presence of various microhabitats due to the macrophyte richness or to the presence of wood structures. CWpond also presented high morphological differentiation associated to vegetation diversity.

Water quality analyses showed that CWpond and CW-SF had higher concentrations of total phosphorus, total nitrogen and ammonia nitrogen than all the other ponds. They were also characterized by low dissolved oxygen (DO) concentrations, as were RONC and PROV ponds, where the decomposition of massive vegetation was particularly intense. All the other natural and artificial ponds presented more similar water quality; the DO oversaturation in CABI and in CAST was due to relevant phytoplankton blooms, influencing also the COD (Table 3.2).

Table 3.2. Water quality variables determined during macroinvertebrate samplings (mean ± st. dev.). CW= Constructed wetlands, NAT = natural ponds, PARK= artificial pools.

Sites		**DO (mg/L)**	**DO (sat%)**	**T (°C)**	**Cond (µS/cm)**	**pH**
CW	CW-SF	2.57 ± 3.51	26.9 ± 36.0	15.6 ± 4.7	662 ± 300	7.57 ± 0.35
	Cwpond	2.75 ± 2.68	31.1 ± 31.2	17.4 ± 6.8	544 ± 232	7.52 ± 0.5
PARK	CAST	9.25 ± 2.71	101.4 ± 36.6	18.8 ± 7.2	31 ± 14	7.31 ± 0.71
	TRAD	2.38 ± 1.47	26.1 ± 16.9	16.3 ± 8.0	54 ± 20	7.31 ± 0.53
NAT	CABI	10.08 ± 3.12	117 ± 50.0	19.4 ± 8.0	71 ± 9	7.30 ± 0.9
	PROV	2.63 ± 1.67	25.7 ± 14.6	15.7 ± 6.4	38 ± 18	6.55 ± 0.64
	RONC	2.98 ± 1.95	33.3 ± 23.7	18.5 ± 8.4	128 ± 13	7.36 ± 0.67
	SSIR	7.39 ± 1.07	80.4 ± 14.2	16.9 ± 4.0	74 ± 11	7.46 ± 0.30

Table 3.2 (continued). Water quality variables determined during macroinvertebrate samplings (mean ± st. dev.). CW= Constructed wetlands, NAT = natural ponds, PARK= artificial pools.

Sites		**TP (mg/L)**	**TN (mg/L)**	**NH_4+ (mg/L)**	**COD (mg/L)**	**E. coli (CFU/100 ml)**
CW	CW-SF	1.093 ± 0.871	11.985 ± 9.769	8.936 ± 8.997	19 ± 13	6201 ± 12188
	Cwpond	0.573 ± 0.506	9.016 ± 7.835	5.649 ± 5.583	19 ± 11	798 ± 1454
PARK	CAST	0.081 ± 0.046	1.879 ± 1.235	0.158 ± 0.203	64 ± 20	15 ± 17
	TRAD	0.034 ± 0.021	1.218 ± 0.543	0.079 ± 0.082	33 ± 14	94 ± 199
NAT	CABI	0.125 ± 0.044	1.981 ± 0.906	0.053 ± 0.049	51 ± 35	230 ± 424
	PROV	0.075 ± 0.053	2.096 ± 1.287	0.315 ± 0.52	35 ± 21	4 ± 4
	RONC	0.112 ± 0.074	1.926 ± 0.736	0.237 ± 0.209	34 ± 7	16 ± 21
	SSIR	0.025 ± 0.012	1.675 ± 0.934	0.06 ± 0.018	5 ± 2	10 ± 6

A total of 30 macroinvertebrate taxa were collected from the whole pond set. For a preliminary comparison of NAT, CW and PARK categories, the mean percentages of macroinvertebrate classes (subclasses for Clitellata) have been calculated (Fig. 3.2). In CW category 90.78% of sampled organisms were Insecta, while the remaining part was constituted by Gastropoda (8.87%) and Oligochaeta (0.35%). A similar percent distribution has been observed in PARK category, where 91.77% of organisms were Insecta, 3.01% were Gastropoda and 5.22% were Oligochaeta. The macroinvertebrate assemblage in NAT group seemed to be more complex, with 60.73% of Insecta, 5.78% of Gastropoda, 8.64% of Hirudinea and 24.85% of Oligochaeta. The Insecta turned out to be the most relevant class in the three ecosystem groups, although there were differences among the composition in the macroinvertebrate orders (Fig. 3.3).

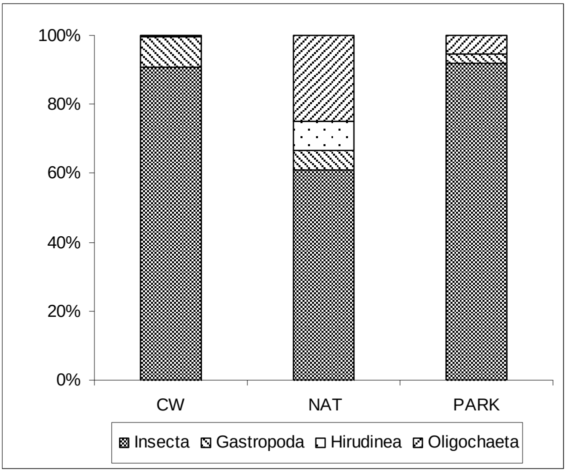

Fig. 3.2. Histograms representing the mean percentage of macroinvertebrate individuals belonging to different classes and Clitellata subclasses in each ecosystem category (CW=Constructed wetlands, NAT=natural ponds, PARK=artificial pools).

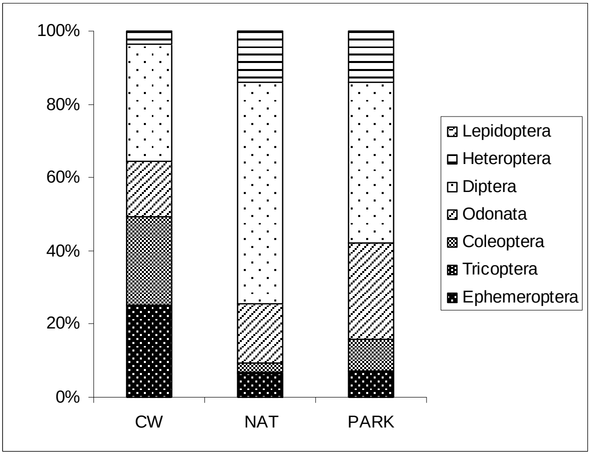

Fig. 3.3. Histograms representing the mean percentages of Insecta class composition (orders or suborders) in each ecosystem category (CW= Constructed wetlands, NAT = natural ponds, PARK= artificial pools).

In CW category, Insecta were mainly constituted by Ephemeroptera (belonging only to Baetidae family - 25.3%), Coleoptera (23.9%), Diptera (31.9%) and Odonata (15.3%) orders, while in NAT category the prevailing orders were Diptera (60.7%), Odonata (16.1%) and Hemiptera (suborder Heteroptera - 13.6%). In PARK group the prevailing orders were Diptera (43.7%), Odonata (26.2%) and Hemiptera (14.0%).

For every pond Taxa Richness, Shannon Index and Pielou Index have been calculated for each sampling campaign: Table 3.3 reports the indices mean values for NAT, CW and PARK ecosystem categories. The values of the biodiversity indices for the three ecosystem categories were comparable, but slightly higher for the PARK group. In the ANOVA model evaluating the biodiversity indices differences there was no significant variation ($\alpha = 0.05$) among and within the three groups of sites and the null hypothesis (no treatment effect) had to be considered true (Table 3.3). Any pair of means among the three treatments for each biodiversity index resulted not significantly different (Tukey's HSD test; $\alpha = 0.05$).

Table 3.3. One-way ANOVA table showing the sum of squares (SS), mean square (MS), the F-ratio and the *P*-value calculated for each biodiversity index. *P*-value standard alpha level = 0.05. CW= Constructed wetlands, NAT = natural ponds, PARK= artificial pools.

			ANOVA					
	Group of sites	**Mean ± St. Dev.**		**dF**	**SS**	**MS**	**F-ratio**	***P-value***
Taxa Richness	**CW**	11.8 ± 2.7	Model	2	67.15	33.58	1.33	0.294
	NAT	8.1 ± 6.5	Residual	15	378.46	25.23		
	PARK	12.3 ± 3.9	Total	17	445.61			
Number of individuals	**CW**	428 ± 495	Model	2	19.06×10^4	95309.24	0.57	0.578
	NAT	202 ± 389	Residual	15	25.13×10^5	167537.10		
	PARK	227 ± 278	Total	17	27.04×10^5			
Shannon Index	**CW**	2.30 ± 0.62	Model	2	3.16	1.58	1.70	0.215
	NAT	1.66 ± 1.29	Residual	15	13.93	0.93		
	PARK	2.69 ± 0.31	Total	17	17.10			
Pielou Index	**CW**	0.65 ± 0.14	Model	2	0.19	0.09	1.24	0.317
	NAT	0.51 ± 0.38	Residual	15	1.15	0.08		
	PARK	0.77 ± 0.15	Total	17	1.34			

In the PCA analysis of environmental and water quality variables, the emergent macrophytes variable was excluded because of its ubiquity in each considered ecosystem. As shown in Fig. 3.4, the 66.13% of variance was explained by the first two PCA axes together. Morphological ecosystem differentiation showed a significant (bilateral *t*-test; α=0.05) positive relationship with the total available microhabitat number.

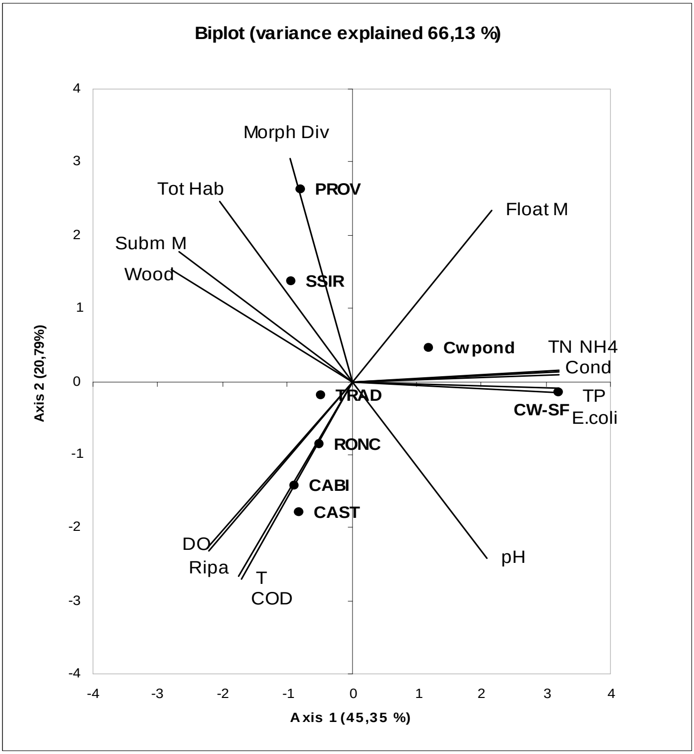

Fig. 3.4. PCA biplot diagram showing relationships (first two axes, 66.13% of the total variance) between some of the environmental variables (lines) and sites (dots). Morph Div=morphological diversity; Tot Hab=number of total microhabitats; Subm M=submergent macrophytes; Float M=floating macrophytes; Wood=woody structures; Ripa=riparian grass.

No significant relationships were found between morphological characteristics and water quality, with the exception of the temperature with riparian grass, probably due to the solar exposition. The whole set of water quality parameters was covariant. On axis 1 CWpond and CW-SF appeared to be clustered and separated from the other ponds, because of their water quality. On the other hand, a correlation could be observed between them and the other ponds on axis 2, that showed a gradient in microhabitats differentiation, in which CWpond and CW-SF have an intermediate position. The two PCA axes showed that ponds and pools were divided into three clusters, based on their relationships with higher morphological diversity (PROV and SSIR), with water nutrient loads (CWpond and CW-SF) and with phytoplankton bloom, related to COD and DO high concentrations (CABI and CAST mainly). Water quality was confirmed as a relevant environmental gradient, that could potentially affect the macroinvertebrate community assemblages.

The first two axes of the CCA exploring the relationship between macroinvertebrate taxa and environmental factors had eigenvalues of 0.473 and 0.300, together explaining 59.9% of the total variation in the data set (Table 3.4). The Monte Carlo permutation test showed a significant result for the sum of all eigenvalues (499 permutations, $P<0.05$). In the preliminary CCA analysis, seven environmental variables (conductivity, total phosphorus, emergent macrophytes, wood presence, morphology, fish presence and droughts) had high variance inflation factors (IF>20), i.e. were highly correlated with other variables and were thus less significant in explaining community assemblages, so they were excluded from the final CCA analysis (see methods).

Table 3.4. Summary of the canonical correspondence analysis. Monte Carlo test run for the sum of all eigenvalues was significant (499 permutations, $P<0.05$).

Axis	**1**	**2**	**Total inertia**
Eigenvalue	0.473	0.300	1.585
Species-environment correlations	0.989	0.978	
Cumulative percentage variance			
of species data	29.9	48.8	
of species-environmental relation	36.7	59.9	
Sum of all canonical eigenvalues			1.290

Environmental variables included in the final analysis were water body surface area, DO, temperature, pH, total nitrogen, ammonia nitrogen, COD, *E. coli,* floating macrophytes and riparian grass. It is worth remembering that, in the examined cases, COD was mostly related to algal blooms rather than to organic load input. This is confirmed by the positive relationship between COD and DO (due to supersaturation caused by photosynthesis) and by the COD values higher in NAT and PARK ponds rather than in CW, fed on domestic sewage.

Although the three ecosystems categories did not present significant differences in the overall biodiversity level, as shown by the ANOVA model, differences in the community assemblages among the three categories were considerable and were underlined by the CCA. The triplot diagram (Fig. 3.5) shows the distribution of the relative abundance of macroinvertebrates (with the Insecta represented at order level) across the sampling sites. CWs appeared clustered because of their different water quality, which seemed to be preferred by Coleoptera, Ephemeroptera and Gastropoda, whose relative frequency is higher in constructed wetland samples. The other macroinvertebrate orders were probably limited by water quality and were related to different habitats unavailable in the CWs, such as the presence of diversified macrophyte communities (in

PARK ponds and some of the NAT wetlands) or the larger surface area (mainly in NAT ponds) leading to the availability of more numerous microhabitats. Hence, these environments support a different macroinvertebrate community. The available habitats can allow the presence of different taxa, such as Odonata, Hemiptera and Trichoptera. In this study, they appear more frequently in NAT and PARK ponds, notwithstanding the overall biodiversity and evenness of the communities is comparable to the one of CWs.

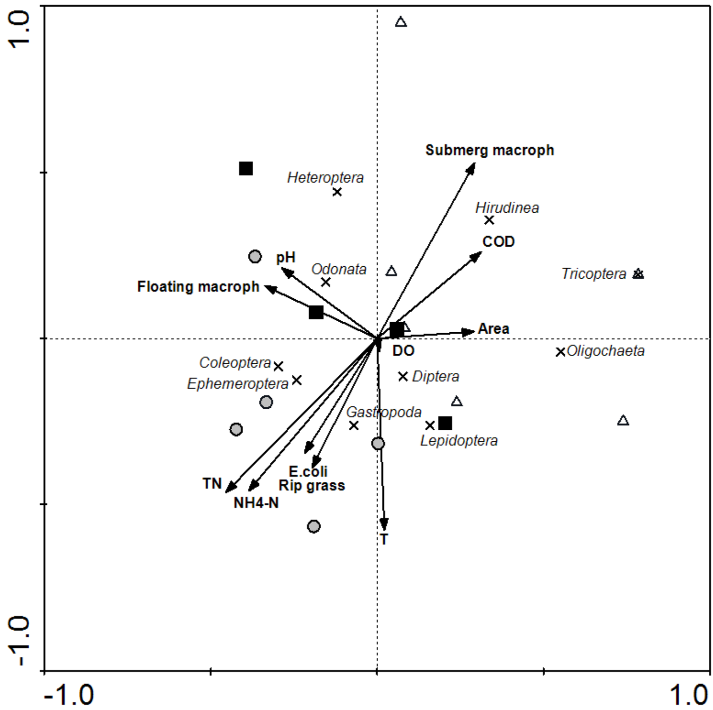

Fig. 3.5. CCA triplot diagram showing the relationships between macroinvertebrates (classes, orders or suborders), environmental variables and sampling sites (natural wetlands are represented by triangles, artificial wetlands by black squares and constructed wetlands by grey dots).

Differences in macroinvertebrate assemblages were observed among sites and were related to the varying environmental, morphological and water quality conditions. Water chemistry and trophic conditions have often been cited as relevant factors affecting macroinvertebrate community assemblage and biomass in lentic ecosystems (Friday 1987, Rasmussen 1988, Brodersen et al. 1998). In addition, the size of the water body and the habitat structure seemed to influence the macroinvertebrate communities. As suggested by Hansson et al. (2005) wetlands characterised by relatively shallow depth, large surface area and high shoreline complexity are more likely to yield higher biodiversity values, also for benthic invertebrates. The preference of many invertebrate taxa for certain vegetation or bottom substrate types (Minshall 1984) may also influence the biodiversity. Many studies have found positive relationships between taxa richness, habitat heterogeneity and area for many invertebrate orders (Huston 1994, Rosenzweig 1995, Heino 2000). Wetland morphological differentiation and water quality as a limiting factor were probably the most relevant factors that could explain the different community structure among the sites we monitored. A diversified macrophyte community and/or the availability of other habitats can support more specialized taxa such as Odonata, Hemiptera and Trichoptera, that in this study appeared more frequently in natural ponds and in the artificial pools that were built by the Park with a high vegetation complexity.

Water quality also plays a role: constructed wetlands, that were characterized by the highest nutrient concentrations, showed assemblages composed mainly by fast colonizing and tolerant families of Diptera, Coleoptera and Ephemeroptera, the latter including only individuals belonging to the Baetidae family.

Although water quality improvement is generally the primary objective of treatment wetlands, the creation of habitats is an inevitable outcome of these projects (Knight et al., 2001). Macroinvertebrate are often early colonists of new created wetlands, with abundance and diversity approaching high levels within a few years from wetland construction (Batzer et al. 2006, Stewart and Downing 2008). The two considered constructed wetlands were recently built and were monitored during their first year of working. The overall level of biodiversity was already comparable to the level of the other examined ecosystems, although the composition of the community was different. Some authors have found similar taxa richness in natural wetlands and in 1 to 10 year-old constructed wetlands (Barnes 1983, Stanczak and Keiper 2004, Hansson et al. 2005, Spieles et al. 2006), but critics often argue that certain aspects of created wetlands (e.g., plant communities and soils) can not be similar to natural wetlands for at least almost 5 years (Campbell et al. 2002). However, the creation of constructed wetlands has the potential to provide a habitat that may be unavailable within the surrounding landscape (Bacerra-Jurado et al. 2009). Thus, a more integrated management of water quality and biodiversity enhancement, as suggested by the *integrated constructed wetlands concept* (Harrington and Ryder 2002, Harrington et al. 2005, Scholz et al. 2007), is required.

3.4 Conclusions

In this study we compared the macroinvertebrate assemblages, the water chemistry and the environmental characteristics of a set of natural ponds, artificial pools and constructed wetlands spread within Pineta Park (Northern Italy). We found that wetland morphological differentiation and water quality were probably the most relevant factors that could explain the different community structure among the sites we monitored. In our opinion, the constructed wetlands we examined showed the potential, right from the start of their insertion in the environment, to be valuable elements of the local ecological network. The pioneer invertebrate communities let them reach an overall biodiversity level similar to the other ponds in the park within a year. However, it will be necessary to assess in the long term if they could support a comparable community composition. This will probably happen if the improvement of the treatment efficiency and the development of a more complex macrophyte community could take place with proficient management actions.

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Chapter 4

Influence of environmental variables on wetland macroinvertebrate biodiversity

Abstract

Many researches have significantly correlated habitat heterogeneity and environmental conditions in wetland ecosystems with macroinvertebrate diversity and community assemblages.
Within *Parco Pineta di Appiano Gentile e Tradate* (Lombardy, Northern Italy) 24 ponds and pools spread within the park territory have been chosen to analyse wetlands biodiversity. They are both artificial and natural ecosystems and two of them are part of a constructed wetland system. Environmental characteristics of each pond, as well as morphology and taxonomic biodiversity in macroinvertebrates communities have been monitored. Physico-chemical parameters were analysed in conjunction with macroinvertebrate samplings, that have been carried out in summer and autumn 2010 through semiquantitative surber sampling, considering the presence/absence and the proportional abundance of each *taxon*. Depth, area, vegetation species, bottom characteristics, fish presence, solar exposition and coverage, banks morphology, tributary presence and phytoplankton blooms are the other environmental variables considered to describe each ecosystem.
The purpose of the study was to consider the relative importance of several variables in explaining the patterns in the structure of macroinvertebrate assemblages in each lentic ecosystem.
According to canonical correspondence analysis (CCA), the most important environmental factors related to assemblage community composition were the water body area and the habitat heterogeneity, intended as the number of available microhabitats in each pond. In general, species composition in small pools differed from that in larger ponds, because of their morphological homogeneity. Total species richness was also explained through a principal components analysis (PCA), that shows a significant positive correlation between

habitat availability (area and hydromorphological diversity) and the Taxa Richness and Shannon indices estimated on families. Significant correlation (p<0.0001) between habitat availability and *taxa* richness was explained also by linear regressions, which shown that communities differentiation is mostly unrelated to water quality. Further investigations showed that the presence of fish acted as a limiting factor.

Keywords: wetlands, invertebrate biodiversity, community assemblage, environmental variables, habitat heterogeneity.

Submitted manuscript.
Part of the results has been presented at the 7th Meeting for PhD students in Ecological Science, Siena, Italy, 11-13 May 2011.

4.1 Introduction

Wetlands are among the most important ecosystems on Earth and they have been valuable as sources, sinks and transformers of a multitude of chemical, biological and genetic materials (Mitsch and Gosselink 2007).

Aquatic macroinvertebrates are used in many wetlands bioassessment programs because difference in environmental requirements among taxa produces community assemblages that reflect ecological conditions (Wissinger 1999, USEPA 2002). Indeed, benthic macroinvertebrates respond to a complex of localised environmental factors in hierarchical arrangement (Winterbourn 1981). Many researches have demonstrated the importance of water chemistry or trophic conditions in influencing the structure of freshwater macroinvertebrate assemblages (e.g. Friday 1987, Rasmussen 1988, Jeffries 1991, Brodersen et al. 1998); extreme water chemistry can cause a decrease in invertebrate species richness and even affect the abundance and diversity of macrophytes, thus reducing the amount of substrates and food resources available to invertebrates (e.g. Friday 1987). Wetland plant diversity is important for determining macroinvertebrate associations (De Szalay and Resh 2000) and wildlife diversity (Knight et al. 2001) because of the creation of habitats and food resources. Wetzel (2001) noted that the most effective wetland ecosystems "are those that possess maximum biodiversity of higher aquatic plants and periphyton associated with the living and dead plant tissue".

Moreover, water body size and habitat heterogeneity have been significantly correlated with macroinvertebrate diversity, thus explaining a high proportion of variation in species richness (Heino 2000). Biotic indices based on species richness and dominant taxa respond to variation in baseline habitat conditions as well as water quality related factors (Collier et al. 1998).

The structural complexity of lentic habitats should have important consequences for macroinvertebrate assemblage structure by, for example, ameliorating the effects of fish predation on invertebrates (e.g. Gilinsky 1984, Diehl 1992). The preference of many invertebrate species for certain vegetation or bottom substratum types (Minshall 1984, Hoffman et al. 1996) may also affect the occurrence of species and lead to patterns observed at the community level.

Even in highly interconnected ponds, local environmental constraints can be strong enough to prevail over regional homogenizing forces and structure local communities (Cottenie et al. 2003).

Total species richness increased with habitat heterogeneity, which was apparently due to the positive effects of spatial heterogeneity on resource diversity (Heino 2000).

One of the most fundamental ecological relationships is that as the area of a region increases, so does the number of different species encountered: the number of species found in a region is a positive function of the area of that region (Connor and McCoy 2001). The "habitat diversity hypothesis" (Williams 1964) proposes that in large areas species richness increases more than in small areas because large areas have a greater variety of available habitats. This availability permits the presence of species that are only found in specific habitats and species that require multiple habitats to persist in large areas. The habitat diversity hypothesis views area as affecting species richness indirectly because of its association with habitat diversity rather than any direct effect of area on the ability of species to colonize or persist in large areas (Connor and McCoy 2001).

The purpose of the study was (1) to assess macroinvertebrate biodiversity, (2) to consider the relative importance of several variables in explaining the patterns in the structure of macroinvertebrate assemblages in each lentic ecosystem and (3) to verify the influence of pond size and habitat availability in macroinvertebrate biodiversity.

4.2 Materials and methods

4.2.1 Study site

Within *Parco Pineta di Appiano Gentile e Tradate* (Fig. 4.1), a regional park located in the foothill 35 km northwest of Milan, between the provinces of Como and Varese, numerous water bodies are present, some of which can be defined as real ponds, with the presence of biological communities typical of wetlands. 24 ponds and pools spread within the park territory were chosen to analyse wetlands biodiversity. They were both artificial and natural ecosystems and two of them were part of a constructed wetland system. The wetlands considered for this study have been classified as natural (16), artificial (6) and constructed (2), depending on their origin. We considered as artificial wetlands the oval-shaped pools introduced by the Park management for naturalistic purposes, mainly to provide habitat and recovery for amphibian species.

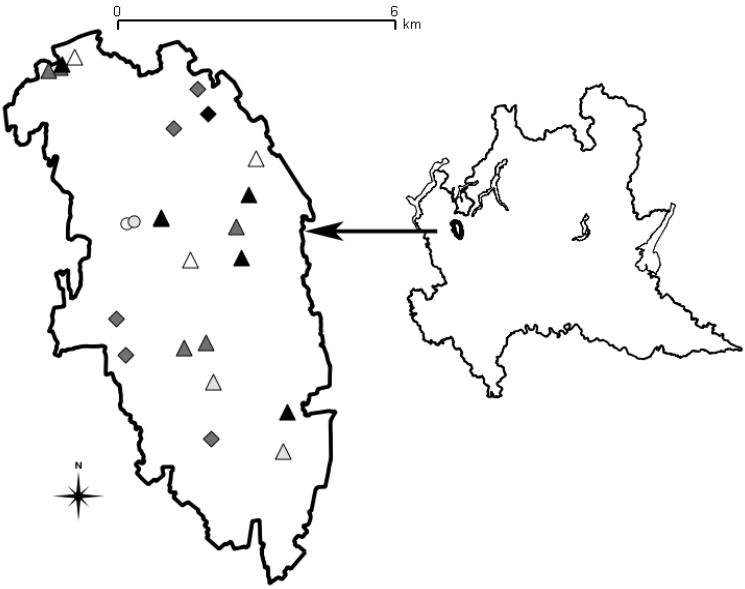


Fig. 4.1. Parco Pineta border and its localization in Lombardy region. Within the park territory are represented the considered wetlands (triangle=natural, diamond=artificial, circle=constructed wetland).

4.2.2 Environmental description and analysis

Wetland definition and description often include three main components: hydrology, physicochemical environment and biota. These components are linked in a cycle in which hydrology affects the physicochemical environments, including the soil, which, in turn, determines with the hydrology what and how much biota is found in the wetland ecosystem (Mitsch and Gosselink 2007).

In this study we considered all these components in order to describe and compare different ecosystems. Hydrology was considered taking into account if the wetland experiences drought period during the year. Physicochemical characteristics were determined seasonally, such as the biotic characterization, that was pointed in particular at macroinvertebrates, but also considering vegetal communities and the presence of uncommon vertebrate predators (fish and turtles – amphibians have been found in all the monitored wetlands). Since the ponds were characterized by many morphological differences, we took into account also other environmental variables, as described below.

Within each lotic or lentic habitat it is possible to distinguish different microhabitats, that can be grouped in three categories (Tachet et al. 2010):

1. Mineral microhabitats, that include the mineral substrates, from silt to boulders.
2. Organic microhabitats, that include all kind of organic debris, such as tree trunks and branches, leafs stored on the bottom and sludge.
3. Vegetal microhabitats, that include all the living plants, essentially hydrophytes.

All these kinds of microhabitats are important in determine the macroinvertebrate community structure, that adapts to the available resources and refuges.

In this study for each water body maximum depth and area were measured seasonally. In order to describe each ecosystem and to identify the available microhabitats, macrophytes community, bottom characteristics, banks morphology, fish presence, solar exposition and shade, tributary presence and phytoplankton blooms were the other environmental variables considered.

- *Macrophytes community* – Hydrophytes were divided into submerged, emergent and floating. The presence of the various kind of macrophytes was considered as significant if they covered the wetland surface for at least 20%. The presence of riparian vegetation in a 1 m buffer from the water edge was also evaluated. Each macrophytes group was considered as presence/absence. Even the presence of living trees and roots in the riparian buffer was taken into account.
- *Bottom characteristics* – The bottom feature and the substratum granulometry were observed. In particular the presence of stones or boulders was noted.
- *Solar exposition* – The solar exposition can influence many parameters, such as temperature, pH, vegetation growth and phytoplankton blooms. In this study it was considered as presence of always sunny zones or always shade zones within the total wetland area.
- *Banks morphology* – It was observed if banks had a windy perimeter and if they gently sloped from the littoral to the pelagic zone.
- *Tributary presence* – It was intended as the presence of inflowing or out flowing water, thus creating also habitat of low running water.
- *Branches/trunks presence* – They were considered as organic wood material fallen into the water. Their presence represent a food resources and a refuge.
- *Drought periods* – Some of the smaller ponds dried up during the hot season. For this reason the hydroperiod was considered as drought period during the year.

Multivariate data analyses have been carried out using XLSTAT 7 (for Principal Component Analysis - PCA) and CANOCO 4.5 (for Canonical Correspondence analysis - CCA) software.

4.2.3 Physicochemical analyses

Water physicochemical and microbiological parameters were analysed in samples collected at the same time as macroinvertebrate qualitative samplings. Some water parameters (temperature, dissolved oxygen, oxygen saturation and conductivity) were measured in situ, using a Hach-Lange probe with a LDO oxygen sensor. For the other parameters (pH, COD, total phosphorus, total nitrogen, ammonia nitrogen, *Escherichia coli),* water samples were collected in bottles and analysed in laboratory within the following 24 hours, according to Standard Methods (APHA, AWWA, WEF, 1998).

4.2.4 Macroinvertebrate sampling

Macroinvertebrate samplings have been carried out in summer and autumn 2010 through qualitative surber sampling (500 µm mesh net, 0.05 m^2), considering the presence/absence and the proportional abundance of each taxon. Samples were taken from each identified microhabitat in every pond with sweeps in rapid sequence (two replicates for the smallest ponds; four replicates for the bigger ponds, as suggested in Gascón et al. 2008).

Sampled macroinvertebrates were preserved in 90% ethanol before being sorted, counted, identified at the lowest taxonomical level possible (usually species or genus; family for Diptera and Oligochaeta) and then conserved in 4% formaldehyde. Taxa richness and Shannon Index were evaluated using the same taxonomical level in all the water bodies, for the purpose of comparison. The overall methodology was followed in each considered wetland.

4.3 Results and discussion

In this study environmental characteristics of each pond, in particular water quality, morphology and taxonomic biodiversity in macroinvertebrates communities have been monitored. All the environmental variables were observed in correspondence of

macroinvertebrate samplings, in order to define in detail the microhabitat and resources available in each ecosystem. The considered wetlands were characterized by different dimensions and environmental variables, that are reported as presence/absence in Table 4.1. The considered pond set was composed by wetland of different origin (natural, artificial and constructed wetlands). In general, the bigger wetlands were characterized by a higher morphological differentiation, mainly due to the bank shape and slope and the presence of different depth. The park artificial pools were created with low morphological differentiation, but with a diversified macrophyte community, planted by the park management. The constructed wetlands presented environmental features similar to artificial pool, with less macrophytes species (mainly *Phragmites australis* and *Typha latifolia*), but bigger size.
According to the “habitat diversity hypothesis” (Williams 1964), considering the environmental variables of each pond, we observed that in general larger ponds were characterized by a higher microhabitat availability. Bigger water body surface allowed higher morphological heterogeneity, intended as the number of available microhabitats in each pond. Nevertheless, considering the bigger wetlands, area being equal, differences in microhabitats were present.

Table 4.1. Environmental variables observed as presence/absence in each wetland ("Type"column: N=natural; A=artificial; CW=constructed wetland).

Pond ID	Type	Max Depth (m)	Area (m^2)	Submerged macroph.	Emergent macroph.	Floating macroph.
A1	N	0,50	200		✓	
A4	N	0,30	18			
A6	N	0,40	25			
A14	N	0,50	5			
B1	N	> 1,50	300	✓	✓	✓
BF1	N	> 1,50	1600		✓	✓
C5	A	1,00	40		✓	✓
CB1	N	0,80	2			
CB3	N	0,35	2			
CB7	A	1,00	40	✓	✓	✓
CB8	A	1,00	40	✓	✓	
CB9	A	0,30	5			
CW1	CW	0,60	105		✓	
CW2	CW	0,50	306		✓	✓
LM1	N	0,25	15			
LM3	N	0,60	70		✓	
T12	N	0,50	30			
T14	N	0,50	35			
T17	N	0,70	200	✓	✓	✓
T27	A	1,00	48	✓	✓	
T28	A	1,00	48		✓	✓
VO1	N	0,30	48		✓	
VO3	N	0,25	20	✓		
VO5	N	0,50	3			

Table 4.1 (continued). Environmental variables observed in each wetland.

Pond ID	Gently sloping banks	Winding banks	Stumps and/or roots	Riparian vegetation	Wood and trunks	Living trees
A1	✓	✓	✓	✓	✓	✓
A4			✓		✓	
A6			✓			
A14			✓			
B1	✓	✓	✓	✓	✓	✓
BF1		✓		✓		✓
C5				✓		
CB1				✓		
CB3				✓		
CB7	✓			✓		
CB8				✓		
CB9						
CW1	✓			✓		
CW2						
LM1			✓	✓		✓
LM3	✓	✓	✓		✓	✓
T12	✓					
T14					✓	
T17	✓	✓	✓	✓	✓	
T27				✓		
T28				✓		
VO1	✓	✓	✓	✓		
VO3	✓					
VO5			✓			

Table 4.1(continued). Environmental variables observed in each wetland.

Pond ID	Boulders	Sunny zone	Shade zone	Flowing water	Different depth
A1					✓
A4			✓		
A6			✓		
A14			✓		
B1				✓	✓
BF1		✓			✓
C5					
CB1			✓		
CB3			✓		
CB7					
CB8					
CB9	✓			✓	
CW1		✓			✓
CW2		✓			
LM1					
LM3				✓	
T12					
T14			✓		
T17			✓		✓
T27			✓		
T28		✓			
VO1			✓	✓	
VO3	✓			✓	
VO5			✓	✓	

During the two sampling campaigns carried out in summer and autumn 2010 a total number of 90 macroinvertebrate taxa have been collected. Biodiversity was expressed as Taxa Richness and Shannon Index calculated as mean within the three category (natural, artificial and constructed wetlands) considering both the two sampling campaigns (Table 4.2). No significant differences (Student's *t*-test; $\alpha=0.05$) in biodiversity indices between wetlands of different origin were observed. The only significant difference ($p=0.026$) was between natural ponds and constructed wetlands Taxa Richness, that was higher in the last category.

Table 4.2. Biodiversity indices calculated for each wetland category (mean ± st. dev.).

	Taxa Richness	Shannon Index
Natural wetlands	8.4 ± 4.2	1.76 ± 0.72
Artificial wetlands	10.7 ± 2.3	2.00 ± 0.54
Constructed wetlands	13.5 ± 2.5	2.26 ± 0.60

A preliminary PCA analysis was carried out to evaluate the importance and the relative correlations (bilateral *t*-tests) between the considered environmental variables (Fig. 4.2). The water basin area resulted significantly correlated to the number of microhabitats ($R=0.393$; $p<0.05$) and to the presence of different kinds of macrophytes (riparian macrophytes: $R=0.349$, floating macrophytes: $R=0.457$; $p<0.05$). Moreover the area resulted significantly correlated to the presence of winding banks ($R=0.514$; $p<0.05$), the presence of living trees ($R=0.526$; $p<0.05$) and the presence of different water depth ($R=0.558$; $p<0.05$), which were all features that characterized the biggest wetlands in the considered pond set. These preliminary results seemed to agree with the concept that large areas have a greater variety of available habitats, intended both as physical features and plant richness. However, due to the observed habitat differences among wetlands of the same size, we have been looking for a more comprehensive kind of information that could explain a higher part of total data variability.

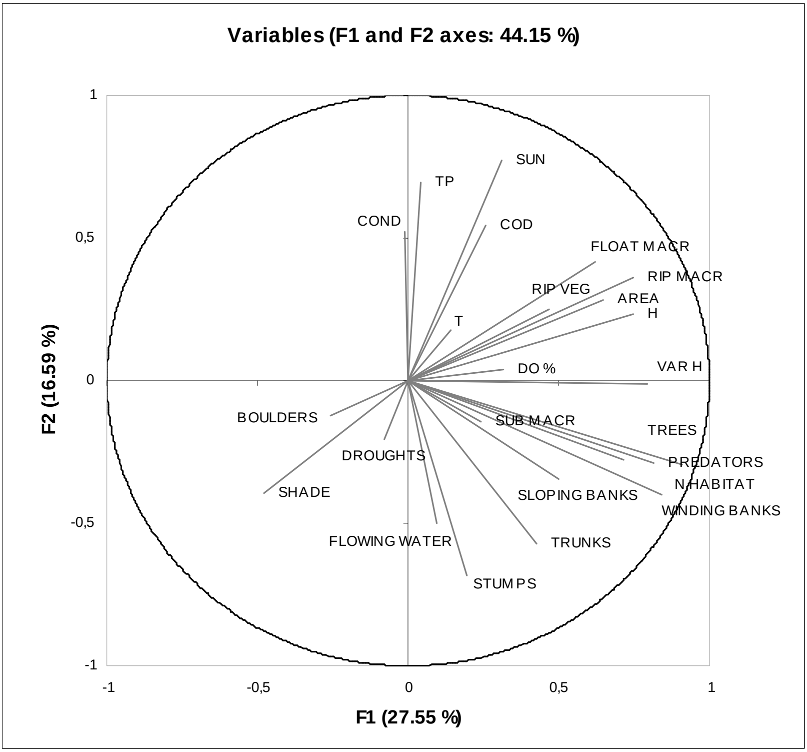


Fig. 4.2. PCA Loadings plot showing the relationship between environmental variables (morphological characteristics and some water quality properties).

The correlation between the water body area and the habitat heterogeneity (intended as the number of available microhabitats) could be effective expressed through a single index, that includes all the significant correlations found between water body dimension and its morphological characteristics. This index, called Area-Morphology Index (A-M Index), was calculated as:

$$\textit{Area - Morphology Index} = \log\,[\textit{habitats number} + \log\,(\textit{area})]$$

A-M index was calculated for each considered pond and then another PCA analysis was performed in order to evaluate if this index could be really representative in the morphological description of each ecosystem. The first two PCA axes together explained 45.70% of the total variance. The corresponding loading plot is represented in Figure 4.3. The A-M Index resulted positively and significantly correlated to most of the considered environmental variables (in particular water body area and depth, vegetation, banks morphology and predator presence).

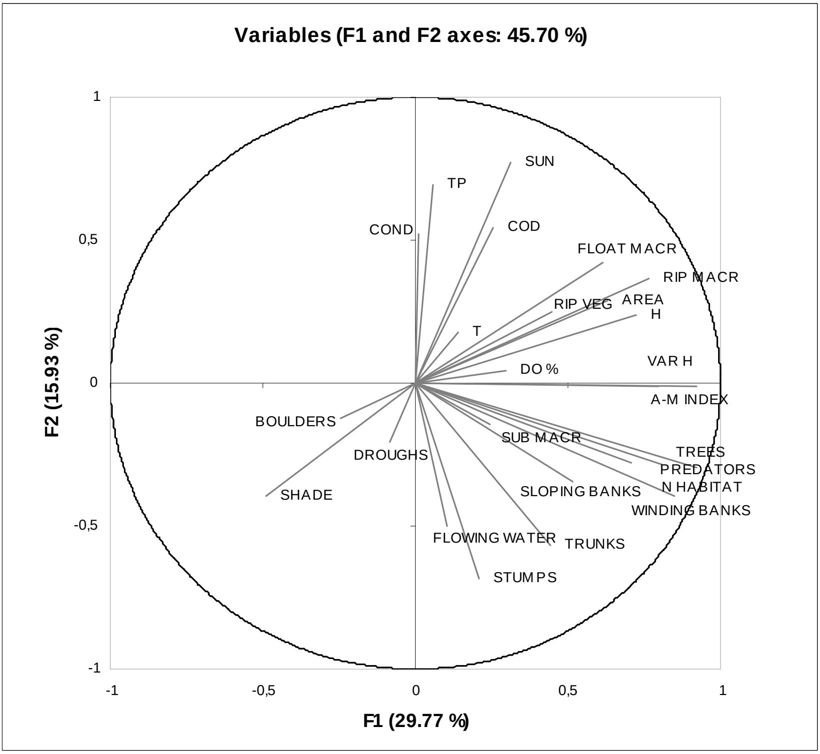


Fig. 4.3. PCA Loadings plot showing the relationship between environmental variables (morphological characteristics and some water quality properties) and the A-M Index.

Considering the significance of the A-M Index as indicator of morphological and environmental differentiation, it was evaluated in relationship to the macroinvertebrate biodiversity measured in each wetland. According to canonical correspondence analysis (CCA, Fig. 4.4), the most important environmental factors related to macroinvertebrate orders and superfamilies (Lumbricoidea) accounting for most of the biodiversity in assemblage community composition were the water body area and the habitat heterogeneity, expressed together as A-M Index..

The first two axes of the CCA exploring the relationship between macroinvertebrate taxa and environmental factors together explained 50.07% of the total variation in the data set. The Monte Carlo permutation test showed a significant result for the sum of all eigenvalues (500 permutations, $p<0.05$).

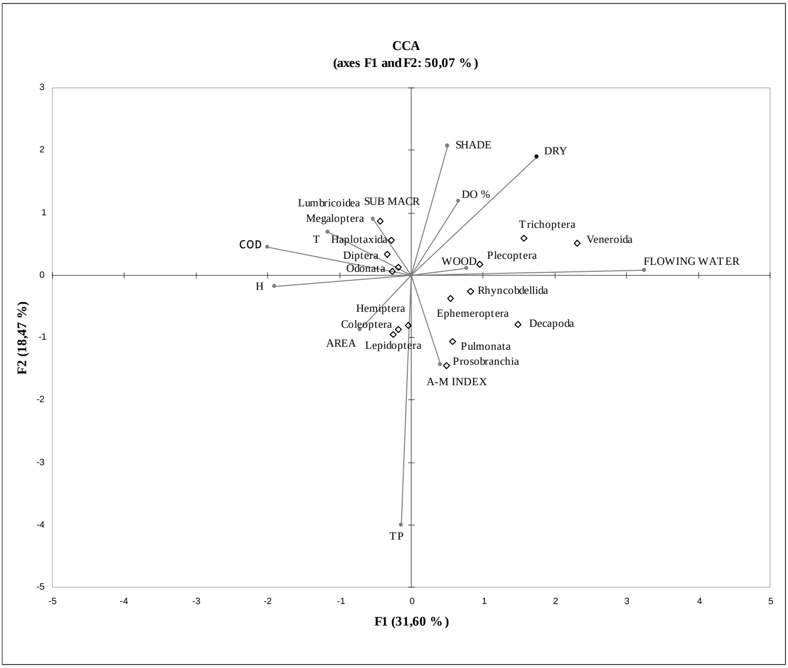


Fig. 4.4. CCA Loadings plot showing relationships between environmental variables and macroinvertebrate orders and superfamiliy (Lumbricoidea).

Total taxa richness was also explained through PCA (fig. 4.5), that was performed to evaluate the relationships and the relative importance of each environmental and water quality variable in influencing biodiversity, expressed as taxa richness and Shannon Index (calculated at family level). Shannon Index calculated on families showed a significant positive correlation ($R=0.350$, $p<0.0001$) with A-M Index. Community diversity appeared to be mostly unrelated to water quality and to the wetland type. Artificial wetlands indeed showed biodiversity indices similar to those calculated for natural wetlands.
Nevertheless, as it is possible to observe in Figure 4.5, biodiversity indices and A-M Index resulted significant on different PCA axes. In order to deepen and better understand this relationship, linear regressions were performed.

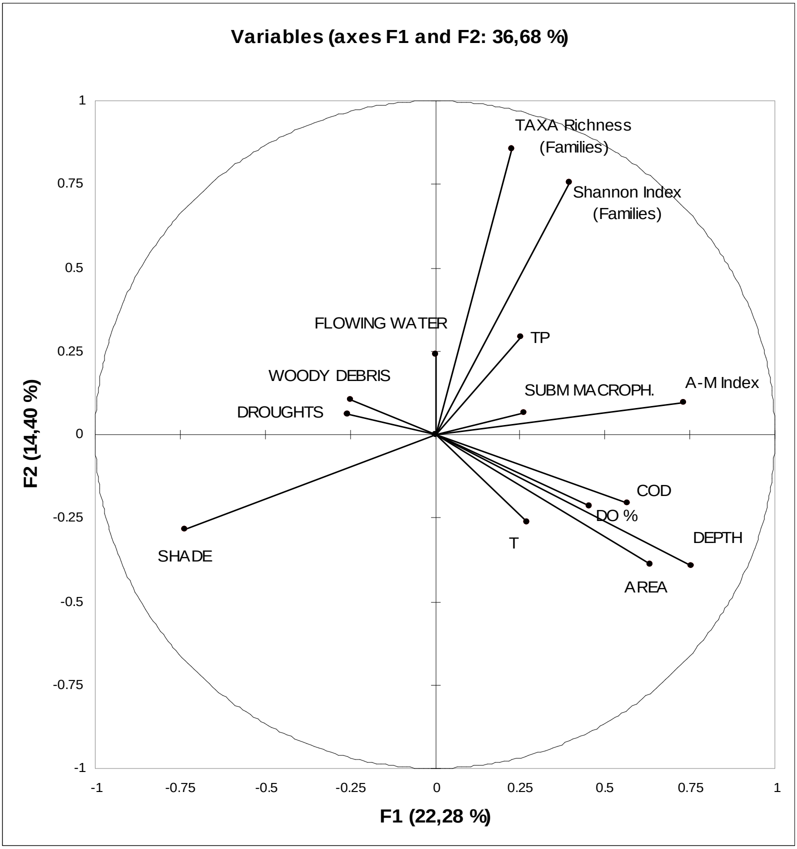
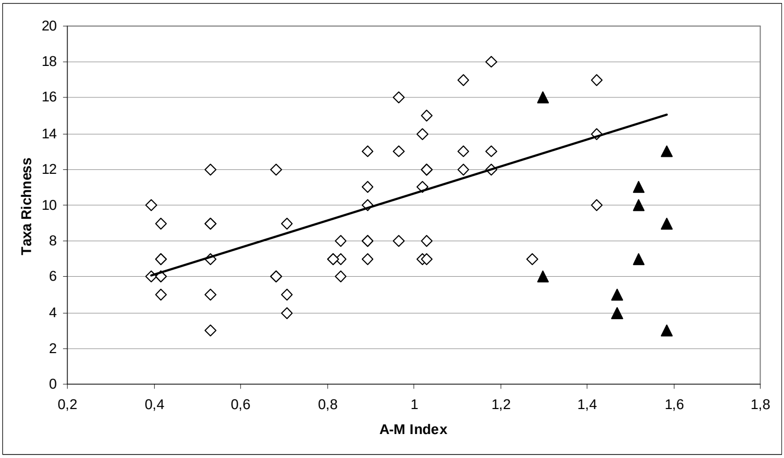


Fig. 4.5. PCA Loadings plot showing the relationship between significant environmental variables and biodiversity indices (Taxa Richness and Shannon Index).

Significant correlation ($p<0.0001$) between habitat availability and taxa richness was explained also by linear regressions (Fig. 4.6 a,b), which showed that the only consistent limiting factor for biodiversity seemed to be the vertebrate predator presence (mainly exotic fish and turtles) other than amphibian presence (which was ubiquitous). Wetlands characterised by fish and turtles presence (shown with black triangles in figures 4.6 a,b) do not comply with the same trend as the other ponds.

a)



b)

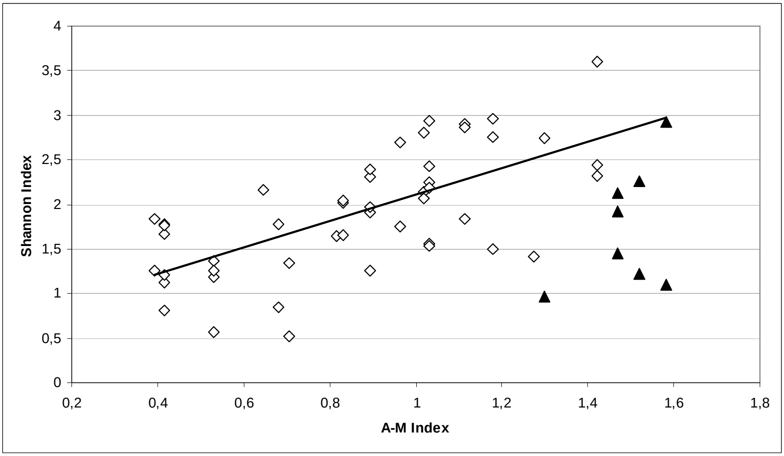


Fig. 4.6 a, b. Linear regressions showing the relationship between the A-M Index and two biodiversity indices: Taxa Richness (R^2=0.481, p<0.0001) and Shannon Index (R^2=0.463, p<0.0001). Black triangles represent wetlands with fish.

To confirm and better define the fish presence and abundance, electrofishing was carried out in autumn 2010. The fish census in the biggest wetlands confirmed the presence of many exotic species, in some cases associated to exotic turtles, too. The check list of fish species is reported in Table 4.3.
Although water chemistry variables and trophic conditions have often been cited as important factors influencing macroinvertebrate community structure and biomass in lentic ecosystems (Friday 1987, Rasmussen and Kalff 1987, Rasmussen 1988, Jeffries 1991, Brodersen et al. 1998), the macroinvertebrate communities analyzed in the present study did not seem to be influenced significantly by physicochemical water characteristics. This is relevant considering that two of the considered wetlands were part of a wastewater treatment system, hence being characterized by high pollutants concentrations, low dissolved oxygen and, occasionally, reducing red-ox conditions.
Many studies have found positive relationships between species richness, habitat diversity and area for various taxa (e.g. Huston 1994, Hill et al. 1994, Rosenzweig 1995, Begon et al. 1996). Pond size has previously been identified as a factor determining species richness in many invertebrate orders such as Mollusca (Lassen 1975, Aho 1978, Brönmark 1985), Diptera (Driver 1977), Hemiptera (Savage 1982), Coleoptera (Nilsson, 1984) and Crustacea (Fryer 1985). Gee et al. (1997) noticed that the most strongest and most significant species number/area relationships occur when the area of the vegetated margin is used, rather than the surface area of the entire pond, thus supporting the assertion that is the pond margins that contain most species (e.g. Kirby 1992, Sansom 1993, Hine 1995). Even the presence of shaded banks can influence the presence of some taxa. Gee et al. (1997) underlined that the species number of Odonata, Ephemeroptera and Trichoptera decrease linearly as the percentage of the shaded pond margin increases. The presence of riparian trees can influence the water temperature, at least in summer, especially in small pond, thus influencing species richness.

Table 4.3. Fish checklist in the wetlands characterized by fish presence (BF1, T17, LM3, B1).

Order	**Family**	**Genus**	**Species**	**Origin**	**Wetlands**
Cypriniformes	Cyprinidae	Scardinius	*S. erythrophtalmus*	native	BF1, T17, LM3, B1
		Carassius	*C. sp.*	exotic	BF1, T17, LM3
		Rutilus	*R. erythrophtalmus*	native	B1
		Leuciscus	*L. cephalus*	native	LM3, B1
Cyprinodontiformes	Poeciliidae	Gambusia	*G. holbrooki*	exotic	LM3
Perciformes	Centrachidae	Lepomis	*L. gibbosus*	exotic	BF1, B1
		Micropterus	*M. salmoides*	exotic	LM3, B1
Siluriformes	Ictaluridae	Ameiurus	*A. melas*	exotic	BF1, LM3,

According to the statistical analyses, the most important environmental factors related to macroinvertebrate assemblage composition were water body area and the availability of different microhabitat, related to variables such as macrophyte cover, banks morphology and refuge availability. As observed by Heino (2000), patterns in species richness were better explained by internal wetland habitat variables and area than by water chemistry. Some authors found that in some cases small ponds could retain greater biodiversity than a single large ponds of similar total area (Gee et al. 1997). Total species richness and biodiversity increased with habitat heterogeneity, which was apparently due to the positive effects of spatial heterogeneity on resource diversity. These correlations emerged in the present study. However, it should be noted that the present study includes ponds separated by few kilometres, and the similar overall biodiversity could be also influenced by macroinvertebrate dispersal.
Even though some authors did not find that fishless ponds could contain more invertebrate taxa than those with fish (Gee et al. 1997), in this study the predator presence was an important factor that influenced the community assemblage and the biodiversity. Similarly to what has been reported by Wellborn et al. (1996), small fishless ponds can support invertebrate assemblages different from those in water bodies containing benthic-feeding fish; fish-containing habitats may in part help explain differences in assemblage structure between small and large water bodies.

4.4 Conclusions

It is well known how numerous are the environmental factors that can influence the macroinvertebrate community assemblages. In this study considerable differences were observable between different ponds and even in the various microhabitats within the same wetland.
In general community composition in small pools differed from that in larger ponds, because of their morphological homogeneity.

Independently of their natural or artificial origin, all the wetlands considered had comparable biodiversity, that in some cases was even higher in artificial ponds and pools. Water quality properties and wetland artificial origin did not influence taxa richness as much as the number of available microhabitats and the water body dimension. For these reasons, constructed wetlands and artificial pools can be considered part of the local ecological network and they significantly contribute to the Park biodiversity. Moreover, the planning of new artificial wetland ecosystems should take into account habitat availability and area in order to allow the constitution of a well-framed ecosystem.

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Chapter 5

Analysis of macroinvertebrate community structure considering life-strategy groups and dispersal mode

Abstract

Continental wetlands usually consist of isolated units of temporary or permanently flooded areas in a context of habitats not suitable for aquatic organisms. Despite this apparent lack of connectivity among sites, many freshwater taxa can move between discrete habitat patches and have broad geographical distribution. Some organisms are wide distributed because of their capacity of active dispersion, and many organisms that are not able of active dispersion depend on biotic or abiotic agents to provide passive transport between sites. Dispersal mechanisms have important consequences for many ecological processes, such as colonization ability and distribution of species. Ecosystem level processes are influenced by the functional characteristics of the organisms involved, rather than by their taxonomic identity. For this reason trait-based analyses are considered to be a better candidate for the monitoring of ecosystem dynamics than taxon-based analyses.

In order to evaluate if newly created ponds and constructed wetlands can be really considered part of the regional ecological network, the macroinvertebrate community assemblages of a wetland set within a regional Park has been analyzed. The wetlands were divided into three categories (natural, artificial and constructed) based on their origin. The macroinvertebrate community of each pond was characterized considering the presence and the abundance of four life strategy groups, which are based on the taxa dispersal mode, their capacity to survive basin desiccation and the need of water to reproduce. The three different categories of wetlands presented a comparable biodiversity level (tested through analyses of variance - ANOVA - and principal components analysis - PCA), although they showed significant differences in community composition considering the life-strategy groups abundances. The relationship between Shannon index and the relative abundance of each group was also deepened through linear regressions, showing that some groups could significantly influence the metapopulation biodiversity.

Keywords: wetlands, macroinvertebrates, community assemblage, dispersal mode, life-strategy group, metapopulation, biodiversity.

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5.1 Introduction

Freshwater invertebrates live in habitats that represent discrete sites surrounded by an inhospitable terrestrial landscape (Bilton et al. 2001). In particular, continental wetlands usually consist of isolated units of temporary or permanently flooded areas in a context of habitats not suitable for aquatic organisms (Figuerola and Green 2002). Despite this apparent lack of connectivity among sites, many freshwater taxa can move between discrete habitat patches and have broad geographical distribution (Brown and Gibson 1983, WCMC 1998), obtained through different strategies. Some organisms are wide distributed because of their capacity of active dispersion, that is possible mainly trough aerial flight across the surrounding landscape. However, many organisms are not able of active dispersion and depend on agents such as animal vectors, wind or water flow to provide passive transport between sites (Maguire 1963, Bilton et al. 2001). In lotic habitats the most common ways of invertebrate passive dispersal are by water current or downstream drift, that can displace from 1% to 2% of benthic stream organisms (Waters 1972), while in lentic habitats dislocation of eggs and larvae can more often be provided by other animals.
Active dispersal may be triggered by changing environmental conditions, such as the increase of temperature or the decrease or water depth (Velasco et al. 1998), but in general the real causes that trigger insects to disperse are poorly understood. However some researches have considered also human activities between the mechanisms that cause, mediate or even impede dispersion (reviewed by Claudi and Leach 2000). Dispersal mechanisms have important consequences for many processes, such as colonization ability, distribution of species, and gene flow (Hanski et al. 1993). Moreover, they have a fundamental ecological importance, influencing population demography, food web dynamics, community succession and evolution (Clobert et al. 2001). Dispersal may also alter the probability of extinction within local population by introducing new colonists and increasing genetic diversity (Freeland

et al. 2000). Dispersal capacity is also very important in determining responses to climatic changes (Hogg and Williams 1996, Hogg et al. 1998). From an individual's point of view, there are both advantages and disadvantages to dispersing from one site to another (Stenseth and Lidicker 1992). Advantages include inbreeding avoidance, the possibility to find a new site with low density occupation and few resources competitors, and potential escape from adverse conditions such as limited resources, predators, pathogens and parasites. Disadvantages include the possibility to not find a suitable new site, the predation during the transfer, the failure to locate a mate and the outbreeding depression.

Besides the spatial aspects, dispersal processes can be considered a mechanism that takes place also through time. Some invertebrate taxa may achieve temporal dispersal through the accumulation and the subsequent release of dormant propagules (eggs, statoblasts, or cells against desiccation) that create a reserve of genetic material analogous to seed banks in plant. Such dispersal in time is a function of the dormant period of the propagules and therefore can vary considerably between taxa (Bilton et al. 2001). Dormancy, or the occurrence of hypometabolism at certain stages of the life cycle, is an ubiquitous strategy used by organisms to bridge unfavourable periods (Brendonck and De Meester 2003). Because dormant propagules can remain viable for long periods (Hairston et al. 1995), they can theoretically hatch and recolonize a local habitat long after the active population has become extinct due to temporary unsuitability of ecological conditions (Mergeay et al. 2007).

Species traits determine the ability of a species to deal with environmental problems and opportunities, so they can potentially be used to explain occurrence under particular environmental conditions (e.g. Keddy 1992, McGill et al. 2006). Many studies have successfully related species traits to differences in species occurrence between locations or periods (Statzner et al. 1994, Bremner et al. 2006, Van Kleef et al. 2006). Consequently, trait-based analyses are considered to be a better candidate for the monitoring of ecosystem dynamics than taxon-based analyses (Statzner et al. 2001, Bonada et al. 2006, Mouillot et al. 2006). Ecosystem level processes are influenced by the functional characteristics of the organisms involved, rather than by their taxonomic identity (Hooper et al. 2002). Functional groups have been defined as sets of species showing either similar responses to the environment or similar effects on major ecosystem processes (Gitay and Noble 1997). Thus, two types of functional groups can be used. Functional effect groups are used when the goal is to investigate the effects of species on ecosystem properties (e.g. trophic groups); functional response groups are used when the goal is to investigate the response of species to changes in the environment, such as disturbance, resource availability or climate (e.g. life strategies - Gascón et al. 2008).

Freshwater biology has placed an increasing importance on processes at “mesoscale” (Holt 1993) between community ecology and biogeography. This perspective is central in studies of spatial and temporal interactions in communities and ecosystems, and a more refined understanding of significant biotic interfaces between terrestrial and aquatic systems (Bohonak and Jenkins 2003).

Considering a metapopulation as a set of local populations linked by dispersal (Hanski 1999), metapopulation theory can contribute to explain if the dynamics of individual population depend at least partially on interactions among populations, and can not be predicted from single population parameters alone. Freshwater invertebrate populations vary in time and space in terms of size or recruitment to a diapausing egg bank, and may be subjected to local extinctions (e. g. Cáceres 1997, Berendonk and Bonsall 2002). If dispersal in space or recruitment from a long-lived egg bank appreciably influences community dynamics (Cáceres and Hairston 1998), freshwater invertebrates can be considered metapopulations and metacommunities under the broadest definition. Community structure and function can be influenced by local or regional processes, but also by processes that involve particular local and regional effects in a specific combinations (Fig. 5.1, from Bohonak and Jenkins 2003).

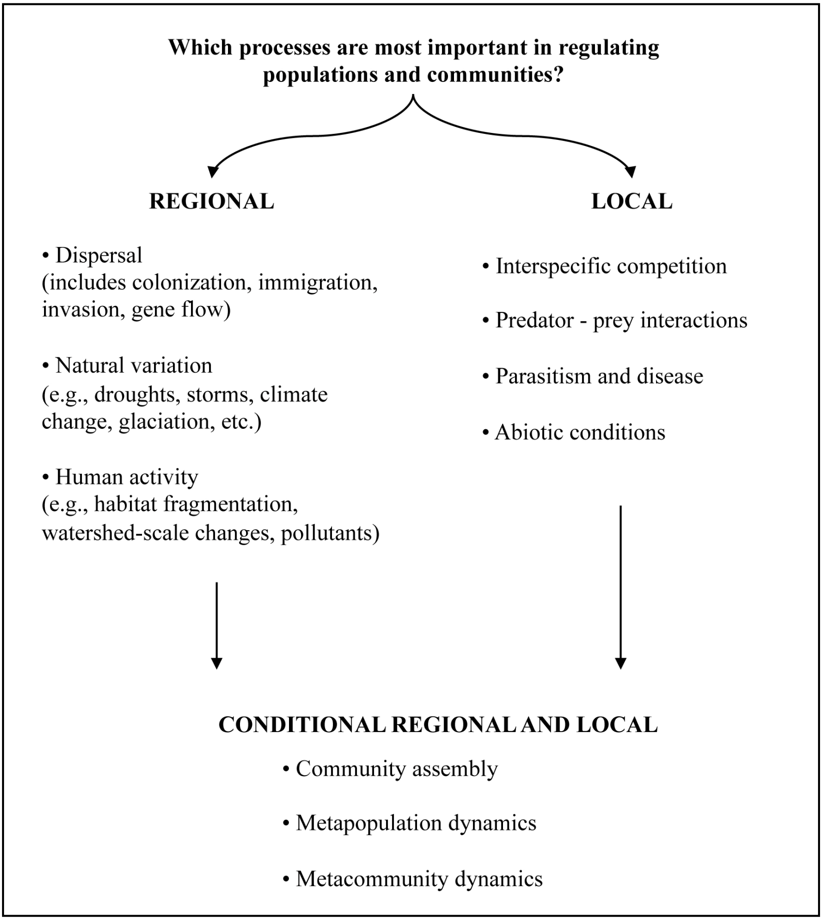

Fig. 5.1. Conceptual framework of regional and local processes that influence community assemblage and regulation (from Bohonak and Jenkins 2003).

In order to evaluate if newly created pond and constructed wetlands can be really considered part of the regional ecological network, the macroinvertebrate community assemblages of a wetland set has been analyzed. The communities were characterized considering the presence and the abundance of four life-strategy groups (Wiggins et al. 1980, Gascón et al. 2008), which are based on the taxa dispersal mode, its capacity to survive basin desiccation and the need of water to reproduce. Macroinvertebrate taxa were recognized at the deeper level possible, in order to accurately describe each ecosystem.
The objectives were to investigate if macroinvertebrate community are influenced by pond type (classified as natural, artificial and constructed), to evaluate if there were seasonal differences in community assemblages and to analyze the pond set through a functional approach, evaluating the patterns in life-strategy groups abundances.

5.2 Methods

5.2.1 Study site

Within Parco Pineta 24 ponds and pools were selected to analyse the macroinvertebrate community composition considering the life-strategy groups and the dispersal mode. The wetlands considered for this study have been classified as natural (16), artificial (6) and constructed (2), depending on their origin (Fig. 5.2). They were characterized by different morphology, environmental variables and dimensions (as described in Chapter 4).

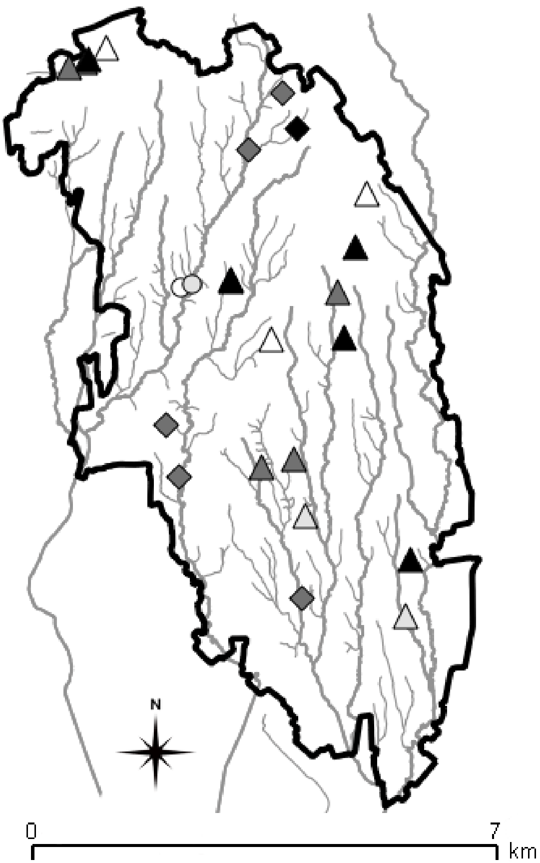

Fig. 5.2. Location of the wetlands within the park territory. Wetlands are represented as categories (triangle=natural pond, diamond=artificial Park pool, circle=constructed wetland). The colour represents the water body dimension (black $\leq$ 20 m^2, dark-grey=21-50 m^2, light-grey=51-200 m^2, white>200 m^2).

The relative distances between wetlands were measured through a GIS software. The measures (expressed in meters) represent distances between the centroids of the polygons representing the wetland surfaces. The nearest pond to each other and the distance to it are reported in Table 5.1.

Table 5.1. Distances to the nearest pond in the park territory.

Wetland ID	Distance to the nearest pond (m)	Nearest pond
A01	1090.42	CB03
A04	736.93	A06
A06	691.52	A14
A14	691.52	A06
B01	315.30	VO05
BF01	807.80	A04
C05	1233.44	T17
CB01	19.74	CB03
CB03	19.74	CB01
CB07	816.85	CB09
CB08	575.22	CB09
CB09	575.22	CB08
CW01	27.70	CW01
CW02	27.70	CW02
LM01	866.35	LM03
LM03	866.35	LM01
T12	498.08	T14
T14	498.08	T12
T17	863.45	T14
T27	803.33	T28
T28	803.33	T27
VO01	77.02	VO05
VO03	260.88	VO01

5.2.2 *Macroinvertebrate community analysis*

Macroinvertebrates were sampled seasonally in Summer and Autumn 2010 and in Spring 2011 by semiquantitive samplings. Within each pond samples have been taken with a 500 µm mesh size net (0.05 m^2), with sweeps in rapid sequence (two replicates for the smallest ponds; four replicates for the bigger ponds, as suggested in Gascón et al. 2008) from every different identified habitat. Sampled macroinvertebrates were preserved in 90% ethanol before being sorted, counted, identified at the lowest taxonomical level possible (usually species or genus; family for Diptera and Oligochaeta) and then conserved in 4% formaldehyde.

Taxa richness and Shannon index of each pond were calculated for every considered season. Macroinvertebrates collected during the seasonal qualitative sampling campaigns were divided into four life-strategy groups (see Appendix I), based on dispersal ability, need of water to reproduction and survival capacity during basin desiccation (Wiggins et al. 1980, Tachet et al. 2002). The distinctive features of each life-strategy group (Wiggins et al. 1980) are reported below and resumed in Table 5.2.

- **Group 1:** *Overwintering residents.* These organisms are permanent residents, living in the basin during winter and dry periods. They have life stages resistant to droughts, such as resistant eggs or cysts that lie more or less exposed on the dry pool basin. Among taxa that do not have resistant egg, juveniles or adults find protection in bottom sediments. All these organisms are able to make passive dispersion only. (Examples: Oligochaeta, Hirudinea, Decapoda, Isopoda, Gastropoda).

- **Group 2:** *Overwintering spring recruits.* These organisms aestivate and overwinter in the dry pool basin but they are able to disperse as adult insects, or as parasites on winged adult insects. Dispersal and recruitment are limited to spring. Oviposition depend on water, so it has to happen before drought period. Various stages (eggs, larvae or adults) can survive the dry period. (Examples: Ephemeroptera, Trichoptera, Coleoptera, Diptera such as Chironomidae, Culicidae and Ceratopogonidae).

- **Group 3:** *Overwintering summer recruits.* These colonizer organisms enter the pool basin during the desiccation because oviposition is independent of water. Oviposition may be preceded by a larval or ovarian diapause away from water. For this reason the recruitment happen at various times during the summer. Most species overwinter as eggs, or in same cases (some Trichoptera families) as larvae in a gelatinous eggs matrix. (Examples: Odonata, Trichoptera, Diptera such as Sciomyzidae).

- **Group 4:** *Non-wintering spring migrants.* Their oviposition depend on water and these organisms enter temporary pools in spring. Adults leave pools before dry period which they spend mainly in permanent waters. In this way, their strategy is to avoid rather than tolerate desiccation. They are able to exploit the resources of temporary pools; most of them are predators and their late spring recruitment to the temporary pools coincides with larger prey size and density. Their exploitation of temporary pools is based on enhancement of dispersal and colonizing tendencies. (Examples: some Ephemeroptera families, Diptera such as Chaoboridae, Odonata such as *Anax spp.*, Hemiptera, some Coleoptera families).

Table 5.2. Main characteristics of the four life-strategy groups (Wiggins et al. 1980, Gascón et al. 2008).

Group	Type of dispersion	Need of water to reproduce	Can survive desiccation in the basin
1	passive	yes	yes/no
2	active	yes	yes
3	active	no	yes
4	active	yes	no

5.3 Results and discussion

For every pond taxa richness and Shannon index have been calculated for each seasonal sampling campaign. Then the indices mean values for natural (NAT), constructed (CW) and artificial (PARK) ecosystem categories were calculated in order to compare the biodiversity hosted by the three wetland types.
Also the abundance of each life-strategy group was calculated for every pond and then considered as mean within the wetland categories. The mean composition of each wetland category (NAT, PARK, CW) in terms of life-strategy group abundances is represented in Figure 5.3. It is possible to observe that the relative abundance of each group and consequently the community assemblages varied considerably between wetland categories and between seasons.

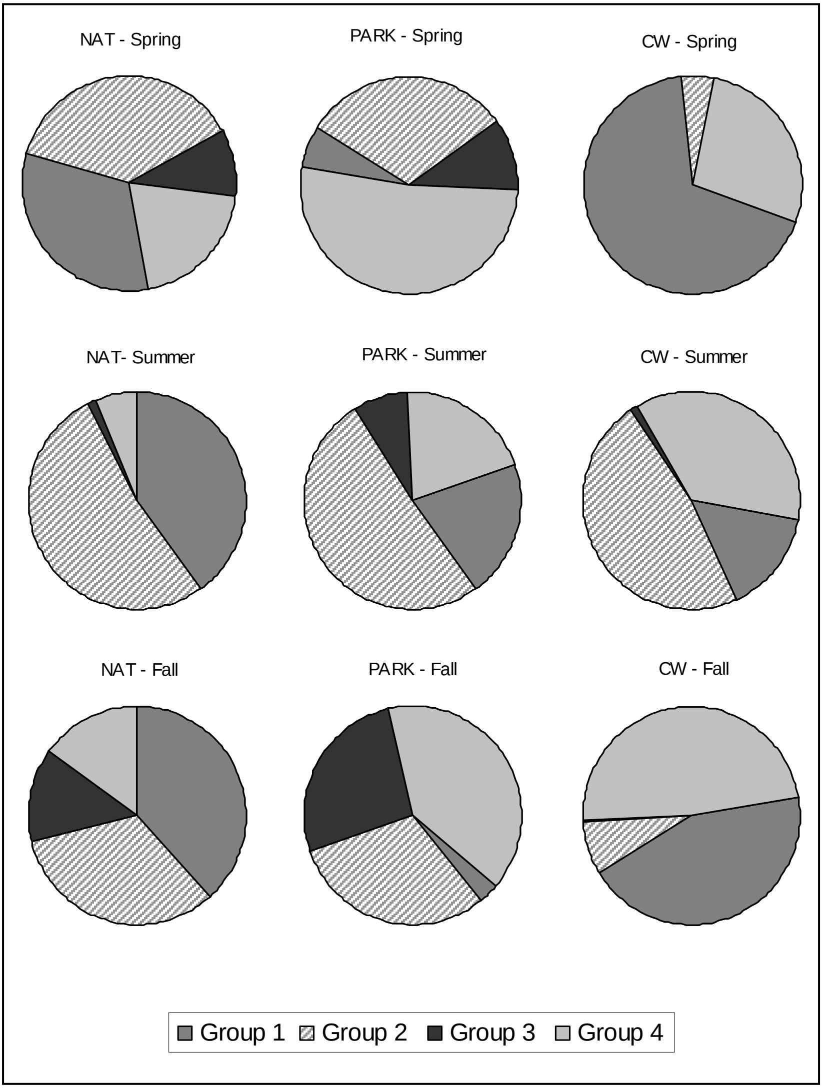

Fig. 5.3. The seasonal community compositions of each wetland category (NAT, PARK, CW) considering the four life-strategy groups.

One-way analysis of variance (ANOVA) was used to check the influence of the wetland type in the macroinvertebrate local biodiversity and in the abundance of each life-strategy group. In the model evaluating the biodiversity differences there was a significant variation ($\alpha=0.05$) among and within the three groups of sites for taxa richness ($p=0.035$). The model resulted also significant for Group 1 ($p<0.0001$) and Group 4 ($p<0.0001$) abundances. All the one-way ANOVA results are presented in Table 5.3.
Subsequent pair-wise comparison were performed using Tukey's HSD post hoc tests (HSD, honestly significant difference). No significant differences in the biodiversity indices were found between NAT, PARK and CW categories. Instead, significant differences ($\alpha = 0.05$) in the abundance of the four life-strategy groups were found between the three wetland types. In particular, the differences were significant for the abundance of Group 1 and Group 4 (Table 5.4). Group 1 seemed to prefer natural and constructed wetlands rather then artificial park pools; Group 4 seemed to be less abundant in natural ponds (Fig. 5.4 a,b).

Table 5.3. One-way ANOVA table for biodiversity indices and for the life-strategy groups abundances in the three wetland types (*P*-value standard alpha level = 0.05).

Metrics		**DF**	**Sum of squares**	**Mean of squares**	**F value**	**P value**
Taxa richness	Model	2	99.539	49.769	3.512	**0.035**
n = 70	Residuals	67	949.604	14.173	-	-
	Total	69	1049.143	-	-	-
Shannon index	Model	2	0.973	0.486	1.031	0.362
n = 70	Residuals	67	31.603	0.472	-	-
	Total	69	32.576	-	-	-
Group 1	Model	2	1.024	0.512	9.865	**<0.0001**
n = 70	Residuals	67	3.477	0.052	-	-
	Total	69	4.500	-	-	-
Group 2	Model	2	0.219	0.109	1.386	0.257
n = 70	Residuals	67	5.281	0.079	-	-
	Total	69	5.500	-	-	-
Group 3	Model	2	0.108	0.054	2.523	0.088
n = 70	Residuals	67	1.436	0.021	-	-
	Total	69	1.544	-	-	-
Group 4	Model	2	0.853	0.426	11.274	**<0.0001**
n = 70	Residuals	67	2.534	0.038	-	-
	Total	69	3.387	-	-	-

Table 5.4. Significant differences (Tukey's HSD post hoc tests, α = 0.05) in the abundance of life-strategy groups between the different wetland category. Only significant *p*-value are reported.

	Group 1	Group 2	Group 3	Group 4
NAT-CW	-	-	-	0.021
NAT-PARK	<0.0001	-	-	<0.0001
CW-PARK	0.010	-	-	-

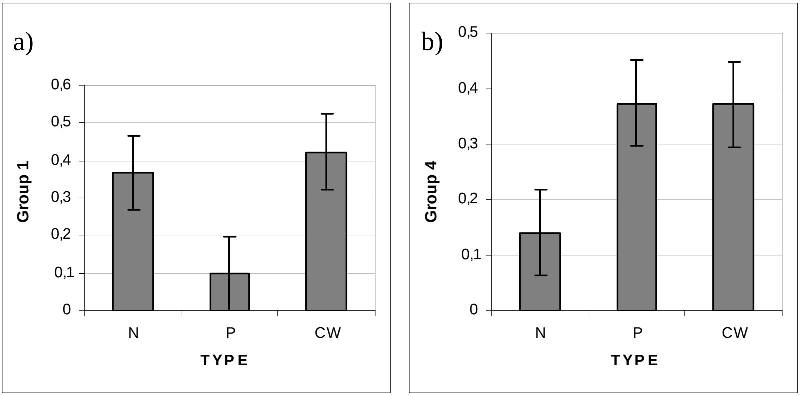

Fig. 5.4 a,b. Abundances (mean and st. error) of Group 1 and Group 4 in the three wetland types (N=natural ponds, P=park artificial pools, CW=constructed wetlands).

In order to evaluate if the differences in biodiversity and life-strategy group abundances were influenced by the season besides the wetland origin, a two-way ANOVA was performed (Table 5.5). The model resulted significant (α=0.05) for Shannon index (p=0.018) but not for taxa richness. The ANOVA model resulted also significant for the abundance of Group 1 (p=0.002) and Group 4 (p<0.0001), which were both mainly influenced by wetland type, and for Group 3 (p=0.034).

Tukey's HSD post hoc tests were performed to obtain pair-wise comparisons. Significant differences ($\alpha=0.05$) in Shannon index resulted between NAT ponds in summer and in spring ($p=0.008$). The Group 1 relative abundance resulted significantly different between wetland types (CW-PARK: $p=0.009$; NAT-PARK: $p<0.0001$), showing low preference for Park artificial pools in all the seasons. The abundance of Group 2 resulted to be not influenced significantly by wetland type and not either by season. Group 3 resulted significantly different mainly for season (autumn-summer: $p=0.008$) showing a decrease of abundance in summer and an increase during autumn. Group 4 resulted to be influenced both by wetland type and season, with less preference for NAT ponds (NAT-PARK: $p<0.0001$; NAT-CW: $p=0.013$) and significant seasonal differences in abundance (spring-summer: $p=0.010$).
All these results agree with the main features of the life-strategy groups. Group 1, which is permanent and drought resistant, seemed to not be influenced by season in the populations of the pond set. Group 2 also was not influenced by seasonality, being composed by resistant and drought tolerant taxa. Group 3 had a seasonal increase in autumn, due to the population raise that happen after the summer eggs deposition. Group 4 is the more exigent and specialized, and showed to be influenced both by season and wetland type. Probably the scarce preference for natural ponds depended on the hydrologic variations and drought periods that characterized those ecosystems, which affect the inability to survive basin desiccation.

Table 5.5. Two-way ANOVA table for biodiversity indices and for the life-strategy groups abundances, considering wetland type and season (*P*-value standard alpha level = 0.05).

Biological metrics		**DF**	**Sum of squares**	**Mean of squares**	**F value**	**P value**
Taxa richness	Model	8	103.679	21.710	1.513	0.172
n = 70	Residuals	61	875.464	14.352	-	-
	Total	69	1049.143	-	-	-
	Type	2	102.244	51.122	3.562	**0.034**
	Season	2	33.257	16.628	1.159	0.321
	Type*Season	4	65.208	16.302	1.136	0.348
Shannon index	Model	8	8.164	1.021	2.550	**0.018**
n = 70	Residuals	61	24.412	0.400	-	-
	Total	69	32.576	-	-	-
	Type	2	1.106	0.553	1.382	0.259
	Season	2	1.248	0.624	1.559	0.219
	Type*Season	4	2.562	0.641	1.601	0.186
Group 1	Model	8	1.457	0.182	3.651	**0.002**
n = 70	Residuals	61	3.043	0.050	-	-
	Total	69	4.500	-	-	-
	Type	2	1.031	0.515	10.332	**<0.0001**
	Season	2	0.066	0.033	0.660	0.520
	Type*Season	4	0.411	0.103	2.061	0.097

Table 5.5 (continued). Two-way ANOVA table for biodiversity indices and for the life-strategy groups abundances, considering wetland type and season (*P*-value standard alpha level = 0.05).

Biological metrics		**DF**	**Sum of squares**	**Mean of squares**	**F value**	**P value**
Group 2	Model	8	0.926	0.116	1.544	0.161
n = 70	Residuals	61	4.574	0.075	-	-
	Total	69	5.500	-	-	-
	Type	2	0.230	0.115	1.536	0.224
	Season	2	0.565	0.282	3.765	**0.029**
	Type*Season	4	0.074	0.019	0.248	0.910
Group 3	Model	8	0.354	0.044	2.265	**0.034**
n = 70	Residuals	61	1.190	0.020	-	-
	Total	69	1.544	-	-	-
	Type	2	0.111	0.055	2.838	0.066
	Season	2	0.066	0.033	1.703	0.191
	Type*Season	4	0.053	0.013	0.674	0.613
Group 4	Model	8	1.357	0.170	5.099	**<0.0001**
n = 70	Residuals	61	2.030	0.033	-	-
	Total	69	3.387	-	-	-
	Type	2	0.876	0.438	13.171	**<0.0001**
	Season	2	0.135	0.068	2.032	0.140
	Type*Season	4	0.160	0.040	1.205	0.318

To deepen the relationships between life-strategy groups and the local biodiversity measured in each pond, linear regressions were performed. Significant relationships (α=0.05) were found between taxa richness and the abundance of Group 2 (p=0.047) and between Shannon index and both Group 2 (p=0.002) and Group 4 (p=0.001). As it is possible to observe in figure 5.5 a, Shannon index decreased when the Group 2 abundance increased. Probably taxa of Group 2 use their adaptability to gain dominance in the local population. Shannon index showed the opposite trend with the abundance of Group 4 (Fig. 5.5 b). Taxa of Group 4, that are more specialized and exigent, made the biodiversity increase.

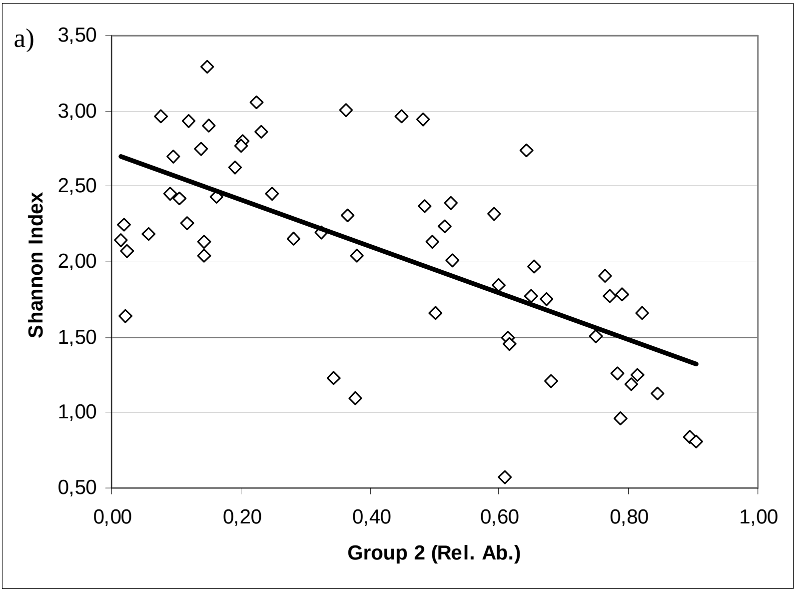

Fig. 5.5 a. Linear regressions showing the significant correlations between Shannon index and the relative abundances of Group 2 (p=0.002, R^2= 0.421) and Group 4 (p=0.001, R^2=0.310).

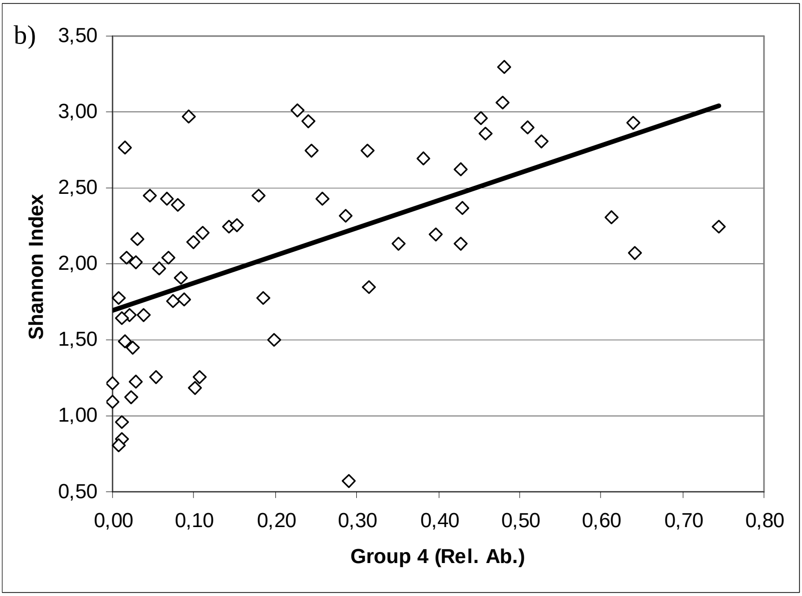

Fig. 5.5 b. Linear regressions showing the significant correlations between Shannon index and the relative abundances of Group 2 (p=0.002, R^2= 0.421) and Group 4 (p=0.001, R^2=0.310).

To evaluate how the environmental variables could influence the macroinvertebrate dispersion and the abundance of each life-strategy group, a principal components analysis (PCA) was performed (Fig. 5.6). Habitat heterogeneity (intended as the number of available microhabitat in each wetland) related to water body area was expressed through the A-M Index (see Chapter 2). The seasons were also considered in the analysis. Between all the observed environmental variables only those that resulted to be the most significant in a preliminary screening were considered in the final PCA analysis. Group 1 did not show any significant correlation with the considered variables. Group 2 resulted to be negatively related to Shannon index (p<0.05, R=-0.371) and A-M Index (p<0.05, R=-0.340), thus confirming the result showed through the linear regression. It also showed a negative correlation (p<0.05, R=-0.332) with summer. Group 3 resulted to be positively correlated to autumn (*p*<0.05, R=0.311) and negatively correlated to summer (*p*<0.05, R=-0.295), but did not show any significant correlation to biodiversity indices. Group 4 resulted to be positively related to taxa richness (*p*<0.05, R=0.238), to Shannon index (*p*<0.05, R=0.398) and even to A-M Index (*p*<0.05, R=0.274). It showed also a negative correlation to summer (*p*<0.05, R=-0.298). All these results agree with the ANOVA models, confirming that Group 4 was composed by specialized and exigent taxa that contributed do increase local biodiversity.

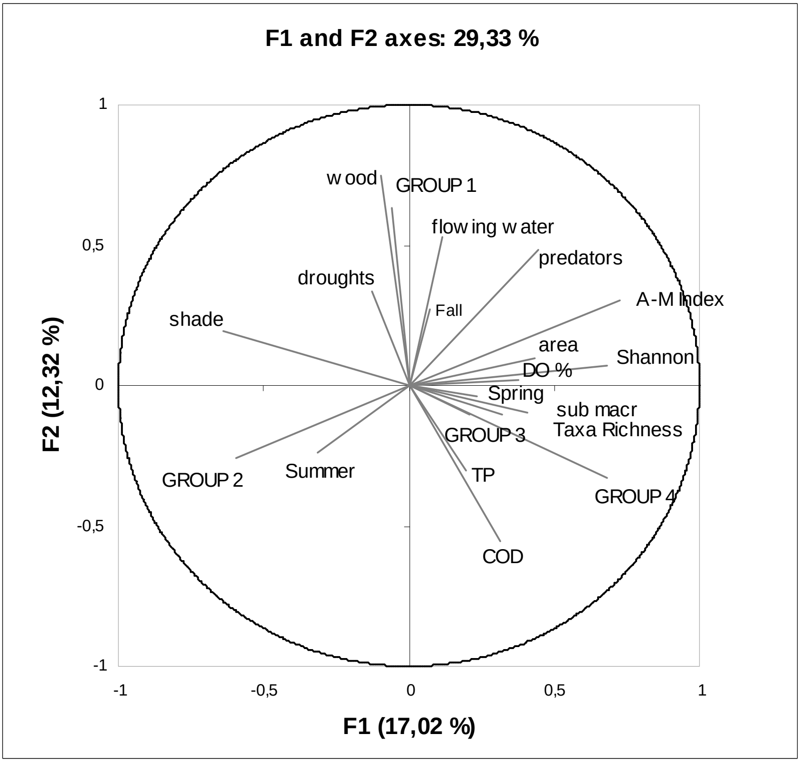

Fig. 5.6. PCA loadindg plot showing the significant correlations between biodiversity (expressed as taxa richness and Shannon index), life-strategy groups, environmental variables and seasons.

To better represent the significant relationships emerged from PCA between A-M Index and the abundance of groups 2 and 4, linear regressions were performed (Fig. 5.7 a,b). The two groups show opposite trends. An increase in habitat heterogeneity correspond to a decrease of the group 2 abundance and an increase of group 4 abundance. In these relationships fish presence did not seem to have any significant influence. In fact, the linear regressions resulted to be significant both considering or not considering vertebrate predators presence.

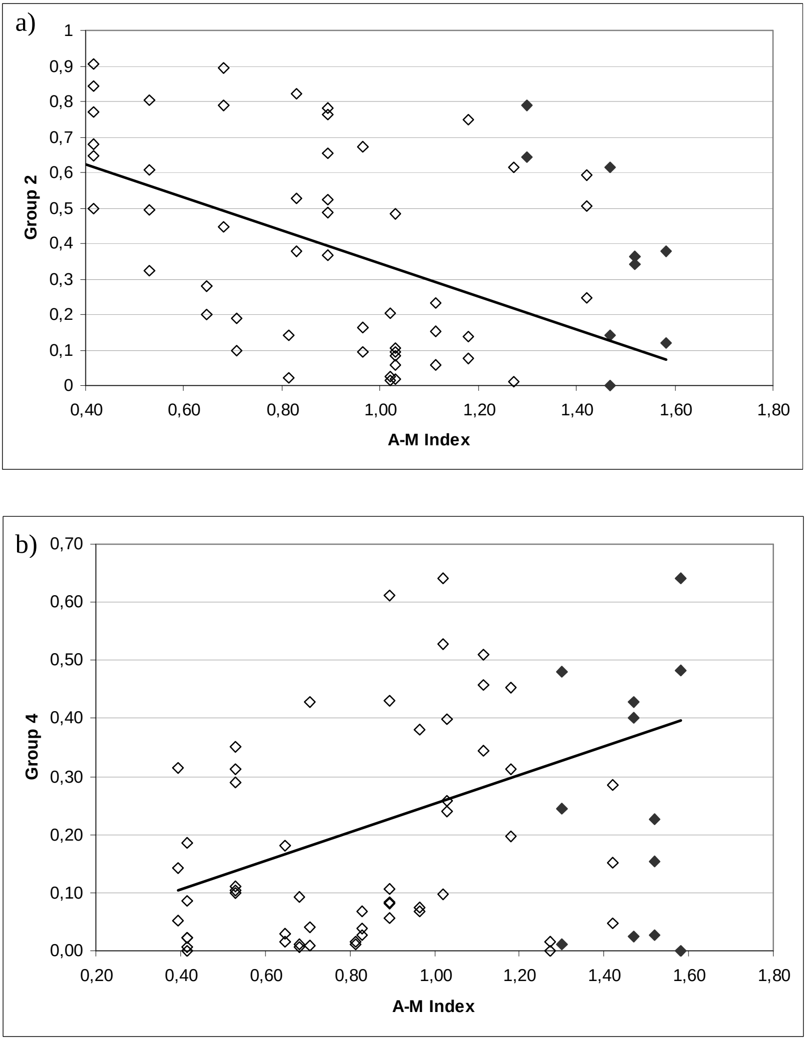

Fig. 5.7 a, b. Linear regressions showing the significant correlations between A-M Index and the relative abundances of Group 2 (p=0.004, R^2= 0.141) and Group 4 (p=0.014, R^2=0.102). Grey diamonds represent wetlands with vertebrate predators.

The functional approach in the community assemblage analysis made the comparison between different wetland types more effective. Although within a giving ecosystem category a multitude of environmental conditions act together, to use an approach based on life-strategy groups means to consider groups of individuals that are able to survive assuming the same ecologically successful strategy. Life-strategies of species provide a functional classification of macroinvertebrates across different systematic groups (Verberk et al. 2008) and have been frequently related to environmental characteristics of both lentic and lotic systems (e.g. Williams 1985, Richards et al. 1997). Previous studies assessed the main stress that influenced the community composition and adaptation is probably the water permanence, that plays an important role in the structure and composition in aquatic systems (e.g. Schneider 1999, Schwartz and Jenkins 2000, Gascón et al 2005), forcing the organism adaptation trough dispersion. However, as water permanence covaried with other environmental pond characteristics, also linked to food resources, it is not possible to split the various effects on the communities. The structure of a metacommunity may depend on specific traits of both the community members and the habitat they live within (Leibold and Miller 2004, Cottenie 2005).

In Pineta Park ponds passive dispersal taxa (Group 1) showed scarce preference for artificial pools, which were isolated and located in wooded areas, with low connection to the other wetlands. These artificial pools, created mainly for amphibian reproduction in areas where water is scarce, are isolated and small, and they are probably difficult to reach by invertebrates showing passive dispersion. Taxa adapted to avoid desiccation, with active dispersion and needing water for reproduction (Group 2), did not show preference for any wetland type but they seemed to hardly tolerate summer season, that lead up to droughts especially in some natural ponds. Group 3 did not showed any preference in wetland type and had a seasonal increase in autumn, due to the population raise that happen after the summer eggs deposition. Group 4 showed negative correlation with natural ponds. This scarce preference was probably related to hydrologic variations and droughts that affect the inability to survive desiccation. Interesting relationships between biodiversity and the relative abundances of each life-strategy group were found. In particular, taxa of Group 4 seemed to increase ecosystem biodiversity, thus having effects also at a regional scale. Because life-strategies have functional relationships with the duration, the degree and the predictability of habitat suitability in space and time (Verberk et al. 2008), they can provide useful information for ecosystems management in order to improve local biodiversity at metapopulation level.

5.4 Conclusions

The three different category of wetlands (natural, artificial and constructed) present a comparable biodiversity level, although they showed significant differences in community composition choosing a functional analysis approach.

Considering the life-strategy groups, the community composition seems to be related to seasonality, as well as wetlands typology. Seasonal variation in hydrological conditions and resource availability influenced the macroinvertebrate dispersal and their feeding habits. Environmental conditions, varying through seasons, created various ecological niches that were exploited in different ways during the year.

It was possible to observe seasonal variation in biodiversity, that seems to be related to life-strategy groups composed by more specialized taxa (mainly Groups 2 and 4).

Wetland biodiversity resulted significantly related to the microhabitat availability, which is connected to the area. The most sensitive group to the availability of microhabitats is 4, and this make sense because it is composed by very specialized taxa, with low resilience.

These aspects are important for planning and management purposes. Microhabitat diversification and appropriate allocation of surface can be useful to increase the overall local and regional biodiversity.

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Chapter 6

General conclusions

Being aware of the distinctive features and processes taking place in wetlands, their insertion is considered useful as restoration measure for different aims and necessities. Within Parco Pineta artificial wetlands have been realized to solve impacts rising from the presence of untreated wastewater discharges and for conservation purposes, creating new ecosystems where water resource was scarce.
The analyses carried out on the constructed wetland system demonstrated that it can be a valid and efficient method to treat wastewater in sparse residential areas where no other treatment services can be implemented, especially in such valuable natural contests. The pollutants removal was in line with results reported in literature for similar plants, also in disinfection mechanisms, that appeared particularly efficient. Although the differences in the efficiency between the various treatment phases and between season for some water quality parameters, the CW system removal permitted to discharge in respect of the national law prescriptions. Moreover these interventions can be considered suitable elements of the local ecological network, creating new resources at many levels.
In fact, considering the macroinvertebrate community and analyzing the biodiversity hosted in these artificial wetlands, no significant differences were found between artificial and natural ecosystems. Even the constructed wetlands, which were characterized by low water quality and higher pollutants concentrations, presented a biodiversity level which in some cases exceeded that one present in natural ecosystems. Even though biodiversity was similar between wetland categories, differences in community compositions have been enlightened. The macroinvertebrate community assemblages seemed to be influenced more by the geographical and hydromorphological variables of the ecosystem rather than the physicochemical water characteristics. In particular, water body area and habitat heterogeneity (intended as the number of available microhabitat) resulted to be the most important variables that

influenced the community composition. These two variables, considered together, could significantly explain the variability within different community even in contiguous and connected ponds. According to the “habitat diversity hypothesis” (Williams 1964), in larger ponds species richness increases more than in smaller ponds. Moreover, in the pond set considered in this study, larger wetlands were the only ones to be characterized by fish and turtles presence, that has resulted to be a limiting factor for macroinvertebrate biodiversity.

Broadening the perspective at the mesoscale population, the spatial and temporal interactions between communities and ecosystems became more relevant than single population parameters considered alone. Dispersal mode and species traits determine the ability to deal with adverse environmental conditions or to successfully exploit resources. The studied wetland categories resulted to be differently preferred by the macroinvertebrate life-strategy groups, defined as those proposed by Wiggins et al. (1980). Seasonal variations in hydrological conditions and resource availability were the main factors that influenced the macroinvertebrate dispersal and their feeding habits. Environmental conditions, varying through seasons, created various ecological niches that were exploited in different ways during the year.

Although the considered ponds and wetlands presented different features, they all contributed to the local ecological network even if they were not all equally interconnected together. Even the more recent ponds can acquire species very rapidly (Gee et al. 1997), and pond creation should be a powerful restoration tool (Williams et al. 2008). At a regional level, ponds can contribute highly to freshwater biodiversity, with recent evidence showing that they often support considerably more species, more unique species and more scarce species than other water body types (Williams et al. 2004). Creation of new ecosystems has to proceed at the same time of conservation of the existing ones (Oertli et al. 2005). Conservation management of wetlands needs to extend beyond the traditional approach of management of protected areas, focused mainly on communities and species; it has to deal with protection or recovery of flow regimes, complicate by effects of climate change (Kingsford 2011).

The present general overview of taxonomic and functional biodiversity of aquatic and riparian ecosystems has produced a faunal checklist of the aquatic ecosystems in the park (reported in Appendix I and Appendix II). The results of this project enlightened the effectiveness of the interventions carried out by the Park management for conservation and restoration of the aquatic and riparian freshwater ecosystems. All the collected information could be useful to design further possible interventions for conservation aims.

6.1 References

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Appendix I

Macroinvertebrate taxa collected during the three years study period in the considered 24 wetlands in Parco Pineta. For each taxon Life-Strategy Group (L-S Group), Dispersal mode (A= active; P= passive) and resistance form have been reported.

Class	**Order**	**Family**	**Genus**	**Species**	**L-S Group**	**Dispersal Mode**	**Resistance Form**
Insecta	Ephemeroptera	Baetidae	*Cloeon*		4	aquatic A aerial A	none
		Baetidae	*Baetis*		4	aerial A.	none
		Siphlonuridae	*Siphlonurus*	*S. lacustris*	2	aerial A.	eggs
	Plecoptera	Nemouridae	*Nemoura*		4	aquatic A aerial A	none
		Taeniopterygidae	*Brachyptera*		4	aquatic A	dormancy
	Trichoptera	Hydropsychidae			4	aquatic A aerial A	none
		Phryganeidae	*Oligotrichia*	*O. striata*	4	aquatic A aerial A	dormancy
		Polycentropodidae	*Plectrocnemia*		4	aerial A	none
		Leptoceridae			4	aquatic A aerial A	none
		Sericostomatidae			4	aquatic A aerial A	none
		Limnephilidae	*Glyphotaelius*	*G. pellucidus*	3	aerial A	dormancy
		Limnephilidae	Limnephilinae (sF)	Limnephilini (tr)	3	aerial A	eggs

Class	Order	Family	Genus	Species	L-S Group	Dispersal Mode	Resistance Form
Insecta	Trichoptera	Brachycentridae			4	aquatic A aerial A	none
	Coleoptera	Dytiscidae	*Dytiscus*		4	aerial A	none
		Dytiscidae	*Acilius*		4	aerial A	none
		Dytiscidae	*Agabus*		2	aerial A	none
		Dytiscidae	*Hydroglyphus*		4	aerial A	none
		Dytiscidae	*Hydroporus*		4	aerial A	none
		Dytiscidae	*Hyphydrus*		4	aerial A	none
		Dytiscidae	*Ilybius*		4	aerial A	none
		Dytiscidae	*Noterus*		2	aerial A	cocoons
		Dytiscidae	*Suphrodytes*		4	aerial A	none
		Elmidae	*Normandia*		4	aerial A	none
		Haliplidae	*Haliplus*		2	aerial A	none
		Helodidae	*Elodes*		2	aerial A	none
		Helodidae	*Microcara*		2	aerial A	none
		Helodidae	*Scirtes*		2	aerial A	none
		Hydraenidae			4	aerial A	none
		Hydrophilidae	*Coelostoma*		4	aerial A	none
		Hydrophilidae	*Enochrus*		4	aerial A	none
		Hydrophilidae	*Helochares*		4	aerial A	none
		Hydrophilidae	*Hydrobius*		2	aerial A	none
		Hydrophilidae	*Hydrochara*		4	aerial A	none

Class	Order	Family	Genus	Species	L-S Group	Dispersal Mode	Resistance Form
Insecta	Coleoptera	Hydrophilidae	*Laccobius*		4	aerial A	none
		Hygrobiidae	*Hygrobia*		4	aerial A	none
		Limnebiidae	*Limnebius*		4	aerial A	none
	Megaloptera	Sialidae	*Sialis*		3	aquatic A	n.a.
	Odonata	Aeshnidae	*Aeshna*	*A. isosceles*	2	aerial A	none
		Aeshnidae	*Aeshna*	*A. cyanea*	2	aerial A	eggs
		Aeshnidae	*Aeshna*	*A. mixta*	2	aerial A	eggs
		Aeshnidae	*Anax*	*A. parthenope*	4	aerial A	eggs, dormancy
		Aeshnidae	*Anax*	*A. imperator*	4	aerial A	eggs, dormancy
		Coenagrionidae	*Coenagrion*	*C.puella/ pulchellum*	4	aerial A	none
		Coenagrionidae	*Ischnura*		4	aerial A	none
		Coenagrionidae	*Ischnura*	*I. elegans*	4	aerial A	none
		Coenagrionidae	*Pyrrhosoma*	*P. nymphula*	4	aerial A	none
		Cordulegasteridae	*Cordulegaster*	*C. boltonii*	4	aerial A	none
		Lestidae	*Lestes*	*L. viridis*	3	aerial A	eggs
		Libellulidae	*Orthetrum*	*O. coerulescens*	4	aerial A	none
		Libellulidae	*Sympetrum*		4	aerial A	none
		Libellulidae	*Libellula*	*L. depressa*	4	aerial A	none
		Ceratopogonidae			2	aerial A	eggs

Class	Order	Family	Genus	Species	L-S Group	Dispersal Mode	Resistance Form
Insecta	Diptera	Chaoboridae			3	aquatic A	eggs, dormancy
		Chironomidae		Chironomini (tr)	2	aquatic A, P aerial A, P	dormancy
		Culicidae			3	aerial A	eggs, dormancy
		Muscidae	*Lispe*		2	aquatic A	none
		Sciomyzidae			3	aquatic A aerial A	dormancy
		Simuliidae			1	aquatic P aerial P	eggs, dormancy
		Stratiomyidae			2	aerial A	dormancy
		Limoniidae			1	aerial P	none
		Tipulidae			2	aquatic A aerial A	cocoons
		Dixidae	*Dixa*		1	aquatic P	dormancy
		Psychodidae			4	aquatic A, P aerial A	none
		Athericidae	*Atherix*		3	aquatic A aerial A	none
		Empididae			3	aerial A	n.a.
		Tabanidae			2	aerial A	dormancy
		Dolichopodidae			3	aerial A, P	cocoons, dormancy

Class	Order	Family	Genus	Species	L-S Group	Dispersal Mode	Resistance Form
Insecta	Hemiptera	Hydrometridae	*Hydrometra*		4	aquatic A	dormancy
		Gerridae	*Gerris*		4	aquatic A aerial A	eggs, dormancy
		Nepidae	*Nepa*	*N. cinerea*	4	aquatic A aerial A	eggs
		Nepidae	*Ranatra*		4	aquatic A aerial A	eggs, dormancy
		Notonectidae	*Notonecta*		4	aquatic A aerial A	eggs, dormancy
		Veliidae	*Velia*		4	aquatic A	dormancy
		Veliidae	*Microvelia*		4	aquatic A	dormancy
		Mesoveliidae	*Mesovelia*		4	aquatic A	eggs, dormancy
		Corixidae	*Corixina*		4	aquatic A aerial A	dormancy
		Pleidae	*Plea*	*P. minutissima*	4	aquatic A aerial A	dormancy
		Naucoridae	*Naucoris*		4	aquatic A aerial A	dormancy
	Lepidoptera	Crambidae	*Paraponyx*	*P. stagnata*	3	aerial A	cocoons, dormancy
		Crambidae	*Nymphula*		3	aerial A	cocoons, dormancy
		Crambidae	*Acentria*		3	aquatic A aerial A	dormancy

Class	Order	Family	Genus	Species	L-S Group	Dispersal Mode	Resistance Form
Insecta	Lepidoptera	Crambidae	*Cataclysta*	*C. lemnata*	4	aerial A	cocoons, dormancy
Gastropoda	Prosobranchia	Bithyniidae	*Bithynia*		1	aquatic P	dormancy
	Pulmonata	Ancylidae	*Ancylus*	*A. fluviatilis*	1	aquatic P	none
		Lymnaeidae	*Lymnaea*		1	aquatic P	dormancy
		Physidae	*Physa*		1	aquatic P	dormancy
		Physidae	*Aplexa*	*A. hypnorum*	1	aquatic P	dormancy
		Planorbidae	*Gyraulus*		1	aquatic P	dormancy
	Pulmonata	Planorbidae	*Planorbis*		1	aquatic P	dormancy, cells
		Acroloxidae	*Acroloxus*	*A. lacustris*	1	aquatic P	none
Bivalva	Veneroida	Sphaeriidae	*Sphaerium*	*S. corneum*	1	aquatic P	dormancy
		Pisidiidae	*Pisidium*		1	aquatic P	eggs, dormancy
Clitellata-Hirudinea	Arhynchobdellida	Erpobdellidae	*Dina*		1	aquatic P	cocoons
		Erpobdellidae	*Helobdella*		1	aquatic P	cocoons
		Erpobdellidae	*Erpobdella*		1	aquatic P	cocoons
		Haemopidae	*Haemopis*	*H. sanguisuga*	1	aquatic P	cocoons
Clitellata-Oligochaeta	Haplotaxida	Tubificidae			1	aquatic P	none
		Naididae			1	aquatic P	cocoons

Class	Order	Family	Genus	Species	L-S Group	Dispersal Mode	Resistance Form
Clitellata-Oligochaeta	Haplotaxida	Enchytraeidae			1	aquatic P	none
		Lumbricidae			1	aquatic P	none
		Haplotaxidae	*Haplotaxis*		1	aquatic P	cocoons, dormancy
	Lumbriculida	Lumbriculidae			1	aquatic P	cocoons
Adenophorea-Enoplia	Mermithida	Mermithidae			1	aquatic P	none
Malacostraca	Decapoda	Cambaridae	*Procambarus*	*P. clarkii*	2	aquatic A	none
	Amphipoda-Gammaridea	Niphargidae			2	aquatic P	none
	Isopoda	Asellidae	*Asellus*	*A. aqauticus*	1	aquatic P	dormancy

Appendix II

Fish (Table A), Reptiles (Table B) and Amphibians (Table C) present in Parco Pineta. Reptile and Amphibian data were provided by the park staff.

Table A. Parco Pineta fish checklist.

Fish				
Order	**Family**	**Genus**	**Species**	**Origin**
Cypriniformes	Cyprinidae	*Scardinius*	*S. erythrophtalmus*	native
		Carassius	*C. sp.*	exotic
		Rutilus	*R. erythrophtalmus*	native
		Leuciscus	*L. cephalus*	native
Cyprinodontiformes	Poeciliidae	*Gambusia*	*G. holbrooki*	exotic
Perciformes	Centrachidae	*Lepomis*	*L. gibbosus*	exotic
		Micropterus	*M. salmoides*	exotic
Siluriformes	Ictaluridae	*Ameiurus*	*A. melas*	exotic

Table B. Parco Pineta reptiles checklist.

Reptiles			
Order	**Family**	**Genus**	**Species**
Chelonia	Emydidae	*Trachemys*	*T. scripta*
Squamata	Anguidae	*Anguis*	*A. fragilis*
	Lacertidae	*Podarcis*	*P. muralis*
		Lacerta	*L. bilineata*
	Colubridae	*Hierophis*	*H. viridiflavus*
		Coronella	*C. austriaca*
		Zamenis	*Z. longissima*
		Natrix	*N. natrix*
	Viperidae	*Vipera*	*V. aspis*

Table C. Parco Pineta amphibians checklist.

Amphibians			
Order	**Family**	**Genus**	**Species**
Urodela	Salamandridae	*Salamandra*	*S. salamandra*
		Triturus	*T. carnifex*
		Triturus	*T. vulgaris*
Anura	Pelobatidae	*Pelobates*	*P. fuscus*
	Bufonidae	*Bufo*	*B. bufo*
	Hylidae	*Hyla*	*H. intermedia*
	Ranidae	*Rana*	*R. dalmatina*
			R. synklepton esculenta