The Central Mediterranean Naturalist	3(2): 69 -78	Malta, September, 2000

PRELIMINARY INVESTIGATIONS INTO THE VERTICAL STRUCTURE OF PROPAGULE BANKS OF TEMPORARY FRESHWATER ROCKPOOLS IN THE MALTESE ISLANDS

Sandro Lanfranco[1], James Callus[2], Stephen Cilia[3], Howard J. Zammit[4]

ABSTRACT

The vertical structure of the propagule bank of five angiosperms (*Elatine gussonei, Damasonium bourgaei, Callitriche truncata, Ranunculus saniculaefolius, Zannichellia palustris*) and one charophyte (*Chara vulgaris*) was studied in eight ephemeral autumnal rockpools from two localities in the Maltese Islands. Distribution of seeds and oöspores was distinctly non-uniform, with 94% of propagules being located in the top 4cm of sediment. Propagules that were recovered from depths exceeding 4cm are unlikely to have been buried *in situ* but are probably due to percolation from the surface during the dry season when cracks and fissures in desiccated sediment provide such a route. Operation of this process is indicated by over-representation of the smallest propagules in the deepest strata. The presence of large numbers of propagules in the surface layers is adaptive, facilitating reception of germination cues and attainment of the soil surface by young shoots. However this strategy also exposes propagules to predation as well as to high temperatures during the dry season. The data obtained in the present study suggest that quantification of contributions to and losses from the propagule bank should be the focus of subsequent work aimed at constructing a predictive model of the population dynamics of these species.

INTRODUCTION

Temporary freshwater pools of the Maltese Islands are restricted to outcrops of karst terrain. The distribution of pools is limited by the relative scarcity of such terrain while the high insularity of the pool habitat implies that resident organisms restricted to these pools would also be relatively infrequent in occurrence and therefore of significance for conservation. As such, a high proportion of obligate hydrophytes recorded from temporary freshwater rockpools in the Maltese Islands are included in the Red Data Book for the Maltese Islands. Allocation of these hydrophytes to IUCN Red List categories (Species Survival Commission, 1994) would require estimation of the mean time to extinction (T_e) of known populations. The construction of models that may be used to predict T_e of aquatic macrophytes necessitates investigation of all the life-cycle stages of these organisms.

Studies concerning the ecological dynamics of macrophytes in temporary freshwater rockpools of the Maltese Islands [Lanfranco (1990, 1995), Vassallo (1998)] have tended to focus on the vegetative stage of the life-cycle without considering the role of the propagule bank in these processes. Whilst investigations of this form can certainly generate valuable ecological data, they are inherently limited by that lack of attention paid to the dynamics of the propagule bank. The only studies concerning propagule banks of temporary freshwater pools in the Maltese Islands are those of Zammit (1999), who worked on pools in Malta and Gozo, and Callus & Cilia (2000), who assessed a number of pools in Malta as part of a wider study concerning the ecology of hydrophytes.

All hydrophyte genera recorded from temporary freshwater pools in the Maltese Islands have also been recorded from other ephemeral wetlands along the north-western coast of the Mediterranean and the structure and composition of local propagule banks would therefore be expected to exhibit considerable overlap with propagule banks in other parts of the Mediterranean. Investigations of propagule banks in temporary wetlands of the Mediterranean region include Grillas *et al.* (1992) in the Camargue, Grillas *et al.* (1993) in the Doñana, Bonis and Lepart (1994) in the Camargue and Bonis and others (1995) in the Camargue.

---

[1] Department of Environmental Science, GF Abela Junior College, University of Malta, Msida, Malta
[2] "Cherie", 78, Triq l-Imriekeb, Marsascala, Malta
[3] Block I Flat 2, Triq l-Ghansar, Santa Lucija, Malta
[4] "Fiorella", Triq P.P. Castagna, Balzan, Malta

The present study aims to provide a preliminary characterisation of the vertical structure of the propagule bank of eight temporary freshwater rockpools with a view to generating foundations for testable hypotheses and ecological models in subsequent investigations.

MATERIALS AND METHODS

The sites of study - The material included in the present study was derived from propagule banks of eight pools in two localities. Five pools were situated at San Pawl tat-Tarġa, Malta and three at il-Blat tal-Kapuċċel, Munxar, Gozo. Rock outcrops at both localities comprised karstified lower coralline limestone characterised by several kamenitzas within which rainwater accumulated during the wet season (September/October - March/April). Identification codes given to each pool follow nomenclature in previous studies and are summarised in Table 1. The longest axis of the study pools ranged from 1.17m to 2.10m, maximum water depth from 0.06m to 0.24m and maximum depth of sediment from 0.06m to 0.21m.

Choice of species - Characterisation of propagule banks of the study pools was limited to the five angiosperms (*Elatine gussonei, Damasonium bourgaei, Callitriche truncata, Ranunculus saniculaefolius, Zannichellia palustris*) and one charophyte (*Chara vulgaris*) for which reliable reference material was available at the time of the study.

Field methods - Coring of sediment from the study pools was carried out in November 1998, immediately following the first wetting (but not flooding) of the pools. No cores were taken during the dry season due to the friable texture of the substratum when desiccated. As such, coring was only practical when the sediment was moist and therefore consolidated. Timing of sediment collection was therefore crucial, since this had to take place shortly after wetting and before the onset of germination.

Seed and oöspore samples were collected by pushing a corer (internal diameter 3cm) through the sediment. The propagule bank of each pool was characterised by collecting a number of replicated samples from randomly-selected points in each pool. The number of replicates varied according to the size of the pool. Details relating to sample size are recorded in Table 2.

Laboratory Methods - Seeds and oöspores were isolated from the soil samples using the method outlined in Grillas *et al.* (1993). Each core section was dried to constant weight at 70°C and subsequently washed for one hour in a 1% solution of H_2O_2 amended with 10g of sodium metaphosphate per litre. The treated sediment was then sifted through two separate sieves of mesh 2mm and 125µm using low-intensity jets of tapwater. The sediment retained by the 125µm mesh was placed on filter paper and dried overnight at 70°C. The dried sediment was subsequently examined under a dissecting microscope and all seeds and oöspores present identified and tallied. Only propagules that are viable contribute to the active reserve, and all seeds and oöspores observed were therefore punctured to test for viability.

Table 1: Identification codes of study pools.

Identification code	Locality	Nomenclature
Pool 1	San Pawl tat-Tarġa, Malta	Lanfranco (1990, 1995) Callus & Cilia (2000)
Pool 2	San Pawl tat-Tarġa, Malta	Zammit (1999)
Pool C	San Pawl tat-Tarġa, Malta	Callus & Cilia (2000)
Pool D	San Pawl tat-Tarġa, Malta	Callus & Cilia (2000)
Pool E	San Pawl tat-Tarġa, Malta	Callus & Cilia (2000)
Munxar 6	Munxar, Gozo	Zammit (1999)
Munxar 15	Munxar, Gozo	Zammit (1999)
Munxar "New Pool"	Munxar, Gozo	Zammit (1999)

Management and analysis of data - Descriptions of propagule banks were based on the numerical abundance of seeds and oöspores. Numerical abundance was standardised in order to permit comparison of abundance between seeds of different species. Standardisation of abundance for propagules of a single species in a single core segment was carried out by dividing the number of propagules recovered from a segment by the total number of propagules in the entire core.

The variance of seed and oöspore abundance within pools and between pools was assessed using One-way

Table 2: Samples taken from study pools.

Pool	Number of replicate cores	Number of core sections (replicate 1)	Number of core sections (replicate 2)	Number of core sections (replicate 3)	Number of core sections (replicate 4)	Number of core sections (replicate 5)
Pool 1	5	11	4	3	7	4
Pool 2	1	4	-	-	-	-
Pool C	4	7	3	2	6	-
Pool D	5	8	6	5	5	2
Pool E	5	5	5	5	4	3
Munxar 6	2	4	6	-	-	-
Munxar 15	1	2	-	-	-	-
Munxar "New Pool"	2	4	2	-	-	-

ANOVA. Comparison of seed and oöspore counts from different strata within a core was carried out using Student's unpaired *t*-test and Welch's approximate *t* while comparison of propagule numbers in the 0-4cm depth range with propagule numbers from strata deeper than 4cm was carried out using a Mann-Whitney Test. Correlation of propagule abundance with depth of burial was carried out using Pearson's product-moment correlation coefficient. All statistical tests were performed using GraphPad InStat version 3.02 for Windows 95 (GraphPad Software, 1998).

RESULTS

Representation of species in propagule banks - The most frequently recorded propagules, in terms of raw numerical abundance, were seeds of *Elatine gussonei* and oöspores belonging to *Chara vulgaris*. Seeds of *Elatine gussonei* were recorded from all pools, all cores and from 92% of core sections. Oöspores attributed to *Chara vulgaris* were present in five pools, 80% of cores and 72% of core sections. Larger seeds (*Damasonium bourgaei*, *Ranunculus saniculaefolius*, *Callitriche truncata* and *Zannichellia palustris*) were noted less frequently. These data are summarised in Table 3.

Within-pool and between-pool variance - The results of One-way ANOVA indicated that the variance of mean seed abundance (for *Elatine gussonei*) and mean oöspore abundance (*Chara vulgaris*) in the top layer of sediment within pools was not significantly different from the variance between pools (*E.gussonei*, P=0.1511; *C.vulgaris*, P=0.0687). This suggested that seed and oöspore counts from different pools could be combined in order to derive generalisations about the vertical structure of the propagule bank. Data from the other four species were too scant to fulfil the assumptions of One-way ANOVA and this probability was therefore not calculated.

Vertical structure of the propagule bank (all species) - The top four centimetres of sediment across all pools contained 94% of all macrophyte propagules recorded. These data are summarised in Figures 1 and 2. A One-way ANOVA with Tukey-Kramer Multiple Comparisons post test indicated that the variation in the mean standardised abundance across the top six sediment strata (0-1cm to 5-6cm) was significantly greater than expected by chance (P=0.0041). The standardised total abundance of propagules in the top layer of sediment was significantly higher than that in strata below 2cm (Table 4).

Vertical structure of the propagule bank (individual species) - The vertical distribution of seeds (oöspores, in the case of *Chara vulgaris*) of the six macrophytes under consideration is summarised in Figures 3 and 4. Inspection of the data suggests that the general trend for all species is repeated at the level of individual species, with more propagules being recorded from surface horizons. These data were however characterised by large variance and the mean abundance of propagules in the topmost horizon was consequently not significantly larger than that from deeper sediment for any species. These results are summarised in Table 5.

When strata down to the 7-8cm level were considered, negative correlation between sediment depth and number of propagules was noted for all species (Table 6). Nevertheless, only two correlations (*E. gussonei* seeds with depth and *C. vulgaris* oöspores with depth) are statistically significant.

Table 1: Presence of macrophyte propagules in samples from study pools.

Species	Frequency in pools	Frequency in cores	Frequency in core sections
Elatine gussonei	100%	100%	92%
Chara vulgaris	63%	80%	77%
Damasonium bourgaei	75%	72%	39%
Callitriche truncata	50%	40%	20%
Ranunculus saniculaefolius	88%	52%	21%
Zannichellia palustris	50%	20%	12%

Table 4: Comparison of standardised total abundance of propagules in top layer and deeper layers.

Strata compared	q statistic*	P value
0-1cm with 1-2cm	3.582	P >0.05
0-1cm with 2-3cm	4.347	P<0.05
0-1cm with 3-4cm	4.562	P<0.05
0-1cm with 4-5cm	4.522	P<0.05
0-1cm with 5-6cm	4.301	P<0.05

* The q statistic is derived from the Tukey-Kramer Multiple Comparisons Test.

Table 5: Comparison of propagule abundance in top layer with deeper layers for each species. Comparisons performed using Student's unpaired t-test and Welch's approximate t.

Species	Strata compared		
	0-1cm with 1-2cm	0-1cm with 2-3cm	0-1cm with 3-4cm
E. gussonei	P=0.9624	P=0.3835	P=0.7015
C. vulgaris	P=0.8492	P=0.2270	P=0.0883
D. bourgaei	P=0.5193	P=0.5184	P=0.5652
C. truncata	P=0.8057	P=0.8952	P=0.4405
R. saniculaefolius	P=0.3940	P=0.4188	P=0.4138
Z. palustris	P=0.2451	P=0.2527	P=0.2853

Table 6: Correlation of mean propagule abundance with depth of burial (all pools)

Species	Correlation coefficient (r)	P value
Elatine gussonei	-0.8994	P=0.0024
Chara vulgaris	-0.8705	P=0.0049
Damasonium bourgaei	-0.6095	P=0.1462
Callitriche truncata	-0.7915	P=0.2085
Ranunculus saniculaefolius	-0.6114	P= 0.1972
Zannichellia palustris	-0.7880	P= 0.2120

Table 7: Ratio of larger seeds to smaller propagules in all sediment strata from all pools.

Stratum	10-11cm	9-10cm	8-9cm	7-8cm	6-7cm	5-6cm	4-5cm	3-4cm	2-3cm	1-2cm	0-1cm
Ratio	0.00	0.00	0.00	0.00	1.39	0.29	0.59	2.58	1.37	1.14	3.63

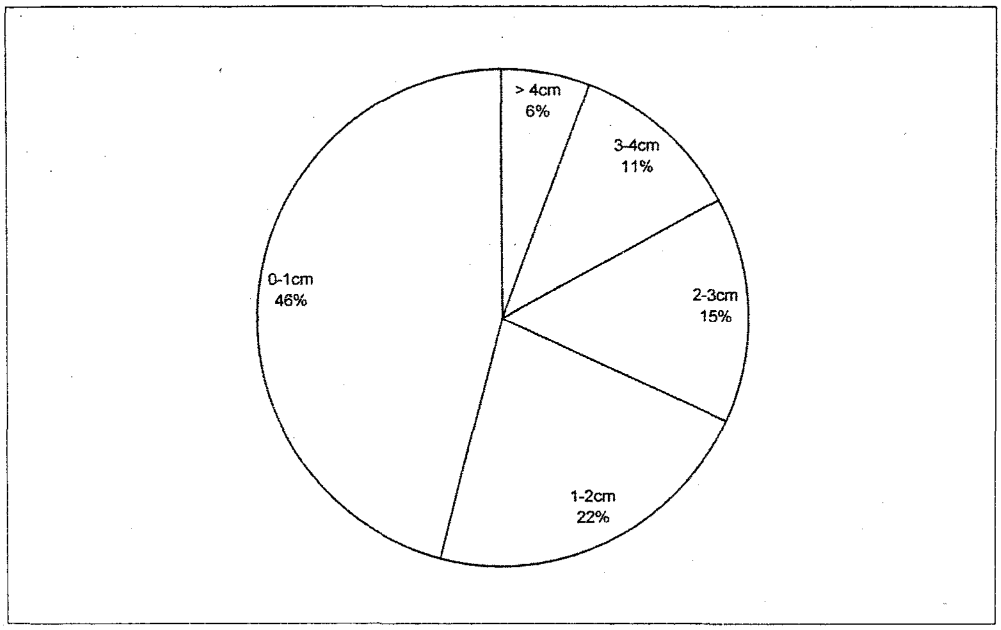


Figure 1: Vertical distribution of seeds and oöspores based on standardised total abundance (all species and all pools).

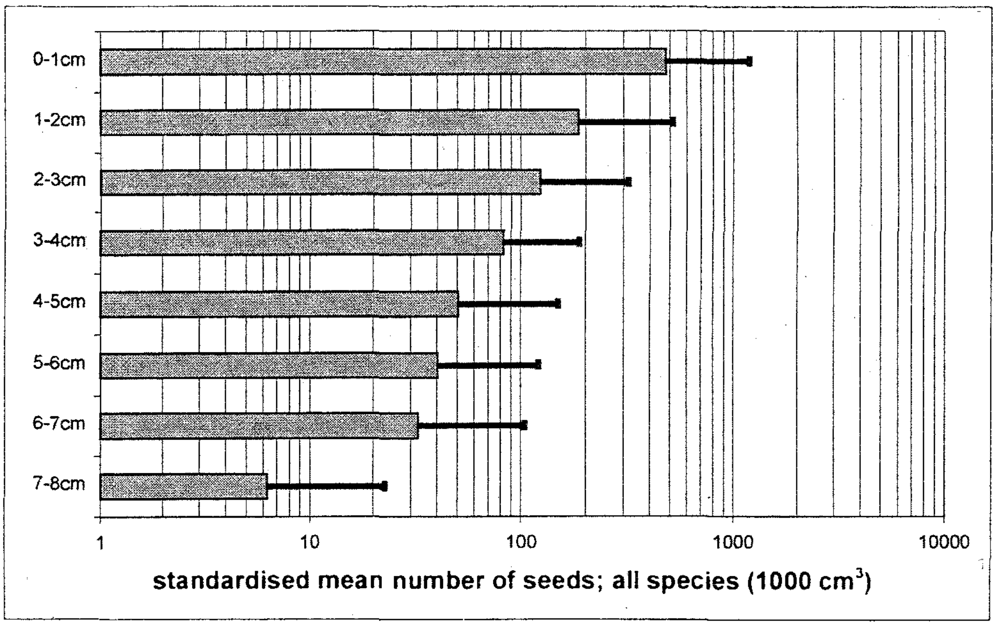


Figure 2: Vertical distribution of seeds and oöspores based on standardised mean abundance (all species and all pools). Error bars represent one standard deviation from the mean.

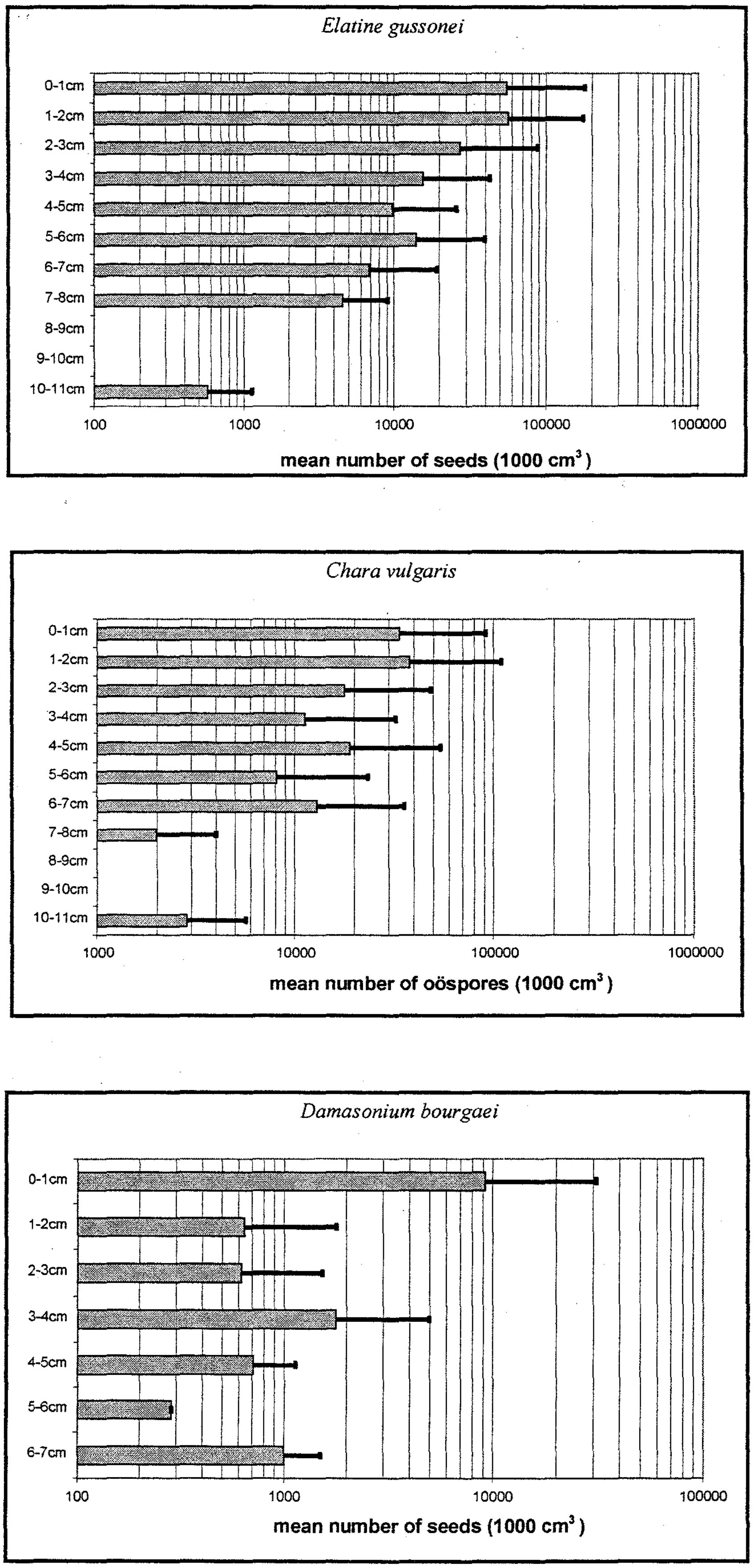
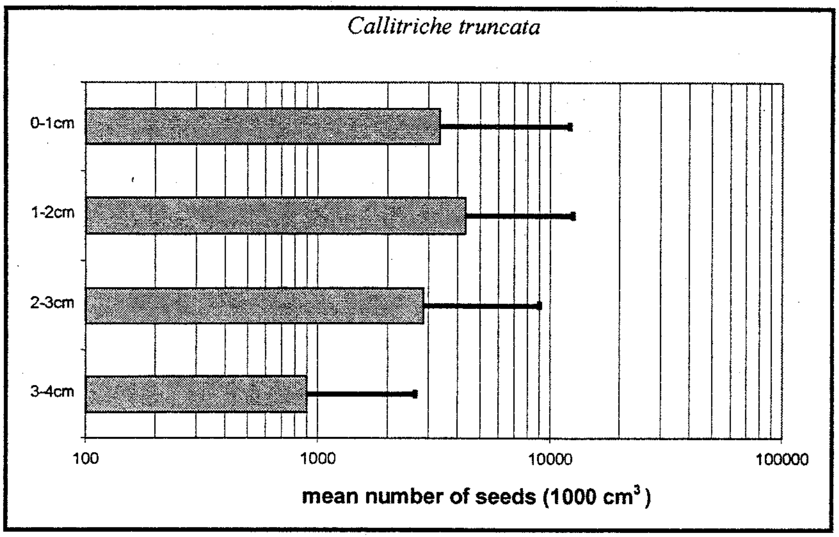
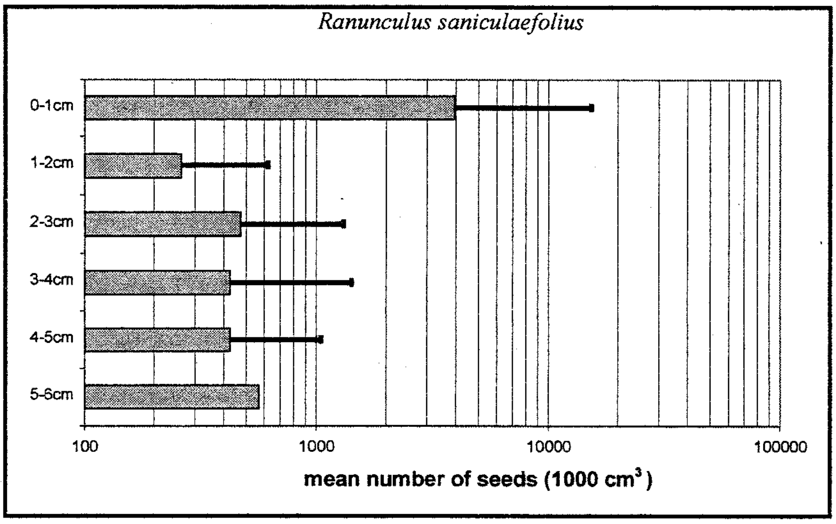


Figure 3. Vertical distribution of propagules attributed to *E. gussonei*, *C. vulgaris* and *D. bourgaei* (all pools). Error bars represent one standard deviation from the mean.





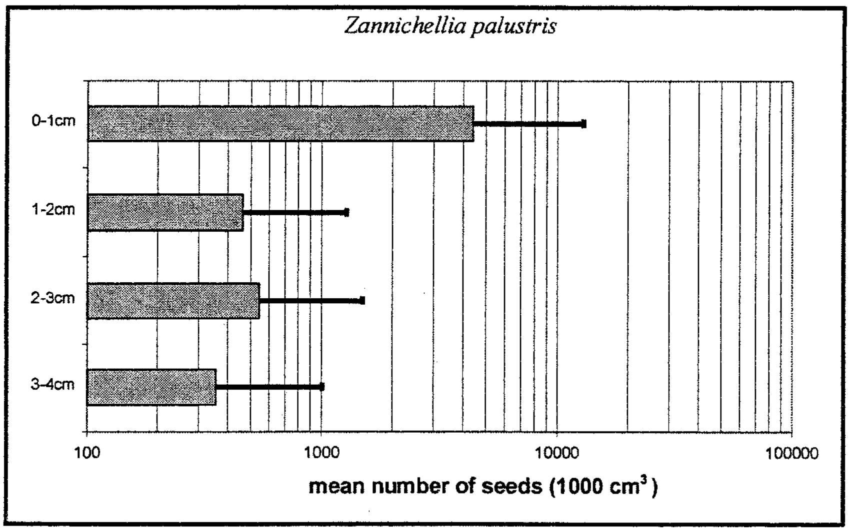


Figure 4: Vertical distribution of propagules attributed to *C. truncata*, *R. saniculaefolius and Z. palustris* (all pools). Error bars represent one standard deviation from the mea

Representation of smaller seeds in deeper sediment strata - The ratio of larger seeds (*D. bourgaei*, *R. saniculeifolius*, *C. truncata*, *Z. palustris*) to smaller propagules (*C. vulgaris*, *E. gussonei*) was calculated for all sediment strata by comparing total standardised abundance for the two size classes. These results are summarised in Table 7 and indicate that smaller propagules are over-represented in deeper sediment strata (>4cm) relative to shallower layers (P=0.0242).

Vegetative stages of aquatic macrophytes - The species represented by propagules in the propagule bank were generally also present in a vegetative form during the wet season. In six pools from the eight under study, all the species represented in the seed/oöspore bank were also present in vegetative form. In Pool 2, *Chara vulgaris* was not recorded in vegetative form although its oöspores were present in the propagule bank. In Munxar "New Pool", *Damasonium bourgaei* and *Zannichellia palustris* were present in the propagule bank but were not recorded in vegetative form. These results are summarised in Table 8.

The pronounced asymmetric vertical distribution in the pools under study is attributable to the low rates of net sediment accumulation in the pools under observation. Although not directly quantified, these are inferred to be very low, facilitating the accumulation of propagules in the surface horizons.

Low rates of sediment accumulation suggest that seeds and oöspores recovered from deeper strata (below 4-5cm) are therefore unlikely to have been buried *in situ*, but may be the product of downward transport through the sediment. Opportunities for such downward movement would occur during the dry season when cracks and fissures in desiccated sediment provide such a route. This suggests that the over-representation of smaller propagules (seeds of *Elatine gussonei* and oöspores of *Chara vulgaris*) in the deeper strata is a consequence of this process. Various authors (Leck (1989), Bonis and Lepart (1994b)) suggest that disturbance by earthworms, mammals and waterfowl may be responsible for transporting buried seeds to the surface. These processes are unlikely to be significant in the temporary pools included in the present study since earthworms are not present in pools for much of the year, if at all, while waterfowl and large mammals are uncommon or altogether absent.

Table 8: Representation of species by seeds (S), oöspores (O) and vegetative forms (V) in the pools under study.

Species	**Pool 1**		**Pool 2**		**Pool C**		**Pool D**		**Pool E**		**Munxar 6**		**Munxar 15**		**Munxar "New Pool"**	
E. gussonei	S	V	S	V	S	V	S	V	S	V	S	V	S	V	S	V
C. vulgaris	O	V	O	-	O	V	O	V	O	V	-	-	-	-	-	-
D. bourgaei	S	V	-	-	S	V	S	V	S	V	S	V	-	-	S	-
C. truncata	S	V	S	V	-	-	S	V	S	V	-	-	-	-	-	-
R. saniculaefolius	S	V	-	-	S	V	S	V	S	V	S	V	S	V	S	V
Z. palustris	-	-	S	V	-	-	-	-	-	-	S	V	S	V	S	-

DISCUSSION

Vertical structure of propagule banks - The propagule banks followed in this study were characterised by distinct vertical distribution of seeds and oöspores, with 94% of propagules being located in the top 4cm of sediment. This is comparable to the results obtained by Grillas *et al.* (1993) from Doñana marshland (55% of propagules in top 4cm) and by Bonis and Lepart (1994b) from the Camargue (76% to 97% of propagules in top 4cm). The distribution of propagules in these wetlands should be contrasted with the structure of the propagule bank in other habitats such as swamps, prairie marshes and bogs, where only 20% to 50% of propagules occur in the top 5cm (Leck (1989)). This trend is not merely general but has been observed for all species included in the present study.

Storage of large numbers of propagules in a surficial bank is an adaptive strategy for the species concerned since this facilitates reception of germination cues and attainment of the soil surface by young shoots. However, this strategy exposes propagules to attack by predators, to high daytime temperatures throughout the dry season and to possible transport out of the sediment by surface runoff.

No direct assessment of loss of propagules due to predation was made during the present study although removal of seeds by ants had been noted from Pool 1 in March 1988. High sediment temperatures during the dry season may also be a source of stress for propagules. The upper lethal temperatures for seeds are species-specific and depend upon duration of exposure (Baskin & Baskin, 1998). Upper lethal temperatures for the species under study are not known although it should be noted that temperatures of desiccated pool sediment during the dry season may attain 45°C (Lanfranco, 1990). Surface runoff following heavy rainfall may remove propagules from the sediment surface and deposit them elsewhere. Although this may function as a mode of dispersal, it should be borne in mind that freshwater rockpools are ecological islands surrounded by inhospitable habitat and displacement of propagules would therefore result in erosion of the metapopulation propagule reserve.

Relation of propagule bank to established vegetation - Patterns of established vegetation mostly reflected the species diversity of propagules in the pool sediment. The absence of *Chara vulgaris* from the established vegetation of Pool 2 and of *Damasonium bourgaei* and *Zannichellia palustris* from Munxar "New Pool" cannot be commented on definitively until germination patterns of these species in such habitats are studied.

General comments - The results obtained in the present work are ultimately based on the numerical abundance of propagules. Whilst such a measure is appropriate for comparisons involving the same species, it is not ideal for comparing among species. Different species allocate reproductive effort into producing numerous small seeds or few large ones and comparison based on numerical abundance is therefore not satisfactory, especially if the propagules involved are characterised by large variance in size. Although standardising numerical abundance across species alleviates this difficulty, it probably does not remove it entirely. This indicates that subsequent studies should focus on biomass of seeds and oöspores rather than numerical abundance in isolation.

The general results of this study indicate that quantification of contributions to and losses from the propagule bank should be the focus of subsequent work aimed at constructing a predictive model of the population dynamics of the species in these pools.

ACKNOWEDGEMENTS

One of the authors (SL) would like to express his most sincere thanks to Dr Patrick Grillas (Station Biologique de la Tour du Valat) for his patient assisstance regarding field and laboratory methodology and for several long discussions on the dynamics of seed banks. SL would also like to thank Professor PJ Schembri (Department of Biology, University of Malta) for several relevant points and interpretations raised during the examination of HJZ's, JC's and SC's dissertations and subsequently amended in this work

(Received 3rd September 2000)

REFERENCES

Baskin, C.C. & Baskin, J.M. (1998). *Seeds*. Academic Press. London. 666pp.

Bonis, A. & Lepart J. (1994). Vertical structure of seed banks and the impact of depth of burial on recruitment in two temporary marshes. *Vegetatio* 112: 127-39.

Bonis, A., Lepart, J. & Grillas P. (1995). Seed bank dynamics and coexistence of annual macrophytes in a temporary and variable habitat. *Oikos* 74: 81-92.

Callus, J. & Cilia, S. (2000) *Aspects of the ecology of hydrophytes in ephemeral wetlands in Malta.*(unpublished dissertation) University of Malta, Faculty of Education. 198 pp.

Grillas, P., Garcia-Murillo, P., Geertz-Hansen, O., Marba, N., Montes, C., Duarte, C.M., Tan-Ham, L., & Grossmann A. (1993). Submerged macrophyte seed bank in a Mediterranean temporary marsh: abundance and relationship with established vegetation. *Oecologia* 94: 1-6.

Grillas, P., van Wijck, C. & Boy, V. (1992). Transferring sediment containing intact seed banks: a method for studying plant community ecology. *Hydrobiologia* 228: 29-36.

Lanfranco, S. (1990). *The Ecology of Freshwater Rockpools in Malta.* (unpublished dissertation) University of Malta, Faculty of Education. 195 pp.

Lanfranco, S. (1995). *Ecological Succession in Maltese Freshwater Rockpools* (unpublished dissertation) University of Malta, Faculty of Science. 205 pp.

Leck, M.A. (1989). Wetland seed banks. In: Leck MA, Parker VT, Simpson RL, (eds.) *Ecology of soil seed banks.* p 283-305. Academic Press Inc.

Species Survival Commission (1994). *IUCN Red List Categories.* International Union for Conservationist Nature and Natural Resources Gland.

Vassallo, G. (1998). *Aspects of the ecology of hydrophytes in temporary freshwater rockpools in Gozo* (unpublished dissertation) University of Malta, Faculty of Education. 89 pp.

Zammit, H. (1999). *Investigations into the seed banks of ephemeral wetlands in relation to environmental factors* (unpublished dissertation) International Environment Institute, Foundation for International Studies, University of Malta. 40pp.