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Population parameters of two water scavenger beetles: Derallus angustus Sharp and Enochrus vulgaris (Steinheil) (Coleoptera: Hydrophilidae) in permanent ponds: spatial distribution and microhabitat preference.

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Population parameters of two water scavenger beetles: *Derallus angustus* Sharp and *Enochrus vulgaris* (Steinheil) (Coleoptera: Hydrophilidae) in permanent ponds: spatial distribution and microhabitat preference.

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Running title: Population dynamics of two aquatic Coleoptera.

Abstract

The spatial distribution, temporal variation, and microhabitat preference of two populations of aquatic Coleoptera were analysed in permanent ponds located in the province of Corrientes, Argentina. Samples were collected every 15 days, between October 2010 and October 2011. The dispersion index and distribution models (Poisson Series, positive binomial and negative binomial) were used to evaluate the spatial distribution. For the evaluation of the microhabitat preference we used non-parametric Kruskal-Wallis test and then the Dunn test with the Bonferroni's correction. In the test of the use of different microhabitats, we used a K proportion test and, when significant, a two proportion Z test was applied between pairs of microhabitats. The species analysed were the hydrophilids *Derallus angustus* Sharp and *Enochrus vulgaris* (Steinheil). The results indicate that the spatial distribution of individuals was mostly related to the homogeneity or heterogeneity of the ecosystem. However, when species were analysed individually, the spatial distribution and the use of microhabitat by each of them were different with respect to preference and behavior. The macrophytes also play a role in the dynamics of these species, as they favour or limit the abundance by modifying the structure thereof.

Keywords*:* *Derallus angustus*; *Enochrus vulgaris*; habitat selection; macrophyte; population dynamics; Argentina

Introduction

Among the water beetles of the Neotropical region, the family Hydrophilidae is one of the most representative; it has about 600 species distributed in 58 genera, of which about 35 are aquatic or riparian (Archangelsky et al. 2009). They live in different lotic (rivers, streams) and lentic habitats (lakes, ponds, pools, phytotelmata) (Hansen 1995; Archangelsky et al. 2009). The presence of species in different environments seems to be influenced by biotic and abiotic factors such as chemical characteristics of the water body (Cuppen 1986), vegetation structure (Eyre et al. 1992; Archangelsky and Fernández 1994; Fernández and Kehr 1995; De Szalay and Resh 2000), or predation (Larson 1990; Resetarits 2001).

The genus *Derallus* Sharp, 1882 comprises 15 species (Oliva 1981, 1983, 1995; Hansen 1999; Oliva et al. 2002) of which eight are registered from Argentina and neighbouring areas (Oliva et al. 2002). They are characterized by a convex body shape, a pronotum with rounded posterior angles, and a black to glossy back colour. *Derallus angustus* Sharp, 1882

(Coleoptera: Hydrophilidae) widely distributed throughout South and Central America to the South of the United States. It has been registered in the Paraná Basin and Uruguay (Oliva et al. 2002). In Argentina, there are records of the species from Salta, Formosa, Chaco, Santa Fe, Corrientes, Entre Rios and Buenos Aires Provinces (Fernández and Bachmann 1998). Generally, *Derallus* species inhabit lentic environments covered with aquatic plants to which larvae and adults are associated.

Enochrus Thomson, 1859 on the other hand, includes 215 species distributed in all biogeographic regions (Hansen 1999; Short and Hebauer 2006). About 60 species are found in the Neotropical Region, and 17 species are known so far from Argentina. *Enochrus (Methydrus) vulgaris* (Steinheil, 1869) (Coleoptera: Hydrophilidae) is a very common species in Argentina; it is registered in the North of the Colorado River and also in neighbouring countries such as Paraguay, Brazil, and Uruguay (Oliva et al. 2002).

The studies mentioned above are mostly concerned with taxonomy and community ecology, although some of them are focused on population ecology. Fernández (1990) studied the spatial distribution, age structure and sex ratio in *Helochares talarum* Fernández, 1983. Thereafter, Fernández and Kehr (1994) studied the annual life cycle of *Helochares femoratus* (Brullé, 1938), and Fernandez and Kehr (1995) analysed the spatial distribution and variability over time in the same species. Fernandez (2006) studied the population dynamics of *Derallus angustus*. Byttebier et al. (2012) studied the seasonal dynamics of *Enochrus vulgaris* (Steinheil, 1869) and *E. variegatus* (Steinheil), and their reproductive strategies in temporary and permanent urban water bodies of Buenos Aires city. Finally, Gómez Lutz et al. (2015a) studied the spatial distribution, temporal variation, and specificity of microhabitat of five *Tropisternus* species.

According to Michela et al. (2000), the examination of the spatial disposition of the individuals in the population could reach conclusions about the natural dispersion and the biological process of the disposition. There are many environmental and genetic factors affecting the special disposition of individuals. Among these factors, the most influential are environmental homogeneity and/or heterogeneity (Fernández and Kehr 1995), shelter sites, natural enemies, habits of oviposition and feeding, food availability, feasibility, and age structure of the population (Alvarez et al. 1992).

The main goals of this study are: (1) to analyse the relative abundance and to assess the temporal variation of *Derallus angustus* and *Enochrus vulgaris* in two ponds of Corrientes Province, Argentina, (2) to determine the spatial distribution of the two species and the possible factors affecting it, and (3) to recognise the microhabitats occupied by the species analysed.

Material and Methods

Between October 2010 and October 2011, fortnightly samples of aquatic beetles were collected in two permanent ponds located approximately 12 km from the city of Corrientes, Argentina. Both ponds are medium size and differ in vegetation cover. In pond nº1 ("Tendalero Pond", LT, 27°29'07.3'' S 58°43'46.3''W) it is characterized by its circular shape, and it is about 80 m diameter and 0.80 m in depth in the central part. Three microhabitats were differentiated, two are formed by different macrophytes and the other one composed of a loose vegetation zone. This pond was composed by *Hydrocleys nymphoides* (Willd.) Buchenau and *Limnobium laevigatum* (Humb. and Bonpl. ex Willd.) Heine, both covering approximately 70 % of the surface whereas the rest of the pond was an area of open water, without macrophytes, considered as a different microhabitat. On the other hand, pond nº2

(“Don Luis Pond”, LDL, (27°28'26.5" S 58°43'35.1"W) have a circular shape, 100 diameters and 1.2 m depth. It does not have different strata of vegetation on the surface. However, it was entirely composed of *Ludwigia* sp. and different grass species homogeneously distributed. Therefore, this site does not show different microhabitats, and the whole surface was considered a single microhabitat.

Aquatic insects were collected with an aquatic hand net (mesh size 300 μm, diameter 30 cm and a handle 2 meters long) by dragging 2 m. It was carried out by a two meters’ crawl in straight line collecting the macrophytes found in the gap between the falling aquatic hand net in the pond to the collecting site, essentially, two meters drag along the length by the network handle. The sampling units were taken at 3 m from each other, placed into plastic bags, fixed *in situ* in 5% formaldehyde and moved to the laboratory. The coleopterans were preserved in 70% ethanol. Species were identified following Oliva et al. (2002). The studied material was placed in the collection of the Centro de Ecología Aplicada del Litoral (CECOAL (CONICET-UNNE)), Corrientes. Only adult specimens were taken into consideration for the different analyses.

In order to analyse the spatial distribution of both population, samples with at least five individuals were considered. The dispersion index (DI) and spatial pattern according to Chiquadrat distribution, (Elliott 1971) and distribution models were used to evaluate the spatial distribution. The models applied were: Poisson series, positive binomial and negative binomial according to the distribution (Pielou 1977), and later the same models were used to evaluate the difference between the expected (obtained through the models of best solution) and the observed data with a chi-squared test (Southwood 1978).

In identifying the possible causes of the aggregations of recorded individuals, the average number of individuals in aggregations are calculated from the formula proposed by Arbus and Kerrich described in Southwood (1978). From this formula, we can determine the causes of aggregations (aggregations may be due to environmental factors or active processes of individuals). This analysis was calculated only for sampling units that had an aggregate spatial distribution and fitted the negative binomial.

$$\lambda = \frac{\bar{x}}{2K} \, v$$

λ = number of individuals in the aggregation for the probability level allocated to *v*, $\bar{x}$ = the mean, K = aggregation index of the probability function of the negative binomial; υ= is a function with a X^2 distribution with 2K degrees of freedom (Arbus and Kerrich, 1951). According the formula the critical value in 2, when the result in lower than 2= environmental effect and higher than 2= active process.

We used non-parametric Kruskal-Wallis test and then the Dunn test with the Bonferroni's correction to calculate the Microhabitat specificity and preference of the species studied. The use of the three microhabitats was tested through a K proportion test and when it was significant, a two proportion Z-test was applied between pairs of microhabitats.

Results

Relative abundance and temporal variation.

Pond LT: In total, 914 beetles were collected (*E. vulgaris: n* = 263; *D. angustus: n* = 651). *Derallus angustus* was recorded in all samples while *E. vulgaris* was recorded in 70% of the samples. The temporal dynamics of *E. vulgaris* in both ponds can be observed in Figure 1(a). In this pond, this species showed a very marked abundance peak in late winter and spring.

During the rest of the year, the abundance was low. On the other hand, *Derallus angustus* dynamics can be seen in Figure 1(b). A high relative abundance was recorded throughout the cycle, albeit with some fluctuations and a downward trend towards winter and early spring.

Pond LDL: In total, 142 beetles were collected (*E. vulgaris:* n=94; *D. angustus:* n=48). *Derallus angustus* was present in 70% of the samples while *E. vulgaris* was present in 60% of the samples. The temporal dynamics of both species can be seen in Figures 1(a) and (b). Both were recorded with low relative abundance in most samples.

***Spatial distribution*.**

Pond LT: The obtained values for each of the analysed species in this pond are summarized in Table 1. In both species, an aggregate arrangement prevailed (70% of the samples in *E. vulgaris* and 61% in *D. angustus*), whereas a random arrangement was observed in the remaining samples. The K values of the negative binomial were variable.

K values below 1 predominated *E. vulgaris* showing strong aggregations, while K values registered for *D. angustus* individual were in most cases higher than 1 indicating lax aggregations. The values of λ indicate that the aggregation of individuals of *E. vulgaris* in this pond is mainly due to external factors (71% of samples), whereas aggregation of individuals of *D. angustus* is predominantly due to active processes (64% of samples).

Pond LDL: A random spatial distribution was registered for *D. angustus* (100% of the samples), whereas *E. vulgaris* showed mainly an aggregated spatial distribution (57% of samples), which 75% were due to external factors (Table 3). In the spatial arrangement of *E. vulgaris*, K values were generally low, indicating strong aggregation (Table 3).

Specificity and preference of microhabitat.

The three microhabitats defined in pond LT were differently used by the two studied species (Kruskal-Wallis test K-W= 18.34; *df* = 2; $p < 0.0001$). An *a posteriori* Dunn's test with Bonferroni's correction (α= 0.016) indicated significant differences between *L. laevigatum* and the open water area. The abundance of individuals of the two species of beetles in relation to microhabitat was distributed as follows: the microhabitat with more individual abundance was that composed of *L. laevigatum* (*E. vulgaris: n*= 62; *D. angustus: n*=117), followed by that formed by *H. nymphoides* (*E. vulgaris: n* = 21; *D. angustus: n* = 94), and finally the open water area, free of aquatic vegetation (*E. vulgaris: n* = 13; *D. angustus*: *n* = 56). *Derallus angustus* and *E. vulgaris* differently occupied the three microhabitats (*D. angustus:* X^2= 31.99; *df* = 2; $p < 0.05$; *E. vulgaris:* X^2 = 64.78; *df* = 2; $p < 0.05$). The microhabitat preference order for *D. angustus* was: *L. laevigatum*, *H. nymphoides*, and open water area. *Enochrus vulgaris*, on its part, preferentially used the microhabitats *H. nymphoides* and open water area, in comparison to that shaped by *L. laevigatum*. The results of the Z- test for two proportions, made from different pairs of microhabitats present in pond LT are shown in Table 2.

Discussion

Both analysed populations were regular in the area of study and recently were registered in a faunal assessment of the aquatic Coleoptera of Mburucuyá National Park, Corrientes Province (Torres et al. 2012) and in a study of the community of aquatic Coleoptera in a rice field of the same Province (Gomez Lutz et al. 2015b). According to the results of this paper, the presence of *D. angustus* and *E. vulgaris* in both studied ponds is associated with aspects of their biology, such as the preference for lentic habitats covered by aquatic plants. A similar result was registered for von Ellenrieder and Fernandez (2000), Fernández (2006) in other population of aquatic coleopteran. Byttebier et al. (2012) postulated that the sites with permanent characteristics and vegetation can be more suitable habitats for some species of aquatic beetles.

This study was performed in two lentic water bodies that differ in structure related to the percentage of coverage of vegetation in the surface and the aquatic vegetation. *Derallus angustus* and *E. vulgaris* were registered in both ponds, despite their different structure. Pond LT, however, had a higher abundance of individuals (*n* =914) related to pond LDL (*n*= 142), which was characterized by a generally homogeneous habitat. Despite being present in both sites, *D. angustus* showed a marked preference for pond LT, as an evidence in the values of abundance and frequency in the habitat with heterogeneous characteristics in relation to aquatic vegetation. According to von Ellenrieder and Fernández (2000), the scarcity of registration of individuals of population in fleeting environments is due to the condition of them, preventing them from developing macrophytes, however, individuals have a preference for permanent or fleeting environments, but, where the presence of macrophytes is registered. This is also reflected in this analysis, where the species was more abundant in pond LT, where much of its percentages coverage of macrophyte on the surface of the ponds is higher.

On the other hand, the population of *E. vulgaris* in pond LT had low abundance in almost all samples, with a slight increase in late winter and early spring, whereas in pond LDL a low abundance prevailed throughout the sampling period.

Knowledge about the disposition of aquatic Coleoptera is very scarce. However, according to Taylor (1984), the spatial distribution of individuals is one of the most characteristic ecological properties of the species. Overall, the spatial distribution pattern of *D. angustus* was different in both ponds. In pond LT it was mostly aggregated due to active processes, whereas in pond LDL it was random. Fernández (1990) noted that a population of *Helochares talarum* had a random distribution, and this was attributed to the environmental homogeneity. Furthermore, the spatial distribution of *E. vulgaris* in pond LT was mainly aggregated due to external factors, indicating that the effect of the substrate in the spatial arrangement of individuals may be substantial. In pond LDL, however, the population of *E. vulgaris* did not show a clear pattern of spatial distribution, as two types of the arrangement were observed, random and aggregate, and in the cases of aggregated arrangement, two possible factors (active process or external influence) are involved. It is probably as observed in other species that *E. vulgaris* occurrence is directly related to the distribution of macrophytes. The spatial arrangement of organisms due to external factors such as the distribution of macrophytes was previously documented by Fernández and Kehr (1995). Gomez Lutz et al. (2015a) also determined that the spatial distribution of species of *Tropisternus* was related to characteristics of the habitat. In general, the aggregate arrangement of individuals is characteristic of environments with heterogeneous characteristics and distinct microhabitats. On the other hand, a random arrangement was observed in environments with more homogeneous characteristics.

Previous studies have suggested that macrophytes influence in various ways on macroinvertebrates. In particular, the substrate and biomass of aquatic vegetation are the factors that most influence the abundance and richness of macroinvertebrates (Poi de Neiff and Neiff 2006; Damborsky et al. 2012). Other authors report that the structure of the macroinvertebrate community is affected by the complexity or heterogeneity of the habitats provided by macrophytes (Thomaz et al. 2008; Thomaz and Ribeiro da Cunha 2010), and by the size and structure of the roots and leaves of aquatic plants (Fontanarrosa et al. 2013).

However, Batzer and Wissinger (1996) attribute the composition and abundance of invertebrates to a combination of biotic factors, date of sampling, hydrological period, and physicochemical factors of the aquatic environment. In this study, in general, the greater abundance and aggregate spatial distribution of individuals observed in pond LT could be related to the heterogeneity of plant substrate (i.e. increased number of macrophytes). Similar results were obtained by Byttebier et al. (2012) for two *Enochrus* populations (*E. vulgaris* and *E. variegatus*), noting that the individuals were mainly associated with greater vegetation cover with an availability of shelters to avoid predation by fish.

Aquatic macrophytes are important because they favour or limit the abundance of some species of water beetles by modifying the structure of the population (Tomaz and Ribeiro da Cunha, 2010), especially in warm climate environments where the vegetation cover of the ponds is abundant. In addition, macrophytes influence the spatial arrangement of aquatic beetles, and favour the aggregation of specimens, some species showed a marked preference for sectors of the water bodies occupied by plants. This study shows that the habitats and microhabitats directly influence the spatial and temporal variation of water beetles.

The results of this study have allowed us to determine certain patterns of distribution in relation to microhabitat preference in two species of water beetles widely distributed in South America. Although both species were recorded in both ponds, and the three microhabitats marked preference for strata formed by *L. laevigatum* was observed. A possible explanation for this pattern is that the substrate provides protection, stability, food or support apparently since they are more adapted to live in this habitat than in others such as *H. nymphoides* or sectors without floating vegetation (both also present in similar proportions in the water environment studied). This indicates that macrophytes provide excellent microhabitats for this population of aquatic beetles. Aquatic plant communities are the support for aquatic

Coleoptera assemblages, and their abundance and richness depends on the abundance and distribution of the vegetation. The monitoring of the population of aquatic beetles in the different present macrophytes in the area of study would be interesting to continue studying and to be able to efficiently predict, conserve and manage aquatic insect biodiversity.

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Table 1 Species sampled in pond LT and sampling dates [n: number of specimens; $\bar{x}$: arithmetic mean; s^2: variance; DI: Dispersion Index; Prob.: probability according to X^2 distribution; Dist.: distribution type; A: aggregate distribution (the best fit model is the negative binomial); R: random distribution (the best fit model is the Poisson series); K: Aggregation Index; df.: degrees of freedom; Prob. X^2: probability of X^2; λ*: mean aggregation, calculated with the formula proposed by Arbous and Kerrich (1951)].

Species	Date	n	$\bar{x}$	s^2	DI	Prob.	Dist.	K	df	Prob. X^2	λ*
Enochrus vulgaris	12/10/10	8	1.14	2.14	11.25	0.08095044	R.		3	> 0.05	
	12/11/10	10	1.43	6.29	26.40	0.0001875	A.	0.42	4	> 0.05	0.65
	25/11/10	10	1.43	3.29	13.80	0.03195184	R.		3	< 0.05	
	20/12/10	24	3.43	22.62	39.58	5.4997E-07	A.	0.61	4	> 0.05	1.27
	22/03/11	5	0.71	1.24	10.40	0.10878665	R.		2	> 0.05	
	14/04/11	12	1.71	4.24	14.83	0.02159338	A.	1,16	4	> 0.05	1.02
	21/07/11	13	1.86	4.81	15.54	0.01645774	A.	1.17	4	> 0.05	1.10
	26/08/11	12	1.71	13.90	48.67	8.6936E-09	A.	0.24	4	> 0.05	0.78
	20/09/11	114	16.29	388.90	143.28	2.0342E-28	A.	0.71	4	> 0.05	5.20
	05/10/11	46	6.57	25.29	23.09	0.00076787	A.	2.31	4	> 0.05	4.78
Derallus angustus	12/10/10	14	2.00	8.00	24.00	0.00052226	A.	0.67	4	> 0.05	0.68
	28/10/10	9	1.29	0.90	4.22	0.64663199	R.		2	> 0.05	
	12/11/10	58	8.29	20.57	14.90	0.02107675	A.	5.59	4	> 0.05	7.67
	25/11/10	60	8.57	28.62	20.03	0.00273181	A.	3.66	4	> 0.05	7.42
	07/12/10	44	6.29	12.57	12.00	0.0619688	R.		9	< 0.05	
	20/12/10	23	3.29	1.90	3.48	0.74685971	R.		4	> 0.05	
	07/01/11	20	2.86	2.14	4.50	0.60933927	R.		4	> 0.05	
	21/01/11	29	4.14	24.48	35.45	3.5279E-06	A.	0.84	4	> 0.05	1.12
	03/02/11	13	1.86	3.14	10.15	0.11832095	R.		4	> 0.05	
	16/02/11	16	2.29	3.90	10.25	0.11451114	R.		4	> 0.05	

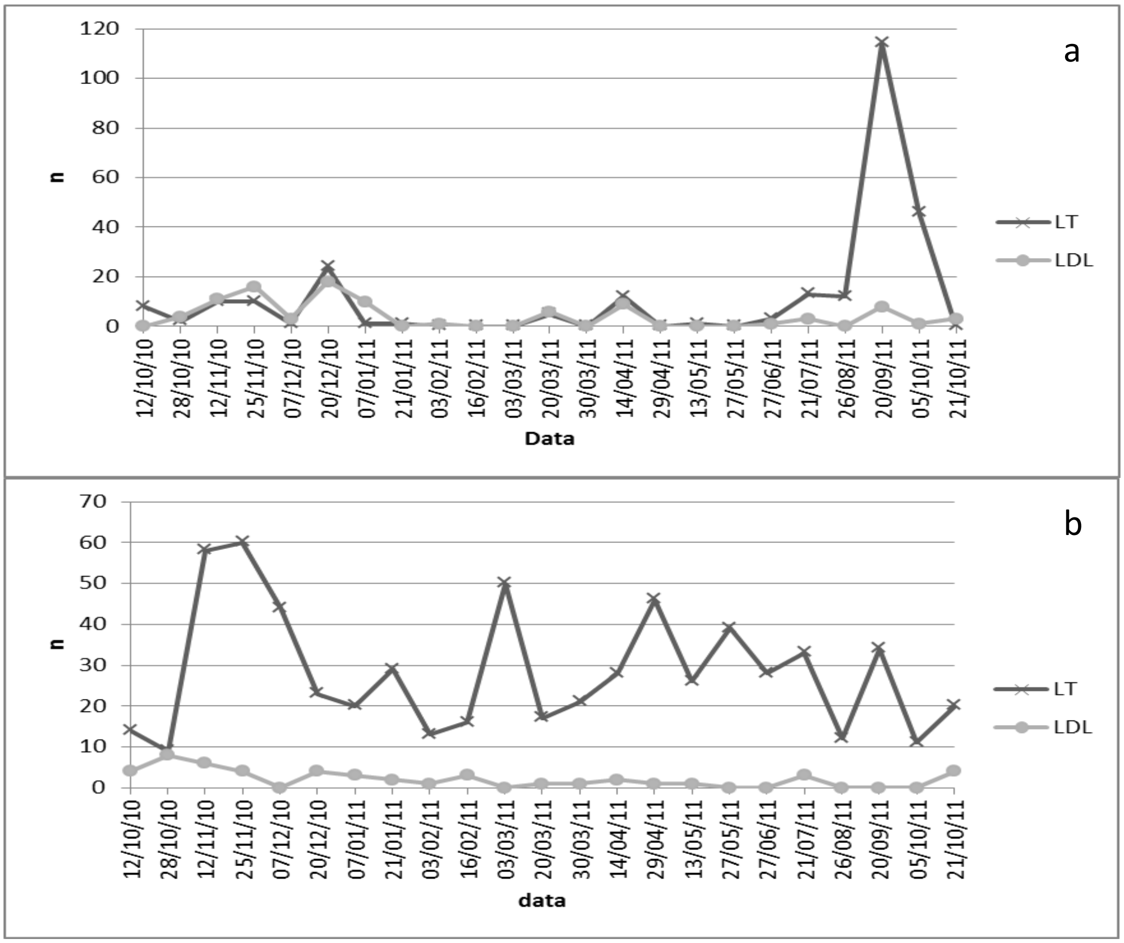
	03/03/11	50	7.14	29.14	24.48	0.00042604	A.	2.32	4	> 0.05	5.17
	20/03/11	17	2.43	4.62	11.41	0.07645405	R.		4	< 0.05	
	30/03/11	21	3.00	12.00	24.00	0.00052226	A.	1.00	4	> 0.05	2.08
	14/04/11	28	4.00	12.00	18.00	0.0062322	A.	2.00	4	> 0.05	3.36
	29/04/11	46	6.57	35.95	32.83	1.1325E-05	A.	1.47	4	> 0.05	3.10
	13/05/11	26	3.71	26.57	42.92	1.208E-07	A.	0.60	4	> 0.05	1.40
	27/05/11	39	5.57	14.29	15.38	0.01746721	A.	3.56	4	> 0.05	4.96
	27/06/11	28	4.00	14.00	21.00	0.00183462	A.	1.60	4	> 0.05	2.96
	21/07/11	33	4.71	21.24	27.03	0.00014293	A.	1.34	4	> 0.05	2.43
	26/08/11	12	1.71	1.90	6.67	0.35277616	R.		3	> 0.05	
	20/09/11	34	4.86	9.48	11.71	0.06886119	R.		8	< 0.05	
	05/10/11	11	1.57	3.95	15.09	0.01956131	A.	1.04	4	> 0.05	1.05
	21/10/11	20	2.86	11.81	24.80	0.00037183	A.	0.91	4	> 0.05	0.71

Table 2 Summary of the values obtained from Z test two for proportions considering microhabitat present in pond LT, in pairs and for each species. Microhabitats: (Lim) *Limnobium laevigatum*, (H.n.) *Hydrocleis nimfoides*, (L) water without floating vegetation; z (V.O) = z adjusted (observed value); z (V.C) = z theoretical (critical value).

		E. vulgaris	*D. angustus*
Lim. - L-	Diference	0.51	0.228
	z (V.O)	7.248	5.641
	z (V.C)	1.96	1.96
	p-value	< 0.0001	< 0.0001
	alpha	0.05	0.05
Lim. - H.n.	Diference	0.427	0.086
	z (V.O)	5.973	2.036
	z (V.C)	1.96	1.96
	p-value	< 0.0001	0.042
	alpha	0.05	0.05
H.n. - L.	Diference	0.083	0.142
	z (V.O)	1.512	3.659
	z (V.C)	1.96	1.96
	p-value	0.13	0
	alpha	0.05	0.05

Table 3 Species sampled in pond LDL and sampling dates [n: number of specimens; $\bar{x}$: arithmetic mean; s²: variance; DI: Dispersion Index; Prob.: probability according to X^2 distribution; Dist.: distribution type; A: aggregate distribution (the best fit model is the negative binomial); R: random distribution (the best fit model is the Poisson series); K: Aggregation Index; df.: degrees of freedom; Prob. X^2: probability of X^2; λ*: mean aggregation, calculated with the formula proposed by Arbous and Kerrich (1951)].

Species	Data	n	$\bar{x}$	s^2	DI	Prob.	Dist.	K	df	Prob. X^2	λ*
Enochrus vulgaris	12/11/10	11	2.75	2.25	2.45	0.48356177	R.		3	> 0.05	
	25/11/10	16	4	16.67	12.50	0.00585266	A.	1.26	1	< 0.05	2.19
	20/12/10	18	4.5	9.67	6.44	0.09187942	R.		8	> 0.05	
	07/01/11	10	2.5	19.00	22.80	4.4451E-05	A.	0.38	1	> 0.05	1.14
	20/03/11	6	1.5	5.67	11.33	0.01005344	A.	0.54	1	> 0.05	0.63
	14/04/11	9	2.25	6.92	9.22	0.02647768	R.		5	< 0.05	
	20/09/11	8	2	8.00	12.00	0.00738316	A.	0.67	1	> 0.05	0.68
Derallus angustus	28/10/10	8	2	3.33	5.00	0.17179714	R.		3	> 0.05	
	12/11/10	6	1.5	3.00	6.00	0.11161023	R.		2	< 0.05	


a
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Data
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26/08/11
20/09/11
05/10/11
21/10/11
data
LT
LDL

Caption

Fig 1 Abundance of (a) *Enochrus vulgaris* and (b) *Derallus angustus* in ponds LT and LDL.