

What you get is what they have? Detectability of intestinal parasites in reptiles using faeces

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Abstract Parasitological analyses are often based on invasive methodologies, involving host sacrifice, raising ethical and conservation issues. However, alternative non-invasive approaches may not be always applicable due to the location of the parasite in the host tissue or the quality and reliability of the non-invasive sample per se. In this study, we compare the differences in detectability of intestinal parasites in reptiles using the classical invasive approach (intestine dissection), versus a non-invasive procedure (faecal examination), collected from the same individual host. Our results showed significantly lower detectability of helminths in faeces versus the intestine. Moreover, the number of parasites found in faeces was not explained either by the intensities found in the respective intestine or by the host identity. Several factors may explain the lack of association between the two types of samples, but more importantly, our results highlight the randomness of the presence of parasites in faeces. Even if it is not recommended that comparative studies of either parasite abundance or parasite communities be conducted on the basis of faecal samples, there are other types of studies (i.e. genetic)

that can be performed with this source of information, thus avoiding the sacrifice of the host. Due to their wide spectrum of life stages and localization in the host tissue, parasites are challenging candidates for non-invasive sampling and consequently, parasitological methodologies should be carefully selected according to the objective of the study.

Introduction

Parasite assessment represents one of the biggest challenges in the inventory of the overall species present on the planet. Due to their small size and the high variety and complexity of life cycles and corresponding life stages, detection and identification of parasites are not straightforward. Estimation of parasite abundances, through the measurement of prevalence (the percentage of infected hosts in a population) and intensity of infection (the mean number of parasites per infected hosts), can be used to determine some basal characteristics of parasite species even when accounting for temporal variation (Poulin 2007). However, such studies are still missing for the majority of species of no economic importance. One of the major impediments for parasitological studies is that the sacrifice of the host is usually required because of parasite location in host tissues. In this context, metazoan parasites may be separated into ectoparasites (such as ticks and mites) and endoparasites (i.e. nematodes and trematodes). While ectoparasites are found on the external surface of their host, hence, being more accessible for collection, endoparasites are found in practically all host internal organs, requiring the sacrifice of the host for their collection. Thus, due to ethical considerations, especially in the case of threatened and rare species or species with low population sizes, when possible, non-invasive methodologies are recommended for parasite assessment. Non-invasive sampling has been implemented for a variety of purposes: from diet studies (Carretero et al. 2006) to genetic assessment of

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rare or threatened species (Busby et al. 2009; Torre et al. 2013) and also for parasite surveys (Wimmer et al. 2004; Richter et al. 2011). However, such alternative methods may not be applicable for all parasite species, being restricted to parasites whose life stages occur in host body locations that allow their direct collection, like skin or that are accessible indirectly, i.e. through faeces and blood.

Due to the effects of parasites on host behaviour and fitness, which may impact community structure, they are increasingly becoming an important aspect to be considered in ecological studies (Poulin 1999; Preston and Johnson 2012). In this context, non-invasive sampling may be particularly advantageous since it does not interfere or jeopardize the structure of host communities, as sampling involving sacrifice would. Faeces from vertebrates have already been used for detection of gastrointestinal parasites such as coccidians (Couch et al. 1996; Daszak et al. 2011; Richter et al. 2011) and intestinal nematodes (Fenner et al. 2011; Jorge et al. 2011, 2012; Gyawali et al. 2013). Intestinal parasite communities have been traditionally assessed through direct intestinal analysis (i.e. Martin and Roca 2005; Diaz et al. 2013) but may also be accessed via non-invasive sampling through the identification of eggs, larvae and the adult forms that may be evacuated in the defaecation process (Couch et al. 1996; Millán et al. 2008; Acosta et al. 2011; Meijer et al. 2011; Zhang et al. 2011). However, intestinal parasites may be under-represented in the faeces due to their location in the intestine, their intensities of infection, presence of adult females shedding eggs and/or the erratic nature of faeces formation and release. Hence, the trade-off between the objectives of the study, the accuracy of the survey and the conservation status of the host must be considered carefully before selecting the appropriate sampling methodology.

In this study, we compare the differences in detectability of intestinal parasites in reptile hosts between two types of samples, faecal pellets and the whole intestinal tract, collected from the same individual hosts, focusing on prevalence and abundance of infection. We then discuss the advantages and disadvantages of the invasive versus non-invasive approaches.

Material and methods

Parasitological procedures

Fifty-two lizard specimens, from eleven species, were collected from different localities (Table 1) between 2008 and 2012. For each individual, two types of samples, faecal pellets and intestines, were obtained. Faeces were collected first either through spontaneous defaecation of the reptiles when captured or by gentle abdominal massage. Intestinal samples were obtained from the same specimens, which died accidentally during fieldwork or were later taken to the laboratory and

ethanized through inhalation of ether vapours, dissected and the intestine removed. Research protocols were approved by the responsible regional authorities [Cabildos Insulares licences: Lanzarote (no. 4889), Fuerteventura (no. 3298 and no. 12570), Gran Canaria (no. 10983), Tenerife (no. 358/2009), La Palma (no. 2009006659), La Gomera (no. 5145) and El Hierro (ref. CGO/rsh) from Spain; Servicio de Protección y Conservación de la Naturaleza, Dirección General del Medio Natural, Consejería de Desarrollo Sostenible y Ordenación del Territorio de la Región de Murcia (licence no. Sol/CPA/ASO/156-08) and Junta de Andalucía (licence no. SGB/FOA/AFR 2010, reg. 17461) from Spain; ICNB from Portugal (licence no. 69/2011/CAPT); Direcção Geral do Ambiente, MAA, from Cape Verde (licence no. 07/2008) and Haut Commissariat Eaux et forêts et à la lutte contre la Desertification from Morocco (licence no. 14/HCEFLCD/DLCDPN/DPRN/CFF)]. Both types of samples were preserved in 96 % ethanol and further analysed using a stereomicroscope in search of helminth parasites. The parasites were then separated, counted and identified to the generic level. Egg forms were not considered because they were few in numbers and are difficult to identify properly.

Statistical analyses

Presence analysis To determine if detectability of intestinal parasites was similar between the two types of samples, faeces and intestines, McNemar's chi-squared test with continuity correction (function `mcnemar.test` of the R package) was applied on the 2×2 contingency table for the presence and/or absence of parasites in matched pairs of intestine and faeces [i.e. total number of hosts where parasites were found in the intestine, but not in the faeces (I+/F-), total number of hosts where parasites were found in both samples types (I+/F+)]. Analyses were performed for all parasites and for the most common parasite genera (*Spauligodon*, *Thelandros* and *Parapharyngodon*). All analyses were performed using the package R version 2.15.1 (R Core Team Development 2011).

Intensity analysis The relationship between the number of parasites found in the intestine and in the faeces from the same host individual was tested using a nonparametric Spearman correlation (function `rcorr`, R package `Hmisc`, Harrel et al. 2013). In order to determine which factors might explain the number of parasites, we performed a repeated analysis of variance (function `ezANOVA`, R package `ez`, Lawrence 2012) using *number of parasites* as dependent variable, *type of sample* as within-subjects factor and *parasite genus* or *host species* as between-subjects factors. Repeated analysis of variance was also performed for each of the three more common parasite genera (*Spauligodon*, *Thelandros* and *Parapharyngodon*), but using *type of sample* as within-subjects factor and *host species* as between-subject factor.

Table 1 Prevalence, intensity and mean intensity of the total intestinal parasites (all species pooled) detected in the two types of samples (I: intestine, F: faeces) from each respective host species

Host family	Host species	Sample type	N samples	N infected	Prevalence (%)	Total intensity	Mean intensity
Lacertidae	<i>Gallotia atlantica mahoratae</i>	I	2	1	50	1	1
		F	2	0	0	0	0
Lacertidae	<i>Gallotia caesaris caesaris</i>	I	2	2	100	149	74.5
		F	2	0	0	0	0
Lacertidae	<i>Gallotia caesaris gomeræ</i>	I	12	11	92.7	407	37
		F	12	9	75	206	22.9
Lacertidae	<i>Gallotia galloti eisentrauti</i>	I	2	2	100	364	182
		F	2	1	50	1	1
Lacertidae	<i>Gallotia galloti palmae</i>	I	2	2	100	104	52
		F	2	0	0	0	0
Lacertidae	<i>Gallotia stehlini</i>	I	1	1	100	10	10
		F	1	0	0	0	0
Lacertidae	<i>Atlantolacerta andreanskyi</i>	I	1	0	0	0	0
		F	1	0	0	0	0
Lacertidae	<i>Podarcis carbonelli</i>	I	1	0	0	0	0
		F	1	0	0	0	0
Lacertidae	<i>Podarcis hispanica</i> PHGal ^a	I	1	0	0	0	0
		F	1	0	0	0	0
Lacertidae	<i>Podarcis hispanica</i> PH2 ^a	I	12	12	100	273	22.75
		F	12	4	33.3	4	1
Lacertidae	<i>Podarcis sicula</i>	I	13	5	38.5	18	3.6
		F	13	2	15.4	8	4
Lacertidae	<i>Podarcis vaucheri</i>	I	1	0	0	0	0
		F	1	0	0	0	0
Phyllodactylidae	<i>Tarentola angustimentalis</i>	I	1	0	0	0	0
		F	1	0	0	0	0
Phyllodactylidae	<i>Tarentola rudis</i>	I	1	1	100	38	38
		F	1	0	0	0	0
	Intestines samples total		52	37	71.1	1,364	36.9
	Faecal samples total		52	16	30.8	219	13.7
	Total		104	53		1,583	

^a See Kaliontzopoulou et al. (2011) for information on *Podarcis* lineages

Similarly, the same analysis was also performed for the most common hosts (*Gallotia caesaris gomeræ*, *Podarcis sicula* and *Podarcis hispanica* PH2), this time using *type of sample* as within-subjects factor and *parasite genus* as between-subjects factor. All analyses were performed using the package R version 2.15.1 (R Core Team Development 2011).

Results

From the 52 lizards (lacertid lizards and geckos) analysed, 39 individuals were found infected with intestinal parasites (parasites found in the intestine and/or in the faeces). A total of

1,583 parasites from six different genera were detected from which 86 % were found in the intestines and 14 % in faeces. One parasite was identified as Cestoda, while all the others were nematodes of the family Pharyngodonidae (Oxyurida). *Spauligodon*, *Thelandros* and *Parapharyngodon* were the most common genera (46, 39 and 13 % of the total number of parasites, respectively), while *Tachygonetria* (0.6 %) and *Skrjabinodon* (0.2 %) were less frequent. In addition, four Pharyngodonidae larvae (0.2 %) were found but they could not be assigned to any genus. Intensities and prevalences for each host taxon are detailed in Table 1.

Our analysis showed strong discrepancies in the detection of parasites depending on the type of sample analysed (Fig. 1). From the total of 39 specimens infected, 37 (95 % of cases)

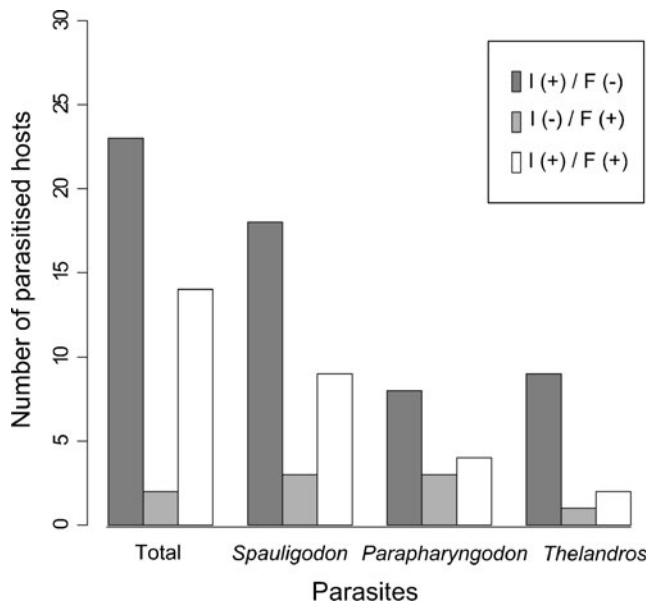


Fig. 1 Bar plot representing the host frequency for the different levels of detectability of matched pairs of intestine and faeces samples for all the parasites species (pooled) and for the three most common parasites. *Dark grey bars* represent hosts with parasites in the intestine but not in the faeces [I(+)/F(-)], *light grey bars* include the number of hosts with parasites in faeces but not in the intestine [I(-)/F(+)] and *white bars* represent hosts with parasites observed both in the intestine and respective faeces [I(+)/F(+)]

contained parasites in their intestine, but in only 14 of these specimens (36%), parasites were also found in the respective faeces. This pattern was similar in separate analyses for each of the three most common parasite genera, i.e. *Spauligodon*, *Parapharyngodon* and *Thelandros* (Figs. 1 and 2). In two host specimens, intestinal parasites were detected only in the faeces, with no parasite been detected in the corresponding intestine (total intensities: 2 and 15).

There was significant discordance between the detectability of helminths in intestines and faeces, considering that the marginal probabilities of each type of sample should be the same (McNemar chi-squared=16, $df=1$, $p<0.001$). Differences in detectability were also significant when considering each nematode genus separately, namely, *Spauligodon* (McNemar's chi-squared=9.33, $df=1$, $p<0.005$), *Thelandros* (McNemar's chi-squared=4.9, $df=1$, $p<0.05$) but not for *Parapharyngodon* (McNemar's chi-squared=1.45, $df=1$, $p=0.2278$; the other genera were not analysed due to the small sample size).

Regarding intensities, the number of parasites found in the intestines was higher than in the faecal samples (repeated measures ANOVA, within-subjects factor=type of sample, $F_{1,63}=8.20$, $p=0.006$) but the correlation between them was not significant (Spearman correlation, $\rho=-0.19$, $p=0.24$). However, variation in the number of parasites did not depend either on the host species (repeated measures ANOVA, within-subjects factor=type of sample, $F_{1,55}=8.80$,

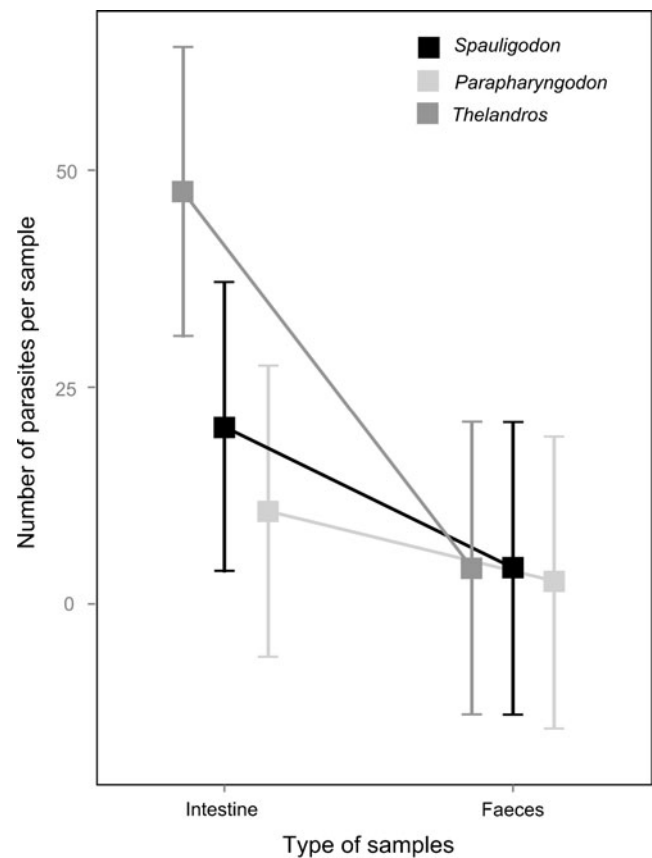


Fig. 2 Differences in the abundance of the three main parasite groups between intestinal and faecal samples. For the three genera, mean (and standard error) number of parasites per sample are shown

$p=0.004$; between-subjects factor=host species, $F_{8,55}=1.24$, $p=0.292$; interaction, $F_{8,55}=1.57$, $p=0.156$) or on parasite genus (within-subjects factor=type of sample, $F_{1,57}=7.98$, $p=0.006$; between-subjects factor=parasite genus, $F_{6,57}=0.95$, $p=0.465$; interaction, $F_{6,57}=0.72$, $p=0.635$). Similar results were obtained when the three most common parasite genera were individually analysed. *Spauligodon* was more abundant in the intestine than in the faeces (repeated measures ANOVA, within-subjects factor=type of sample, $F_{1,24}=11.85$, $p=0.002$; between-subjects factor=host species, $F_{5,24}=1.58$, $p=0.203$; interaction, $F_{5,24}=1.72$, $p=0.169$) and showed a negative, though not significant trend in the correlation of abundances between both types of samples (Spearman, $\rho=-0.32$, $p=0.08$). For *Parapharyngodon*, the number of parasites was similar in intestines and faeces and for host species (repeated measures ANOVA, within-subjects factor=type of sample, $F_{1,10}=3.11$, $p=0.108$; between-subjects factor=host species, $F_{5,10}=1.48$, $p=0.280$; interaction, $F_{5,10}=0.79$, $p=0.577$), although again, a negative, not significant, correlation between sample types was detected (Spearman, $\rho=-0.28$, $p=0.31$). For *Thelandros*, we did find significant differences in all comparisons (repeated measures ANOVA, within-subjects factor=type of sample, $F_{1,8}=22.45$, $p=0.0015$;

between-subjects factor=host species, $F_{3,8}=49.67$, $p=0.00002$; interaction, $F_{3,8}=37.13$, $p=0.0005$). Also in this case, no correlation between the numbers of parasites in intestines and faeces was detected (Spearman correlation, $\rho=-0.46$, $p=0.13$).

Within each of the most common host species (*G. caesaris gomeræ*, *P. hispanica* PH2 and *P. sicula*), there were no differences between the number of parasites found in intestines and faeces and no effect of parasite genus on numbers of parasites per sample (in both species, repeated measures ANOVA, within-subjects factor=type of sample, $p>0.05$; between-subjects factor=parasite genus, $p>0.05$; interaction, $p>0.05$), the only exception being *P. hispanica* PH2. For this host species, there was a significantly higher number of parasites in the intestines than in the faeces but no effect of the parasite genus (repeated measures ANOVA, within-subjects factor=type of sample, $F_{1,11}=53.10$, $p=0.00005$; between-subjects factor=parasite genus, $F_{1,11}=4.22$, $p=0.064$; interaction, $F_{1,11}=4.86$, $p=0.0496$). No correlation between the number of parasites detected in both types of samples was observed in any of the three host species analysed (Spearman correlation, in all cases $p>0.05$).

Discussion

The majority of helminths found in both types of samples, faeces and intestines, were adult forms of nematodes belonging to the family Pharyngodonidae (Oxyurida), which are commonly found in the intestinal helminth communities of reptiles (i.e. Martin et al. 2005), with the genus *Spauligodon* being the most common. Pharyngodonidae nematodes inhabit the last part of the intestine of their host and they live free in it, while other helminths such as cestodes and trematodes are usually found in the upper part of the intestine fixed to the intestinal mucosa and thus are less likely to be dislodged in faeces. However, the detectability of Pharyngodonidae nematodes was significantly lower in faeces than in the intestine. Faeces were collected before the intestine content was analysed, and the helminths retrieved from the faeces were probably dislodged and evacuated when faeces were expelled. It has been reported that in oxyurids, gravid females may pass out of the host and function as oothecae (Adamson 1990), which may be an additional reason for the presence of adult nematode females in the faeces. Contrary to our expectation, no positive correlation was found between the helminth intensities found in the faeces and that in the paired intestine, i.e. a higher intensity of helminths in the intestine was not accompanied by a higher intensity of helminths in faeces. We only detected an effect of the type of sample (detecting a higher number of parasites in the intestine than in faeces), but no effect of the host or parasite taxonomic identities on the number of parasites found. The absence of correlation may

be due to the spatial distribution of the parasites within the intestine and to the random deposition of parasites in the forming faeces. The same pattern was uncovered in separate analyses for each of the three more common parasites. Depending on the total length of the host or their diet, the intestinal tract can vary in size and shape (i.e. length and presence of a caecum; Stevens and Hume 1995; Carretero 2004) which may influence the spatial distribution of parasites and consequently influence their probability of being dislodged during the defaecation process. Although *G. caesaris gomeræ* presents a larger body size and a greater tendency to herbivory compared with *Podarcis* lizards (Roca 1999; Martin et al. 2005), no effect of host species was detected on the intensity of parasites found in faeces. The only exception was in the separate analysis for the nematode genus *Thelandros*, where a significant effect of host species was detected. However, this was probably due to the finding of a single host intestine containing 359 individuals of this parasite.

Considering our results regarding the lack of correlation between both types of samples, we conclude that comparative studies on parasite abundance or parasite community studies performed on the basis of faecal samples should be interpreted very cautiously. Similar results were obtained regarding diet studies performed on stomach versus intestine samples (Carretero and Llorente 2001), highlighting that different source of samples, even if a priori related, may not yield comparable information. The presence of nematode parasites or their eggs in faeces has been used to estimate parasite prevalence, intensities or abundances in several organisms, especially for those large mammals in which obtaining large sample sizes involving necropsy are not possible due to their endangered status or sampling difficulties (Ashford et al. 1996; Millán et al. 2008; Acosta et al. 2011; Zhang et al. 2011). Ecological studies based solely on faeces most probably underestimate the true abundance and diversity present at the community level and consequently will not reflect the real helminth community. Other methodologies of faeces examination, i.e. faecal floatation or molecular based techniques, could yield higher parasite detection. However, the same uncorrelated pattern may still be found regarding the deposition or ability of faeces to drag adult parasites or other parasite stages while forming or during defaecation.

Nevertheless, faeces are still a reasonable alternative for baseline surveys. The variety of studies that can be conducted on helminths retrieved from faeces will depend on the collection technique. Fresh samples, collected directly and stored in ethanol, will allow both morphological (even given some degree of shrinkage) and genetic analyses to be conducted. Based on our results and the typical low prevalence of reptile intestinal helminths, we recommend that a large number of faecal samples should be collected to increase the likelihood of detection of intestinal parasites. Similarly, it can also be

important to aim at temporal replication, which would allow greater insight into the true parasite fauna present in a given host population and/or locality. Another advantage of using faecal samples is that parasite surveys using this method do not alter the population structure of the host, such that repeated sampling of the same host individual (i.e. for monitoring seasonal variation) is possible. Nevertheless, preliminary studies may be first performed in order to assess the reliability of non-invasive sampling for each group of parasite and host. In fact, the best methodology would be combining the two types of samples, i.e. faeces that may be expelled during the capture process and the intestinal content, since, as seen in some specimens, faeces sometimes may provide the best representation of an infracommunity. Several studies have also been conducted on museum specimens of hosts (i.e. Hartigan et al. 2010; Bursey and Goldberg 2012). However, these types of samples are usually not viable for genetic analysis due to the preservation medium since helminths are located in tissues that are more prone to degradation. In conclusion, parasitological methodologies should be carefully selected according to the objective of the study. However, different parasites with different life stages and with different prevalence and intensities in a given host will inevitably present different sampling challenges. Unfortunately, because of the internal location of most parasites, non-invasive sampling approaches are not always possible and the sacrifice of the host will often be required for accurate estimates of abundance or diversity.

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References

- Acosta L, León-Quinto T, Bornay-Llinares FJ, Simón MA, Esteban JG (2011) Helminth parasites in faecal samples from the endangered Iberian lynx (*Lynx pardinus*). *Vet Parasitol* 179:175–179
- Adamson ML (1990) Haplodiploidy in the Oxyurida: decoupling the evolutionary processes of adaptation and speciation. *Ann Parasitol Hum Comp* 65(Suppl 1):31–35
- Ashford RW, Lawson H, Butynski TM, Reid GDF (1996) Patterns of intestinal parasitism in the mountain gorilla *Gorilla gorilla* in the Bwindi-Impenetrable Forest, Uganda. *J Zool* 239:504–517
- Bursey CR, Goldberg SR (2012) A new species of *Spauligodon* (Nematoda: Oxyuroidea: Pharyngodonidae) in *Gonatodes antillensis* (Squamata: Sphaerodactylidae) from Bonaire, Lesser Antilles. *J Parasitol* 98:344–346
- Busby GBJ, Gottelli D, Wachter T, Marker L, Belbachir F, Smet K, Belbachir-Bazi A, Fellous A, Belghoul M, Durant SM (2009) Genetic analysis of scat reveals leopard *Panthera pardus* and cheetah *Acinonyx jubatus* in southern Algeria. *Oryx* 43(3):412–415
- Carretero MA (2004) From set menu to a la carte. Linking issues in trophic ecology of Mediterranean lacertids. *Ital J Zool* 74(2):121–133
- Carretero MA, Llorente GA (2001) What are they really eating? Stomach versus intestine as sources of diet information in lacertids. In: Vicente L, Crespo EG (eds) *Mediterranean Basin Lacertid Lizards: a Biological Approach*. ICN, Lisboa, pp 105–112
- Carretero MA, Perera A, Harris DJ, Batista V, Pinho C (2006) Spring diet and resource partitioning in an alpine lizard community from Morocco. *Afr Zool* 41(1):113–122
- Couch L, Stone PA, Duszynski DW, Snell HL, Snell HM (1996) A survey of the coccidian parasites of reptiles from islands of the Galápagos archipelago: 1990–1994. *J Parasitol* 82(3):432–437
- Daszak P, Ball SJ, Streicker DG, Jones CG, Snow KR (2011) A new species of *Caryospora* Leger, 1904 (Apicomplexa: Eimeriidae) from the endangered Round Island boa *Casarea dussumieri* (Schlegel) (Serpentes: Bolyeridae) of Round Island, Mauritius: an endangered parasite? *Syst Parasitol* 78:117–122
- Díaz JI, Fusaro B, Longarzo L, Coria NR, Vidal V, Jerez S, Ortiz J, Barbosa A (2013) Gastrointestinal helminths of Gentoo penguins (*Pygoscelis papua*) from Stranger Point, 25 de Mayo/King George Island, Antarctica. *Parasitol Res* 112:1877–1881
- Fenner AL, Smales LR, Bull CM (2011) Using social networks to deduce whether residents or dispersers spread parasites in a lizard population. *J Anim Ecol* 80(4):835–843
- Gywali P, Khanal S, Shrestha B (2013) Intestinal helminth fauna in sleepy lizard (*Tiliqua rugosa*) in Australia. *Inter J Vet Sci* 2(1):17–20
- Harrell JrFE, with contributions from Dupont C and many others (2013) Hmisc: Harrell Miscellaneous. R package version 3.10-1.1. <http://CRAN.R-project.org/package=Hmisc>
- Hartigan A, Phalen DN, Šlapeta J (2010) Museum material reveals a frog parasite emergence after the invasion of the cane toad in Australia. *Parasit Vectors* 3:50
- Jorge F, Roca V, Perera A, Harris DJ, Carretero MA (2011) A phylogenetic assessment of the colonisation patterns in *Spauligodon atlanticus* Astasio-Arbiza et al., 1987 (Nematoda: Oxyurida: Pharyngodonidae), a parasite of lizards of the genus *Gallotia* Boulenger: no simple answers. *Syst Parasitol* 80:53–66
- Jorge F, Carretero MA, Perera A, Harris DJ, Roca V (2012) A new species of *Spauligodon* (Nematoda: Oxyurida: Pharyngodonidae) in geckos from São Nicolau island (Cape Verde) and its phylogenetic assessment. *J Parasitol* 98(1):160–166
- Kaliontzopoulou A, Pinho C, Harris DJ, Carretero MA (2011) When cryptic diversity blurs the picture: a cautionary tale from Iberian and North African *Podarcis* wall lizards. *Biol J Linn Soc* 103:779–800
- Lawrence MA (2012) ez: Easy analysis and visualization of factorial experiments. R package version 4.1-1. <http://CRAN.R-project.org/package=ez>
- Martin JE, Roca V (2005) Helminths of the Atlantic lizard, *Gallotia atlantica* (Reptilia: Lacertidae), in the Canary Islands (Eastern Atlantic): composition and structure of component communities. *Acta Parasitol* 50(1):85–89
- Martin JE, Roca V, Carretero MA, Llorente GA, Montori A, Santos X (2005) Relationships between diet and helminths in *Gallotia caesaris* (Sauria: Lacertidae). *Zoology* 118:121–130
- Meijer T, Mattsson R, Angerbjörn A, Osterman-Lind E, Fernández-Aguilar X, Gaviera-Widén D (2011) Endoparasites in the endangered

- Fennoscandian population of arctic foxes (*Vulpes lagopus*). *Eur J Wildl Res* 57:923–927
- Millán J, Gortazar C, Ballesteros F (2008) Parasites of the endangered Cantabrian capercaillie (*Tetrao urogallus cantabricus*): correlates with host abundance host family and lek site characteristics. *Parasitol Res* 103:709–712
- Poulin R (1999) The functional importance of parasites in animal communities: many roles at many levels? *Int J Parasitol* 29(6):903–914
- Poulin R (2007) Evolutionary ecology of parasites. Princeton University Press, Princeton
- Preston D, Johnson P (2012) Ecological consequences of parasitism. *Nat Educ Knowl* 3(10):47
- R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. <http://www.R-project.org/>
- Richter B, Nedorost N, Maderner A, Weissenböck H (2011) Detection of *Cryptosporidium* species in feces or gastric contents from snakes and lizards as determined by polymerase chain reaction analysis and partial sequencing of the 18S ribosomal RNA gene. *J Vet Diagn Invest* 23:430–435
- Roca V (1999) Relación entre las faunas endoparásitas de reptiles y su tipo de alimentación. *Rev Esp Herpetol* 13:101–121
- Stevens CE, Hume ID (1995) Comparative physiology of the vertebrate digestive system. Cambridge University Press, Cambridge
- Torre I, Arrizabalaga A, Freixas L, Ribas A, Flaquer C, Díaz M (2013) Using scats of a generalist carnivore as a tool to monitor small mammal communities in Mediterranean habitats. *Basic Appl Ecol* 14:155–164
- Wimmer B, Craig BH, Pilkington JG, Pemberton JM (2004) Non-invasive assessment of parasitic nematode species diversity in wild Soay sheep using molecular markers. *Int J Parasitol* 34:625–631
- Zhang L, Yang X, Wu H, Gu X, Hu Y, Wei F (2011) The parasites of giant pandas: individual-based measurement in wild animals. *J Wildl Dis* 47:164–171