E-maill address: jpriotto@exa.unrc.edu.ar, Departamento de Ciencias Naturales, Universidad Nacional de Río Cuarto. Agencia Postal N°3; 5800 Río Cuarto, Córdoba, Argentina. Tel.: +5403584676836. Fax-number: ++5403584676230

Running title: Movement in linear habitats

CORN MICE (*CALOMYS MUSCULINUS*) MOVEMENT IN LINEAR HABITATS OF AGRICULTURAL ECOSYSTEMS

Lucía Sommaro[a], Daniela Gómez[a], Florencia Bonatto[a], Andrea Steinmann[a,b], Marina Chiappero[a,c], José W. Priotto[a,b,*].

[a]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ciudad Autónoma de Buenos Aires, Avda. Rivadavia 1917 – CP C1033AAJ, Argentina.*

[b]*Departamento de Ciencias Naturales, Universidad Nacional de Río Cuarto. Agencia Postal N°3; 5800 Río Cuarto, Córdoba, Argentina.*

[c] *Cátedra de Genética de Poblaciones y Evolución, FCEyN Universidad Nacional de Córdoba, Argentina.*

Movement patterns of *Calomys musculinus* in linear habitats were studied in relation to sex, season, year, abundance, and width of border. Movement distances (MD) were measured by seasonal capture, mark and recapture (CMR) samples during two years in wide and narrow borders in agroecosystems. The smallest MD were registered in autumn and the highest in spring. In breeding period, MD of males were larger than those of females. In non-breeding period MD were similar between sexes. Effects of sex on MD were consistent with *C. musculinus* promiscuous mating system. MD were greater in narrow than in wide borders. The narrowness of suitable habitats would force the mice to extend foraging trips in narrow borders. The opportunistic

behavior of *C. musculinus* allows it to use linear habitats in a similar way to the two-dimensional natural habitats.

Key words: Argentina agriculture ecosystems, border habitats, corn mice, edges, Generalized Linear Mixed Models, movement distances

*Correspondent: **jpriotto@exa.unrc.edu.ar**

Fragmentation of natural landscapes as a result of agriculture, increasing urbanization and other human activities, is a current global concern. Many spatial parameters are involved in these landscape changes, including the composition (e.g. habitat patches), spatial configuration (e.g. fragmentation and isolation), and shape (geometry) (Forman and Godron 1986; Forman 1995; Kareiva and Wennergren 1995) of landscape elements. Habitat remnants persist as linear strips along rivers, property borders, roads, and railways as a consequence of large surfaces of continuous wildlife habitats (e.g. forest and grassland areas) which are converted to urban and agricultural areas (Forman 1995). Wildlife species are restricted to such linear habitats in many heavily altered landscapes. Therefore, the functioning of populations in linear as opposed to two-dimensional habitat area has become a concern in environmental management (Bennett 1990; Hobbs 1992; Fauske et al. 1997), with the emphasis upon the role of linear habitats as dispersal corridors (Forman and Godron 1986; Bennett 1990). However, linear habitat structures may contain potential resources for long –term survival and reproduction as well (Fauske et al. 1997). Thus, these habitats play a role as buffer zones where width and vegetation height in relation to surrounding habitats determine the attractiveness of these zones to herbivores and the extent to which permanent populations can exist (Yletyinen and Norrdahl 2008).

One of the most important consequences of agricultural ecosystem development is the fragmentation by roads. Roads have been found to act as barriers to small mammal movements (Oxley et al. 1974; Kozel and Fleharty 1979; Garland and Brandley 1984; Mader 1984; Swihart and Slade 1984; Cittadino et al. 1998; Clarke et al. 2001). These barriers can affect the colonization of habitat patches and consequently population persistence (Kozakiewicz 1993).

77 Central Argentina agriculture ecosystems consist of a matrix of crop areas (corn,
78 soybean, sunflower, wheat, linen) surrounded by a network of roadsides and fencelines,
79 railways and other edge habitats that support a plant community with some remnants of
80 native flora and many introduced weeds, that provide a more stable cover than crop
81 fields (Bonaventura and Cagnoni 1995; Busch et al. 1997, 2000). These non-crop linear
82 areas provide habitat for a variety of small mammal species and their predators
83 including birds of prey. One of the most abundant species in these habitats is the corn
84 mouse, *Calomys musculinus* (Cricetidae, Sigmodontinae). This species was mainly
85 studied due to its role as reservoir of Junin virus, an etiological agent of the Argentine
86 Hemorragic Fever (AHF) (Mills and Childs 1998). *C. musculinus* may live in a variety
87 of habitats including natural pastures, crop fields, cultivated fields undisturbed after
88 harvest, border areas protected by wire fences with little agricultural disturbance, road
89 borders, borders between cultivated fields or pastures, and railway banks (Busch et al.
90 2000; Castillo et al. 2003). *C. musculinus* individuals live primarily in border habitats
91 and they use crop fields only when there is high weed cover and vertical vegetation
92 density (Ellis et al. 1997). However, at present weed cover is almost null in crop fields
93 due to the increasing use of agrochemicals (Bilenca et al. 2007). *C. musculinus*
94 populations are characterized by seasonal density changes with low density during
95 winter (16/ha) and peaks during late summer or early autumn (260/h); a 1:1 sex ratio is
96 usual (Mills and Childs 1998). The reproductive period has an average length of 8
97 months (from mid-September to April; Mills and Childs 1998).
98 In studies performed in natural grasslands (two-dimensional habitat), *C.*
99 *musculinus* has a promiscuous mating system; females typically mate with more than
100 one male during each estrous period (Steinmann et al. 2009). During the breeding
101 period, females keep exclusive home ranges and are territorial, whereas males have

home ranges that are more than twice as large as those of females and are shared fully with both sexes. In non-breeding period male and female home range sizes are similar (Steinmann et al. 2005; Steinmann et al. 2009).

C. musculinus is one of the most abundant species in linear habitats, and it shows a wider habitat and trophic niche than other coexisting rodent species in rural habitat (Busch et al. 2000). Its space use pattern has been studied principally in two-dimensional habitats (Steinmann et al. 2005; 2006a, 2006b). The aim of our study was to explore *C. musculinus* movement patterns in linear habitats of Central Argentina agricultural ecosystems. The following questions were addressed: (1) Do males have higher movement distances than females? (2) Do movement distances depend on the reproductive period? (3) Does the width of linear habitat (border) affect movement distances of mice? (4) Do rural roads act as barriers to *C. musculinus* movement?

MATERIALS AND METHODS

Field methods. -- This study was carried out from November 2005 to August 2007 in linear habitats of agriculture ecosystems in Río Cuarto Department (Córdoba Province, Argentina). Phytogeographically, this region corresponds to "Provincia del Espinal, Distrito del Algarrobo" (Cabrera 1953), which is a low elevation plain (600-900 m) with vegetation dominated by algarrobo (*Prosopis alba*, *P. nigra*), accompanied by quebracho blanco (*Aspidosperma quebracho blanco*), mistol (*Zizyphus mistol*) and itin (*Portulaca kuntzie*). The vegetation, however, has undergone marked alterations as a result of agriculture and cattle farming. At present, the landscape mainly consists of individual crop fields, surrounded by wire fences with borders dominated by weedy species. Despite the influence of crop fields, borders have a homogeneous plant cover of

126 about 85 % throughout the year and a high availability of seed in soil (Priotto et al.
127 2002).

128 Four sampling transects located in field borders were used as study sites, 1
129 placed in a wide border (range of width: 10 - 30 m, transect A) and 3 placed in narrow
130 borders (≤2 m, transects B, C and D). Sampling transects were set up on opposite sides
131 of 7 m wide secondary roads (A opposite to B; C opposite to D). Each transect was
132 composed of 300 m trap lines with 30 Sherman live traps. Trap lines were separated by
133 500 m. Transects A and B had 4 trap lines (A1, A2, A3, A4, B1, B2, B3 and B4) and
134 transects C and D had 2 trap lines (C1, C2, D1 and D2) (Fig. 1). Seasonal samplings
135 were carried out during two consecutive years. Capture, mark and recapture (CMR)
136 trapping sessions were conducted for 5 consecutive days in November (spring), March
137 (summer), May (autumn) and July/August (winter). Trapped animals were weighed and
138 marked with ear tags with numerical codes. Sex and reproductive state (males: scrotal or
139 abdominal testicles, females: perforated or imperforated vulva, pregnancy evidence,
140 visible nipples or not) were also recorded. Small pieces of the tail tip were taken and
141 preserved in ethanol for other studies that include DNA analyses. The research on live
142 animals was performed in a humane manner and followed guidelines for the care and
143 use of animals approved by the American Society of Mammalogists (Gannon et al.
144 2007).

145 We calculated the largest movement distance among capture points of an
146 individual captured at least twice during a trapping session. Movement distance was
147 considered to provide an index of home range size (Gaines and Johnson 1982; Pusenius
148 and Viitala 1995). Because of small sample sizes for juveniles, all analyses used only
149 adult males and females.

150 *Data analysis*.--The primary focus of this study was to investigate the influence
151 of sex, season and year on movement distances of *C. musculinus* in linear habitats. We
152 also test for the effects of abundance index as a covariate because this variable could
153 potentially influence movement distances. Subsequently we analyzed the effect of
154 border width on movement distances, considering separately those individuals captured
155 in wide and narrow borders.
156 Because distance measurements are likely to be non-normal, a generalized linear
157 mixed model (GLMM) with Negative Binomial distribution in R 2.9.0 software (R
158 Development Core Team 2009) was used to identify the factors that determined
159 movement distances. We used repeated measure analysis. The repeated measures were
160 transects (in each transect individual movement distances were registered in three
161 seasons and two years). Individuals were not considered as repeated measures because
162 there were no movement distances of the same individual in different seasons and years.
163 Akaike Information Criterion, corrected for small sample size (AIC_c), was used as a
164 measure of the fit of a model (the smaller the AIC_c, the better the fit). We wanted to
165 identify the effect of abundance, season and sex on movement distances. We considered
166 sex, season, abundance and year as main effects and their 2-way interactions. Seasonal
167 analysis of movement distances were based on the following categories: spring
168 (November), summer (March), autumn (May). Winters were discarded from the
169 statistical analyses because few movement distances could be calculated during the first
170 winter and borders were burnt during the second one. The low number of recaptures for
171 each individual did not allow us to obtain reliable results in the abundance estimations
172 using CMR analysis. For this reason the number of individuals captured in the line
173 where each movement distance was calculated was used as an index of abundance.
174

RESULTS

From November 2005 to August 2007 there were 1152 captures of 808 individual *C. musculinus* being caught. Four hundred and twelve females and 396 males were ear-tagged. *C. musculinus* abundance index was greater during the second year, and the mean number of individuals varied among seasons with a peak in summer and a minimum in winter (Fig. 2).

A total of 82 and 97 movement distances were calculated for females and males respectively. *C. musculinus* individuals moved within the limits of each line; there were no long distance movements (among lines within a transect). This species was reluctant to cross roads; only 8 (4 males and 4 females) marked individuals crossed roads.

GLMM analysis based on AIC_c, revealed that sex and season were the most important factors affecting movement distances. AIC_c never decreased in any model that included abundance or year with or without interactions (Table 1). The best model included the interaction between sex and season. Coefficients for the best model are shown in Table 2. The greatest movement distances for both sexes were in spring. Male movements were larger than those of females in spring and summer, whereas the inverse pattern was observed in autumn (Fig. 3). In spite of these divergences, only the difference between spring and autumn was statistically significant (Table 2). Having examined the effect of sex, season and year, we investigated, using the same analysis, the effect of border width on movement distances. In this analysis we only included seasons with high number of individuals (spring and summer). Movement distances were greater in the narrow than in the wide border, but this difference was not statistically significant ($z = 1.8040$, $P = 0.0712$; Fig. 4).

DISCUSSION

Generally home range size is regarded as one indicator of habitat quality (Tufto et al. 1996). Since length of movements are a good index of home range size in small mammals (Slade and Russell 1998), and length of foraging trips affects both the energetic cost of food acquisition and the risk to be predated (Norrdahl and Korpimäki 1998; Banks et al. 2000; Yletyinen and Norrdahl 2008), movement distances can be used as indicator of habitat quality. Habitat quality is affected heavily by the shape of habitat patch, because it affects the maximum diameter of a given habitat area; the diameter increases as the habitat becomes more linear. Habitat quality would increase in relation to habitat width, because natural bi-dimensional habitats have higher quality than crop borders of agricultural ecosystems (one-dimensional habitats).

The factors that influenced the movement patterns of *C. musculinus* in linear habitats were similar to those observed in two-dimensional habitats (grasslands) by de Villafañe and Bonaventura (1987) and Steinmann et al. (2005). Movement distances varied seasonally, smallest in autumn and highest in spring. They were determined mainly by sex and reproduction. Autumn trapping session was conducted in May, non-breeding period, when the first frosts were registered. Cold periods are energetically demanding for small mammals, and energetic costs can be reduced by restricting to a minimum the movement activities outside the insulated nest (Yletyinen and Norrdahl 2008). During the breeding period, movement distances of males were larger than those of females, whereas in the non-breeding period movement distances were similar between sexes. Main effects of sex on movement distances of *C. musculinus* were consistent with the promiscuous mating system of the species. In promiscuous rodents, longer movement distances of males reflect a strategy that maximizes access to fertile females, whereas lower movements of females reveal territorial behavior directed at

protection of nestlings from infanticidal females (Wolff and Peterson 1998; Bond and Wolff 1999; Steinmann et al. 2005; Steinmann et al. 2009) or defense of food resource (Ims 1987; Ostfeld 1985; 1990). During the breeding period, differences in movement distances between spring and summer can be explained by spacing behaviors of males and females. At low population densities (spring) there are many vacant areas in borders where females can establish sparse and exclusive reproductive spaces. Thus, larger movement distances of males would be in relation to an active search for estrous females. Meanwhile, in summer (high population densities) there are few vacant areas in borders due to the fact that all spaces are occupied by estrous females. Thus, movement distances of males searching for females would be diminished.

Taking into account that the length of movement can be considered an index of habitat quality in relation to energetic costs of food acquisition and predation risk (Norrdahl and Korpimäki 1998; Banks et al. 2000; Yletyinen and Norrdahl 2008), longer movement distances of mice in narrow borders than in wide borders indicate that the latter were better habitats for *C. musculinus* than the former. Due to the fact that mice mainly reside in borders, the narrowness of suitable habitat could force mice to extend foraging trips in narrow borders whereas in wide borders movements in several directions are possible. Similar results were found in *Microtus agrestis* in agricultural ecosystems of Finland (Yletyinen and Norrdahl 2008). However, the movement pattern related to habitat shape is not clear in *C. musculinus*. Movement distances in two-dimensional habitats are similar (30 m in average from data obtained by Steinmann et al. 2005 and de Villafañe and Bonaventura 1987) to those observed in narrow borders in our study, where there were fewer recaptures per individuals than in the former publications. Movement length could be used as an indicator of quality in linear habitats, but it could not allow to differentiate between one- and two- dimensional

habitats. Future research should be developed, using similar methodologies, to evaluate the effect of habitat shape (one- vs. two- dimensional habitats) in movement length to address this assessment.

Roads acted as barriers to *C. musculinus* movements, as observed in many other small mammal species (Oxley et al. 1974; Kozel and Fleharty 1979; Garland and Brandley 1984; Mader 1984; Swihart and Slade 1984; Cittadino et al. 1998; Clarke et al. 2001). Mice would be averse to crossing areas without cover to avoid predation (Cittadino et al. 1998; Sheffield et al. 2001).

Ecological changes together with the agriculture development of the Humid Pampa have favored *C. musculinus* since this species is captured in higher proportion in the current rodent assemblage than it was in the undisturbed original grassland assemblage (Crespo 1966; Bilenca and Kravets 1995). This is because *C. musculinus* has a wider habitat and trophic niche than other coexisting rodent species in agrarian systems of Central Argentina (Busch et al. 2000). The opportunistic behavior of this species allows it to use linear habitats in a similar way to the two-dimensional natural habitats.

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RESUMEN

Se estudiaron los patrones de movimiento de *Calomys musculinus* en hábitat lineales en relación al sexo, la estación, el año, la abundancia y el ancho del borde. Las distancias de movimiento (DM) fueron registradas a través de muestreos estacionales de CMR durante dos años en bordes anchos y angostos de agroecosistemas. Las menores DM fueron registradas en otoño y las mayores en primavera. En el periodo reproductivo las DM de los machos fueron mayores que las de las hembras. Las DM fueron similares entre sexos en el periodo no reproductivo. El efecto del sexo sobre las DM es consistente con el sistema de apareamiento promiscuo de *C. musculinus*. Las DM fueron mayores en los bordes angostos que en los anchos. La estrechez de los hábitats adecuados forzaría a los ratones a extender los viajes de forrajeo en los bordes angostos. El comportamiento oportunista de *C. musculinus* le permite utilizar los hábitats lineales de una manera similar a los hábitats naturales bidimensionales.

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Fig. 1. Distribution and schema of transects studied across agriculture ecosystem in Río Cuarto Department (Córdoba Province, Argentina). 2009 Map Link/Tele Atlas (www.google.com).

Fig. 2. Mean number of individuals (±SE) of *Calomys musculinus* by season and year in linear habitats.

Fig. 3. Seasonal movement distances ($\overline{X}$ ± SE) of males and females of *Calomys musculinus* in linear habitats.

Fig. 4. Movement distances ($\overline{X}$ ± SE) of *Calomys musculinus* in wide and narrow linear habitats.

Table 1. Analysis for the generalized linear mixed model (GLMM) describing movement distances of *Calomys musculinus*. The best model is indicated in bold typeface.

Variable	AICc	ΔAICc	N° parameters	Deviance
Season x sex	**1327.994**	**0.000**	**7**	**1313.994**
Season	1330.768	2.774	4	1322.768
Season + sex	1330.806	2.812	5	1320.806
Season + year	1332.230	4.236	5	1322.230
Abundance + sex	1332.622	4.628	4	1324.622
Abundance x sex	1332.962	4.968	5	1322.962
Abundance	1335.122	7.128	3	1329.122
Season x year	1335.608	7.614	7	1321.608
Sex	1337.746	9.752	3	1331.746
Year	1340.744	12.750	3	1334.744

436 **Table 2.** Coefficients (β) for the best model of GLMM analysis of movement distances
437 of *Calomys musculinus*. Standard errors (in parentheses) and the significance of the
438 coefficients are also shown; p< 0.05 shown in bold typeface. The effect of each term in
439 the model is tested. P values tested the null hypothesis that β = 0. The Terms with
440 significant values less than 0.05 have some discernable effect. Since there is an intercept
441 term, the third level of season (spring) and the second of sex (male) are redundant. The
442 estimates for the first two levels contrast the effects of the first two seasons to the third
443 and the estimates for the females contrast their effects to males.

Factors	β (SE)	*z* value	*P* value
Intercept	3.443 (0.23)	0.148	0.000
Summer	-0.306 (0.36)	-0.846	0.398
Autumn	**-2.028 (0.46)**	**-4.280**	**0.000**
Female	-0.337 (0.37)	-0.979	0.328
Summer x female	-0.522 (0.52)	-0.966	0.334
Autumn x female	1.407 (0.74)	1.788	0.074

444

Fig. 1.

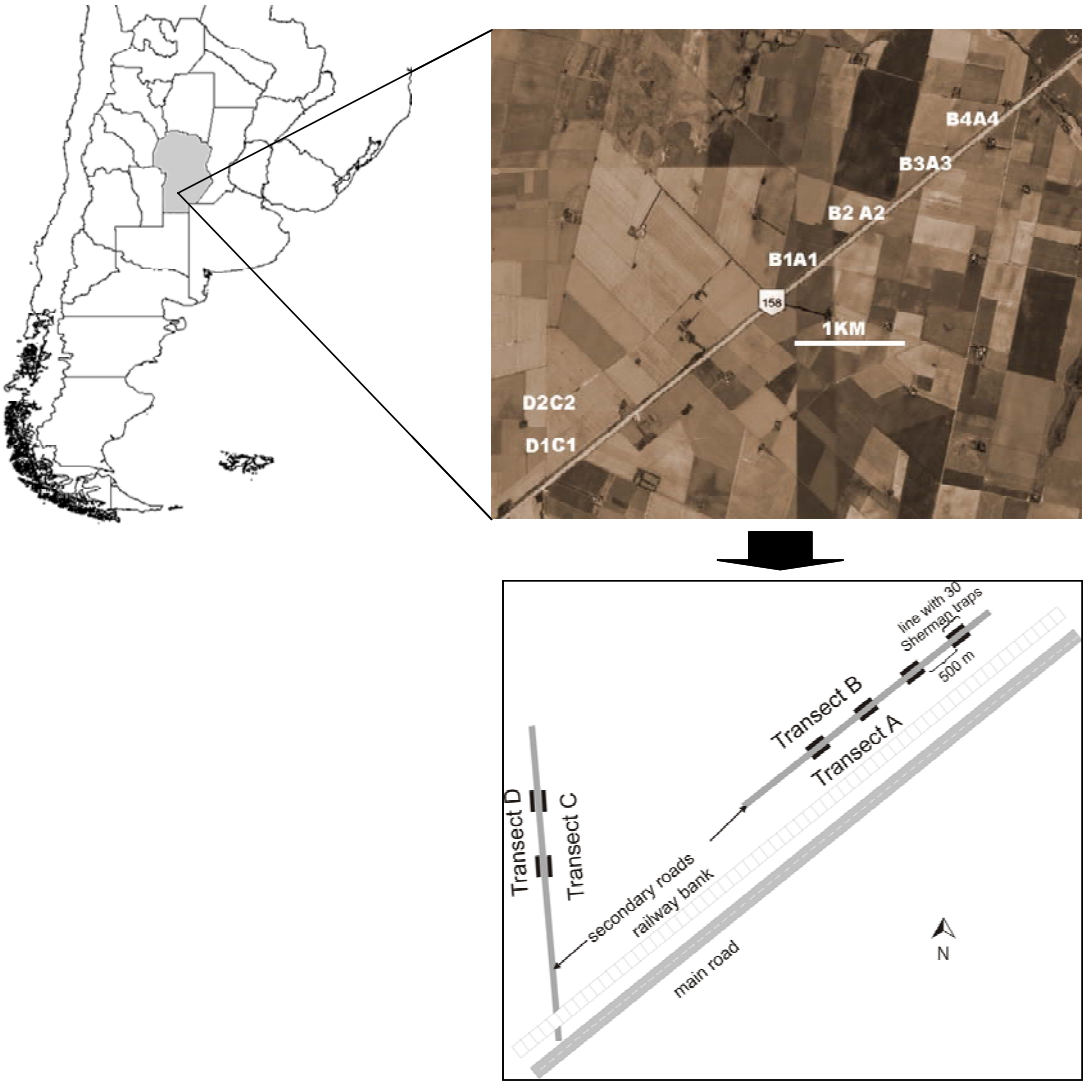

B4A4
B3A3
B2 A2
B1A1
158
1KM
D2C2
D1C1
line with 30 Sherman traps
500 m
Transect B
Transect A
Transect D
Transect C
secondary roads
railway bank
main road
N

Fig. 2.

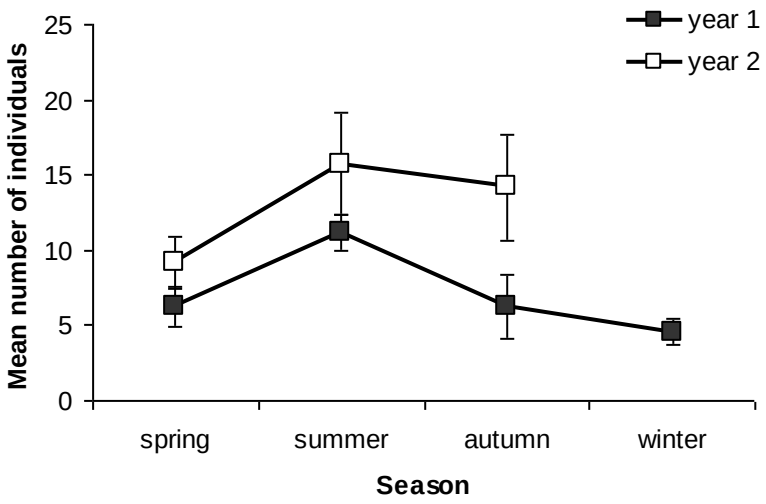

year 1
year 2
Mean number of individuals
25
20
15
10
5
0
spring
summer
autumn
winter
Season

Fig. 3.

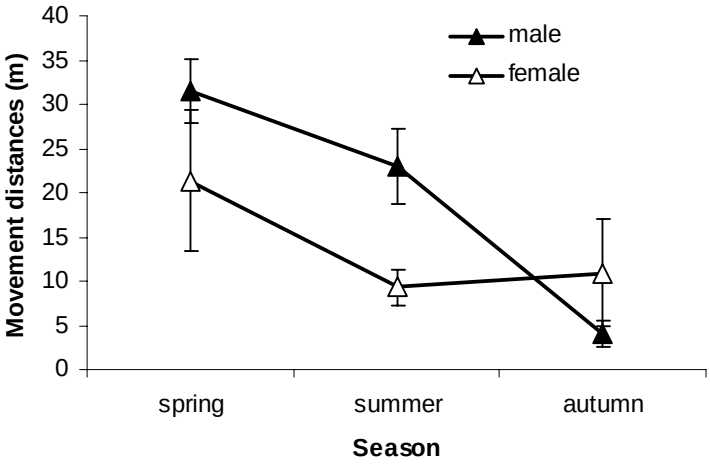
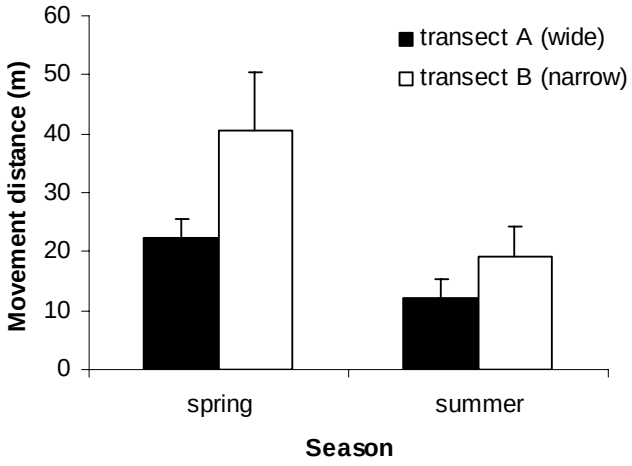

40
35
30
25
20
15
10
5
0
Movement distances (m)
male
female
spring
summer
autumn
Season

Fig. 4.


60
50
40
30
20
10
0
Movement distance (m)
transect A (wide)
transect B (narrow)
spring
summer
Season