

Original Contribution

Environmental Variables Associated with Hantavirus Reservoirs and Other Small Rodent Species in Two National Parks in the Paraná Delta, Argentina: Implications for Disease Prevention

María Victoria Vadell¹ and Isabel Elisa Gómez Villafañe²

¹Grupo de Ecología de Enfermedades Transmitidas por Vectores, Instituto de Investigación e Ingeniería Ambiental, Universidad Nacional de San Martín, Buenos Aires, Argentina

²Laboratorio de Ecología de Poblaciones, Departamento de Ecología, Genética y Evolución, IEGEBA (CONICET-UBA), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Intendente Güiraldes 2160, Ciudad Universitaria, C1428EGA Nuñez, Buenos Aires, Argentina

Abstract: Hantavirus pulmonary syndrome (HPS) is a severe zoonotic disease caused by hantaviruses hosted in various rodents species. In Argentina, its transmission to humans has been associated to exposure during activities such as farming, recreation, and tourism which are carried out in wild and rural areas. The aim of this study was to analyze the macro- and micro-habitat use and spatio-temporal variation of small sylvan rodents in Pre Delta and Islas de Santa Fe national parks, located in an HPS-endemic area of Argentina. Rodent communities were studied at six sites: two islands, a riparian forest, an inland forest, a marsh, and the margins of a pond. A total of 453 individuals of five species were captured with a trapping effort of 9471 trap-nights. Maximum species richness was found at the marsh and the pond margin sites. Abundance of rodents was influenced by flooding events. Two hantavirus reservoirs, *Oligoryzomys flavescens* and *Akodon azarae*, were identified in the area. *O. flavescens* was captured in every habitat, but it was dominant in Islas de Santa Fe National Park where its abundance was strongly influenced by flooding. *A. azarae* was captured in every habitat except on the islands. *A. azarae* behaved as a generalist species at a micro-habitat scale in every habitat of Pre Delta National Park except for the marsh where it selected patches with low vegetation height. Based on these results, several disease prevention measures, including the use of rodent-proof containers for food, and keeping the grass short in the camp site, are proposed in order to reduce the risk to visitors and residents of contracting HPS.

Keywords: ecology, habitat use, host, mammals, national parks, zoonoses

INTRODUCTION

The control and prevention of rodent-borne diseases largely depends upon understanding the ecology of their

hosts, including its spatial and temporal distribution (Mills et al. 1999). Hantavirus pulmonary syndrome (HPS) is a severe endemic disease of the Americas, characterized by a rapid onset of pulmonary edema, followed by respiratory failure and cardiogenic shock (Jenison et al. 1995; Peters and Khan 2002; Martinez et al. 2010). Transmission between humans and rodents occurs via inhalation of virus

material in aerosolized excreta of infected rodents (Peters and Khan 2002). In Argentina, seven native sigmodontine species have been identified as hantavirus hosts and five of them have been associated with hantavirus genotypes that cause HPS in humans (Levis et al. 2004; Padula et al. 2007; Palma et al. 2012).

Several studies on habitat use and selection have highlighted the importance of vegetation on abundance, spatial and temporal distribution, and composition of small rodent communities in HPS-endemic areas of central-east Argentina (Busch et al. 1997; Suárez and Bonaventura 2001; Hodara and Busch 2010). However, most of these studies have been conducted in agro-ecosystems and little is known about the environmental and vegetation factors that influence abundance and distribution of small rodent species in natural environments, such as protected areas.

This research was carried out in two national parks located in the floodplain of the Paraná River, where islands are an important geomorphological element (Drago 1981). As part of the floodplain of the Paraná River, these islands are subject to the influence of hydro-sedimentological pulses of the river (Neiff 1990, 1999), triggering substantial changes in vegetation (Franceschi et al. 2010). Depending on the degree and duration of the flood, small mammals may suffer direct mortality, emigrate to non-flooded areas, or remain in temporary refugia (Williams et al. 2001; Jacob 2003; Wijnhoven et al. 2005). Five sigmodontine species have been previously described in the study area (Vadell et al. 2011), two of which are known hantavirus reservoir species (Levis et al. 2004; Padula et al. 2007; Vadell et al. 2011). These species are *O. flavescens*, an omnivorous species that inhabits grasslands, forests, and irrigated crop-field borders, and *A. azarae*, an omnivorous species that inhabits principally grasslands and other habitats with high herbaceous cover (Ellis et al. 1997; Gómez Villafañe et al. 2005, 2012; Andreo et al. 2009).

Human activities in and around the islands of the Paraná delta include sustainable and recreational fishing, cattle farming, crop farming, and eco-tourism. Fishermen and farmers often live in temporary, precarious settlements, which sometimes consist of just a plastic roof with no walls, placed near the shore of rivers and streams. These activities and life style involve a very close contact with the natural environment which increases the risk of contact with rodents. Knowledge of the environmental factors that affect rodent abundance can help predict the population dynamics of hantavirus reservoir species, and be used to guide protective measures to prevent human disease (Mills and Childs 1998).

The aim of this study was to evaluate the influence of environmental variables on the macro- and micro-habitat use of small wild rodents in Pre Delta National Park and Islas de Santa Fe National Park, central-east Argentina. In doing so, we address the following questions:

Are all small rodent species influenced by the same environmental variables?

Which are the main environmental factors determining patterns of rodent distribution and abundance?

Are hantavirus reservoir species restricted to a specific habitat type?

METHODS

Study Area

The study was conducted in Pre Delta (PDNP; 32°08'S; 60°38'W) and Islas de Santa Fe (ISFNP; 32°16'S; 60°43'W) national parks located in the floodplain of the Paraná River, Argentina, at the upper section of its delta. PDNP occupies an area of 24.58 km² consisting of both continental and insular areas, while ISFNP occupies 49.60 km² of islands located mainly in the main channel of the Paraná River (Fig. 1). The climate is temperate humid, with mean monthly temperatures ranging from 1°C during the coldest month to 38°C during the warmest month. Mean annual precipitation is 900 mm, and it is mostly concentrated between October and April. Almost all of the study area is subjected to periodic flooding pulses resulting from water level changes in the Paraná River and its tributaries. These pulses do not occur regularly throughout seasons and years, and they vary in intensity and duration (Neiff and Malvárez 2004; Neiff 2004). The intensity of each flooding also varies according to the location and altitude of each individual site within the study area (Aceñolaza et al. 2003; Neiff 2004).

Small mammals were studied at six sites within the study area (two within ISFNP and four within PDNP):

1. Island 1 (ISFNP) is dominated by mixed forests of *Salix humboldtiana* and *Tessaria integrifolia* on the main levees, prairies of grasses on slopes, and broadleaf herbs and grasses around permanent or almost permanent inner ponds in the central part of the island. This island has been heavily grazed by domestic cattle for decades.
2. Island 2 (ISFNP) is similar to Island 1 but with a small farm (a house and a barn with some domestic and barnyard animals).

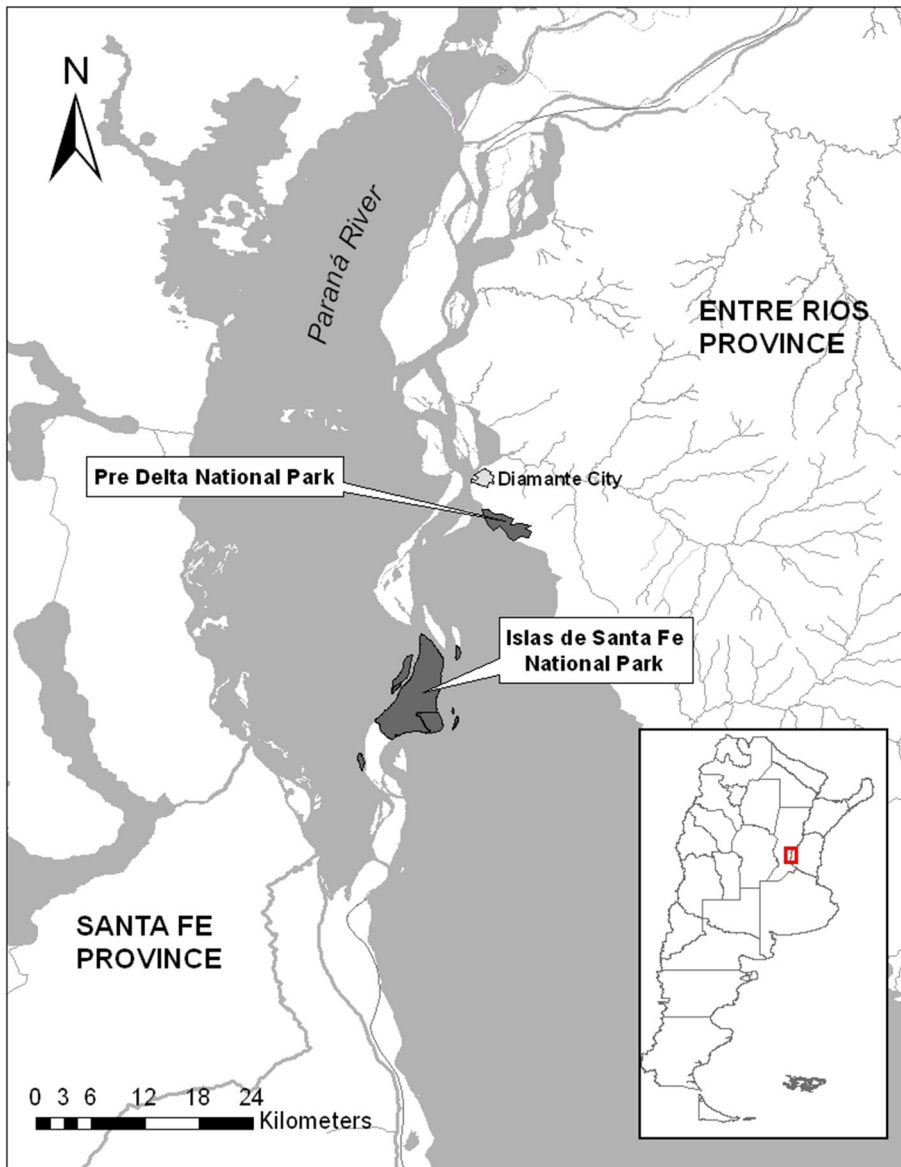


Figure 1. Pre Delta and Islas de Santa Fe national parks.

3. The riparian forest (PDNP) is a forest dominated by *S. humboldtiana* on the main levees, and *Sapium haematospermum*, *Albizia inundata*, and *Erythrina crista-galli* in the low land. Grasses and forbs form a dense understory.
4. The inland forest (PDNP) is a low-diversity forest dominated by *S. humboldtiana* and *T. integrifolia*. Grasses and forbs form a dense understory.
5. The marsh (PDNP) is a low-diversity wetland dominated by *Cortaderia selloana* and *Typha latifolia*.
6. The pond margins (PDNP) consist of open forests and shrublands surrounding a permanent inland pond. The understory is dominated by grasses of the genus *Panicum*, *Cortaderia*, and *Eryngium*.

Rodent Survey

At each of the six sites, small rodents were live-trapped once each season (Spring: October–December; Summer: January–March; Autumn: April–June; Winter: July–September) from July 2008 to September 2011 (12 trapping sessions in PDNP and 11 trapping sessions in ISFNP). Trapping was conducted at all sites during each trapping session except for the pond margins (PDNP) which was not included in the trapping design until winter 2009 (only eight trapping sessions), and the marsh which was not sampled during spring 2009 because it was inaccessible due to a flooding event. Between 25 and 50 Sherman live traps (15 × 16 × 31 cm) baited with a mixture of peanut butter, fat, and rolled oats were placed at

10-m intervals along transects in each site for three consecutive nights a season. The area covered by traps at each site varied between 2500 and 5000 m².

For each animal captured, we recorded species according to external or skull morphology (when necessary). Individuals were tagged with a uniquely numbered ear tag and were released at the point of capture.

In October 2008, all *O. flavescens* captured on the islands of ISFNP were killed in order to reduce the risk of HPS for fishermen and park workers given the occurrence of a fatal case that same year. In the following trapping sessions, only *O. flavescens* individuals captured in or around the house and the barn on island 2 (where park workers lived) were removed.

For each trapping session at a site, we calculated a capture rate (CR) as an index of population abundance. CR was estimated as

$$CR = \frac{\text{number of individual captured}}{\text{(number of traps} \times \text{number of active nights)}}$$

A mark-recapture estimate was not used given the low number of recaptured individuals.

Vegetation, Weather, and Hydrological Characteristics

Vegetation

In order to characterize the habitat used by small rodents, we recorded the characteristics of vegetation and substrate using a 1 × 1 m quadrant placed around each trap station. We recorded the percentage of the quadrant covered by green (GGrass) and dry grasses (DGrass), and green (GBroad) and dry broadleaves (DBroad) up to 1 m tall, and the percentage of bareground (Bare). We also measured maximum vegetation height (Height) at each trap station. The percentage of each quadrant covered by each vegetation type (or bare ground) was assigned to one of five cover-classes (0–12.5%, 12.5–25%, 25–50%, 50–75%, and 75–100%). Maximum vegetation height was assigned to one of four classes (<1 m, 1–2 m, 2–3 m, and >3 m). Vegetation was surveyed once during each trapping session from June 2009 to September 2011.

Weather

Weather and hydrological variables were also used to characterize the habitat used by small rodents. Based on the

literature (Suárez and Bonaventura 2001; Williams et al. 2001; Bonaventura et al. 2003; Jacob 2003; Hodara and Busch 2010; Gómez Villafañe et al. 2012), we recorded 16 environmental variables that could potentially influence the abundance of small rodents: mean, maximum, and minimum water level during the month previous to each trapping session (MaxWat, MinWat, and MeanWat); number of months since last flooding event (TimeFlood); mean, maximum, and minimum temperature during the month previous to each trapping session (T, MaxT, MinT); and monthly accumulated rainfall during the month previous to each trapping session (Rain). This analysis was also performed applying time lags of one (T30, MaxT30, MinT30, Rain30) and two months (T60, MaxT60, MinT60, Rain60) for temperature and rainfall variables.

Hydrology

Water level data for the Paraná River were provided by Prefectura Naval Argentina (Argentinean National Coast Guard; <http://www.prefecturanaval.gov.ar>) at Diamante port, 4 km away from Pre Delta National Park. Temperature and rainfall data were obtained from a weather station located in Diamante city. Weather and hydrological variables were recorded throughout the trapping period (July 2008–September 2011).

Statistical Analyses

In order to study habitat use by small rodents, two scales of analysis were defined: at the site scale (macro-habitat) and at the trap station scale (micro-habitat). At macro-habitat scale, the average of each vegetation variable recorded at each trapping station within a site was used, except for the maximum height in which the median value was used. At micro-habitat scale, the data used corresponded to each trapping station. Models at the micro-habitat scale were run separately for each study site. At the macro-habitat scale, site was included as an explanatory variable.

Logistic regressions (McCullagh and Nelder 1989; Nicholls 1991; Crawley 1993) were used to examine macro- and micro-habitat use by small rodents by means of the Generalized Linear Models procedure of R (R-Core-Team 2013) based on a priori models with a combination of explanatory variables (Tables 1, 2). Multiple correlation tests (Zar 1996) were performed among all the explanatory variables in order to avoid redundancy in the models.

Table 1. Macro-habitat Models Showing QAICc Values and Akaike Weights (w_i) for All Small Rodents and for Each Separated Species (*Aa*, *Cc*, *Or*, and *Of*) from Pre Delta and Islas de Santa Fe National Parks.

Candidate models	All species		<i>Or</i>		<i>Of</i>		<i>Cc</i>		<i>Aa</i>		
	QAICc	w_i	QAICc	w_i	QAICc	w_i	QAICc	w_i	QAICc	w_i	
Weather and hydrological models											
0	<i>null</i>	141.47	0.00	68.44	0.09	34.81	0.28	71.37	0.01	56.41	0.48
1	Site	143.55	0.00	64.07	0.83	37.80	0.06	76.11	0.00	61.21	0.04
2	Season	146.08	0.00	74.64	0.00	39.21	0.03	77.05	0.00	60.13	0.07
3	MaxT	143.51	0.00	70.71	0.03	37.01	0.09	73.12	0.00	58.70	0.15
4	TimeFlood	130.88	0.02	70.39	0.04	33.84	0.45	62.30	0.77	58.24	0.19
5	MaxT+TimeFlood	144.05	0.00	72.81	0.01	38.66	0.04	64.78	0.22	60.68	0.06
6	Site+MaxT+Site×MaxT	155.88	0.00	–	–	51.85	0.00	–	–	–	–
7	Site+TimeFlood+Site×TimeFlood	144.92	0.00	–	–	48.78	0.00	–	–	–	–
8	Season+TimeFlood+Season×TimeFlood	123.20	0.98	–	–	38.10	0.05	–	–	–	–
Vegetation models											
0	<i>null</i>	74.06	0.47	56.36	0.44	64.00	0.00	38.45	0.50	54.00	0.20
1	GBroad	76.02	0.18	58.78	0.13	52.73	0.92	40.61	0.17	54.53	0.15
2	GGrass	75.90	0.19	57.39	0.26	66.03	0.00	40.59	0.17	56.37	0.06
3	Height	76.32	0.15	58.57	0.15	65.77	0.00	40.85	0.15	52.01	0.53
4	GGrass+GBroad+Height	80.51	0.02	62.51	0.02	57.68	0.08	45.83	0.01	56.32	0.06
5	Site+GBroads+Site×GBroad	87.86	0.00	–	–	–	–	–	–	–	–
6	Site+GGrass+Site×GGrass	87.89	0.00	–	–	–	–	–	–	–	–
7	Site+Height+Site×Height	86.73	0.00	–	–	–	–	–	–	–	–

Data used for weather–hydrological models are from the period between July 2008 and September 2011, while the data used for the vegetation models are from June 2009 to September 2011. QAICc values lower than the null models are shown in bold letters. Akaike weights (w_i) for each model are also shown.

At a macro-habitat scale, we explored the relationship between CR (total and per species) and explanatory variables by means of two groups of candidate models: weather–hydrological models (eight models) and vegetation models (seven models; Table 1). Site and season were also included as explanatory variables in both groups of candidate models. Models of both groups were built using the binomial family distribution and the logit link function (Zuur 2009). The number of parameters was limited by the number of observations (Burnham and Anderson 2002).

Models were based on a Quasi-Akaike's information criterion corrected for small sample size and over-dispersion data (QAICc) (Burnham and Anderson 2002). Models with smaller QAICc values than the null model, and with variables that have a parameter different from zero, were considered selected models. The final model was based on the average of the selected models (Symonds and Moussalli 2011).

For the micro-habitat analysis, the relationship between the presence of rodents (total and by species) at each trap station, and the vegetation variables and season was explored for each site using the binomial family distribu-

tion and the clog-log link function (Zuur 2009). Between three and seven candidate models per site derived from combinations of up to three variables were used (Table 2). Model selection was based on Akaike's information criterion (AIC) (Burnham and Anderson 2002). Models with smaller AIC values than the null model, and with variables that have a parameter different from zero, were considered selected models. The final model was based on the average of the selected models (Symonds and Moussalli 2011).

We consider trapping a valid method to study habitat selection by small rodents because all traps are equally baited and hence their attraction effect on rodents should be equal. Thus, the capture event should be determined by the characteristics of the micro-habitat.

RESULTS

Small Rodent Richness and Composition

A total of 453 individuals of five species were captured 512 times with a trapping effort of 9471 trap-nights. The species

Table 2. Micro-habitat Models for Each Site Showing AIC Values and Akaike Weights (w_i) for All Small Rodents and for Each Separated Species (*Or*, *Of*, *Cc*, and *Aa*) Caught in Pre Delta and Islas de Santa Fe National Parks Between June 2009 and September 2011.

Candidate models	All species		<i>Or</i>		<i>Of</i>		<i>Cc</i>		<i>Aa</i>		
	AIC	w_i	AIC	w_i	AIC	w_i	AIC	w_i	AIC	w_i	
Island 1											
0	<i>null</i>	109.92	0.44	–	–	104.73	0.46	–	–	–	–
1	Ggrass	110.83	0.28	–	–	105.87	0.26	–	–	–	–
2	Dbroad	111.83	0.17	–	–	106.56	0.18	–	–	–	–
3	Ggrass+Dbroad	112.78	0.11	–	–	107.75	0.10	–	–	–	–
Island 2											
0	<i>null</i>	199.58	0.03	–	–	195.70	0.04	–	–	–	–
1	Ggrass	195.12	0.31	–	–	191.44	0.36	–	–	–	–
2	DBroad	199.88	0.03	–	–	196.77	0.02	–	–	–	–
3	GBroad	200.04	0.03	–	–	196.37	0.03	–	–	–	–
4	Ggrass+DBroad	195.92	0.21	–	–	192.87	0.17	–	–	–	–
5	Ggrass+GBroad	195.72	0.23	–	–	192.24	0.24	–	–	–	–
6	Dbroad+GBroad	200.22	0.02	–	–	197.36	0.02	–	–	–	–
7	Ggrass+Dbroad+GBroad	196.53	0.15	–	–	193.67	0.12	–	–	–	–
Riparian forest											
0	<i>null</i>	259.44	0.03	134.20	0.15	65.99	0.45	113.19	0.08	90.63	0.05
1	Height	261.27	0.01	134.65	0.12	67.22	0.24	113.64	0.06	91.13	0.04
2	GBroad	253.19	0.69	132.09	0.43	67.55	0.20	109.84	0.43	85.76	0.56
3	Height+GBroad	255.12	0.26	132.83	0.30	68.78	0.11	109.89	0.42	86.69	0.35
Continental forest											
0	<i>null</i>	500.06	0.28	404.01	0.20	101.2	0.51	172.2	0.41	93.17	0.30
1	Height	500.01	0.29	405.68	0.09	103.09	0.20	173.32	0.24	92.62	0.40
2	GBroad	500.72	0.20	402.20	0.49	102.93	0.21	173.48	0.22	94.83	0.13
3	Height+GBroad	500.40	0.24	403.71	0.23	104.86	0.08	174.42	0.14	94.43	0.16
Marsh											
0	<i>null</i>	259.56	0.06	183.87	0.19	64.68	0.25	96.23	0.00	101.95	0.11
1	Height	255.85	0.35	181.77	0.53	66.58	0.10	84.73	0.43	98.42	0.62
2	Dgrass	259.06	0.07	185.68	0.08	63.40	0.47	96.40	0.00	103.85	0.04
3	Height+DGrass	255.09	0.52	183.63	0.21	65.28	0.18	84.17	0.57	100.34	0.24
Pond margins											
0	<i>null</i>	210.20	0.34	171.1	0.38	–	–	41.21	0.08	62.68	0.50
1	GGram	211.84	0.15	173.08	0.14	–	–	37.08	0.65	64.68	0.18
2	GBroad	210.06	0.36	171.22	0.35	–	–	43.21	0.03	64.26	0.23
3	GGram+GBroad	211.88	0.15	173.19	0.13	–	–	39.08	0.24	66.26	0.08

AIC values lower than the null models are shown in *bold letters*. A dash shows that the analysis was not carried out due to low sample size.

were *Oxymycterus rufus* (167 individuals), *Oligoryzomys flavescens* (160), *Calomys callidus* (68), *Akodon azarae* (42), and the genus *Holochilus* (15, including 3 *H. chacarius* determined to the species level).

Maximum rodent species richness was found at the marsh and the pond margins where all small rodent species were captured, followed by the inland and riparian forests and the two islands belonging to ISFNP (Fig. 2).

O. flavescens was captured at every site in both parks, and was numerically dominant on the islands. In PDNP, *O. rufus* was the most abundant species and was numerically dominant at the marsh, the pond margins and the inland forest. *C. callidus* was the most abundant species in the riparian forest. *A. azarae* was captured in all the sites (Fig. 2).

After the removal of captured *O. flavescens* during October 2008 on the two islands of ISFNP, these popula-

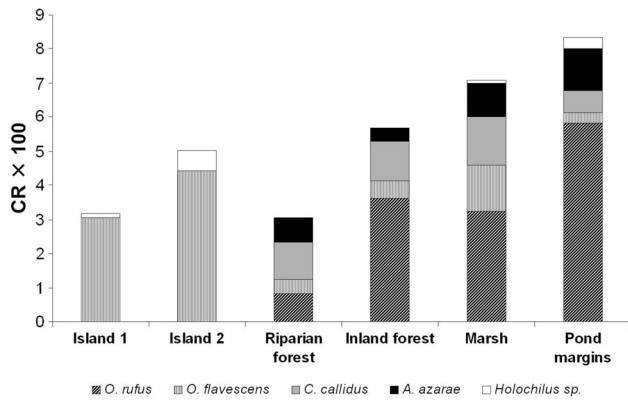


Figure 2. Capture rate (CR) of each species captured from July 2008 to September 2011 in Islas de Santa Fe (island 1 and island 2) and Pre Delta national parks (riparian forest, inland forest, marsh, and pond margins). Trapping on the islands started in October 2008 and in the pond margins in September 2009.

tions crashed abruptly (Fig. 3). By June 2009, both populations showed a recovery after which they declined reaching zero abundance in September 2009 (island 1) and December 2009 (island 2), and did not begin recovering until March 2011 (Fig. 3).

Habitat Use

The selected weather–hydrological models described the CR of small rodents at PDNP and ISFNP as a function of season and the time elapsed since last flooding (Table 1). Model averaging also showed a positive association (stronger in spring and autumn than in winter) between CR of small rodents and the time since last flooding, contrary to summer where the association was negative (Table 3). The near absence of captures in May 2010 is probably related to the large flooding event which began at the end of 2009 and reached the maximum water level by February 2010 (Fig. 4). The peaks of CR occurred in spring, late winter, and early autumn, and they were related mainly to an increase in the abundance of *O. flavescens* and *O. rufus*, which were the numerically dominant species.

At the micro-habitat scale, small rodents selected patches with low cover of green grasses on island 2, selected habitat patches with high cover of green broadleaves in the riparian forest, and preferred patches with high dry grass cover and high vegetation in the marsh (Tables 2, 4).

CR of *O. flavescens* at the macro-habitat scale was positively affected by the time elapsed since the last flood (estimator: 0.07; SE: 0.01; CI 95%: 0.05; 0.09; Table 1) and

by green broadleaf cover (estimator: -0.04 ; SE: 0.01; CI 95%: -0.05 ; -0.03 ; Table 1).

O. flavescens selected patches with low green grass cover on island 2 (estimator: -0.02 ; SE: 0.01; CI 95%: -0.05 ; 0.00; Table 2), and with high dry grass cover in the marsh (estimator: 0.03; SE: 0.01; CI 95%: 0.00; 0.05; Table 2). None of the candidate models explained the presence of *O. flavescens* on island 1 (Table 2). This analysis was not carried out in the pond margins due to the low number of *O. flavescens* captured in this site.

CR of *A. azarae* at the macro-habitat scale was negatively affected by vegetation height (estimator: -0.63 ; SE: 0.21; CI 95%: -0.07 ; -0.24 ; Table 1). No weather–hydrological model was selected for *A. azarae* (Table 1), suggesting that the temperature and the time elapsed since the last flooding did not have an effect on the CR of this species, and that the season and sites do not explain the observed differences within PDNP.

At the micro-habitat scale, *A. azarae* selected patches with low vegetation at the marsh (estimator: -0.84 ; SE: 0.40; CI 95%: -1.63 ; -0.05 ; Table 2). At the other sites, this species did not select patches based on the variables considered in this study.

CRs of *O. rufus* differed among sites (Tables 1, 5), with the greatest values in the pond margins followed by the continental forest and the marsh (Fig. 2 and Table 5). This species was least captured in the riparian forest and was not captured on the islands (Fig. 1 and Table 2). Maximum temperature, time since the last flooding, season, and vegetation at a macro-habitat scale did not seem to affect its abundance (Tables 1, 5).

At a micro-habitat scale, *O. rufus* selected patches with high cover of green broadleaves at the riparian (estimator: 0.02; SE: 0.01; CI 95%: 0.0; 0.05) and continental forests (estimator: 0.01; SE: 0.01; CI 95%: 0.00; 0.02; Table 2). At the marsh, this species selected patches with high vegetation (estimator: 0.36; SE: 0.18; CI 95%: 0.01; 0.72; Table 2).

CR of *C. callidus* was associated with the time elapsed since the last flooding event (estimator: 0.08; SE: 0.02; CI 95%: 0.05; 0.12; Table 1). This species was not captured on the islands (ISFNP) and no significant difference was observed in its CR among the sites where it was captured (Fig. 2 and Table 1). Vegetation analyses suggest that the abundance of this species was not associated with the vegetation variables at the macro-habitat scale (Table 1).

At a micro-habitat scale, *C. callidus* selected patches with high green broadleaf cover at the riparian forest (estimator: 0.04; SE: 0.01; CI 95%: 0.00; 0.08; Table 2), and

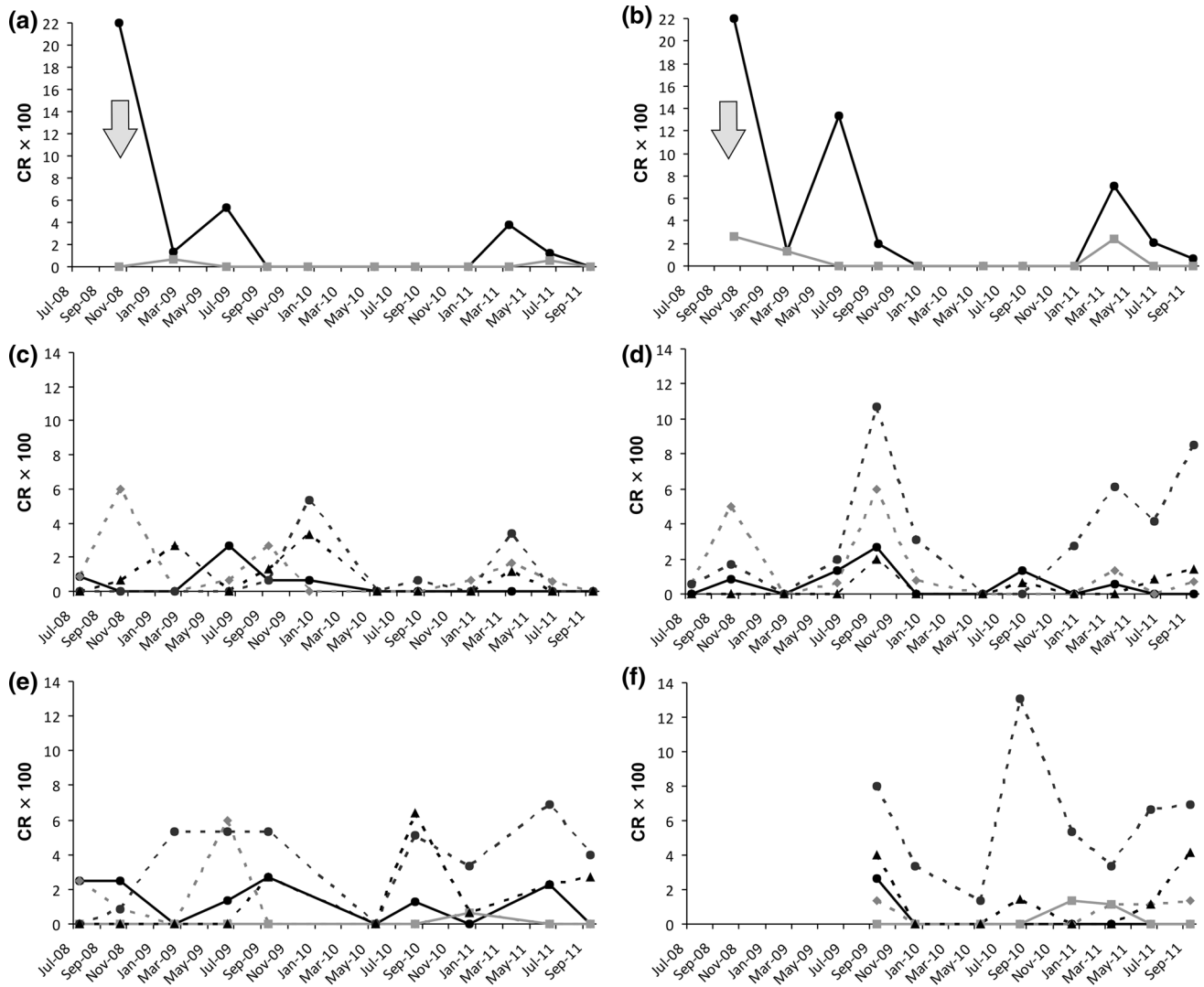


Figure 3. Capture rate (CR) of *O. flavescens*, *C. callidus*, *Holochilus* sp., *O. rufus*, and *A. azarae* in Islas de Santa Fe National Park: **a** island 1, **b** island 2, and Pre Delta National Park: **c** riparian forest, **d** inland forest, **e** marsh, and **f** pond margins from July 2008 to September 2011. Arrows show removal of all captured *O. flavescens* individuals. Note different scaling of y-axis for the islands.

patches with tall vegetation (estimator: 1.11; SE: 0.32; CI 95%: 0.48; 1.74; Table 2) and high cover of dry grasses (estimator: 0.02; SE: 0.01 CI 95%: 0.00; 0.05; Table 2) at the marsh.

Holochilus sp. was captured on the islands, in the marsh, and in the pond margins (Fig. 2). Individuals were captured during every season except winter (Fig. 3).

DISCUSSION

Two hantavirus reservoir species, *O. flavescens* and *A. azarae*, were found in the study area. *O. flavescens* was captured at every studied habitat and was the most abun-

dant species in Islas de Santa Fe National Park. This species is the reservoir of Lechiguanas genotype, one of the two known genotypes that cause HPS in central-east Argentina (Padula et al. 2007). The presence and high abundance of *O. flavescens* is of epidemiological importance in the area because the presence and circulation of Lechiguanas pathogenic genotype has been demonstrated in the populations of this species in Pre Delta and Islas de Santa Fe national parks (Vadell et al. 2011). The human activities that take place in this portion of the Paraná delta, such as fishing, cattle, crop farming, and eco-tourism (Gómez Villafañe, pers. com.) encourage the contact between people and sylvan rodents, increasing the risk of contracting

Table 3. Averaged Estimates of Relevant Variables for the Macro-habitat Model for Small Rodents from Pre Delta and Islas de Santa Fe National Parks.

	Estimator	SE	CI 95%
Weather and hydrological models			
Intercept	-4.74	0.29	-5.31 to -4.16
Spring	0.77	0.35	0.07 to 1.48
Summer	3.04	0.42	2.21 to 3.87
Winter	0.87	0.36	0.15 to 1.59
TimeFlood	0.09	0.01	0.06 to 0.12
TimeFlood×Spring	0.02	0.02	-0.01 to 0.06
TimeFlood×Summer	-0.18	0.03	-0.23 to -0.13
TimeFlood×Winter	-0.04	0.02	-0.08 to -0.01

Data used for weather–hydrological models are from the period between July 2008 and September 2011, while the data used for the vegetation models are from June 2009 to September 2011.

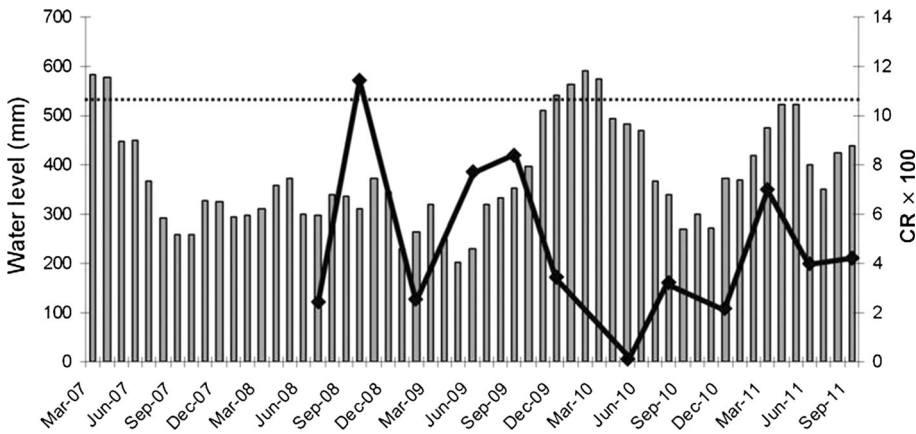


Figure 4. Water level of the Paraná River (gray bars) and total capture rate of rodents (solid line) at Pre Delta and Islas de Santa Fe national parks. Water levels are shown since March 2007, when the last flood before the start of rodent trapping occurred. The dashed line indicates the value over which it was considered a flood event (530 mm).

HPS. The death of a fisherman who contracted HPS while living in Islas de Santa Fe National Park (island 1) highlights the importance of studying the hantavirus–rodent system, and the need for implementation of prevention measures to reduce virus exposure.

O. flavescens and *C. callidus* were negatively influenced by flooding. *O. flavescens* was found in highest numbers on the islands, areas strongly influenced by floods, and was the only species trapped there except for few individuals of the genus *Holochilus*. As part of the upper delta of the Paraná River, these islands are very recently formed and have a lower altitude than the continental areas. Hence, they are frequently flooded by intermediate and high pulses that surpass the main levees, leaving the whole ground area covered by water. *O. flavescens* is known as a good colonizer and has been described as a species adapted to arboreal habits (Massoia and Fornes 1965; Udrizar Sauthier et al.

2010; Maroli et al. 2015) and wetlands (Suárez and Bonaventura 2001). This species has been seen nesting in trees during flooding events in Pre Delta National Park (Pre Delta National Park forest rangers, pers. com.) and on other plants at heights between 1 and 2 m above ground level at the border of small channels (Udrizar Sauthier et al. 2010). The great increases in water level of the Paraná River, as occurred in this study, generate floods that sometimes surpass 100 cm above ground level. These floods restrict the access of small rodents to the vegetation at ground level during several days, weeks, or even months. Hence, great floods could affect small rodent populations in a negative way by destruction of their habitat, direct mortality, and by migration of survivors to non-flooded areas (Jacob 2003).

A. azarae, host of Pergamino hantavirus genotype in the area (Vadell et al. 2011; Palma et al. 2012), was present

Table 4. Average Estimates of Final Micro-habitat Models for Small Rodents from Pre Delta and Islas de Santa Fe National Parks Between June 2009 and September 2011.

	Estimator	SE	CI 95%
Island 2			
Intercept	-1.70	0.25	-2.19 to -1.21
GGrass	-0.02	0.01	-0.05 to 0.00
Riparian forest			
Intercept	-3.99	0.71	-5.37 to -2.60
GBroad	0.02	0.01	0.00 to 0.04
Marsh			
Intercept	-2.06	0.42	-2.88 to -1.23
Height	0.33	0.13	0.07 to 0.59
DGrass	0.01	0.01	0.00 to 0.03

in every habitat of Pre Delta National Park and behaved as a generalist species at a micro-habitat scale in every habitat except for the marsh where it selected patches with low vegetation height. This lack of micro-habitat selection is in agreement with results obtained for this species by other authors in Otamendi Natural Reserve (Buenos Aires province) (Gómez Villafañe et al. 2012; Maroli et al. 2015). In addition, Hodara and Busch (2010) found selection by *A. azarae* at a micro-habitat scale in maize fields but not in field edges, and suggested an increased selectivity in the “poor habitat” (maize fields), in which individuals can perceive suitable and unsuitable patches. Under this hypothesis, the studied habitats at Pre Delta National Park (inland forest, pond margins, and riparian forest) might all be suitable for *A. azarae*. While this species has not been associated with a pathogenic genotype of hantavirus, studies should be continued because a potential risk to humans cannot be discarded. High mutation rates and the existence of multiple genotypes give RNA viruses such as hantaviruses the ability to become adapted to changing environments and to overcome barriers to spread (Cleaveland et al. 2007). In this scenario, the fact that *A. azarae* behaves as a generalist species in Pre Delta National Park is of epidemiological importance and should be taken into account if this species becomes a host of a pathogenic hantavirus in the future.

O. rufus was the most abundant species in every habitat except for the islands. *O. rufus* used the habitats differently at Pre Delta National Park, with a higher abundance in the pond margins, followed by the continental forest and the marsh, and with a minimum abundance in the riparian forest. This differential use of habitat does not seem due to

differences in the general physiognomy of these habitats because they are all mainly moist and close to water bodies, nor to their vegetation because it was not included in the models as a relevant variable to explain *O. rufus* abundance.

The low number of *Holochilus* sp. captured in both parks could be due to a sub-estimation produced by the trapping method. This species is considered a strict herbivore and hence might not be attracted to the bait used during this study. This hypothesis is supported by the slight increase in the number of captures seen in autumn 2011, the period in which a high amount of both dead and alive individuals were observed across the middle delta of the Paraná River, which may have resulted from an outbreak of this species which trapping failed to detect. Moreover, two individuals from the genus *Holochilus* were seen swimming and moving around and over aquatic plants of the family Pontederiaceae in a stream that crosses Islas de Santa Fe National Park, and several others were also observed by park rangers in previous days (M.V. Vadell, personal observation). The construction of nests on plants of the family Pontederiaceae has been reported for *Holochilus brasiliensis* in the region (Udrizur Sauthier et al. 2010). This genus is considered closely associated with mesic microenvironments, such as grassy marshes, wetlands, and riparian forests (Pardiñas et al. 2010, 2013), as can be found in Pre Delta and Islas de Santa Fe national parks. Two species of this genus, *H. brasiliensis* and *H. chacarius*, have overlapping distributions in the study area (Pardiñas et al. 2013), but in this study we could only confirm the presence of *H. chacarius* in Islas de Santa Fe national park, based on the morphology of the jaws of three specimens. Pre Delta National Park contained a high diversity and abundance of small rodents, while Islas de Santa Fe National Park

Table 5. Averaged Estimates of Relevant Variables of the Macro-habitat Model for *O. rufus* from Pre Delta and Islas de Santa Fe National Parks Between July 2008 and September 2011.

	Estimator	SE	CI 95%
Weather and hydrological models			
Intercept	-3.30	0.12	-3.54 to -3.08
Riparian forest	-1.47	0.28	-2.04 to -0.95
Marsh	-0.09	0.20	-0.50 to 0.30
Pond margins	0.52	0.21	0.10 to 0.91

had a very low diversity. The low diversity in Islas de Santa Fe National Park could be related to its low diversity of habitats or to the intensity and frequency of the floods which could prevent the establishment of some species less adapted to flooding. In Pre Delta National Park, all the species with known distribution in this area were captured with the exception of *Oligoryzomys nigripes* and *Scapteromys aquaticus* (IUCN 2014). Massa et al. (2013) found that these species were absent (or in very low numbers) in barn owl's pellets from different localities close to Pre Delta and Islas de Santa Fe national parks.

Given the susceptibility of hantaviruses to ultraviolet rays (Mills et al. 1995), we suggest creating open camping areas with mowed lawns (approximately 5 cm long) and trimmed shrubs in Islas de Santa Fe National Park. Tents should be placed at least 10 m away from the perimeter of the camping area to reduce contact with rodents living in the surrounding vegetation. We also suggest placing signs warning people about HPS and the risk of camping in densely vegetated areas. Campers should also be warned against keeping food inside tents and touching live or dead rodents, as suggested by Mills et al. (2002). Because Islas de Santa Fe National Park has been recently created and has not been officially open to the public yet, we suggest designing trails and walks that avoid very shadowy sectors in order to minimize the risk of contracting HPS. We also suggest paying special attention to the rodent proofing of buildings, and the use of rodent-proof containers for food and garbage, especially during flooding events when rodents are more prone to seek refuge (Zhang et al. 2007). In addition, special prevention measures should be taken during and after floods in order to minimize the risk of leptospirosis (Bharti et al. 2003; Ahern et al. 2005). Leptospirosis is a very common bacterial zoonosis caused by the spirochetes of the genus *Leptospira* that can be present in a wide range of animals including rodents, livestock, and domestic pets (Haake and Levett 2010). In spite of the fact

that rodent numbers are expected to decrease after floods, the risk of leptospirosis is known to increase with flooding because of the bacteria's ability to survive in wet soil and water (Bharti et al. 2003; Vanasco et al. 2008). Educational brochures and leaflets should be designed and given to tourist agents and local people in order to create awareness of disease prevention in natural environments.

ACKNOWLEDGEMENTS

We would like to thank all the personnel of Pre Delta National Park for their support and help in the field. We are also indebted to Francisco García Erize, Mariano Feldman, Carolina Massa, and Malena Maroli for their help with the field work. We are also grateful to Richard Douglass for his useful revision of the manuscript. This research was funded by CONICET (Argentina), Universidad de Buenos Aires, Administración de Parques Nacionales and Fundación Bunge y Born.

REFERENCES

- Aceñolaza PG, Povedano HE, Manzano AS, de Dios Muñoz J, Areta JJ, Virgolini ALR (2003) Biodiversidad del Parque Nacional Pre-Delta. *Miscelánea* 12:169–184
- Ahern M, Kovats RS, Wilkinson P, Few R, Matthies F (2005) Global health impacts of floods: epidemiologic evidence. *Epidemiologic Reviews* 27:36–46
- Andreo V, Lima M, Provencal C, Priotto J, Polop J (2009) Population dynamics of two rodent species in agro-ecosystems of central Argentina: intra-specific competition, land-use, and climate effects. *Population Ecology* 51:297–306
- Bharti AR, Nally JE, Ricaldi JN, Matthias MA, Diaz MM, Lovett MA, et al. (2003) Leptospirosis: a zoonotic disease of global importance. *The Lancet Infectious Diseases* 3:757–771
- Bonaventura SM, Pancotto V, Madanes N, Vicari R (2003) Microhabitat use and density of sigmodontine rodents in *Spartina densiflora* freshwater marshes, Argentina. *Mammalia* 67:367–377

- Burnham KP, Anderson DR (2002) *Model Selection and Multi-model Inference: A Practical Information-Theoretic Approach*, 2nd ed., New York: Springer
- Busch M, Alvarez MR, Cittadino EA, Kravetz FO (1997) Habitat selection and interspecific competition in rodents in pampean agroecosystems. *Mammalia* 1:167–184
- Cleaveland S, Haydon D, Taylor L (2007) Overviews of pathogen emergence: which pathogens emerge, when and why?. In: *Wildlife and Emerging Zoonotic Diseases: The Biology, Circumstances and Consequences of Cross-Species Transmission*, Childs JE, Mackenzie JS, Richt JA (editors), New York: Springer, pp 85–111
- Crawley MJ (1993) *GLIM for Ecologists*, Oxford: Blackwell Scientific Publications
- Drago EC (1981) Grados de conexión y fases hidrológicas en ambientes leníticos de la llanura aluvial del río Paraná (Argentina)[hidrología]. *Ecología* 6:27–33
- Ellis B, Mills J, Childs J, Muzzini M, McKee K, Enria D, et al. (1997) Structure and floristics of habitats associated with five rodent species in an agroecosystem in Central Argentina. *Journal of Zoology* 243:437–460
- Franceschi EA, Torres PS, Lewis JP (2010) Diversidad de la vegetación durante su recuperación tras una creciente extraordinaria del Río Paraná Medio (Argentina). *Revista de Biología Tropical* 58:707–716
- Gómez Villafañe IE, Miño MH, Cavia R, Hodara K, Courtalón P, Suárez OV, et al. (2005) *Guía de Roedores de la Provincia de Buenos Aires*, Buenos Aires: LOLA
- Gómez Villafañe IE, Expósito Y, San Martín Á, Picca P, Busch M (2012) Rodent diversity and habitat use in a protected area of Buenos Aires province, Argentina. *Revista Mexicana de Biodiversidad* 83:762–771
- Haake DA, Levett PN (2010) *Leptospira* species (leptospirosis). In: *Principles and Practice of Infectious Diseases*, Bennet JE, Dolin R, Blaser MJ (editors), Philadelphia: Elsevier, pp 2714–2720
- Hodara K, Busch M (2010) Patterns of macro and microhabitat use of two rodent species in relation to agricultural practices. *Ecol Res* 25:113–121
- IUCN (2014). The IUCN red list of threatened species. Accessed 12 Nov 2014.
- Jacob J (2003) The response of small mammal populations to flooding. *Mammalian Biology - Zeitschrift für Säugetierkunde* 68:102–111
- Jenison S, Hjelle B, Simpson S, Hallin G, Feddersen R, Koster F (1995) Hantavirus pulmonary syndrome: clinical, diagnostic, and virologic aspects. *Seminars in Respiratory Infections* 10:259–269
- Levis S, Garcia J, Pini N, Calderon G, Ramirez J, Bravo D, et al. (2004) Hantavirus pulmonary syndrome in northwestern Argentina: circulation of Laguna Negra virus associated with *Calomys callosus*. *The American Journal of Tropical Medicine and Hygiene* 71:658–663
- Maroli M, Vadell MV, Iglesias A, Padula P (2015) Daily movements and microhabitat selection of hantavirus reservoirs and other sigmodontine rodent species that inhabit a protected natural area of Argentina. *EcoHealth* 12:1–11
- Martinez VP, Bellomo CM, Cacace ML, Suárez P, Bogno L, Padula PJ (2010) Hantavirus pulmonary syndrome in Argentina, 1995–2008. *Emerging Infectious Diseases* 16:8
- Massa C, Teta P, Cueto GR (2013) Effects of regional context and landscape composition on diversity and composition of small rodent assemblages in Argentinian temperate grasslands and wetlands. *Mammalia* 78:1–12
- Massoia E, Fornes A (1965) Nuevos datos sistemáticos, biológicos y etoecológicos de *Oryzomys (Oligoryzomys) delticola* Thomas (Rodentia-Cricetidae). *Delta del Paraná, Investigaciones Agrícolas* 4:27–34
- McCullagh P, Nelder JA (1989) *Generalized Linear Models*, 2nd ed., London: Chapman and Hall
- Mills JN, Childs JE (1998) Ecologic studies of rodent reservoirs: their relevance for human health. *Emerging Infectious Diseases* 4:529–537
- Mills JN, Yates TL, Childs JE, Parmenter RR, Ksiazek TG, Rollin PE, et al. (1995) Guidelines for working with rodents potentially infected with hantavirus. *Journal of Mammalogy* 76:716–722
- Mills JN, Ksiazek TG, Peters C, Childs JE (1999) Long-term studies of hantavirus reservoir populations in the southwestern United States: a synthesis. *Emerging Infectious Diseases* 5:135
- Mills J, Corneli A, Young JC, Garrison L, Khan A, Ksiazek TG (2002) *Hantavirus Pulmonary Syndrome—United States: Updated Recommendations for Risk Reduction*. National Center for Infectious Diseases. <http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5109a1.htm>. Accessed 15 Sept 2015.
- Neiff JJ (1990) Ideas para la interpretación ecológica del Paraná. *Interciencia* 15:424–441
- Neiff JJ (1999) El régimen de pulsos en ríos y grandes humedales de Sudamérica. *Tópicos Sobre Humedales Subtropicales y Templados de Sudamérica* 1:99–150
- Neiff JJ (2004). Bosques fluviales de la cuenca del Paraná. In: *Ecología & Manejo de los bosques de Argentina*, Arturi MF, Frangi JL, Goya JF (editors), La Plata, pp 1–26
- Neiff JJ, and Malvárez AI (2004) Grandes humedales fluviales. *Documentos del Curso Taller Bases Ecológicas para la clasificación e inventario de humedales en Argentina*, Buenos Aires
- Nicholls AO (1991) Examples of the use of generalised linear models in analysis of survey data for conservation evaluation. In: *Nature Conservation: Cost Effective Biological Surveys and Data Analysis*, Margules CR, Austin MP (editors), East Melbourne: CSIRO Australia, pp 54–63
- Padula P, Martinez VP, Bellomo C, Maidana S, San Juan J, Tagliaferri P, et al. (2007) Pathogenic hantaviruses, northeastern Argentina and eastern Paraguay. *Emerging Infectious Diseases* 13:1211–1214
- Palma RE, Polop JJ, Owen RD, Mills JN (2012) Ecology of rodent-associated hantaviruses in the Southern Cone of South America: Argentina, Chile, Paraguay and Uruguay. *Journal of Wildlife Diseases* 48:267–281
- Pardiñas UFJ, Teta P, D'Elia G (2010) Roedores sigmodontinos de la región pampeana: historia evolutiva, sistemática y taxonomía. In: *Biología y Ecología de Pequeños Roedores en la Región Pampeana de Argentina*, Polop FJ, Busch M (editors), Córdoba: Universidad Nacional de Córdoba
- Pardiñas UF, Teta P, Voglino D, Fernández FJ (2013) Enlarging rodent diversity in west-central Argentina: a new species of the genus *Holochilus* (Cricetidae, Sigmodontinae). *Journal of Mammalogy* 94:231–240
- Peters CJ, Khan AS (2002) Hantavirus pulmonary syndrome: the new American hemorrhagic fever. *Clinical Infectious Diseases* 34:1224
- R-Core-Team (2013) *R: A Language and Environment for Statistical Computing*, Vienna

- Suárez OV, Bonaventura SM (2001) Habitat use and diet in sympatric species of rodents of the low Paraná delta, Argentina. *Mammalia* 65:167–176
- Symonds MR, Moussalli A (2011) A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. *Behavioral Ecology and Sociobiology* 65:13–21
- Udrizar Sauthier WO, Abba AM, Udrizar Sauthier DE (2010) Nests of *Oligoryzomys* sp. and *Holochilus brasiliensis* (Rodentia, Cricetidae) in eastern Entre Ríos Province, Argentina. *Mastozoología Neotropical* 17:207–211
- Vadell M, Bellomo C, San Martín A, Padula P, Gómez Villafañe I (2011) Hantavirus ecology in rodent populations in three protected areas of Argentina. *Tropical Medicine & International Health* 16:1342–1352
- Vanasco N, Schmeling M, Lottersberger J, Costa F, Ko A, Tarabla HD (2008) Clinical characteristics and risk factors of human leptospirosis in Argentina (1999–2005). *Acta Tropica* 107:255–258
- Wijnhoven S, Van Der Velde G, Leuven RS, Smits AJ (2005) Flooding ecology of voles, mice and shrews: the importance of geomorphological and vegetational heterogeneity in river floodplains. *Acta Theriologica* 50:453–472
- Williams AK, Ratnaswamy MJ, Renken RB (2001) Impacts of a flood on small mammal populations of lower Missouri River floodplain forests. *The American Midland Naturalist* 146:217–221
- Zar JR (1996) *Biostatistical Analysis*, 3rd ed., New Jersey: Prentice Hall
- Zhang M, Wang K, Wang Y, Guo C, Li B, Huang H (2007) Recovery of a rodent community in an agro-ecosystem after flooding. *Journal of Zoology* 272:138–147
- Zuur AF (2009) *Mixed Effects Models and Extensions in Ecology with R*, Nueva York: Springer