# Mediterranean Temporary Ponds in South Portugal: key faunal

# groups as management tools?

Luís Cancela da Fonseca<sup>1,3</sup>, Margarida Cristo<sup>1,2</sup>, Margarida Machado<sup>1,2</sup>, Jordi Sala<sup>4</sup>, João Reis<sup>1,2</sup>, Rita Alcazar<sup>5</sup> and Pedro Beja<sup>6</sup>

#### (lfonseca@ualg.pt)

<sup>1</sup> FCMA, Universidade do Algarve, campus de Gambelas, 8005-139 Faro, Portugal;

<sup>2</sup> CCMar, Universidade do Algarve, campus de Gambelas, 8005-139 Faro, Portugal;

<sup>3</sup> Laboratório Marítimo da Guia/IMAR, Estrada do Guincho, 2750-374 Cascais, Portugal;

<sup>4</sup> Institute of Aquatic Ecology, University of Girona, campus Montilivi, E-17071 Girona, Spain;

<sup>5</sup>.LPN, Estrada do Calhariz de Benfica, 187, P-1500-124 Lisboa, Portugal;

<sup>6</sup> ERENA, Av. Visconde Valmôr, 11 – 3°, P-1000-289 Lisboa, Portugal

## ABSTRACT

Temporary ponds, in spite of being protected by the Habitats Directive (92/43/CEE), are presently disappearing at a fast rate. They urge management measures, but which parameters to use to establish management procedures? Knowing that most large branchiopod species (LBS) inhabit exclusively temporary lentic ecosystems, and also an important number of amphibian species (AS) use those habitats for reproduction, these groups may be used as key faunal groups to assess the value of Mediterranean Temporary Ponds for conservation purposes. 11 *taxa* deserve particular conservation concern and their habitats need special management decisions (LBS: *Branchipus schaefferi, Streptocephalus torvicornis, Tanymastix* sp, *Maghrebestheria maroccana, Cyzicus grubei* and *Triturus marmoratus pygmaeus*). Each has different ecological requirements (total area, depth, hydroperiod length...). The aim of this work is to propose the use of life cycles of the referred LBS with no protection status, and reproduction period (pairing, fertilization and larval development) of AS with protection status, as management tools for conservation purposes.

## **RESUMO:**

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.

# INTRODUCTION

Temporary ponds are, mostly, small and shallow water bodies, easily overlooked and vulnerable to a large range 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. 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 (Collinson *et al.*, 1995; Grillas & Roché, 1997).

Knowing that most large branchiopod species (LBS) inhabit exclusively these temporary lentic ecosystems (Alonso, 1996), and also an important number of amphibian species (AS) use those habitats for reproduction (Barbadillo *et al.*, 1999; Ferrand de Almeida *et al.*, 2001), these groups 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* and *Bufo bufo* have a preference for permanent waters. In Portugal, Large Branchiopoda has no conservation status, in contrast of some European countries where several species already have special protection status (Biggs *et al.*, 1994; Mura, 1999; Boix *et al.*, 2002); amphibian species, on the contrary, are protected in Portugal (Ferrand de Almeida *et al.*, 2001).

Among *taxa* inhabiting Mediterranean temporary ponds in South Portugal, 11 deserve particular conservation concern and their habitats special management decisions: i) LBS - *Branchipus schaefferi*, *Streptocephalus torvicornis, Tanymastix* sp, *Maghrebestheria maroccana, Cyzicus grubei* and *Triops mauritanicus*; ii) AS: *Pelodytes* sp and *P. ibericus, Bufo calamita, Hyla meridionalis* and *Triturus marmoratus pygmaeus* (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. The understanding of the most important ecological parameters constraining the presence of these species is one of the main objectives of the present work. The proposal of management tools for conservation of Mediterranean Temporary Ponds, such as life cycles of the referred LBS species, with no protection status, and reproduction period (pairing, fertilization and larval development) of AS species, with conservation status, is the aim of this work.

# MATERIAL AND METHODS

# <u>Study area</u>

Since 1996 several surveys are being carried out mainly on the south region of Portugal, collecting data on LBS and AS. An ecological study covering biological, physical and chemical parameters, allowing a finer collection of information on these habitats was carried out on the Guadiana region (GUAD) around Mértola and the Parque Natural do Vale do Guadiana (Fig.1). This survey was conducted during the wet season of the years 2001-2002 (31 October 2001 to 2 July 2002). Additional ecological data is also presented from the Southwest coast of Portugal.

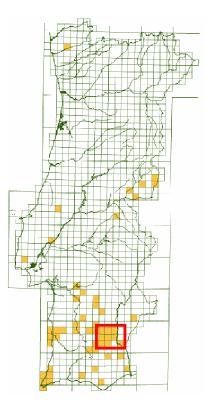


Fig. 1 –Distribution (UTM 10x10 km) of large branchiopod freshwater species sites in Portugal. The Guadiana region (GUAD) around Mértola and the Parque Natural do Vale do Guadiana is highlighted in red.

## Abiotic characterization

For the environmental characterization of pond water, the following parameters were collected *in situ*: oxygen concentration, percentage of dissolved oxygen and temperature (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 (prepositioned 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 to a UTM 10x10 km grid over a Portugal continental map.

Due to the prospective character of the work undertaken, only in GUAD a regular and systematic survey has been carried on. Table 1 presents a list of all the environmental parameters (abiotic and biotic) obtained. Out of this region, only *in situ* determinations and flooded area and plant coverage were registered, in most of the sites where at least one LBS has been found.

Hydroperiod was considered as the sum of periods when the pond was flooded (total wet phase) and was quantified as number of weeks of wet phase extent. One to several flooding periods was registered depending on pond size and depth and rain regime. Relative depth was computed as the maximum depth expressed as a percentage of maximal area of the pond (Wetzel, 1993). Human activity considered were the ploughing regime, intensity of ploughing, and fertilizing. 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 base scheme: 0— not ploughed during the last 10 years; 1— ploughed once; 2—2-5 times; 3—6-10 times. Fertilizing quantification has been done in the same basis according to: 0— without fertilizing during the last 10 years; 1—once; 2—2-4 times; 3— 5-8 times. Water samples were collected once at the end of January 2002, and chemical analysis were done using standard procedures at the DGA certified laboratory (Ministry of the Environment).

## **Biotic characterization**

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

Environmental Parameters	Code	Units	Transformation
Abiotic			
Chloride	CL	mg Cl/l	
Nitrates	NA	mg NO <sub>3</sub> /I	
Silicates	SI	mg SiO <sub>2</sub> /I	
Sulphates	SU	mg SO4/I	
Iron	FE	mg Fe/l	
Potassium	PO	mg K/l	
Sodium	SO	mg Na/l	
Magnesium	MG	Mg Mg/I	
Total hardness	DT	CaCO <sub>3</sub> /I	
Conductivity (average)	Cond	μS/cm	
Dissolved Oxigen	DO	mg/l	
pH	рН	-	
Temperature	Temp	°C	
Turbidity (average)	Turb	NTU	
Depth (average)	Prof	cm	
Relative depth	PrfRel t	%	Arc-sin
Maximal area	AR	m <sup>2</sup>	
Maximal area/potencial maximal area	AR/AP t		Arc-sin
Hydroperiod	Hidrop	Weeks	
Biotic			
Plant coverage (range)	R. Cobert	0-4 scale	
Human activity			
Ploughing	Lavra	0-1	
Intensity of ploughing	Int-lavra	0-3 scale	
Fertilizing	Adubação	0-3 scale	

Table 1 – List of environmental parameters registered during the different surveys (words between brackets and codes refer to what has been considered in the CCA analyses with units and transformation applied).

#### Fauna sampling

Faunal inventories were conducted by qualitative (erratic) or periodic semiquantitative sampling. The first case holds data about species gathered out of GUAD in a binary (presence/absence) basis. Concerning that first area, data on abundances of species was expressed in terms of catch-per-unit-effort (CPUE): mean number of individuals per sweep. Samples were done by one person wading across the pond using a hand round net (305 mm in diameter) of 1mm square mesh. In the last case (GUAD), each sample consisted of 3 replicates of 45 seconds each, conducted from the border to the center of the pond covering all available habitats.

#### Guadiana region - Branchiopod Sampling

In the beginning of the sampling period weekly surveys were conducted, but after the disappearing of the short life cycle species (anostracan species), the periodicity turn to fortnightly. Sampling was conservative, and for samples with a high number of anostracan specimens a Folson sample splitter was used. Identification and counting of rare species (*Cyzicus grubei* and *Triops mauritanicus*) was done at the field before splitting. The juvenile anostracan were preserved (neutral 10 % formaline) to be processed at the laboratory. The adults were also identified and counted in the field.

## Guadiana region - Amphibian sampling

Non destructive monthly sampling was conducted simultaneously with the branchiopod sampling. Identification and counting was done at the field before splitting. For very young larvae which size did not allow the identification, live samples were brought to the lab, maintained alive until possible identification and 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, 2003). 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 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 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

#### <u>Surveys</u>

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.

Half of them exhibit more than one wet period, and length and number of swaps from wet to dry periods is very variable (Fig. 2). This variation in hydroperiods is common in all over the surveyed areas and along the years that our studies were conducted (more than 10 years).

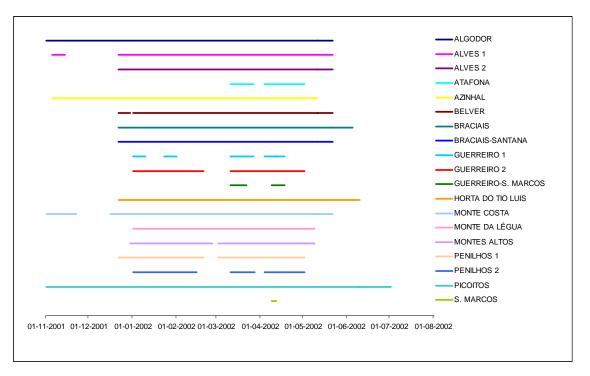


Fig. 2 – Variation of hydroperiod on ponds inhabited by LBS and AS at the Guadiana region.

At present 11 species of LBS crustaceans are known from Portugal, belonging to three orders: Anostraca, Spinicaudata and Notostraca. More than 260 sites were prospected. The LBS were only recorded from 126 of those, where10 of the 11 species present in Portugal were found, as well as 10 species of AS (Table 2).

Three new *taxa* were collected during the sampling surveys: an amphibian of the genus *Pelodytes*, previously known of the southwest Portugal but with an uncertain status till now (Ferrand de Almeida, pers. comm.), and two new and unknown Anostraca (a species of the genus *Tanymastix* and another belonging to genus *Tanymastigites*, a new one for Europe).

 Table 2. Large Branchiopod Species (LBS) and Amphibian Species (AS) found in the sites surveyed in

 Portugal (with codes used in the GUAD data analysis)

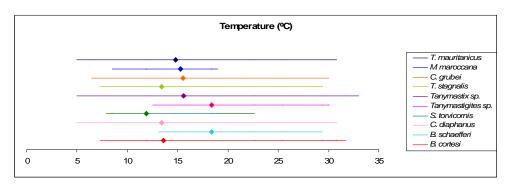
LBS	Code	Code AS		
Anostraca		Urodela		
Branchipus cortesi Alonso & Jaume	BRCO	Pleurodeles waltl Michaelles	PLWA	
Branchipus schaefferi Fischer		Salamandra salamandra crespoi (Malkmus)		
Chirocephalus diaphanus Prevost	CHDI	Triturus boscai (Lataste)		
Streptocephalus torvicornis bucheti Daday	STTO	Triturus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

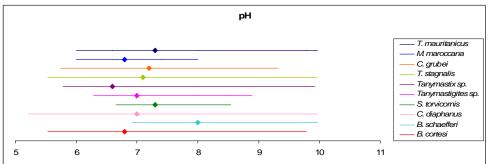
# Large Branchiopod Species

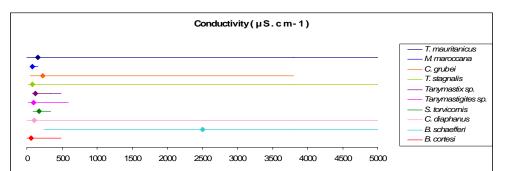
*C. diaphanus* is the commonest LBS species in Portugal and is present in 65 of the 125 freshwater LBS sites. The number of sites where the different species have been recorded are: *T. mauritanicus*, 52; *T. stagnalis*, 41; *B. cortesi*, 40; *C. grubei*, 22; *Tanymastix* sp., 14; *B. schaefferi*, 8; *S. torvicornis*, 8; *Tanymastigites* sp., 6; *M. maroccana*, 5. Out of the geographical area surveyed by us, there is one site confirmed for *Lepidurus apus* in the north-western part of Portugal (Grosso-Silva & Soares Vieira, 2002). These results pointed out that a large and diversified number of conditions may determine their distributions.

The results of the analysis of some of the environmental parameters taken into account in this study are presented in Figures 3 and 4. Ranges and average and 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 more euryecious species.

Differences are also found concerning the parameters considered in Fig. 4, but they are not similar. *C. diaphanus* seems to be again the more tolerant in a global view, but facing different descriptors, diverse responses appear. *Tanymastigites* sp. is the less tolerant to plant cover, preferring lower depths and also smaller areas (together with *Tanymastix* sp.). The shorter hydroperiods faced by *M. maroccana* may be biased by the shortness of data on this species.







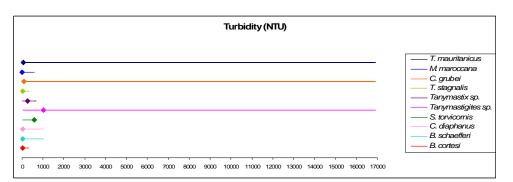
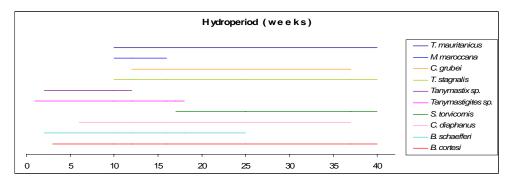
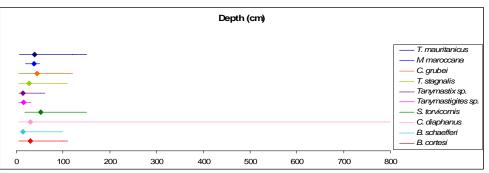
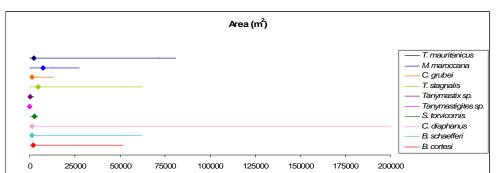


Fig. 3 – Average points (temperature and pH) and Median points (conductivity and turbidity) (•) and their different ranges endured by the different LBS in most of the sites where their presences were registered.







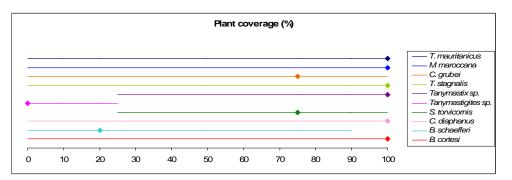


Fig. 4 – Median points (•) of depth, area and plant coverage and different range of hydroperiods, depth, area and plant coverage endured by the different LBS in most of the sites where their presences were registered.

Principal components 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). The variance retained by the three first axes (respectively 53.0%, 22.8% and 10.2%) shows a sufficient difference between axes 2 and 3 and allow the interpretation based on the axes 1 and 2.

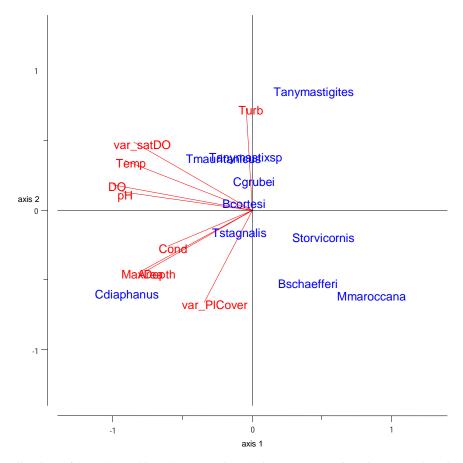


Fig. 5 – Ordination of large branchiopods (PCA) in relation to range of environmental variables. Codes according to Table 1, except for dissolved oxygen saturation (var\_satDO) and plant cover (var\_PlCover) (axis 1 and 2 explicate 75.8% of variance).

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 more euryecious.

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.

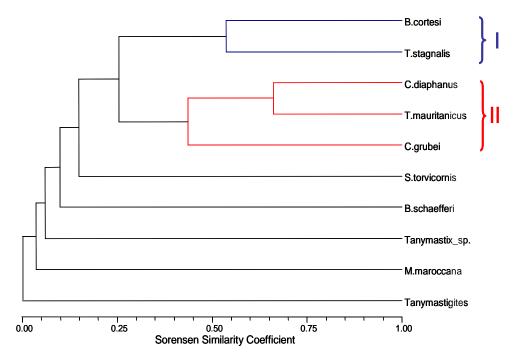


Fig. 6 – Cluster multivariate analysis using the Sørensen Similarity Coefficient (UPGMA method). Two main groups of large branchiopods may be identified (presence/absence LBS per pond data, R mode).

Results of principal components analysis (PCA) in relation to average and median values of environmental variables is showed in Fig. 7.

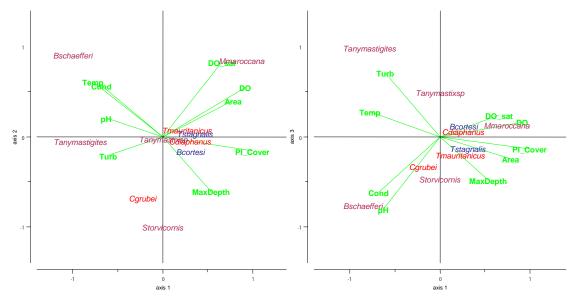


Fig. 7 – Ordination of large branchiopods (PCA) in relation to average and median values of environmental variables. Codes according to Table 1 and/or Fig. 5 except for dissolved oxygen saturation (DO sat) and plant cover (Pl Cover) (Blue and red are those referred as Groups I and II in presence/absence cluster analysis) (axis 1, 2 and 3 explicate 86.0% of variance).

The variance retained by the three first axes (respectively 46.2%, 21.3% and 18.5%) did not reveal sufficient difference 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 the average/median values of the environmental parameters agree with and explain those from the presence/absence analysis (Fig. 6) and their central position emphasise 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. As a rule, Anostraca species are those with shorter life spans, as it was found in the pond Monte de Vale Santo, in the Portuguese Southwest coast, near Sagres (Fig. 8).

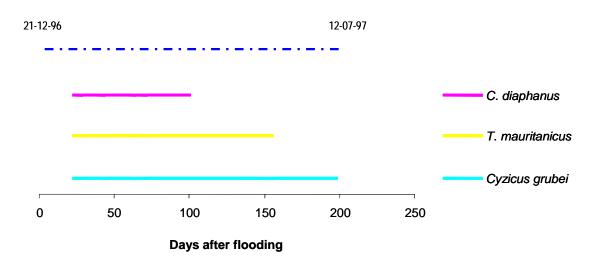


Fig. 8 – Diagram showing large branchiopods (Anostraca, Notostraca and Spinicaudata) life span in relation to flooding period in pond Monte de Vale Santo (Sagres) (Blue dashed line represents hydroperiod).

Concerning the studied ponds in GUAD, the Anostraca life length, with very rare exceptions, is 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 *Tanymastix* sp. are those with shorter life cycles. *T. stagnalis* lives 10-11 weeks, with females maturation after 2-3 weeks; the second species may have mature females 10 to 18 days after hatching and a total life length up to 8 weeks.

Recorded life spans of *C. diaphanus* vary from 1.5 to 4 months with the presence of mature females from 2 weeks (with high water temperatures) to 2 months after hatching.

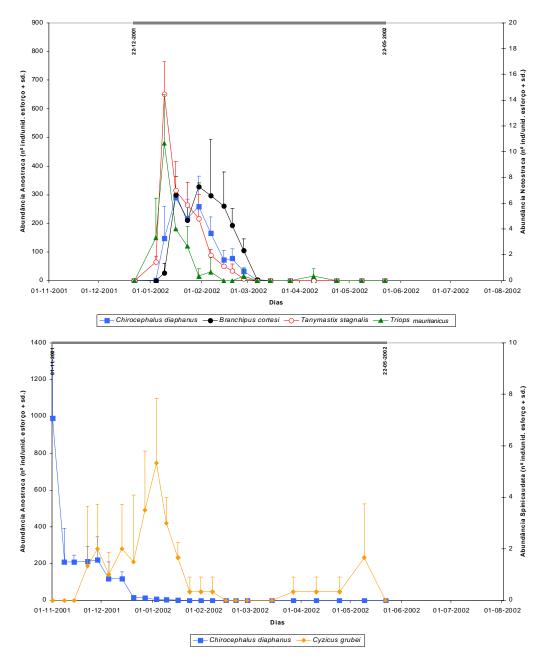


Fig. 9 – Abundance (+ SD) of LBS during the flooding period in ponds "Alves 2" and "Algodor" (GUAD), as an example of populations evolution during life spans (grey patterned lines represents hydroperiod).

Abundances fluctuate during the life span (Fig. 9) and, as a rule, species of the above referred group start early with initial blooms. *C. grubei*, on the other hand, is the

species with the longer life cycle as it was known from the southwest populations (cf. Fig. 8).

The analysis of Fig. 10 supports the idea that the environmental parameters that most influences species distribution might be hydroperiod and depth, either positively conditioning the presence of *C. grubei* and *S. torvicornis* or negatively influencing the distribution of *Tanymastix* 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.

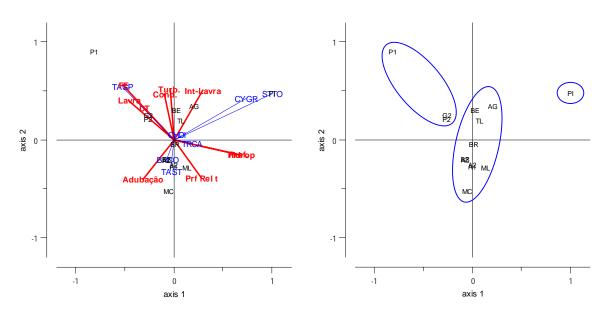


Fig. 10 – Ordination (CCA) of temporary ponds (GUAD) based on LBS average abundances (left: blue) and values of environmental variables (left: red). Right graph: groups of ponds (axis 1, 2 and 3 explicate 74.5% of variance).

The CCA based on the abundances of LBS allowed the separation of three groups of ponds (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 upon their permanence time in ponds (Fig. 11). *Pleurodeles waltl* was registered almost in every studied pond, with the exception of two with very short hydroperiods, and is one of the fastest colonizers and longer remainders. This species, together with *Pelobates cultripes* 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 colonizers and the rarest species.

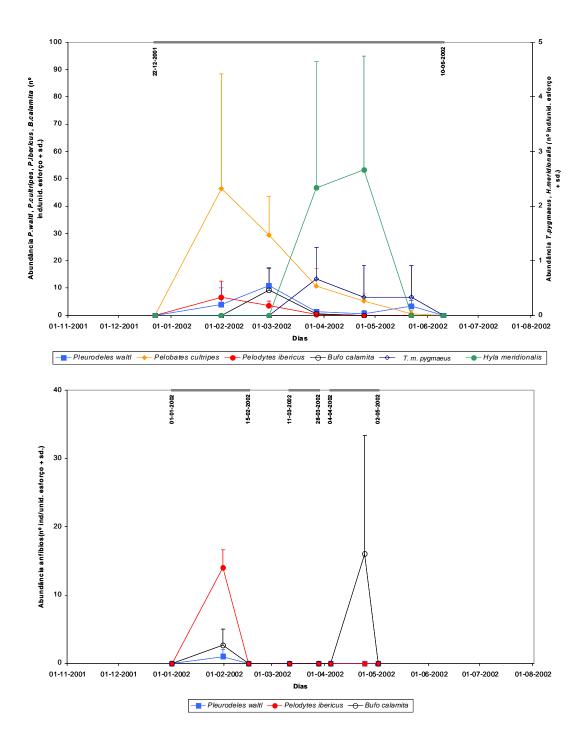


Fig. 11 – Abundance (+ SD) of AS during the flooding period in ponds "Horta do Tio Luís" and "Penilhos 2" (GUAD) as an example of populations evolution during larval phase (grey patterned lines represents hydroperiod).

The analysis of Fig. 12 do not show a clear direct influence of a particular environmental variable on the distribution of species, perhaps with the exception of depth and hydroperiod, which positively influenced the behaviour of *R. perezi and T.m. pygmaeus* and seem to have a negative contribution in relation to both *B. calamita* and *P. ibericus*. Also, fertilizing seems to have a negative weight on the behaviour of the majority of the species.

The CCA based on the average abundances of AS also allowed the separation of three groups of ponds (Fig. 12, right graph) with a very similar clustering to the one obtained with LBS.

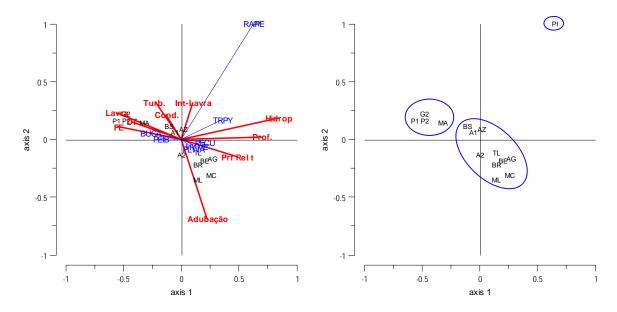


Fig.12 – Ordination (CCA) of temporary ponds (GUAD) based on AS average abundances (left: blue) and values of environmental variables (left: red). Right graph: groups of ponds (axis 1, 2 and 3 explicate 80.3% of variance).

# DISCUSSION

The Portuguese fauna of freshwater large branchiopods, inhabitants of temporary ponds and pools, has been poorly studied. In the 1944 catalogue of the Portuguese invertebrate collection of the Zoological Museum of Coimbra University, one specimen of *Apus cancriformis* Schäffer 1756 (= *Triops cancriformis* (Lamarck, 1801)) is included (Carvalho, 1944). The first published scientific paper, from 1951 by Vianna-Fernandes (1951), refers the occurrence of 3 new species in the Portuguese

fauna – 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), started 45 years later. The inventory of freshwater large branchiopod species we present here updates the knowledge on LBS of Portuguese temporary lentic systems.

Information on Amphibian species on these environments in Portugal is also scarce and data collected in GUAD is an important contribution to the knowledge on the distribution and life cycles of AS in temporary pools and ponds at southeast Portugal. Together with Beja & Alcazar work (2003), the present study joints relevant data to the understanding of these priority habitats leading to more accurate management strategies.

In spite of some research carried out North to 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 years, reporting the presence of *Lepidurus apus* in Portugal. Our own surveys (unpublished data) just have joined 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 were, 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, 3 new LBS *taxa*: *Branchipus schaefferi*, *Tanymastix* sp. and *Tanymastigites* sp., the description of the last two being carried out.

Based on the fact that from 1996 onwards we surveyed a large area of Portugal, and a large number of sites, we should be able to say that our knowledge on these Mediterranean Temporary Ponds is still insufficient, because the variability found leads to the rise of more and more questions that need to be answered, as it was pointed out by Beja (2000).

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

## Large Branchiopod species

The results of the analysis of the cross information on environmental parameters and presence of species, stresses that there are several parameters to which species are more sensitive than to others, namely in what concerns their range of variation. Conductivity, turbidity (Fig. 3), hydroperiod, depth and area (Fig. 4) are the main constraints to the species distribution. It is also possible to observe that the group of species that tolerates wider variations on those parameters are *T. mauritanicus*, *C. grubei*, *C. diaphanus*, *T stagnalis* and *B. cortesi*.

We may also notice that in what concerns medians of environmental parameters, most of the values are located at the low end of the graph with the exception of conductivity preferences of *B. schaefferi*. In fact this species is considered able to endure brackish waters (Lanfranco *et al*, 1991; Petkovski, 1997).

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

The same group formed by the 5 more tolerant species is stressed out 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). The most sensitive species in the other hand are: *Tanymastigites* sp., *M. maroccana, Tanymastix* sp., *B. schaefferi* and *S. torvicornis*. The environmental parameters that mostly explain 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) have reported that at GUAD species richness of large branchiopods is greater in ponds with longer hydroperiods, higher depth 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 situations may be found.

LBS show great variability in their life cycles length. They hatch early and simultaneously, as in Morocco (Thiéry, 1987) but in most cases Anostracan have shorter life cycles and 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 order to be described. Nevertheless the new genus (*Tanymastigites*) discovered for Europe was only known from North Africa and Arabian Peninsula (Belk and Brtek, 1995; Thiéry, 1996).

On the whole, there were tendencies for: i) *Tanymastix* sp. and *Tanymastigites* sp. to avoid deep ponds with extended flooding periods; ii) the previous species and *M. maroccana*, *S. torvicornis* and *C. grubei* to be absent from large ponds; iii) *M. maroccana*, *S. torvicornis*, *Tanymastigites* sp., *Tanymastix* sp. and *B. cortesi* to favour waters with relatively low conductivity; iv) *S. torvicornis* to be associated with deep ponds with moderate to long hydroperiods; v) *T. stagnalis*, *C. diaphanus*, *T. mauritanicus* and *B. schaefferi* to tolerate moderate brackish waters.

# Amphibian Species

Temporary ponds are particularly important for the amphibian survival in general and 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 pioneer species were *P. cultripes, P. ibericus*, and *P. waltl*, and behaving as later colonizers we found *T. m. pygmaeus*, *H. meridionalis* and *R. perezi* (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 the hydroperiod, if only one, and differentiated species occupancy if there was a succession of dry and wet periods. These facts were also registered for the Southwest Portugal by Beja & Alcazar (2003).

Some positive relations with agriculture activities were noticed: *R. perezi* 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 an abundant plant cover.

It also seems that there is a tendency for *R. perezi* 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 Amphibian species 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 constrains 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).

AS found in the studied ponds are considered, like almost all the Portuguese species of amphibians, not 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). *Pelobates cultripes, Bufo calamita* and *Hyla meridionalis* own the more restricted protection status: they belong to the strictly protected species group of Bern Convention.

As general remarks on AS we can say that: i) Salamandra salamandra crespoi and Triturus boscai appears to be very rare in temporary ponds; ii) the species Pelodytes punctatus referred by Beja & Alcazar (2003) is certainly a new taxa (Ferrand de Almeida, pers. comm.); iii) in temporary ponds, more persistent wet phases favour the occurrence of T. m. pygmaeus, H. meridionalis and R. perezi; 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.

## <u>Management</u>

The conservation of biodiversity in areas were water is scarce has an increased importance for the maintenance of species that directly depend on temporary ponds for reproduction – Large Branchiopods and Amphibians 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 contribute to their preservation. Nowadays, with the new strategies for water and soil management relayed to agriculture intensification, their role within a traditional land use logic is being lost. So, Mediterranean Temporary Ponds are under threat and most of them are being reclaimed for agriculture or other human activities. Actually 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.

Farmers tend to either filling them up to use 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 a swap of 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; M. Machado, M. Cristo & L. Cancela da Fonseca, unpublished data) to ensure their survival (LBS: from ca. 20 - Branchipus schaefferi and *Tanymastix* sp. - up to 120 days – *Cyzicus grubei* - to complete their life cycles; AS: from ca. 20 - Bufo calamita – to more than 100 days – *T. marmoratus pygmaeus* – to complete their larval development).

If, on one hand, the knowledge of life cycles of the occurring species is important to define the strategies for maintenance of ponds with shorter or longer hydroperiods, on the other hand, it is also needed to have in mind that the colonization by species with low mobility (as AS are supposed to be) depend on the relative proximity of ponds. For species that dispersal is done by external agents (wind, animals) it is also of major importance that the hydroperiods will allow the reproduction success. Moreover, as LBS population success relays on the cyst bank kept in pond sediment (Belk, 1998) its unusefulness, by silting up, or possible destruction, by shifting to a permanently inundated status, leads to the 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 of its sites during the last years. Situations where the hydroperiod had a short length in some GUAD ponds, prevented *H. meridionalis* and *T. m. pygmaeus* from reaching the adult phase (Cristo *et al.*, 2002). So management of Temporary Mediterranean Ponds should consider these aspects of the bio-ecology of those species and it comes into sight that a conservation strategy for the entire temporary pond biodiversity should preserve ponds representative of the whole hydroperiod gradient.

As it was noticed, ponds with longer hydroperiod 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 needed tools. In any case, length of life cycles of the exclusive species should be considered together with their behaviour and ecological features of the habitat.

Human actions tending to promote habitat changes may have both advantages and shortcomings for LBS and AS (Cristo *et al.*, 2002), depending on 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 be only possible inside a network of ponds representative of the entire environmental gradient, in which 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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