



Mediterranean temporary ponds in Southern Portugal: key faunal groups as management tools?

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Abstract. Temporary ponds, in spite of being protected by the Habitats Directive (92/43/CEE), are presently disappearing at a fast rate. Urgent management measures are thus required, but which parameters should be used to establish management procedures? Since most large branchiopod species (LBS) inhabit exclusively temporary lentic ecosystems, and an important number of amphibian species (AS) also use those habitats for reproduction, they could be used as key faunal groups to assess the value of Mediterranean Temporary Ponds for conservation purposes. In particular, 11 taxa deserve special conservation concern and their habitats special management decisions (LBS: *Branchipus schaefferi*, *Streptocephalus torvicornis*, *Tanymastix* sp, *Maghrebestheria maroccana*, *Cyzicus grubei* and *Triops mauritanicus*; AS: *Pelodytes* sp and *P. ibericus*, *Bufo calamita*, *Hyla meridionalis* and *Triturus marmoratus pygmaeus*). Furthermore, each of them have different ecological requirements (total area, depth, hydroperiod length...). The aim of this work is to propose the use of (i) life cycles of referred LBS with no protection status, and (ii) reproduction periods (pairing, fertilization and larval development) of AS with protection status, as management tools for conservation purposes.

Key words: Freshwater large branchiopods, amphibians, temporary lentic ecosystems, Habitats Directive, hydroperiod.

Resumo: Charcos temporários mediterrânicos do Sul de Portugal: grupos faunísticos chave como ferramentas de gestão? As Lagoas Temporárias Mediterrâneas apesar de protegidas pela Directiva Habitats (92/43/CEE), estão presentemente a desaparecer a um ritmo elevado. Urgem medidas de gestão, mas que parâmetros usar para proceder a essa gestão? Sabendo que a maioria das espécies de grandes branquiópodes (LBS) habitam exclusivamente ecossistemas lênticos temporários e também um importante número de espécies de anfíbios (AS) utilizam aqueles habitats para reprodução, poder-se-ão usar estes como grupos-chave para avaliar o valor das Lagoas Temporárias Mediterrâneas com objectivos de conservação. Neste âmbito, 11 taxa merecem particular atenção, necessitando os seus habitats de decisões relativas à sua gestão. (LBS: *Branchipus schaefferi*, *Streptocephalus torvicornis*, *Tanymastix* sp, *Maghrebestheria maroccana*, *Cyzicus grubei* e *Triops mauritanicus*; AS: *Pelodytes* sp e *P. ibericus*, *Bufo calamita*, *Hyla meridionalis* e *Triturus marmoratus pygmaeus*). Cada espécie tem necessidades ecológicas particulares (área total, profundidade, duração do hidroperíodo...). O objectivo deste trabalho é o de propor o uso dos ciclos de vida das LBS, sem estatuto de protecção e os períodos de reprodução (acasalamento, fertilização e desenvolvimento larvar) das AS, com estatuto de protecção, como instrumentos de gestão que permitam a conservação destes habitats.

Palavras-chave: Grandes branquiópodes dulceaquícolas, anfíbios, ecossistemas lênticos temporários, Directiva Habitats, hidroperíodo.

Introduction

Temporary ponds are in general, small and shallow water bodies, easily overlooked and vulnerable to a large number of human activities. They are presently disappearing at a fast rate, particularly in the Mediterranean region, due to drainage, reclamation for agricultural or urban development, filling up with litter or wastes, pollution by fertilizers, pesticides or garbage, unsustainable agriculture or cattle raising, water subtraction, deepening for conversion into irrigation reservoirs and underground water disturbances and the present climate changes or “desertification/tropicalization” of Mediterranean landscapes. Mediterranean Temporary Ponds are protected under the Habitats Directive 92/43/CEE. However, Portugal still lacks management plans concerning these special and fragile habitats, particularly because the state of knowledge is still insufficient to establish management procedures (cf. Collinson *et al.* 1995, Grillas & Roché 1997).

Knowing that most freshwater large branchiopod species (LBS) inhabit exclusively these temporary lentic ecosystems (Alonso 1996), and also that an important number of amphibian species (AS) uses those habitats for reproduction (Barbadillo *et al.* 1999; Ferrand de Almeida *et al.* 2001), we propose that they may be used as key faunal groups to assess the value of Mediterranean temporary ponds for conservation purposes. From the last group, only few species as *Rana perezi* Seoane and *Bufo bufo* L. have a preference for permanent waters. In Portugal, LBS have no conservation status, in contrast with some European countries where several species already have special protection status (Biggs *et al.* 1994, Mura 1999, Boix *et al.* 2002); AS, on the contrary, are protected in Portugal (Ferrand de Almeida *et al.* 2001).

Among *taxa* inhabiting Mediterranean temporary ponds in Southern Portugal, 11 deserve particular conservation concern and their habitats special management decisions: i) LBS - *Branchipus schaefferi* Fischer, *Streptocephalus torvicornis* (Waga), *Tanyastix* sp, *Maghrebestheria marocana* Thiéry, *Cyzicus grubei* (Simon) and *Triops mauritanicus* (Ghigi); ii) AS: *Pelodytes* sp, and *P. ibericus* Sánchez-Herráiz, Barbadillo, Machordon & Sanchiz; *Bufo calamita* (Laurenti), *Hyla meridionalis* (Boettger), and *Triturus marmoratus pygmaeus* (Wolterstorff) (Barbadillo *et al.* 1999, Sánchez-Herráiz *et al.* 2000, Ferrand de Almeida *et al.* 2001, Cristo *et al.* 2002, Beja & Alcazar 2003). It is easy to realize that all those different species have different environmental demands and so, several different responses have been found. Understanding

the most important ecological parameters constraining the presence of these species is one of the main objectives of the present work. Furthermore, we propose to use life cycles of the referred LBS species (with no protection status), and the reproduction period (pairing, fertilization and larval development) of AS species (with conservation status) as management tools for the Mediterranean Temporary Ponds conservation.

Materials and Methods

Study area

Since 1996 several surveys have been carried out, mainly in the Southern region of Portugal, aiming at collecting data from LBS and AS. An ecological study with the objective of collecting detailed information on these habitats and covering biological, physical and chemical parameters, was carried out on the Guadiana region (GUAD) around Mértola and in the Parque Natural do Vale do Guadiana (Fig.1). This survey was conducted during the wet season of 2001-2002 (from October 31st 2001 to July 2nd 2002). Additional ecological data is also presented from the Southwest coast of Portugal.

Abiotic characterization

For the environmental characterization of ponds, the following parameters were measured *in situ*: temperature, oxygen concentration, and percentage of dissolved oxygen (YSI 55 oxymeter); conductivity (YSI 33 S-C-T conductivity meter); pH (YSI 60 pH meter); turbidity (Hach Chemical, 2100P); depth at the deepest point (pre-positioned ruler (cm) in GUAD).

GPS location and total area was also registered using either a Magellan GPS ProMARK XTM or a Garmin - GPS72, and data analyzed with the software package ArcView GIS 3.2. These data were later plotted into a UTM 10x10 km grid over a continental Portugal geographic map.

Due to the prospective character of the work undertaken, a regular and systematic survey was carried out only in GUAD. Table I presents a list of all environmental parameters (abiotic and biotic) measured. Outside of this region, and for most of the sites where at least one LBS was found, only *in situ* determinations and flooded area and plant coverage were registered.

Hydroperiod (in weeks) was considered to be the sum of all periods when the pond was flooded (total wet phase) during an entire solar year. One to several flooding periods were registered depending on pond size and depth, as well as rain regime.

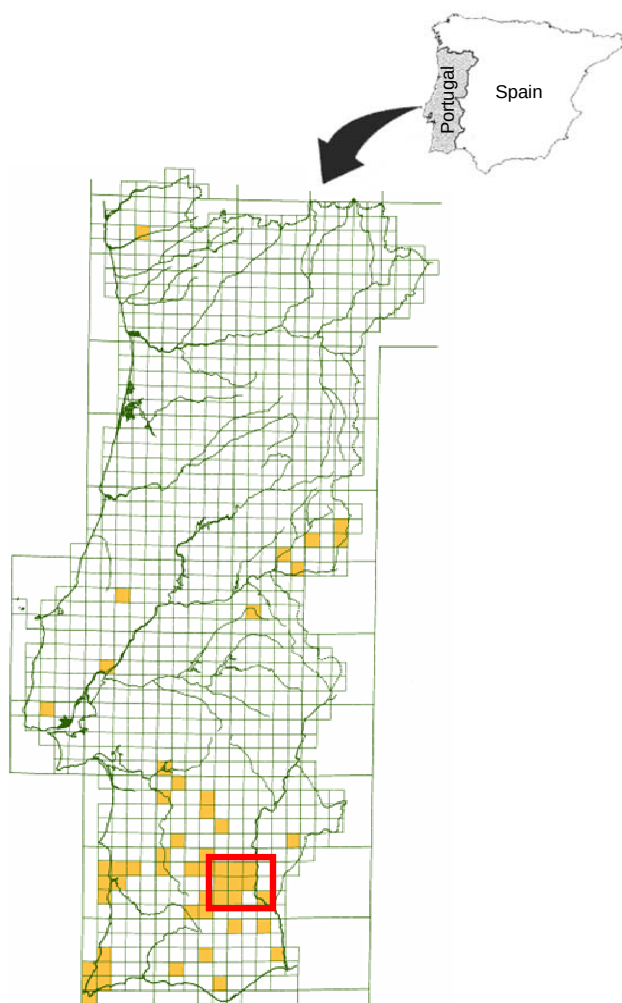


Figure 1. Distribution (UTM 10x10 km) of freshwater LBS sites in Portugal. The Guadiana region (GUAD) around Mértola and the Parque Natural do Vale do Guadiana are highlighted in red.

Relative depth was computed as the maximum depth expressed as a percentage of pond maximal area (Wetzel, 1993). The human activities considered were the ploughing regime and the intensities of ploughing and fertilizing, and they were based on inquiries to farmers and other land owners. Ploughing was quantified using binary categories: ponds not ploughed (0) or ploughed (1) in the farming season running at the time. Ploughing intensity of the pond was scored in a 10 years based scheme: 0 - not ploughed during the last 10 years; 1 - ploughed once; 2- 2-5 times; 3- 6-10 times. Fertilizing quantification was done in the same basis: 0- without fertilizing during the last 10 years; 1- fertilized once; 2- 2-4 times; 3- 5-8 times. Water samples were collected once at the end of January 2002, and chemical analysis performed using standard procedures at the DGA certified laboratory (Ministry of the Environment).

Biotic characterization

Plant coverage censuses were visually estimated, using the following scale: 0 (0 %), 1 (1-25%), 2 (25-50%), 3 (50-75%) e 4 (75-100%).

Fauna sampling

Faunal inventories were conducted by qualitative (erratic) or periodic semi-quantitative sampling. The first case included data about species gathered outside GUAD in a binary (presence/absence) basis. For that area, data on abundance of species was expressed in terms of catch-per-unit-effort (CPUE): mean number of individuals per sweep. Samples were collected by trawling with a hand round net (mouth diameter, 305 mm) with mesh size of 1 mm². In the last case (GUAD), each sample consisted of 3 collections (replicates) of 45 seconds each, conducted from the border to the center of the pond over all possible habitats.

Guadiana region –LBS Sampling

In the beginning of the sampling period weekly surveys were conducted, but after the disappearing of anostracans, which have the shorter life cycles, the periodicity changed to fortnightly. Sampling was conservative, and for samples with a high number of anostracan specimens a Folsom sample splitter was used. Identification and counting of rare species (*Cyzicus grubei* and *Triops mauritanicus*) was done *in situ* before splitting. Juvenile anostracans were preserved (neutral 10 % formaline) to be processed in the laboratory. The adults were also identified and counted *in situ*.

Guadiana region - AS sampling

Non destructive AS monthly samplings were conducted simultaneously with the LBS ones. Identification and counting were done *in situ* before splitting. For very young larvae which size did not allow identification, live specimens were brought to the lab, maintained alive until identification was possible and then returned to their original ponds.

Data analysis

Multivariate analyses were performed on the gathered data. Principal Component Analyses (PCA) and Canonical Correspondence Analyses (CCA) were done on quantitative environmental and biological data using the Brodgar software package (Zuur 2000). Cluster multivariate analysis using the Sorensen Similarity Coefficient and the unweighted pair-group average amalgamation scheme (UPGMA) was done on qualitative data (presence/absence of

Table I. List of environmental parameters measured during the different surveys (words between brackets and codes refer to what has been considered in the CCA analyses with units and transformation applied).

Environmental parameters	Code	Units	Transformation
Abiotic			
Chloride	Cl	mg Cl/l	
Nitrates	NO ₃	mg NO ₃ /l	
Silicates	SiO ₂	mg SiO ₂ /l	
Sulphates	SO ₄	mg SO ₄ /l	
Iron	Fe	mg Fe/l	
Potassium	K	mg K/l	
Sodium	Na	mg Na/l	
Magnesium	Mg	mg Mg/l	
Total hardness (average)	TH	mg CaCO ₃ /l	
Conductivity (average)	Cond	μS/cm	
Dissolved oxygen	DO	mg/l	
DO saturation (range)	R_Sat_DO	%	
pH	pH		
Temperature	Temp	° C	
Turbidity (average)	Turb	NTU	
Average depth	Depth	cm	
Relative depth	R_Dep	%	Arc-sin
Maximal area	AR	m ²	
Maximal area/ potential maximal area	AR/AP		Arc-sin
Hydroperiod	Hydrop	Weeks	
Biotic			
Plant coverage (range)	R._Cover	0 – 4 scale	
Human activity			
Ploughing	Ploug	0 - 1 scale	
Intensity of Ploughing	Int_Ploug	0 – 3 scale	
Fertilizing	Fert	0 – 3 scale	

LBS per pond) in order to investigate relationships between species (R mode analysis – Legendre & Legendre 1984). This hierarchical analysis was undertaken with NTSys 2.0.2 k software (Rohlf 1998).

Analyses were conducted using either raw data, parameter ranges, averages (parameters subject to circadian variations) or medians (parameters not subject to circadian variations).

Results

Surveys

Ecological changes during annual cycle of temporary water bodies are connected to variations of their wet and dry phases. As an example of these, results of the study carried out in the Natural Park of Vale do Guadiana (19 temporary pools and ponds during the 2001/2002 wet season) are presented.

Nine exhibited a fragmented wet period, and length and number of swaps from wet to dry periods

was very variable (Fig. 2). This variation in hydroperiods was common all over the surveyed areas and throughout the years studied (more than 10 years).

More than 260 sites were prospected in Portugal. At present, 11 species of LBS crustaceans are known, belonging to three orders: Anostraca, Spinicaudata and Notostraca. LBS in the present study were only recorded from 128 of those sites, where only 10 species were found, as well as 10 species of AS (Table II).

Three new *taxa* were collected during the sampling surveys: an amphibian of the genus *Pelodytes*, also collected in Southwest Portugal and never reported before because of taxonomic *incertae sedis* (Ferrand de Almeida, pers. comm.), and two previously unknown Anostraca species, never reported before: one belonging to the genus *Tanymastix* and another to the genus *Tanymastigites* (a new genus for Europe).

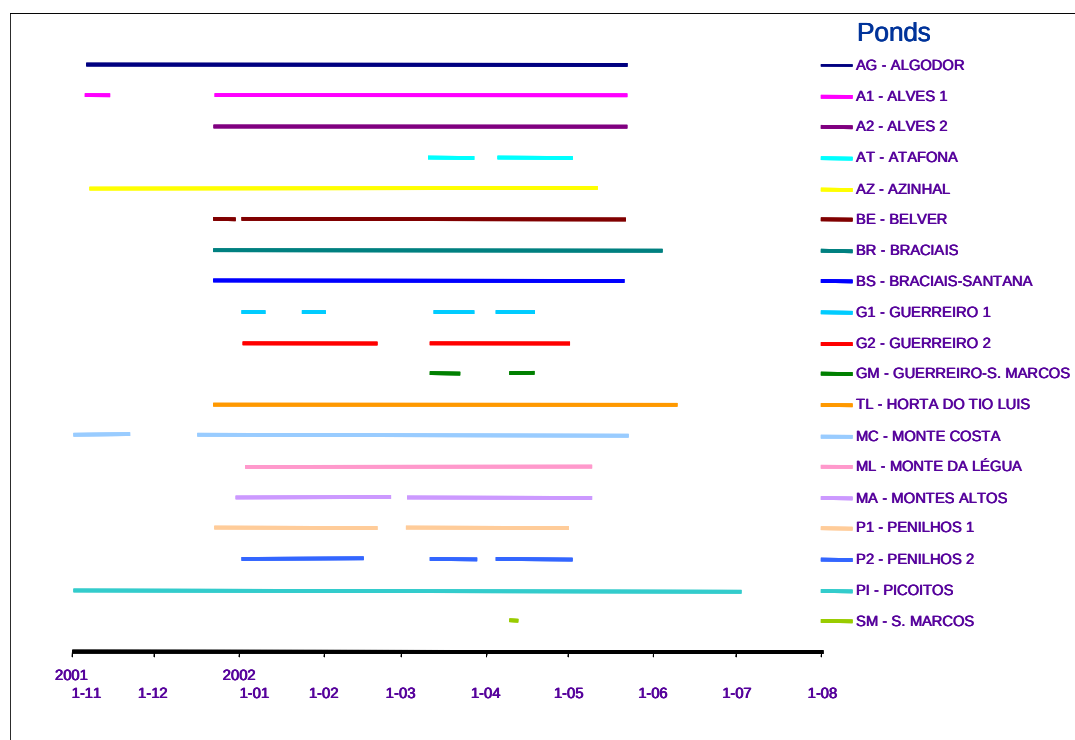


Figure 2. Variation of hydroperiod in ponds inhabited by LBS and AS in the Guadiana region.

Table II. Large Branchiopod Species (LBS) and Amphibian Species (AS) found in the sites surveyed in Portugal (with codes used in the GUAD data analysis).

LBS	Code	AS	Code
Anostraca		Urodela	
<i>Branchipus cortesi</i> Alonso & Jaume	BRCO	<i>Pleurodeles waltl</i> Michaelles	PLWA
<i>Branchipus schaefferi</i> Fischer	BRSC	<i>Salamandra salamandra crespoid</i> (Malkmus)	
<i>Chirocephalus diaphanus</i> Prevost	CHDI	<i>Triturus boscai</i> (Lataste)	
<i>Streptocephalus torvicornis bucheti</i> Daday	STTO	<i>Triturus marmoratus pygmaeus</i> (Wolterstorff)	TRPY
<i>Tanymastigites</i> sp. (new taxon)	TANY	Anura	
<i>Tanymastix stagnalis</i> (L.)	TAST	<i>Bufo calamita</i> (Laurenti)	BUCA
<i>Tanymastix</i> sp. (new taxon)	TASP	<i>Hyla meridionalis</i> (Boettger)	HYME
Spinicaudata		<i>Pelobates cultripes</i> (Cuvier)	PECU
<i>Cyzicus grubei</i> (Simon)	CYGR	<i>Pelodytes ibericus</i> Sánchez-Herráiz, Barbadillo, Machordon & Sanchiz	PEIB
<i>Maghrebestheria maroccana</i> Thiéry	MAMA	<i>Pelodytes</i> sp. (new taxon)	
Notostraca		<i>Rana perezi</i> Seoane	RAPE
<i>Triops mauritanicus</i> (Ghigi)	TRMA		

Large Branchiopod Species

Chirocephalus diaphanus is the commonest LBS species in Portugal and was present in 65 of the freshwater LBS sites analysed. The number of sites where each species was recorded are: *Triops mauritanicus*, 52; *Tanymastix stagnalis*, 41; *Branchipus cortesi*, 40; *Cyzicus grubei*, 22; *Tanymastix* sp., 14; *Branchipus schaefferi*, 8; *Streptocephalus torvicornis*, 8; *Tanymastigites* sp., 6; *Maghrebestheria maroccana*, 5. Out of the geographical area surveyed by us, there is one site confirmed for *Lepidurus apus* (L.) in the North-Western Portugal (Grosso-Silva & Soares Vieira 2002). These results suggest that a large and diversified number of conditions may determine

their distribution.

Results of the analysis of some of the environmental parameters studied are presented in figures 3 and 4. Ranges and average, as well as median values of water descriptors (Fig. 3) emphasise tolerance differentiation among species. Clear differences are more evident in turbidity and conductivity, but they exist also in pH and temperature. In general, *T. mauritanicus*, *C. grubei* and *C. diaphanus* are the most euryecious species.

Differences are also found concerning the parameters depicted in figure 4, but they are not similar to those found above. *C. diaphanus* seems to be again the most tolerant in a global view, but facing different descriptors, diverse responses

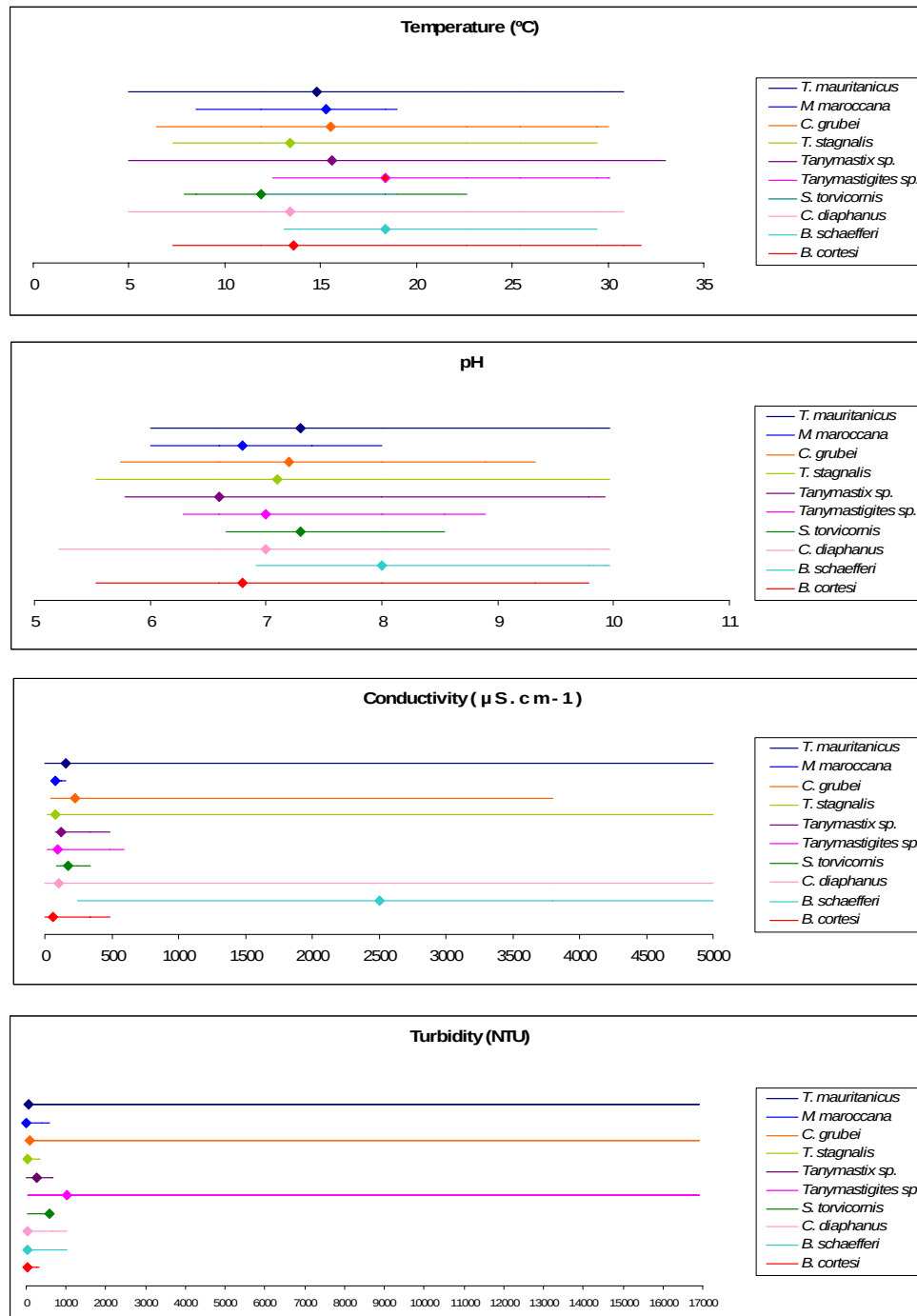


Figure 3. Average points (temperature and pH) and Median points (conductivity and turbidity) (♦) and their different ranges endured by the different LBS in most of the sites where their presence was registered.

appear. *Tanymastigites sp.* is the less tolerant to plant cover, preferring shallower ponds and also smaller areas (together with *Tanymastix sp.*). The short hydroperiod faced by *M. maroccana* may be due to lack of sufficient data collected for this species.

Principal component analysis (PCA) on ranges of environmental variables reveals more (left half of axis 1) and less (right half of axis 1) tolerant species towards ponds ecological conditions (Fig. 5). Variance retained by the first three axes

(respectively 53.0%, 22.8% and 10.2%) shows a satisfactory difference between axes 2 and 3 and allows interpretation based on axes 1 and 2.

A tree clustering, obtained from the Sørensen Similarity Coefficient (R mode, presence/absence data of LBS in ponds), displays two main groups of variables (Fig. 6): (i) An upper group with *B. cortesi* and *T. stagnalis*; (ii) A lower group with *C. grubei*, *C. diaphanus* and *T. mauritanicus*, the species previously considered to be the most tolerant.

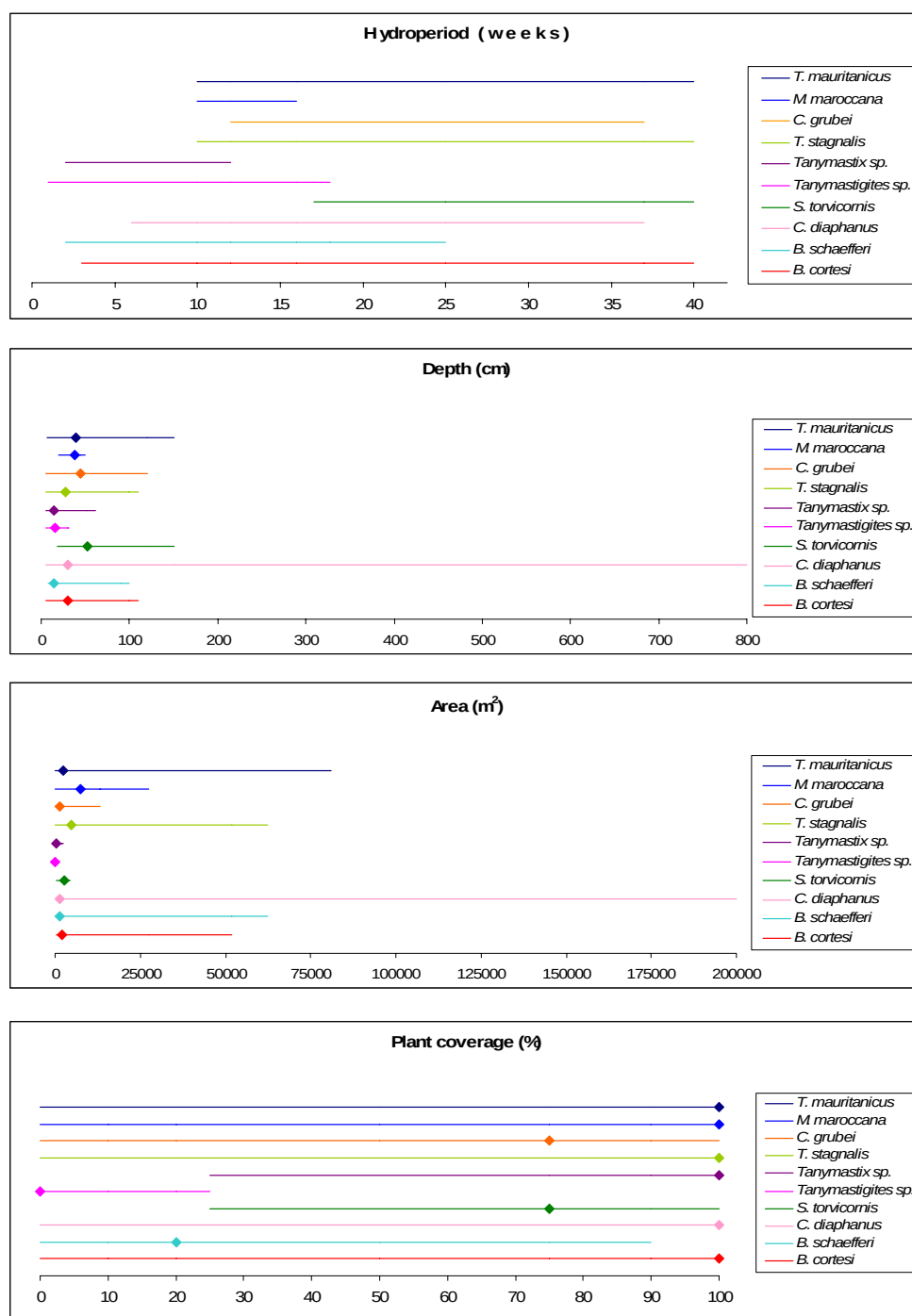


Figure 4. Median points (♦) of depth, area and plant coverage and different range of hydroperiods, depth, area and plant coverage endured by the different LBS in most of the sites where their presence was registered.

Species on the first group are also very tolerant, mainly regarding pH, temperature, hydroperiod, pond area and plant cover. The other five species are separated in this hierarchical cluster analysis and may be considered as outsiders. In fact, they appear in the ponds alone or together with any of the other species, but without a defined pattern.

Results of principal components analysis (PCA) in relation to average and median values of environmental variables are shown in figure 7.

The variance retained by the three first axes (respectively 46.2%, 21.3% and 18.5%) did not reveal satisfactory differences between axes 2 and 3 and recommends interpretation to be based on axes 1, 2 and 3.

This analysis has pointed out that: (i) all but one (*Tanymastix sp.*) of the species ungrouped in the cluster analysis are related to one or two descriptors of the ponds; (ii) the groups revealed by the ordination analysis based on average/median values of the environmental parameters agree with and

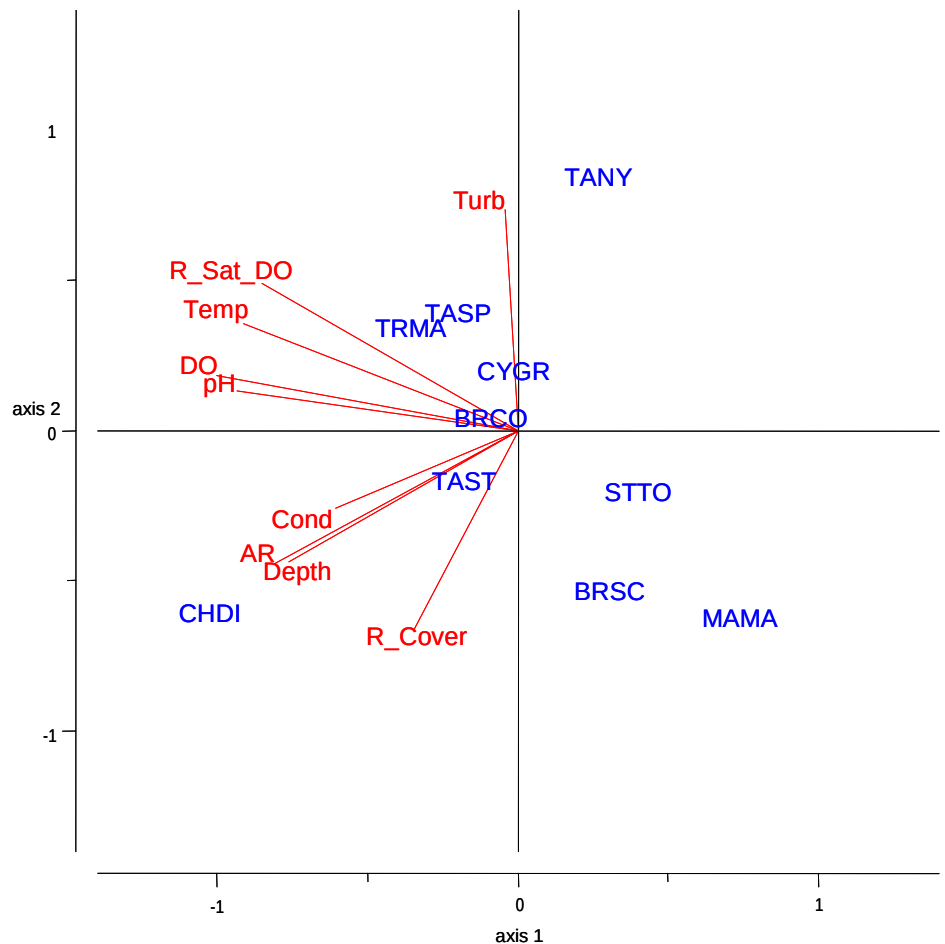


Figure 5. Ordination of large branchiopods (PCA) according to the range of environmental variables. Codes according to Tables I and II (axis 1 and 2 explain 75.8% of the variance).

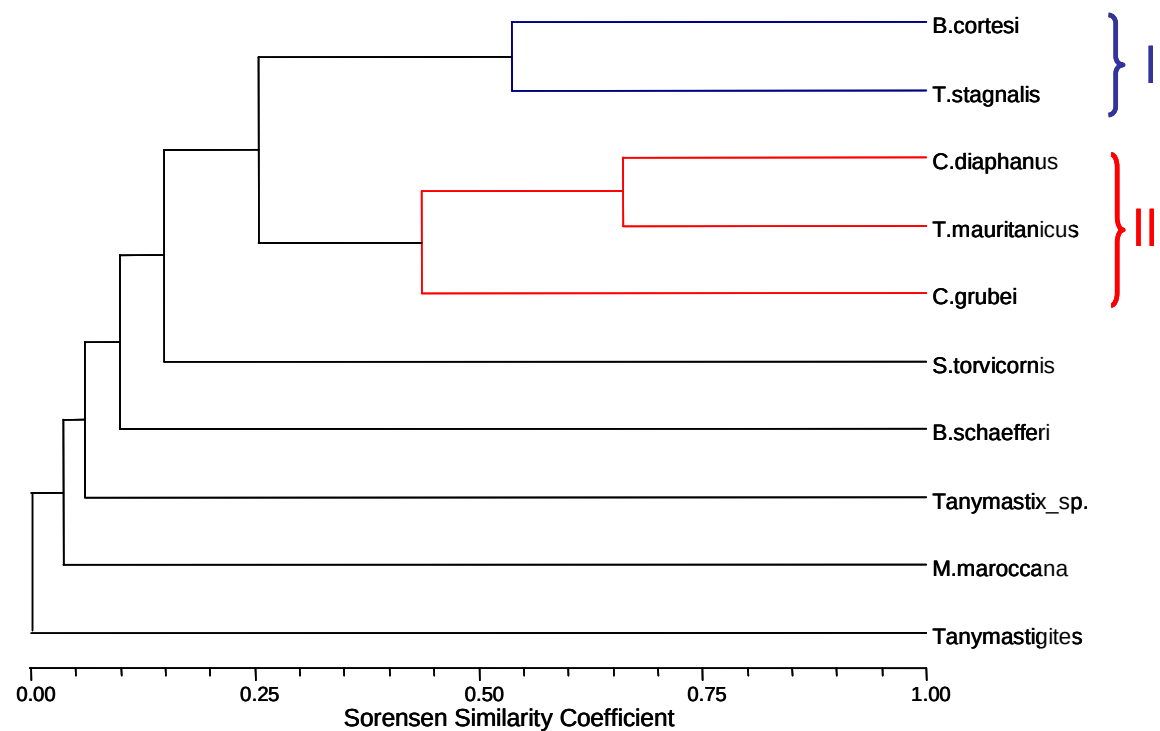


Figure 6. Cluster multivariate analysis using the Sørensen Similarity Coefficient (UPGMA method). Two main groups of large branchiopods can be identified (presence/absence LBS per pond data, R mode).

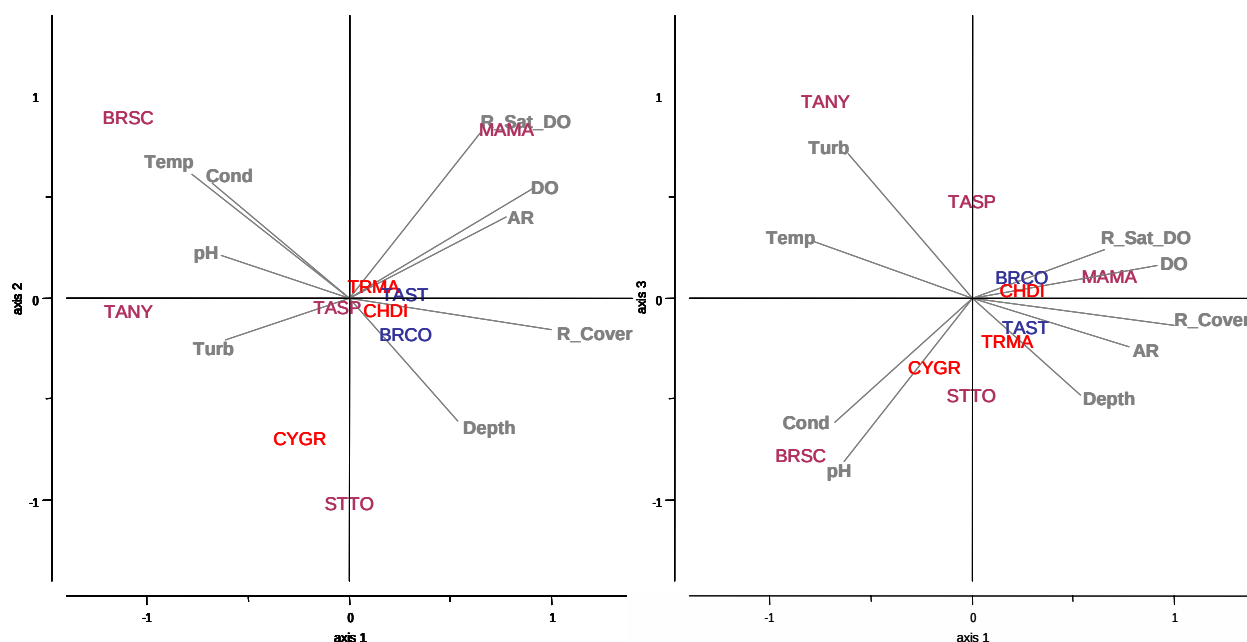


Figure 7. Ordination (PCA) of LBS in relation to average and median values of environmental variables. Codes according to Tables I and II. Blue and red are those referred as Groups I and II in presence/absence cluster analysis (axis 1, 2 and 3 explain 86.0% of the variance).

explain those from the presence/absence analysis (Fig. 6). Furthermore, their central position emphasises their euryecious status and relative independence towards the descriptors utilized for pond characterization.

All LBS hatch in ponds soon after flooding but different species have life cycles more or less extended. In general, among the considered animals, Anostraca species were those with the shortest life span, as it was found in the Monte de Vale Santo pond, in the Portuguese Southwest coast, near Sagres (Fig. 8).

Concerning the studied ponds in GUAD, the length of Anostraca life period, with very rare exceptions, was less or equal to 3 months. That represents, for the bulk of the studied ponds, 1/2 to 2/3 of their hydroperiods.

Among the studied Anostraca, *T. stagnalis* and *Tanyastix* sp. were those with shorter life cycles. *T. stagnalis* lived 10-11 weeks, with female maturation occurring after 2-3 weeks; the second species appeared to have mature females 10 to 18 days after hatching and a total life span of up to 8 weeks.

Recorded life spans of *C. diaphanus* varied from 1.5 to 4 months with presence of mature females appearing from 2 weeks (when the water temperature is higher) to 2 months after hatching (in colder waters).

Abundances fluctuate during life span (Fig. 9) but, in general, species of the above referred group show precocious blooms. *C. grubei*, on the other hand, is the species with the longer life span as

it was already known from the southwest populations (cf. Fig. 8).

Analysis of Fig. 10 supports the idea that environmental parameters that most influence species distribution might be hydroperiod and depth, either positively conditioning the presence of *C. grubei* and *S. torvicornis* or negatively influencing the distribution of *Tanyastix* sp. This species is clearly related to iron content in water and also positively influenced by ploughing, turbidity and conductivity. On the other hand, *B. cortesi* and *T. stagnalis* are negatively influenced by iron content, turbidity, intensity of ploughing and conductivity.

CCA based on LBS abundance allowed the separation of ponds into three distinct groups (Fig. 10, right graph).

Amphibian Species

Data on relationships between environmental parameters and AS are only available from GUAD. Colonization by different species is not synchronous and there is great variation in their permanence time in ponds (Fig. 11). *Pleurodeles waltl* Michaelles was registered almost in every studied pond, with the exception of two with very short hydroperiods, and is one of the fastest colonizers but also one of the species that needs longer periods to accomplish its larval stage. This species, together with *Pelobates cultripes* (Cuvier) and *Pelodytes ibericus*, constitute the group of the early colonizers, followed by *Bufo calamita*. *Triturus marmoratus pygmaeus*, *Hyla meridionalis* and *Rana perezi* were the latest to colonize and also the rarest species.

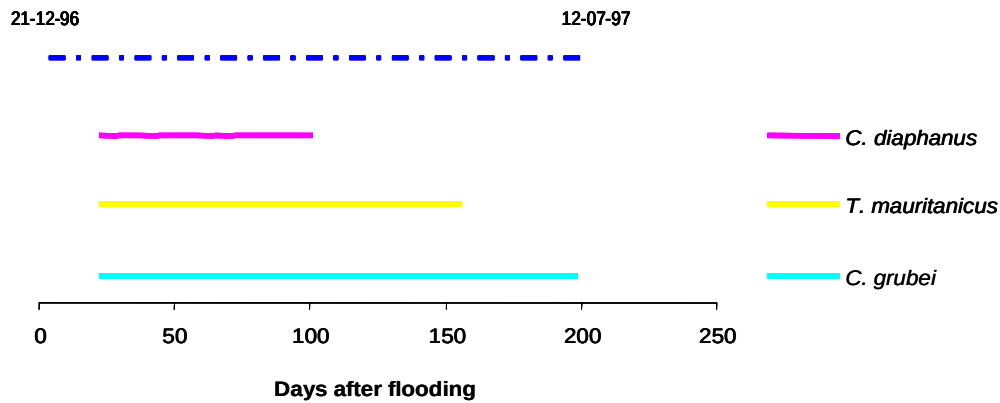


Figure 8. Diagram showing LBS life span in relation to flooding period in Monte de Vale Santo pond (Sagres) (dashed blue line represents hydroperiod).

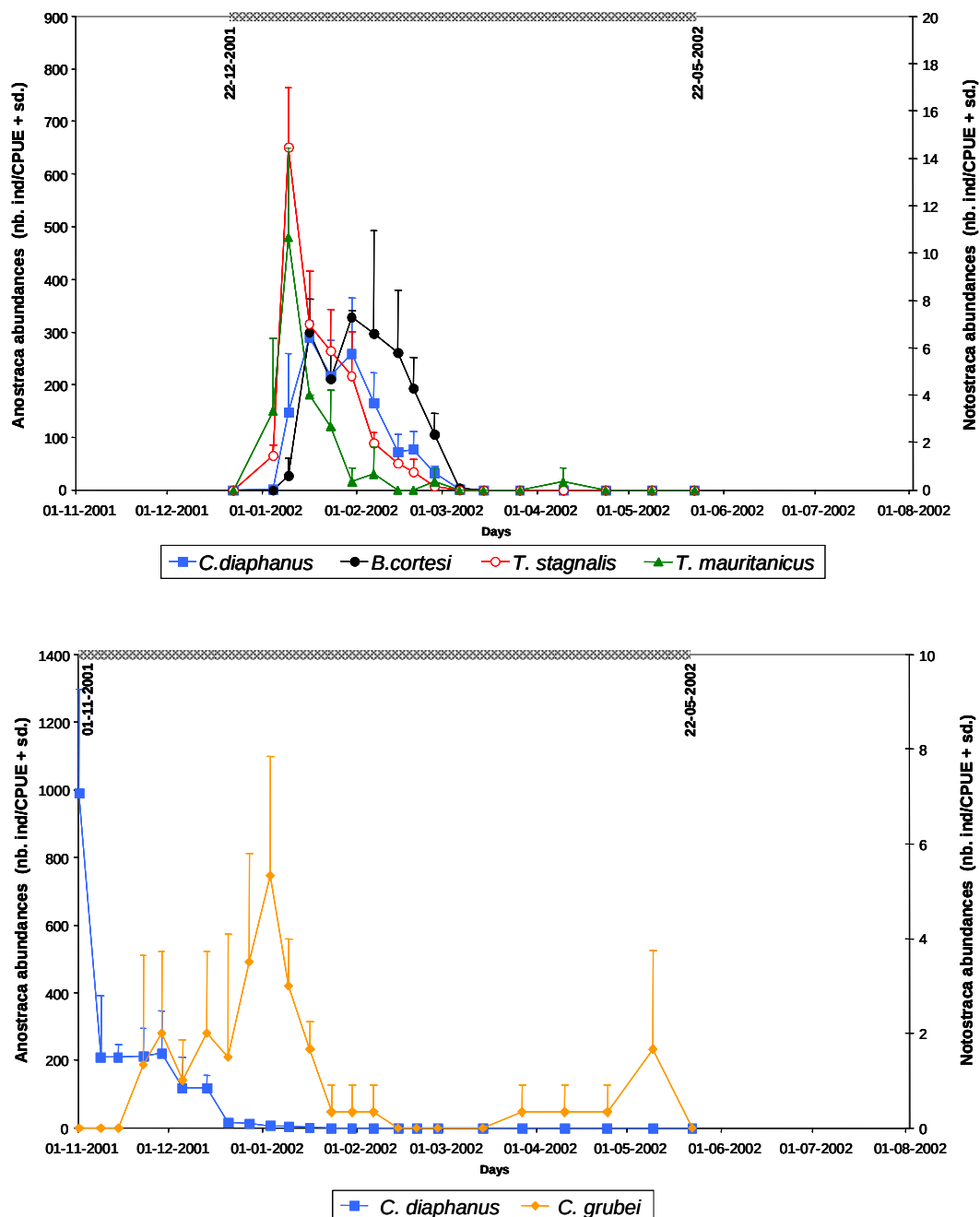


Figure 9. Abundance (+ SD) of LBS during the flooding period in ponds "Alves 2" and "Algodor" (GUAD), as an example of population evolution during life spans (patterned grey lines represent hydroperiods).

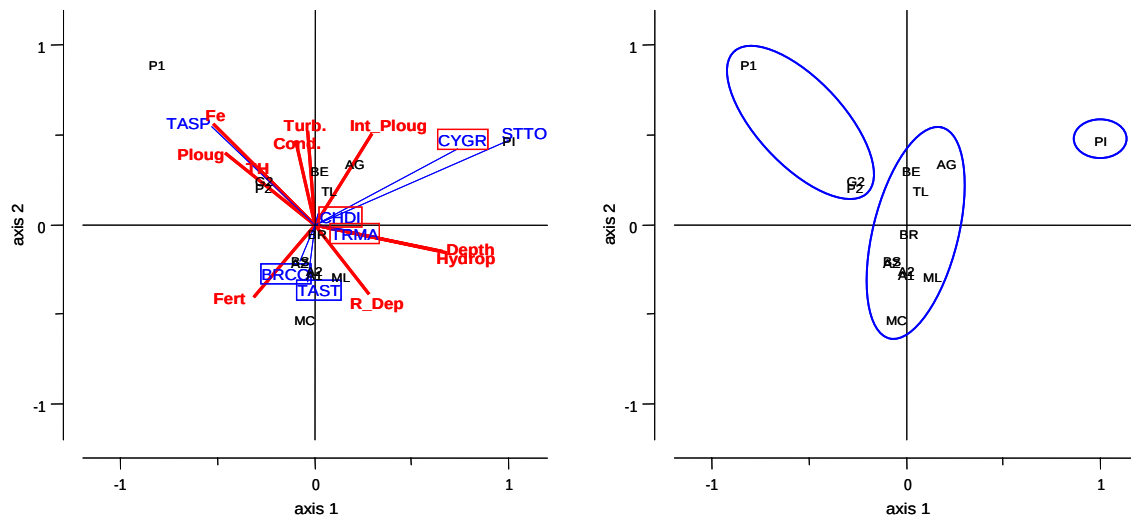


Figure 10. Ordination (CCA) of temporary ponds (GUAD) based on LBS average abundances (left: blue) and values of environmental variables (left: red). Right graph: groups of ponds (axis 1, 2 and 3 explain 74.5% of the variance). Codes according to Tables I and II, and Fig. 2.

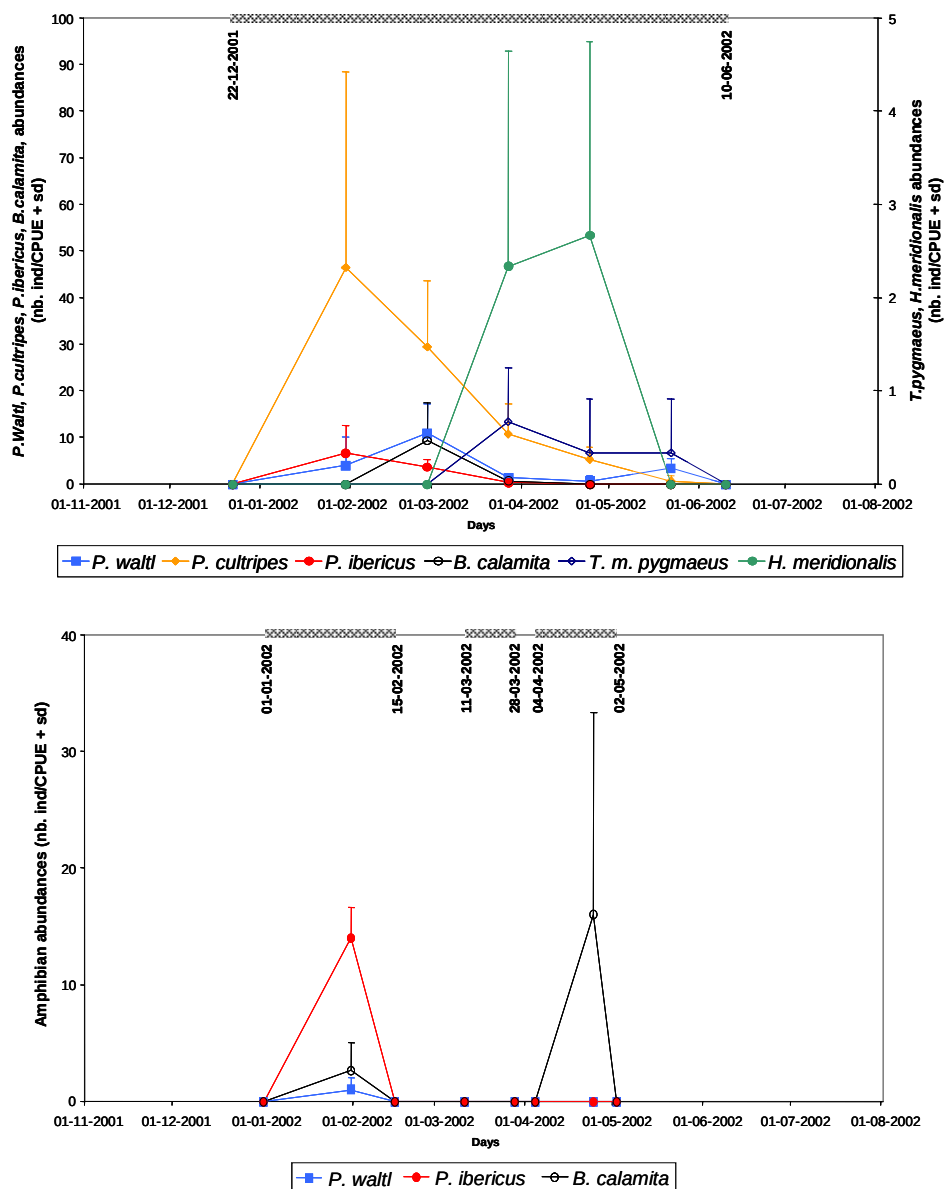


Figure 11. Abundance (+ SD) of AS during the flooding period in ponds "Horta do Tio Luís" and "Penilhos 2" (GUAD) as an example of populations evolution during larval phase (grey patterned lines represents hydroperiod).

Analysis of figure 12 does not show a clear direct influence of any particular environmental variable on species distribution, perhaps with the exception of depth and hydroperiod, which positively influenced the behaviour of *R. perezii* and *T.m. pygmaeus* and seemed to negatively affect both *B. calamita*

and *P. ibericus*. Fertilizing also appeared to play a negative role on most species behaviour.

The CCA based on average abundances of AS also allowed separation of ponds into three groups (Fig. 12, right graph) with an outline very similar to that obtained for LBS.

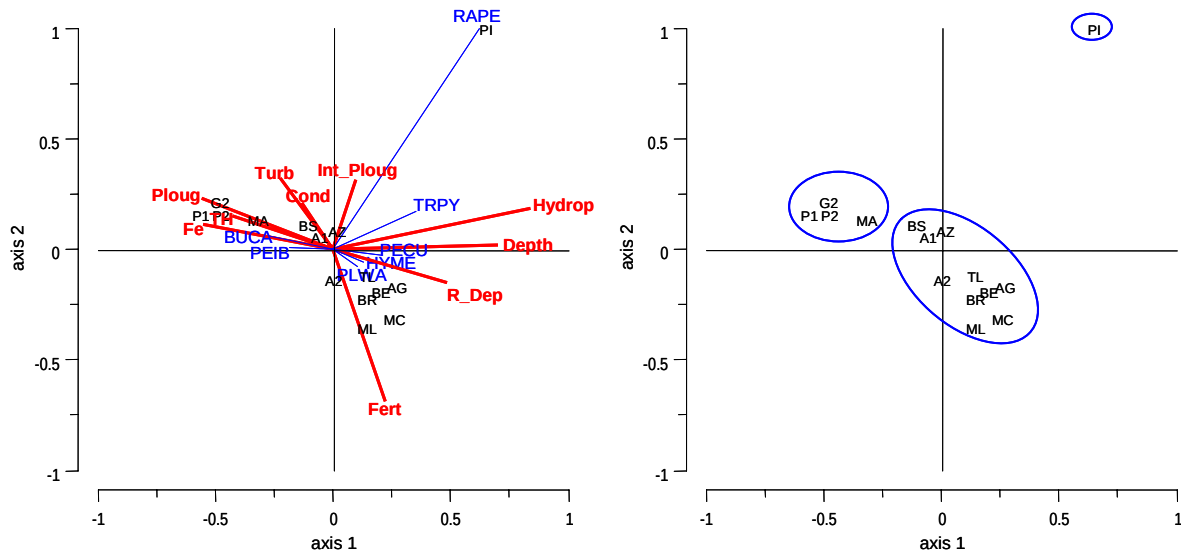


Figure 12. Ordination (CCA) of temporary ponds (GUAD) based on AS average abundances (left: blue) and values of environmental variables (left: red). Right graph: groups of ponds (axis 1, 2 and 3 explain 80.3% of the variance). Codes according to Tables I and II, and Fig. 2.

Discussion

The Portuguese fauna of freshwater LBS, living in temporary ponds and pools, has been poorly studied. In the 1944 catalogue of Portuguese invertebrate collection of the Zoological Museum at Coimbra University, just one specimen of *Apus cancriformis* Schäffer 1756 (= *Triops cancriformis* (Lamarck, 1801)) was included (Carvalho 1944). The first published scientific paper (Vianna-Fernandes 1951), refers the occurrence of 3 new species – *Caenestheriella grubei* Daday, 1915 (= *Cyzicus grubei* (Simon, 1886)), *Streptocephalus torvicornis* (Waga, 1842) and *Tanymastix lacunae* Daday, 1910 (= *Tanymastix stagnalis* (L., 1758)).

The classification of Mediterranean Temporary Ponds as a conservation priority in the EU Habitats Directive (92/43/CEE) has resulted in increased scientific attention to the biological assemblages associated with these habitats in Portugal. This led to a new study of this group of crustaceans (Machado *et al.* 1999a), 45 years later. The inventory of freshwater LBS presented here updates and extends the knowledge on LBS of Portuguese temporary lentic systems.

Information about these environments in Portugal is also scarce and therefore, data collected in GUAD represents an important contribution to increase knowledge on the distribution and life

cycles of AS in temporary pools and ponds from Southeast Portugal. Together with Beja & Alcazar work (2003), the present study presents additional data relevant to improve our understanding of these priority habitats, thus leading to more accurate management strategies.

Although some research was carried out in the North of Lisbon, this part of the Portuguese territory is far less studied. Only Grosso-Silva & Soares-Vieira (2002) have contributed with new data on LBS in the last decade, reporting the presence of *Lepidurus apus* in Portugal. Our own surveys (unpublished data) have already added more sites to the known Portuguese LBS fauna.

Southern Portugal (South of Lisbon) is still the best surveyed part of the country (Vianna-Fernandes 1951, Machado *et al.* 1999a, Machado *et al.* 1999b, Beja & Alcazar 2003, Korn *et al.* 2006), with 128 sites where, at least, one species was found in one occasion. In this area some of us (M. Machado, J. Sala, L. Cancela da Fonseca & M. Cristo, unpublished data) found, since 1999, three new LBS taxa: *Branchipus schaefferi*, *Tanymastix* sp. and *Tanymastigites* sp. The last two are new species for science, never reported previously and their description is ongoing (M. Machado and J. Sala, personal communication).

Because from 1996 onwards we surveyed a large area of Portugal, and a large number of sites, we can now say that our knowledge on these Mediterranean Temporary Ponds is still insufficient, since the variability found leads to more and more questions that remain still answered, as pointed out by Beja (2000).

Differences between ponds colonization emerge as a pertinent fact from our surveys. Even very close ponds may present relevant differences concerning AS and particularly LBS faunas. And these facts remain difficult to explain.

Large Branchiopod Species

Results from analysis of cross information between environmental parameters and presence of a given species, indicate that species are more sensitive to certain parameters than to others, namely in what concerns their ability to tolerate variation of habitat parameters. Conductivity, turbidity (Fig. 3), hydroperiod, depth, and area (Fig. 4) are the main constraints for species distribution. It was also possible to observe that those species that tolerate wider variations of those parameters analysed are *T. mauritanicus*, *C. grubei*, *C. diaphanus*, *T. stagnalis* and *B. cortesi*.

Concerning the mean values for those environmental parameters measured, most of them are located at the low end of the graph, with the exception of conductivity preferences of *B. schaefferi* a species considered capable of enduring brackish waters (Lanfranco *et al.* 1991, Petkovski 1997).

In relation to plant coverage the situation is opposite. Here the exception is *Tanymastigites* sp. which was never found in ponds with high plant coverage. Also *B. schaefferi* avoids highly vegetated ponds. This behaviour was also recorded in German populations (Hössler *et al.* 1995).

The same group formed by the 5 more tolerant species is outlined by the ordination analyses (based on biotic and abiotic descriptors – Figs. 5 and 7) and cluster analysis (in relation to presence/absence of species in ponds – Fig. 6). On the other hand, the most sensitive species are: *Tanymastigites* sp., *M. maroccana*, *Tanymastix* sp., *B. schaefferi* and *S. torvicornis*. The environmental parameters that explain more accurately the presence of each of these species are respectively: turbidity; dissolved oxygen; depth, area, conductivity, and pH (negatively); conductivity and pH; and depth.

As a rule, Cristo *et al.* (2002) reported that at GUAD, LBS richness is greater in ponds with longer hydroperiods and higher water column and/or “relative depth”. Nevertheless, *Tanymastix* sp.

prefers shallow pools with short hydroperiods and, between this situation and the preference of *C. grubei* for deeper ponds with longer hydroperiods, all intermediate situations may be found.

LBS show a large variability in their life span. They hatch early and simultaneously, as in Morocco (Thiéry 1987) but in most cases Anostraca has a shorter life span than the other groups, which need longer hydroperiods. So, hydroperiod is of major importance for conservation purposes. It is important to preserve large ponds with longer hydroperiods, but in order to support the entire biodiversity of LBS in these Mediterranean Temporary ponds, it is also indispensable to preserve small ponds with short hydroperiods.

The new species recorded are still under study, in preparation to be described. Nevertheless the genus *Tanymastigites* is new for Europe (it was only known from North Africa and Arabian Peninsula; Belk and Brtek 1995, Thiéry 1996).

Alltogether, our data provides evidence suggesting that: i) *Tanymastix* sp. and *Tanymastigites* sp. avoid deep ponds with extended flooding periods; ii) these species and also *Tanymastix* sp., *M. maroccana*, *S. torvicornis* and *C. grubei* are absent from large ponds; iii) *M. maroccana*, *S. torvicornis*, *Tanymastigites* sp., *Tanymastix* sp., and *B. cortesi* prefer waters with relatively low conductivity; iv) *S. torvicornis* prefers deep ponds with moderate to long hydroperiods; v) *T. stagnalis*, *C. diaphanus*, *T. mauritanicus*, and *B. schaefferi* tolerate moderate brackish waters.

Amphibian Species

Temporary ponds are particularly important for amphibian survival in general and in particularly for some species that prefer these temporary systems. Flooding of this provisional and widespread water bodies are fundamental in autumn and winter seasons, when mating and reproduction occurs (Beja & Alcazar 2003).

In our surveys, we found *P. cultripes*, *P. ibericus*, and *P. waltl* to be pioneer species where *T. m. pygmaeus*, *H. meridionalis* and *R. perezi* appeared to be later colonizers (Fig. 11). The last one was unable to reach the adult phase in GUAD temporary ponds during the period of the study. Actually, *R. perezi* generally inhabits permanent freshwater systems (Ferrand de Almeida *et al.* 2001, Beja & Alcazar 2003).

In general there was a replacement of species along a single hydroperiod, and differentiated species occupancy if there was a succession of dry and wet periods. Similar facts

were also registered in Southwest Portugal by Beja & Alcazar (2003).

Our results emphasise some positive relationships with agriculture activities, such as: *R. perezii* and ploughing frequency; *P. ibericus* and *B. calamita* with ponds ploughed recently. However, fertilizing has a negative generalised impact on species abundances but *H. meridionalis*, *P. waltl* and *P. cultripes* show an ambivalent behaviour, avoiding fertilizing (Fig. 12) but inhabiting also ponds with abundant plant coverage.

It also seems that there is a tendency for *R. perezii* to prefer deep ponds with long hydroperiods and *P. ibericus* and *B. calamita* to avoid them. Several authors (Barbadillo *et al.* 1999, Sánchez-Herráiz *et al.* 2000, Ferrand de Almeida *et al.* 2001) have referred the preference of these two species for temporary waters and Beja & Alcazar (2003) have pointed out a clear preference of *Pelodytes punctatus* (a species very close to *P. ibericus*) for ponds with low depth and short hydroperiods.

T. m. pygmaeus was found in intermediate deep ponds with high conductivity values and long wet phases. Beja & Alcazar (2003) also reported *T. marmoratus* preferences for temporary ponds with longer hydroperiods, but avoiding permanent ponds.

Cristo *et al.* (2002), for the GUAD study-case, found AS richness positively correlated with area and depth. In Southwest Portugal, Beja & Alcazar (2003) found that AS richness tends to be greater in larger and/or most persistent temporary ponds. As a matter of fact, hydroperiod is undoubtedly one of the major constraints for AS success for the studied areas. It is also considered the main conditioning factor for amphibian colonization by several authors working on temporary ponds under different geographical and climatic conditions (Pechmann *et al.* 1989, Snodgrass *et al.* 2000, Beja & Alcazar 2003).

Like almost all the Portuguese species of amphibians, the AS found in the studied ponds are not considered threatened in the red book of Portuguese vertebrates (Cabral *et al.* 2005). However, all of them have an international protection status due to International Conventions or UE Directives, and even national and regional Portuguese laws (Ferrand de Almeida *et al.* 2001). Among them, *P. cultripes*, *B. calamita* and *H. meridionalis* are those with a more restricted protection status since they belong to the group of strictly protected species of Bern Convention.

As general remarks on AS we can say that: (i) *S. s. crespoides* and *T. boscai* appear to be very rare in temporary ponds; (ii) the species *P. punctatus*, referred by Beja & Alcazar (2003), is likely to be a

new taxon (*Pelodytes* sp. - Ferrand de Almeida, pers. comm.); (iii) in temporary ponds, more persistent wet phases favour the occurrence of *T. m. pygmaeus*, *H. meridionalis* and *R. perezii*; (iv) *B. calamita* and *P. ibericus* prefer shallower pools with shorter hydroperiods; (v) *P. waltl* and *P. cultripes* were the most widespread and the most persistent AS in the majority of the prospected areas.

Management

Conservation of biodiversity in areas where water is scarce has an increased importance for the maintenance of species that directly depend on temporary ponds for reproduction – LBS and AS among others, and indirectly for those feeding on them – storks, gray herons, little grebes and several ducks.

It is of common knowledge that for centuries, wetlands that we now include in the temporary ponds have been used by farmers, as strategic water reserves in dry geographical areas (Grillas & Roché 1997). Their use as drinking points for cattle and remnant green grass beds after drying have contributed to their preservation. Nowadays, with the new strategies for water and soil management in relation to agriculture intensification, their role as part of the traditional ways of land usage is being lost. So, Mediterranean Temporary Ponds are under threat and most of them are being reclaimed for agriculture or other human activities. In fact, these temporary systems are now regarded by land users as not important and only recently the National Authorities for nature conservation gave them the attention they deserve (Grillas & Roché 1997, Beja & Alcazar 2003).

Farmers tend to either fill them up to use the resulting land for agriculture, or if the ponds have already a considerable depth, to dig them to make water reservoirs. In any case, their use by exclusive inhabitants of temporary ponds is lost, even in the last situation, where a shift to permanent water bodies frequently occurs. Maintenance of alternate wet and dry phases is fundamental for the survival of specialized taxa that evolved under this particular regime.

As we have seen, different species have diverse environmental demands. Among these, hydroperiod length is a major ecological requirement. LBS and AS need different flooding periods (Cristo *et al.* 2002, Beja & Alcazar 2003) to ensure their survival (LBS: from ca. 20 days – *Branchipus schaefferi* and *Tanyastix* sp. - up to 120 days – *Cyzicus grubei* - to complete their life cycles; AS: from ca. 20 days – *Bufo calamita* – to more than 100 days – *T. marmoratus pygmaeus* – to

complete their larval development).

If, on one hand, knowledge of the life cycles of occurring species is important to define strategies for maintenance of ponds with shorter or longer hydroperiods, on the other hand it is also important to have in mind that colonization by species with low mobility (as AS are supposed to be) depends on the relative proximity of these ponds. For species whose dispersal is done by external agents (wind, animals) it is also of major importance that hydroperiods will allow reproduction success. Moreover, as LBS population success relays on the cyst bank kept in pond sediment (Belk 1998), its destruction due to silting up or by shifting to a permanently inundated status leads to disappearance of that population.

Recently, a Southwest coast pond, Monte de Vale Santo, was partially drained, risking the survival of *C. grubei*, one of the species with a longer life cycle among LBS. This species showed a population decline in several sites during the last years. Similarly, in some GUAD ponds where the hydroperiod was reduced, this prevented *H. meridionalis* and *T. m. pygmaeus* from reaching the adult phase (Cristo *et al.* 2002). Therefore, management of Temporary Mediterranean Ponds should consider these aspects of the bio-ecology of those species, and implement a conservation strategy for the entire temporary pond biodiversity capable of preserving ponds representative of the entire hydroperiod range.

As it was noticed, ponds with longer hydroperiods and/or bigger areas and/or greater depths have higher LBS and AS richness. So these have a high conservative value. However, the maintenance of their overall biodiversity depends on the preservation of a diversity of pond sizes, since, as already pointed out; some species only develop in

shallow ponds with short hydroperiods (Schneider & Frost 1996, Welborn *et al.* 1996, Cristo *et al.* 2002, Beja & Alcazar 2003).

Instruments for management and conservation of these systems have to be implemented and studies on the species that are exclusive of them can supply the required tools. In any case, length of exclusive species life cycles should be considered, together with their behaviour and the ecological features of their habitat.

Human actions tending to promote habitat changes may have both advantages and disadvantages for LBS and AS (Cristo *et al.* 2002), depending on the species considered. Facing the variety of situations concerned, it is wise to advise that conservation should consider the overall necessary conditions for species survival. And this will only be possible inside a network of ponds representative of the entire environmental gradient, where species as a whole may accomplish their ecological needs, as it was stated by Beja & Alcazar (2003) for the Portuguese Southwest ponds.

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References

- Alonso, M. 1996. Crustacea Branchiopoda. **Fauna Ibérica**, 7. Museo Nacional de Ciencias Naturales, CSIC, Madrid. 486 p.
- Barbadillo, L. J., Lacomba, J. I., Pérez-Mellado, V., Sancho, V. & López-Jurado, L. F. 1999. **Anfíbios y Reptiles de la Península Ibérica, Baleares y Canarias**. Editorial Geoplaneta, S.A., Barcelona. 419 p.
- Beja, P. 2000. Ecology and conservation of Portuguese temporary ponds. Pp. 91–95. *In*: Pond Action (Ed.). **Proceedings of the Ponds Conference 1998**. The Ponds Conservation Trust, Oxford.
- Beja, P. & Alcazar, R. 2003. Conservation of Mediterranean temporary ponds under agricultural intensification: an evaluation using amphibians. **Biological Conservation**, 114: 317–326.
- Belk, D. 1998. Global Status and Trends in Ephemeral Pool Invertebrate Conservation: Implications for Californian Fairy Shrimp. Pp. 147–150. *In*: Witham, C. W., Bauder, E. T., Belk, D., Ferren Jr. W. R. & Ornduff, R. (Eds). **Ecology, Conservation, and Management of Vernal Pool Ecosystems – Proceedings from a 1996 Conference**. California Native Plant Society, Sacramento, C. A.
- Belk, D. & Brtek, J. 1995. Checklist of the Anostraca. **Hydrobiologia**, 298: 315–353.

- Biggs, J., Corfield, A., Walker, D., Whitfield, M. & Williams, P. 1994. New approaches to the management of ponds. **British Wildlife**, 5 (5): 273-287.
- Boix, D., Sala, J. & Moreno-Amich, R. 2002. Population dynamics of *Triops cancriformis* (Crustacea: Branchiopoda: Notostraca) of the Espolla temporary pond in the northeastern Iberian peninsula. **Hydrobiologia**, 486: 175-183.
- Cabral, M. J., Almeida, J., Almeida, P. R., Dellinger, T., Ferrand de Almeida, N., Oliveira, M. E., Palmeirim, J. M., Queiroz, A. I., Rogado, L. & Santos-Reis, M. (Eds). 2005. **Livro Vermelho dos Vertebrados de Portugal**. Instituto da Conservação da Natureza, Lisboa. 660 p.
- Carvalho, R. N. 1944. Catálogo da colecção de invertebrados de Portugal existentes no Museu Zoológico da Universidade de Coimbra. Crustacea. **Memórias e Estudos do Museu zoológico da Universidade de Coimbra**, 160: 1-15.
- Collinson, N. H., Biggs, J., Corfield, A., Hodson, M. J., Walker, D., Whitfield, M. & Williams, P. J. 1995. Temporary and permanent ponds: an assessment of the effects of drying out on the conservation value of aquatic macroinvertebrate communities. **Biological Conservation**, 74: 125-133.
- Cristo, M., Machado, M. & Sala, J. 2002. **Identificação dos elementos de conservação (fauna de crustáceos filópodes e anfíbios) nos charcos temporários do Parque Natural do Vale do Guadiana e áreas limítrofes**. ICN –CCMAR/Universidade do Algarve, Faro. 173pp.
- Ferrand de Almeida, N., Ferrand de Almeida, P., Gonçalves, H., Sequeira, F., Teixeira, J. & Ferrand de Almeida, F. 2001. **Anfíbios e Répteis de Portugal**. Guias FAPAS, Porto. 249 p.
- Grillas, P. & Roché, J. 1997. **Vegetation of Temporary Marshes. Ecology and Management**. Tour du Valat, Arles, France. 86 p.
- Grosso-Silva, J. M. & Soares-Vieira, P. 2002. Primeiro registo de *Lepidurus apus* (Linnaeus, 1758) para Portugal (Crustacea, Branchiopoda, Notostraca, Triopidae). **Boletín de la Sociedad Entomológica Aragonesa**, 30: 176.
- Hössler, J., Meier, G. & Tessenow, U. 1995. Some notes on the ecology of a German *Branchipus schaefferi* population (Crustacea: Anostraca). **Hydrobiologia**, 298: 105-112.
- Korn, M., Marrone, F., Pérez-Bote, J. L., Machado, M., Cristo, M., Cancela da Fonseca, L. & Hundsdoerfer, A. K. 2006. Sister species within the *Triops cancriformis* lineage (Crustacea, Notostraca). **Zoologica Scripta**, 35: 301-322.
- Lanfranco, S., De Walsche, C., Schembri, P. & Mertens, J. 1991. Branchiopods (non-cladocerans) of the Maltese Islands (central Mediterranean). **Hydrobiologia**, 212: 241-243.
- Legendre, L. & Legendre, P. 1984. **Écologie Numérique. I – Le traitement multiple des données écologiques. II – La structure des données écologiques**. (2nd ed.). Masson Editeurs, Paris. 335 p.
- Machado, M., Cristo, M. & Cancela da Fonseca, L. 1999a. Non-Cladoceran Branchiopod Crustaceans from Southwest Portugal. I. Occurrence notes. **Crustaceana**, 72: 591-602.
- Machado, M., Cristo, M., Reis, J. & Cancela da Fonseca, L. 1999b. Biological data on *Triops cancriformis mauritanicus* (Ghigi, 1921) and *Cyzicus grubei* (Simon, 1886) – Crustacea, Branchiopoda – in SW Portugal temporary ponds. **Limnetica**, 16: 1-7.
- Mura, G. 1999. Current status of the Anostraca of Italy. **Hydrobiologia**, 405: 57-65.
- Pechmann, J. H. K., Scott, D. E., Gibbons, J. W. & Semlitsch, R. D. 1989. Influence of wetland hydroperiod on diversity and abundance of metamorphosing juvenile amphibians. **Wetlands Ecology and Management**, 1: 3-11.
- Petkovski, S. 1997. On the presence of the genus *Branchipus* Schaeffer, 1766 (Crustacea: Anostraca) in Macedonia. **Hydrobiologia**, 359: 37-44.
- Rolf, F. J. 1998. **NTSYS-pc version 2.0k Numerical Taxonomy and Multivariate Analysis System. Program manual**. Steauket, Exeter Software, New York.
- Schneider, D. W. & Frost, T. M. 1996. Habitat duration and community structure in temporary ponds. **Journal of the North American Benthological Society**, 15: 64-86.
- Sánchez-Herráiz, M. J., Barbadillo, J. L., Machordom, A. & Sanchiz, B. 2000. A new species of Pelodytid frog from the Iberian Peninsula. **Herpetologica**, 56: 105-118.
- Snodgrass, J. W., Komoroski, M. J., Bryan Jr., A. L. & Burger, J. 2000. Relationships among isolated wetland size, hydroperiod, and amphibian species richness: implications for wetland regulations. **Conservation Biology**, 14: 41-419.

- Thiéry, A. 1987. Les Crustacés Branchiopodes Anostraca, Notostraca & Conchostraca des milieux limniques temporaires (Dayas) au Maroc. Taxonomie, biogéographie, écologie. **Thèse Doc. Ès Sciences**, Université de Droit, d'Économie et des Sciences d'Aix-Marseille III, Marseille. 405 p.
- Thiéry, A. 1996. Large branchiopods (Crustacea: Anostraca, Notostraca, Spinicaudata, Laevicaudata) from temporary inland waters of the Arabian Peninsula. **Fauna of the Saudi Arabia**, 15: 37-98.
- Vianna-Fernandes, A. M. 1951. Contribuições para o estudo dos Filópodes portugueses. I. Primeiras espécies encontradas em Portugal. **Arquivos do Museu Bocage**, 22: 75-85.
- Wellborn, G. A., Skelly, D. K. & Werner, E. E. 1996. Mechanisms creating community structure across a freshwater habitat gradient. **Annual Review of Ecology and Systematics**, 27: 337-363.
- Wetzel, R. G. 1993. **Limnologia**. Fundação Calouste Gulbenkian, Lisboa. 1016 p.
- Zuur, A. 2000. **Software package for Multivariate Analysis and Multivariate Time Series Analysis. Brodgar v. 2⁺**. Highland Statistics, Ltd. Aberdeen. United Kingdom. 255 p.

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