OIKOS

Research

Increasing efficiency and reducing bias in the sampling of seed–dispersal interactions based on mist-netted birds

Beatriz Rumeu, Juan P. González-Varo, Cristina de Castro, Antonio López-Orta, Juan Carlos Illera, Marcos Miñarro and Daniel García

B. Rumeu (https://orcid.org/0000-0002-9017-5466) ✉ *(beatriz.rumeu@uca.es) and J. P. González-Varo (https://orcid.org/0000-0003-1439-6475), Depto de Biología – IVAGRO, Univ. de Cádiz, Puerto Real, Spain. – C. de Castro and D. García (https://orcid.org/0000-0002-7334-7836), Depto de Biología de Organismos y Sistemas (Univ. de Oviedo) and: Inst. Mixto de Investigación en Biodiversidad (Univ. Oviedo-CSIC-Princ. Asturias), Mieres, Spain. – A. López-Orta and J. C. Illera (https://orcid.org/0000-0002-4389-0264), Inst. Mixto de Investigación en Biodiversidad (Univ. Oviedo-CSIC-Princ. Asturias), Univ. de Oviedo, Mieres, Spain. – M. Miñarro (https://orcid.org/0000-0002-5851-6873), Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA), Villaviciosa, Spain.*

Efficient and unbiased sampling of ecological interactions is essential to our understanding of the functions they mediate. Seed dispersal by frugivorous birds is a key mutualism for plant regeneration and community dynamics. Mist-netting is one of the most widely used methods to sample avian seed dispersal through the identification of seeds in droppings of captured birds kept inside cloth bags. However, birds may drop seeds on the ground before being extracted from the net, leading to a fraction of missing information due to ineffective sampling. Worryingly, this fraction could be unevenly distributed across bird and plant species, leading to sampling biases. Here, we assess the effectiveness of using a 1-m wide mesh below mist nets to sample seeds dropped by entangled birds. We used data from birds mist-netted during one-year-round. We sampled nearly 50% of interaction events and 75% of dispersed seeds on the mesh band below the mist nets (i.e. lost information without this optimization). The proportion of seeds sampled on the mesh bands was not evenly distributed among bird species but strongly related to bird size, ranging from 57–63% in warblers to 84–94% in thrushes. Moreover, the proportion of seeds sampled on the mesh was negatively related to seed size, although this relationship was weaker. We also evaluated accumulation curves of species and pairwise interactions with increasing sampling effort, both with and without using the mesh bands. The number of seed species sampled increased by 21% when using the mesh bands and the number of pairwise interactions by 36%. Our findings provide strong evidence on how inefficient and biased traditional mist-netting can be for sampling community-wide seed–dispersal interactions. We thus urge the use of mesh bands in future studies to increase sampling effectiveness and avoid biases, which will ultimately improve our understanding of the seed dispersal function.

Keywords: frugivorous birds, mesh band, mist-netting, method optimization, plant–animal interactions, sampling biases, sampling effectiveness

www.oikosjournal.org

Introduction

Interactions between frugivorous animals and fleshy fruits are essential for the endozoochorous seed dispersal of many plant species across the world's biomes. For example, up to 94% of woody species in tropical rainforests and up to 64% in Mediterranean woodlands produce fleshy fruits and, thus, rely on frugivores to disperse their seeds (Jordano 2014). Frugivorous seed dispersers eat the edible pulp of fleshy fruits to obtain nutrients and eject undamaged seeds away from the parent plants in conditions that are generally suitable for germination (Howe 1986, Herrera 2002). By doing so, they foster the colonisation of vacant areas for seedling establishment and the exchange of individuals among plant populations (Wang and Smith 2002, Jordano 2017). Hence, frugivore-mediated seed dispersal is a pivotal process for the dynamics of plant communities (Wang and Smith 2002, Schupp et al. 2010, 2017), particularly in the Anthropocene, under scenarios of habitat loss and fragmentation (García et al. 2013, González-Varo et al. 2017), biological invasions (Teixido et al. 2022) and climate change (González-Varo et al. 2021).

The empirical study of seed–dispersal interactions requires identifying the animal species that disperse seed species of interest in a plant community, and quantifying the strength of such pairwise interactions (Bascompte and Jordano 2013). Therefore, a robust and complete sampling of the interactions is required to extract reliable information on the identity and relevance of species within interacting plant–frugivore assemblages (Costa et al. 2016, Jordano 2016). The methods used to detect these interactions are diverse and target on different stages of the seed-dispersal process: direct observations of fruit consumption (departure stage), sampling seeds ejected by captured animals (transport stage) and sampling seeds in animal defecations or regurgitations (deposition stage) (Schlautmann et al. 2021, Quintero et al. 2022). None of these approaches are free from biases. For instance, large birds can be more easily detected through focal observations than small ones (Vitorino et al. 2022) and, in the same way, large scats are more easily detected on the ground when searching for seeds defecated or regurgitated by frugivorous animals (Schlautmann et al. 2021, Quintero et al. 2022). Accordingly, there is a growing interest among ecologists regarding which method could be more effective for sampling seed–dispersal interactions, or how data from different sampling methods could be combined (Jordano 2016, Schlautmann et al. 2021, Quintero et al. 2022, Vitorino et al. 2022). In contrast, the optimization of commonly used methodologies has received very little attention.

Birds are the main group of frugivorous seed dispersers for the vast majority of endozoochorous plants (Herrera 2002, Jordano 2014). This explains why mist netting has been – and still is – one of the standard methods used to sample avian seed–dispersal interactions (Herrera 1984a, Jordano 1989, Heleno et al. 2013, Costa et al. 2016, Fricke and Svenning 2020). Collecting the seeds in droppings ejected by captured birds allows species identification of both interacting partners (i.e. plant species i dispersed by

bird species j) as well as quantifying the strength of pairwise interactions (i.e. prevalence or number of seeds of plant species i in droppings of bird species j; Costa et al. 2016). Seeds recovered in bird droppings can also provide data on their viability and hence, on the qualitative component of the seed dispersal process (Schupp et al. 2010, 2017, Nogales et al. 2017). In addition, when compared with focal direct observation of frugivorous birds consuming fruits (Sorensen 1981, Rumeu et al. 2020), the mist-netting method has the advantage of better sampling interactions with rare plants or with plants located outside the boundaries of the study area (Escribano-Ávila et al. 2018).

In mist-netting based studies, captured birds are extracted from the net and kept inside cloth bags for a while (usually about 30 min, before or after ringing), where they can drop seeds by defecating or regurgitating them. However, before being extracted from the net, captured birds may drop seeds that fall on the ground and get easily lost (González-Varo et al. 2014). This can result in the loss of whole interaction events (i.e. interactions between captured birds and fleshy-fruited species that remain unsampled), or the loss of fractions of them (i.e. some seeds of given species are dropped on the ground and some others inside the cloth bag, leading to biases in the strength of the interactions recorded) (Hernández-Dávila et al. 2015). Therefore, the standard procedure of the mist-netting method could be ineffective in sampling interactions and characterising their weight, as previously suggested (González-Varo et al. 2014, Hernández-Dávila et al. 2015). Methods based on animal captures such as mist-netting have intrinsic biases related to species catchability (Quintero et al. 2022). But worryingly, when sampling interspecific seed dispersal interactions, traditional mist-netting could lead to additional sampling biases if the fraction of missing interactions is not evenly distributed across bird and plant species. These biases are in fact expected, because gut passage time of seeds increases with bird body mass, with mean values ranging from 22 to 80 min in frugivorous passerines (Herrera 1984b). Moreover, small seeds tend to have longer passage times than large seeds inside birds' guts (Johnson et al. 1985, Fukui 2003). According to these allometric relationships with gut passage times, the standard sampling of seeds inside cloth bags could lead to the undersampling of interactions involving small birds and large seeds.

The placement of a plastic mesh beneath the mist nets has been used in some studies to avoid the loss of droppings of trapped bats (Galindo-González et al. 2009) and birds (González-Varo et al. 2014). Yet, to our knowledge, only one study has quantified the extent to which this technique improves the sampling of bird droppings with seeds (Hernández-Dávila et al. 2015). Although the study was based on a small sample size (20 droppings with seeds), it showed that cloth bags only included 35% of all seeds dropped by captured birds. However, this report has had little impact among researchers using mist nets to study frugivory and seed dispersal. In addition, as far as we know, no study has yet assessed whether the fraction of

unsampled interactions is related to the size of the interacting bird and seed species.

Here, we carried out a comprehensive one year-round study encompassing a diverse community of bird-dispersed plants in northern Spain to assess how much the sampling of seed-dispersal interactions is optimized by the placement of a 1-m wide plastic mesh on the ground, below mist nets (Fig. 1). This mesh band enables the quick detection of seeds dropped by birds while trapped in the net. First, we assessed the effectiveness of this method by estimating the proportion of interaction events recorded and the proportion of seeds recovered on the mesh. Second, we tested whether the proportion of seeds sampled on the mesh (i.e. presumably lost on the ground and unsampled when the mesh band is not used) was unevenly distributed among birds and seeds of different sizes. Finally, we estimated accumulation curves of species and pairwise interactions to assess the extent to which this technique improves the sampling in relation to standard mist netting. Ultimately, our goal was to provide solid evidence on the effectiveness of using mesh bands below mist nets to perform more complete and unbiased samplings of seed–dispersal interactions.

Material and methods

Study design

Study sites

We conducted our study in three apple *Malus × domestica* orchards located in northern Spain, between 43°28′ 51″–43°27′ 10″ N and 5°28′ 58″–5°26′ 45″ W. Orchards were separated from 1.5 to 4.5 km one from each other, and their elevation ranged between 25 and 97 m a.s.l. They represent replicates of a regional agroecosystem that harbours a diverse community of wild birds and fleshy-fruited plants (García et al. 2018), consisting of small orchards of 0.6–4.0 ha embedded in a highly variegated landscape with abundant patches of natural woody vegetation. In fact, most orchards are surrounded by hedgerows dominated by fleshy-fruited plants (e.g. *Hedera helix*, *Rubus fruticosus*, *Sambucus nigra*), which rely on frugivorous animals for the dissemination of their seeds. The main frugivores known to disperse the seeds of these hedgerow species are small- and medium-sized passerines such as European robins *Erithacus rubecula*, blackcaps *Sylvia atricapilla* and thrushes *Turdus* spp. (Hernández 2007).
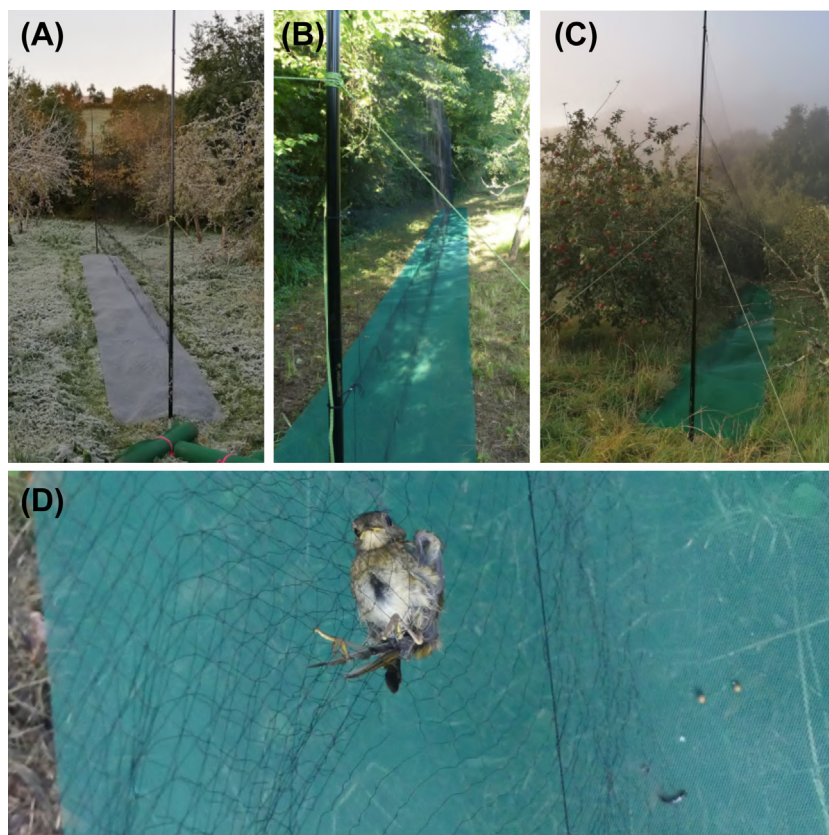

Figure 1. Plastic mesh bands of 1 m-wide and 1 mm pore placed beneath mist nets allow the quick detection of seeds expelled by trapped birds. (A and B) The colour of the mesh bands can be chosen to minimize visual contrast with the ground. (C) Mesh band can be placed in tall-grass conditions. (D) Juvenile of European robin *Erithacus rubecula* trapped in a mist net with a mesh band underneath. Note the presence of one scat and two dogwood *Cornus sanguinea* seeds on the mesh, readily detectable for sampling.

Mist-netting and sampling of bird droppings

The three sites were sampled with equal effort all year-round (August 2019–July 2020), covering the entire fruiting phenology of all local fleshy-fruited species. Each site was visited twice per month. Bird droppings were collected during 72 ringing sessions with five mist nets at each site, which remained open for five consecutive hours. Mist-nets were of different size, ranging between 9–18 m length and 2.5–3 m height, total area of capture being of 178.5 m^2. Net mesh varied between 16 and 19 mm.

We placed a 1 m-wide plastic mesh band of 1 mm pore below each mist net (Fig. 1) to easily locate any seed expelled by birds on the ground while trapped in the nets (between its impact with the net and the subsequent manipulation by the ringer). Mesh bands had exactly the same length as the mist nets above them and were fixed to the ground with pickaxes similar to those used for a camping tent. Mist nets were checked every 45–60 min. Seeds located on the mesh below captured birds were collected and assigned to the specific bird trapped in the vertical, recording that they were seeds 'sampled on the mesh'. It was always straightforward to associate each captured bird with its dropping sample on the mesh. Then, captured birds were kept up to 30–40 min inside ringing cloth bags to obtain droppings (defecations or regurgitations), as done in traditional mist-netting studies (Rumeu et al. 2011, Spotswood et al. 2012a, Heleno et al. 2013, Costa et al. 2022). All captured birds were ringed with a unique numbered aluminium ring (Aranzadi Ringing Scheme) by the same person (CC), who also measured their body weight to the nearest 0.1 g using a portable digital scale. After ringing and releasing the birds where they had been captured, those seeds found in the cloth bags were collected and registered as 'sampled inside the bag'. Regurgitated and defecated seeds were pooled together due to the impossibility of discerning between these two modes of ejection, both on the mesh bands and – particularly – inside cloth bags. The species identification of intact seeds in droppings were later confirmed from external morphology by comparison with a seed reference collection from the study area and a regional guide for seed identification of fleshy-fruited species (Torroba Balmori et al. 2013). To test whether the proportion of seeds sampled on the mesh was unevenly distributed among seeds of different sizes, we obtained data on seed length and seed width from the literature (Torroba Balmori et al. 2013). Seed

dry weight was also obtained by weighting the seed load of 40–100 fruits (21 in the case of *Solanum dulcamara*) from up to seven individuals per fleshy-fruited plant species present in the hedgerows of the study areas. For those plant species absent from the hedgerows but present in our samples (five out of 17 species), seed dry weight was gathered from the literature (Torroba Balmori et al. 2013).

Data analyses

Data from the three nearby localities were pooled for all analyses and seed-dispersal information provided by recaptured birds was kept. We classified seeds from each interaction event (i.e. interactions between a seed species *i* and an individual bird captured *b*) as 1) found only on the mesh, 2) found only in the bag or 3) found in both. We focused on the proportion of interaction events that were only sampled on the mesh, as they represent events that would have been missing by traditional sampling with cloth bags. These data were expressed as a Bernoulli-distributed variable (1: event sampled only on the mesh; 0: event sampled inside the bag). Then, we estimated the proportion of interaction events detected only on the mesh by fitting a general linear model (GLM) with binomial distribution and logit link to the intercept of this response variable (i.e. only an intercept estimate; no predictor variables). The intercept estimated by this GLM does not account for the identity of the captured bird species and thus equals the mean proportion observed in the sample along with its error term. In order to account for clustered measures per bird species, which contributed very unevenly to the data (e.g. from 92 interaction events by *Sylvia atricapilla* to only three by *S. borin*; Table 1), we also fitted a generalized mixed model (GLMM) including bird species as random factor (random intercepts). We followed the same analytical approach to estimate the proportion of seeds sampled on the mesh per interaction event (i.e. fraction of seeds probably lost on the ground without the mesh). In this case, the number of seeds sampled on the mesh were modelled as 'successes' and the number of seeds sampled in the bag as 'failures' in the binomial GLM and GLMM (with bird species as random intercepts) fitted to estimate the intercept (no predictor variables). These analyses were performed using the glmmTMB package ver. 1.1.2.3 (Brooks et al. 2017) in R ver. 4.0.2 (<www.r-project.org>). We used the emmeans package

Table 1. Contribution to seed dispersal, in terms of interaction events and number of dispersed seeds, by the mist-netted bird species that dropped seeds. Note that a single interaction event (i.e. the interaction between a captured bird *b* and a seed species *i*) can be sampled only on the mesh, only inside the cloth bag and in both places (e.g. a bird ejecting multiple seeds of a given plant species both on the mesh and inside the bag). The 108 captures included 106 individuals and two recaptures of *Sylvia atricapilla*.

Bird species	Captures n	Interaction events				Seeds		
		$n_{only\ mesh}$ (%)	$n_{only\ bag}$ (%)	n_{both} (%)	n_{total}	n_{mesh} (%)	n_{bag} (%)	n_{total}
Erithacus rubecula	12	9 (75.0)	2 (16.7)	1 (8.3)	12	46 (74.2)	16 (25.8)	62
Sylvia atricapilla	67	37 (40.2)	34 (37.0)	21 (22.8)	92	238 (59.5)	162 (40.5)	400
Sylvia borin	2	0 (0.0)	0 (0.0)	3 (100.0)	3	3 (42.9)	4 (57.1)	7
Turdus merula	15	12 (63.2)	0 (0.0)	7 (36.8)	19	353 (94.6)	20 (5.4)	373
Turdus philomelos	12	5 (41.7)	5 (41.7)	2 (16.7)	12	53 (77.9)	15 (22.1)	68
Total	108	63 (45.7)	41 (29.7)	34 (24.6)	138	693 (76.2)	217 (23.8)	910

ver. 1.3.5.1 to obtain the estimated means and 95% confidence intervals from the fitted models.

We further explored whether the proportion of seeds sampled on the mesh was related to bird and seed size. In the case of birds, biometric measures from recaptured individuals were excluded. We used individual body weight as a proxy of bird size. For fleshy-fruited species, seed length, seed width and seed dry weight were highly correlated ($r > 0.85$ in all cases, $p < 0.001$). We eventually selected mean seed dry weight as a proxy of seed size because the model with this variable (see model details below) had a better fit than the model including seed length or seed width as a proxy of seed size (ΔAIC > 2.1 in both cases). We tested for the effect of bird and seed size (separately and grouped) on the proportion of seeds sampled on the mesh by fitting GLMMs with binomial error structure and logit link function, using the R package glmmTMB. The models included bird and plant species as random factors (random intercepts) to account for the repeated measures per bird and seed species. We assessed the goodness-of-fit of these models from their AIC and marginal R^2 values ($R^2_{GLMM(m)}$), that is, the variance explained by the fixed-effects variables, using the delta method (Nakagawa et al. 2017); $R^2_{GLMM(m)}$ values were obtained with the R package MuMIn (ver. 1.43.17) (Bartoń 2020).

Finally, we evaluated accumulation curves of bird and seed species, as well as of pairwise interactions, with increasing sampling effort with and without the mesh bands below the mist nets. We used the rarefaction/extrapolation method as proposed by Chao et al. (2014). For this purpose, we built incidence matrices with information on whether each seed species i, each bird species j or each ij pairwise interaction was recorded in seed–dispersal events during each ringing session. These matrices were constructed separately for the subset data collected inside cloth-bags (i.e. obtained through the traditional mist-netting method), and for the whole data collected on the mesh bands and inside cloth bags. The analyses were computed using the iNEXT package (Hsieh et al. 2016) in R (<www.r-project.org>), using the diversity order q = 0 (species richness) and based on incidence biodiversity data.

Results

We captured a total of 108 frugivorous birds (106 individuals and two recaptures) from five different species (Table 1) while dispersing seeds from 17 fleshy-fruited plant species (Supporting information). These captures yielded a total of 138 interaction events (note that a single dropping can include more than one interaction event if it contains seeds from multiple species), corresponding to 34 unique pairwise interactions. Half of the captured birds (52%; 56 out of the 108) dropped seeds only on the mesh. These represent captures that would not have produced data without the mesh. Or, in other words, this percentage means that only 52 captured birds (48%) would have reported data on seed–dispersal interactions with a traditional mist-netting sampling.
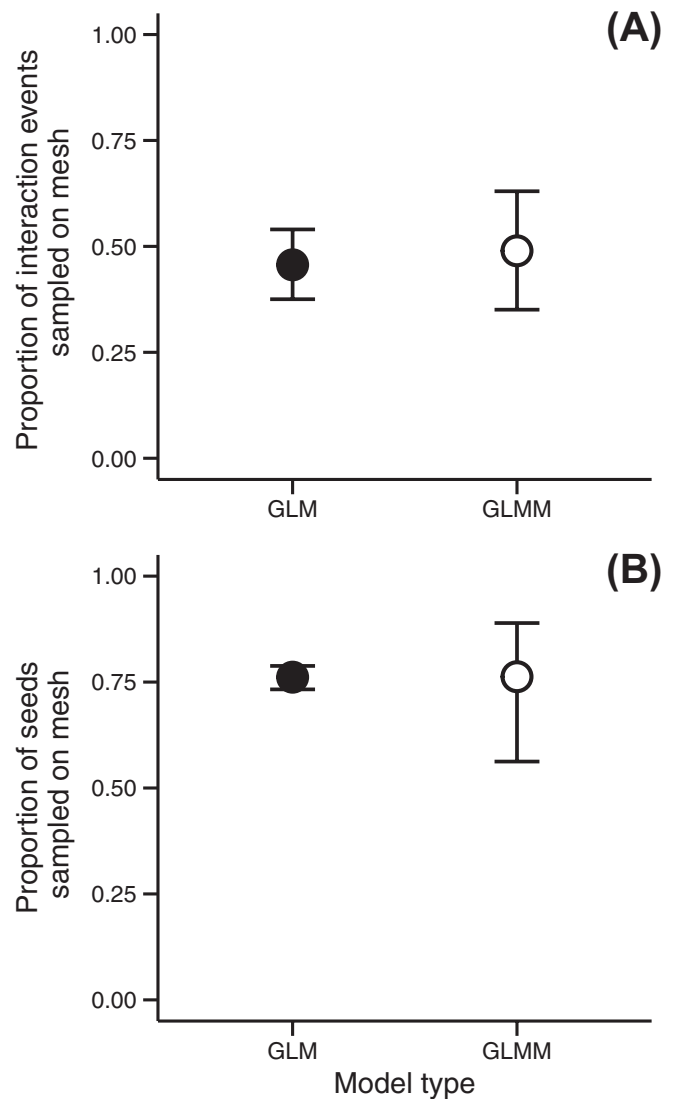

Figure 2. (A) Proportion (mean ± 95% confidence intervals) of interaction events sampled only on the mesh estimated by a GLM (filled circle) and a GLMM (open circle). The GLMM included bird species as a random intercept to account for the uneven contribution of bird species to seed dispersal (repeated measures per bird species). (B) Proportion (mean ± 95% confidence intervals) of seeds sampled on the mesh estimated by a GLM (filled circle) and a GLMM (open circle).

Bird species did not contribute equally to seed dispersal. For instance, *Sylvia atricapilla* was responsible for 92 (67%) out the 138 interaction events, whereas *S. borin* contributed only with three (2%) (Table 1). Strikingly, we found that 46% of all interaction events recorded were sampled only on the mesh (Table 1 and GLM estimate in Fig. 2a), and this value reached 49% when accounting for the uneven contribution of bird species (GLMM estimate in Fig. 2a).

We sampled a total of 910 seeds, from which 693 (76%) were found on the mesh bands below the mist-nets (Table 1 and GLM estimate in Fig. 2b) and only 217 (24%) were recovered inside the cloth bags (Table 1). The estimated

proportion of seeds found on the mesh was the same (76%) when accounting for the repeated measures per bird species, although estimated 95% confidence intervals were much wider (GLMM estimate in Fig. 2b). Indeed, the quantitative contribution of bird species was highly unbalanced. For instance, *Turdus merula* dispersed 373 (41%) out of all 910 seeds sampled, from which 353 were found on the mesh (51% out of the 693 seeds sampled below mist nets). In contrast, *S. borin* dispersed only seven seeds (0.8%) and contributed to the 0.4% of the seeds found on the mesh (Table 1).

We found strong evidence that the proportion of seeds sampled on the mesh was positively related to bird size (Table 2, Fig. 3a). The proportion of seeds sampled on the mesh ranged from 57–63% in small-sized birds like robins and warblers to 84–94% in thrushes *Turdus* sp., our largest species (Fig. 3a). The effect of seed size and its explanatory power were weaker (Table 2, Fig. 3b). The proportion of seeds sampled on the mesh decreased with seed size, from 74 to 14%, but this reduction was only clear for seeds heavier than 100 mg (Fig. 3b). Without using the mesh band, these results indicate that small seeds dispersed by large birds have a higher probability of being dropped before the bird is extracted from the mist net, being potentially lost and unsampled.

Regarding the species and interactions sampled, we found that all the five bird species dropped seeds inside cloth-bags. Accordingly, the accumulation curves with increasing sampling effort were very similar for the sampling without mesh bands and the whole sampling (Fig. 4a). However, we found three seed species on the mesh (n = 17 species, Supporting information) that were not recorded inside the cloth bags (n = 14 species, 82% of the total). This modest difference became consistent after approximately 15 sampling sessions, although extrapolated curves points to convergence after increasing sampling effort (dashed lines, Fig. 4b). With respect to the identity of bird–plant pairwise interactions, we found that the interactions recorded only inside the cloth bags (n = 25) accounted for the 74% of the total pairwise interactions detected with the whole sampling strategy adding the mesh bands (n = 34). Importantly, the lower capacity of cloth bags to sample pairwise interactions was clear after a few sampling days, and the extrapolated curves indicate that the deficit of pairwise interactions would remain after increasing sampling effort (dashed lines, Fig. 4c).

Discussion

There is consensus among ecologists on the importance of a robust and complete sampling of species interactions to acquire a more comprehensive knowledge of complex ecological communities and the functions they mediate (Jordano 2016, Escribano-Ávila et al. 2018, Schlautmann et al. 2021, Quintero et al. 2022). Our results prove that it is necessary to optimize the mist-netting method with mesh bands to improve the sampling of seed–dispersal interactions.

Optimizing the sampling of seed–dispersal interactions

Here we show that the simple placement of a mesh band beneath mist nets doubles the number of bird captures reporting data on seed–dispersal interactions. Strikingly, it avoids the undersampling of ~50% of all interaction events and ~75% of all dispersed seeds (Fig. 2). Hence, our results reveal that by the traditional mist-netting technique, researchers might be sampling only the ~25% of dispersed seeds. The percentage of seeds found on the mesh is close to the 78% reported by Hernández-Dávila et al. (2015) applying a similar methodology to mist-netted passerines in Neotropical cloud forests and pastures. The latter suggests that our findings could be broadly generalizable across ecosystems and regions whenever the frugivores are small- and medium-sized birds.

Overall, our results uncover the vast amount of information on frugivory and seed dispersal by birds that can be lost when the sampling technique is not optimized. The methodology proposed here is not only important in terms of sampling effectiveness but also in ethical terms if we bear in mind that mist-netting is an invasive technique not devoid of risks (Spotswood et al. 2012b). Therefore, it seems mandatory to maximize the information that can be obtained from captured animals. In this line, 56 out of the 108 captured birds (52%) only produced data on the mesh bands. In other words, all seed-dispersal data from half of the birds would have been lost on the ground without the mesh bands.

Table 2. Main results of the generalized linear mixed-models predicting the effect of bird size (body weight) and seed size (mean seed dry weight) on the proportion of seeds found on the mesh band placed beneath the mist nets (response variable). We fitted three different models where the predictor variables were, respectively, (a) bird size, (b) seed size and (c) both bird and seed size. In the three models, bird and plant species were included as random factors (random intercepts). Results include parameter estimates ± SE for the predictors and goodness-of-fit measures for the models (AIC, ΔAIC and R^2_{GLMM}). Asterisks denote significant levels as follows: †, $0.1 < p > 0.05$; *, $p < 0.05$; and ***, $p < 0.001$. Bold values indicate the goodness-of-fit measures of the model with ΔAIC = 0.

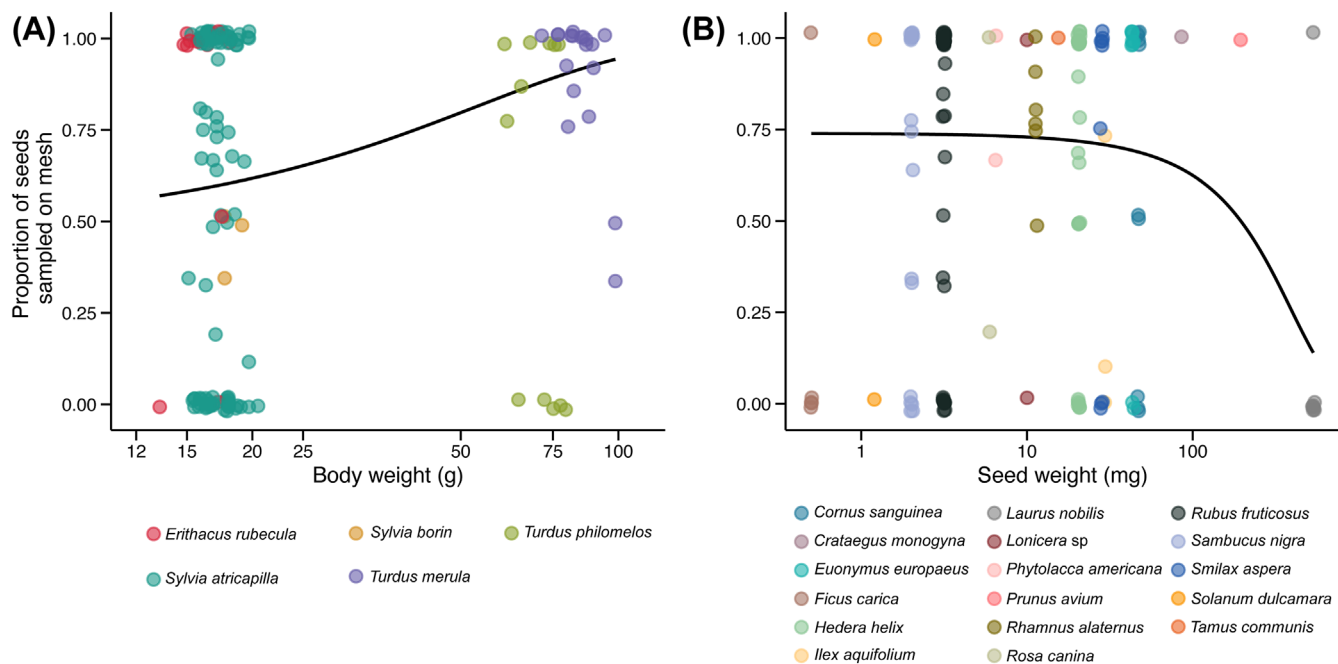| Model predictors and goodness-of-fit	(a) Bird size	(b) Seed size	(c) Bird and seed size
(Intercept)	−0.050 ± 0.441	1.244 ± 0.555*	0.123 ± 0.404
Body weight	0.028 ± 0.007***	–	0.030 ± 0.007***
Seed weight	–	−0.005 ± 0.003†	−0.005 ± 0.003*
AIC	611.0	611.3	**608.6**
ΔAIC	2.4	2.7	**0**
$R^2_{GLMM(m)}$	0.247	0.116	**0.401**
$R^2_{GLMM(c)}$	0.698	0.744	**0.713**

Figure 3. (A) Proportion of seeds sampled on the mesh in relation to bird size (body weight). (B) Proportion of seeds sampled on the mesh and seed size (mean seed dry weight). The line in each panel denotes GLMM predictions (model *c* in Table 2) after keeping constant (mean value) the other covariate. Note that x-axes are log-transformed.

The role of bird and seed size

The proportion of seeds sampled on the mesh was strongly and positively related to bird size while, to a lesser extent, negatively related to seed size (Table 2, Fig. 3). This means that the information lost by traditional sampling inside cloth bags is not evenly distributed across bird and plant species. For instance, warblers and robins dropped 57–63% of seeds on the mesh, whereas thrushes dropped 84–94% of the seeds they dispersed. These percentages illustrate that not using mesh bands produces sampling biases between bird species in the way that data from larger species are underrepresented in relation to data from smaller species. This undersampling is particularly concerning because large birds are already sampled less frequently through mist netting than expected from frugivory observations (Vitorino et al. 2022). Moreover, not using mesh bands can produce also sampling biases between plant species in the way that data from smaller-seeded species are underrepresented in relation to data from larger-seeded species, although this relationship seems to be highly influenced by the heaviest-seeded species occurring in our community (*Laurus nobilis*).

Together, these results indicate that small seeds dispersed by large birds have a much higher probability of being lost and unsampled without using mesh bands. This is the opposite of our expectation that the proportion of seeds dropped on the mesh (i.e. undersampling) would be greater for small birds and large seeds. Indeed, gut passage time increases with bird body mass (Herrera 1984b, Johnson et al. 1985, Levey 1986, Uriarte et al. 2011), and small seeds tend to have longer passage times than large seeds (Johnson et al. 1985, Fukui 2003). Our unexpected results might be explained by the fact

that larger frugivorous birds have larger meal sizes, ingesting more fruits – and thus seeds – than smaller birds (Snow and Snow 1988). For example, Snow and Snow (1988) reported that meal sizes of ivy *Hedera helix* were of 8.3 and 8.8 fruits per visit for our study thrushes (*T. merula* and *T. philomelos*, respectively) but of 2.3 for European robins and 2.5 for blackcaps. In many occasions, birds defecate or regurgitate seeds as a behavioural response when they become entangled in the mist-net (Rumeu et al. unpubl.). If so, the first dropping would contain more seeds in larger than in smaller birds, particularly if the fruits consumed have many small seeds. The latter would also explain why smaller seeds such as *Solanum dulcamara*, *Sambucus nigra* or *Rubus fruticosus*, were more frequently dropped on the mesh bands than larger seeds. Another non-mutually exclusive possibility is related to the fact that large and small birds exhibit behavioural differences when they get trapped in the nets. For example, large species are generally louder than small ones, and smaller birds are more prone to stress (Spotswood et al. 2012b). Differences on bird activity on the net could ultimately affect the rate of defecation and regurgitation and, thereby, the proportion of seeds found earlier on the mesh bands and later on the cloth bags.

Accumulation curves of species and pairwise interactions

Our evaluation of accumulation curves for species and pairwise interactions with increasing sampling effort also evidenced the importance of the mesh band below mist-nets to gather a more comprehensive data in terms of diversity (richness) of the species and interactions recorded. The bird species
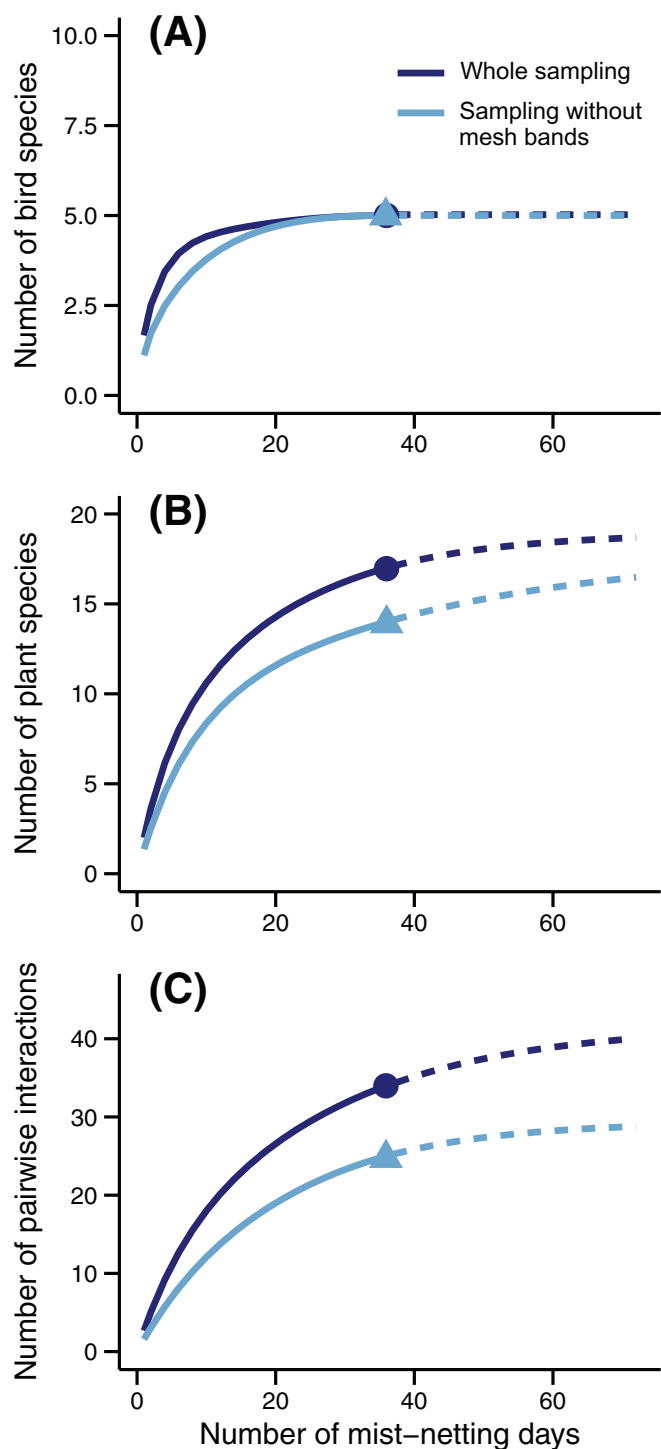
Figure 4. Rarefaction–extrapolation curves of species and pairwise interactions with increasing sampling effort for the whole sampling of bird droppings on mesh bands and cloth bags, and for the traditional sampling without mesh bands. (A) Bird species, (B) plant species and (C) number of pairwise interactions. Symbols denote observed data. Continuous lines represent interpolated data, while discontinuous lines indicate extrapolated estimates.

acting as seed dispersers were equally sampled regardless of the mesh-band (Fig. 4a), but this was not the case for the plant species. Using the mesh-band allowed us to detect the dispersal of a 21% more plant species (17 instead of 14 species; Fig. 4b). However, the most striking result came from the pairwise interactions, as we show that the mesh-band allowed us to detect 36% more pairwise interactions (34 instead of 25). Thus, we can end up with a deficient sampling when the mesh band is not used and, worryingly, this undersampling tends to increase with sampling sessions (Fig. 4c). This result is particularly relevant for mist-netting studies characterizing networks of seed dispersal interactions, because under-sampled networks may result in biased descriptors that affect our understanding of the studied communities (Costa et al. 2016, Acevedo-Quintero et al. 2020, Vitorino et al. 2022).

Final considerations

Here, we show strong evidence on the effectiveness of using a mesh band below mist nets as a simple and low-cost technique to optimize this widely-used method to sample seed–dispersal interactions. We are aware that there can be some challenges associated to the use of mesh bands. For example, if many frugivorous birds are simultaneously caught in the same net, correctly assigning faecal samples to individual birds may become difficult (Hernández-Dávila et al. 2015). Besides, the terrain where mist nets can be placed may vary substantially depending on the local conditions of each particular study site. However, the effectiveness of the methodology here proposed is such that, even on highly irregular or steep terrains, we encourage researchers to find the way of using a mesh band below the mist-nets. The colour of the mesh band can be also chosen to minimize the visual impact (see a grey mesh band on a frozen terrain in Fig. 1A).

To some extent, the percentages reported here could be influenced by the time intervals to check the mist nets, which ranged between 45 and 60 min, a very standard interval in Spain that is not recommended to exceed (Pinilla 2000). Frequency to check mist nets checks usually varies among studies according to environmental conditions (Pinilla 2000, Spotswood et al. 2012b) or the presence of potential predators (Guimarães et al. 2020), but intervals typically range between 30 and 60 min. One might argue that a shorter interval would have produced much lower percentages of interactions and seeds sampled on the mesh bands. However, it is important to note that 30 min already exceeds the peak of the gut passage time of the thrushes, the largest birds of our study (Morales et al. 2013). Moreover, our results suggest that the gut passage time is not an important variable predicting the proportion of seeds sampled on the mesh, given its positive relationship with bird size (Herrera 1984b) and the behavioural responses to net impact discussed above. We thus think that shorter check intervals (e.g. 30 min instead of 45–60 min) would have led to similar results. In fact, Hernández-Dávila et al. (2015) reported a 78% of seeds sampled on plastic sheets placed on the ground with checking periods of 30 min.

Strikingly, previous recommendations on the use of bands below mist nets to collect droppings (Galindo-González et al. 2009, Hernández-Dávila et al. 2015) have gone unnoticed among researchers studying seed-dispersal interactions. In this contribution, we demonstrate the crucial role of using mesh bands to obtain a more comprehensive picture of interacting communities in terms of interaction events, interaction strength (measured as the number of seeds dispersed), and diversity of species and interactions. Most of the current knowledge on frugivory and seed dispersal by birds is framed within network theory and based on data obtained through mist netting (Fricke and Svenning 2020). Therefore, we strongly urge the use of mesh bands for future studies of seed–dispersal interactions based on mist-netted birds as a complementary technique to traditional sampling inside cloth-bags. Finally, it is not only a matter of sampling effectiveness and sampling biases, but also our responsibility to obtain the best possible data quality when using handling procedures that might affect the welfare of animals.

Author contributions

Beatriz Rumeu and **Juan P. González-Varo** contributed equally to this publication. **Beatriz Rumeu**: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Funding acquisition (equal); Methodology (lead); Writing – original draft (lead). **Juan P. González-Varo**: Conceptualization (lead); Formal analysis (lead); Funding acquisition (equal); Methodology (lead); Writing – original draft (lead). **Cristina de Castro**: Data curation (lead); Methodology (lead). **Antonio López-Orta**: Methodology (equal); Writing – review and editing (supporting). **Juan Carlos Illera**: Funding acquisition (equal); Methodology (supporting); Writing – review and editing (supporting). **Marcos Miñarro**: Funding acquisition (equal); Writing – review and editing (supporting). **Daniel García**: Funding acquisition (lead); Investigation (lead); Project administration (lead); Writing – review and editing (supporting).

Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.98sf7m0mx> (Rumeu et al. 2022).

Supporting information

The Supporting information associated with this article is available with the online version.

References

Acevedo-Quintero, J. F. et al. 2020. Sampling bias affects the relationship between structural importance and species body mass in frugivore–plant interaction networks. – Ecol. Complex. 44: 100870.

Bartoń, K. 2020. Package MuMIn: multi-model inference. – R package ver. 1.43.17, <https://cran.r-project.org/web/packages/MuMIn/MuMIn.pdf>.

Bascompte, J. and Jordano, P. 2013. Mutualistic networks. – Princeton Univ. Press.

Brooks, M. E. et al. 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. – R J. 9: 378–400.

Chao, A. et al. 2014. Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. – Ecol. Monogr. 84: 45–67.

Costa, A. et al. 2022. Seed dispersal by frugivores from forest remnants promotes the regeneration of adjacent invaded forests in an oceanic island. – Restor. Ecol. e13654, in press.

Costa, J. M. et al. 2016. Sampling completeness in seed dispersal networks: when enough is enough. – Basic Appl. Ecol. 17: 155–164.

Escribano-Ávila, G. et al. 2018. Tropical seed dispersal networks: emerging patterns, biases and keystone species traits. – In: Dáttilo, W. and Rico-Gray, V. (eds), Ecological networks in the tropics: an integrative overview of species interactions from some of the most species-rich habitats on Earth. Springer International Publishing, pp. 93–110.

Fricke, E. C. and Svenning, J.-C. 2020. Accelerating homogenization of the global plant–frugivore meta-network. – Nature 585: 74–78.

Fukui, A. 2003. Relationship between seed retention time in bird's gut and fruit characteristics. – Ornithol. Sci. 2: 41–48.

Galindo-González, J. et al. 2009. A more efficient technique to collect seeds dispersed by bats. – J. Trop. Ecol. 25: 205–209.

García, D. et al. 2013. Functional heterogeneity in a plant–frugivore assemblage enhances seed dispersal resilience to habitat loss. – Ecography 36: 197–208.

García, D. et al. 2018. Birds as suppliers of pest control in cider apple orchards: avian biodiversity drivers and insectivory effect. – Agric. Ecosyst. Environ. 254: 233–243.

González-Varo, J. P. et al. 2014. Who dispersed the seeds? The use of DNA barcoding in frugivory and seed dispersal studies. – Methods Ecol. Evol. 5: 806–814.

González-Varo, J. P. et al. 2017. Unravelling seed dispersal through fragmented landscapes: frugivore species operate unevenly as mobile links. – Mol. Ecol. 26: 4309–4321.

González-Varo, J. P. et al. 2021. Limited potential for bird migration to disperse plants to cooler latitudes. – Nature 595: 75–79.

Guimaráes, D. P. et al. 2020. Birds trapped in mist nets killed by opportunistic predators in a forest in southwestern Amazonia. – Rev. Bras. Zooci. 21: 1–8.

Heleno, R. et al. 2013. Seed dispersal networks in the Galápagos and the consequences of alien plant invasions. – Proc. R. Soc. B 280: 20122112.

Hernández, A. 2007. Alimentación de aves frugívoras en setos y bordes de bosque del norte de España: importancia de algunas especies de plantas en invierno y primavera. – Ecología 21: 145–156.

Hernández-Dávila, O. A. et al. 2015. Plastic sheets: a new method for collecting faecal samples with seeds dispersed by birds. – J. Trop. Ecol. 31: 187–190.

Herrera, C. M. 1984a. A study of avian frugivores, bird-dispersed plants and their interaction in Mediterranean scrublands. – Ecol. Monogr. 54: 2–23.

Herrera, C. M. 1984b. Adaptation to frugivory of Mediterranean avian seed dispersers. – Ecology 65: 609–617.

Herrera, C. M. 2002. Seed dispersal by vertebrates. – In: Pellmyr, O. and Herrera, C. M. (eds), Plant–animal interactions. An evolutionary approach. Blackwell Science, pp. 185–208.

Howe, H. F. 1986. Seed dispersal by fruit-eating birds and mammals. – In: Murray, D. R. (ed.), Seed dispersal. Academic Press, pp. 123–190.

Hsieh, T. C. et al. 2016. iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). – Methods Ecol. Evol. 7: 1451–1456.

Johnson, R. A. et al. 1985. Nutritional values of wild fruits and consumption by migrant frugivorous birds. – Ecology 66: 819–827.

Jordano, P. 1989. Pre-dispersal biology of *Pistacia lentiscus* (Anacardiaceae): cumulative effects on seed removal by birds. – Oikos 55: 375–386.

Jordano, P. 2014. Fruits and frugivory. – In: Gallagher, R. S. (ed.), Seeds: the ecology of regeneration of plant communities, 3rd edn. CABI, pp. 18–61.

Jordano, P. 2016. Sampling networks of ecological interactions. – Funct. Ecol. 30: 1883–1893.

Jordano, P. 2017. What is long-distance dispersal? And a taxonomy of dispersal events. – J. Ecol. 105: 75–84.

Levey, D. J. 1986. Methods of seed processing by birds and seed deposition patterns. – In: Estrada, A. and Fleming, T. H. (eds), Frugivores and seed dispersal. Tasks for vegetation science. Springer, pp. 147–158.

Morales, J. M. et al. 2013. Frugivore behavioural details matter for seed dispersal: a multi-species model for Cantabrian thrushes and trees. – PLoS One 8: e65216.

Nakagawa, S. et al. 2017. The coefficient of determination R^2 and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. – J. R. Soc. Interface 14: 20170213.

Nogales, M. et al. 2017. Contribution by vertebrates to seed dispersal effectiveness in the Galápagos Islands: a community-wide approach. – Ecology 98: 2049–2058.

Pinilla, J. 2000. Manual para el anillamiento científico de aves. – SEO BirdLife and DGCN-MIMAM.

Quintero, E. et al. 2022. Methodological overview and data-merging approaches in the study of plant–frugivore interactions. – Oikos 2022: e08379. doi: 10.1111/oik.08379

Rumeu, B. et al. 2011. Differential seed dispersal systems of endemic junipers in two oceanic Macaronesian archipelagos: the influence of biogeographic and biological characteristics. – Plant Ecol. 212: 911–921.

Rumeu, B. et al. 2020. Frugivore species maintain their structural role in the trophic and spatial networks of seed dispersal interactions. – J. Anim. Ecol. 89: 2168–2180.

Rumeu, B. et al. 2022. Data from: Increasing efficiency and reducing bias in the detection of seed–dispersal interactions based on mist-netted birds. – Dryad Digital Repository, <https://doi.org/10.5061/dryad.98sf7m0mx>.

Schlautmann, J. et al. 2021. Observing frugivores or collecting scats: a method comparison to construct quantitative seed dispersal networks. – Oikos 130: 1359–1369.

Schupp, E. W. et al. 2010. Seed dispersal effectiveness revisited: a conceptual review. – New Phytol. 188: 333–353.

Schupp, E. W. et al. 2017. A general framework for effectiveness concepts in mutualisms. – Ecol. Lett. 20: 577–590.

Snow, B. and Snow, D. 1988. Birds and berries. – T and A D Poyser.

Sorensen, A. E. 1981. Interactions between birds and fruit in a temperate woodland. – Oecologia 50: 242–249.

Spotswood, E. N. et al. 2012a. An invasive tree alters the structure of seed dispersal networks between birds and plants in French Polynesia. – J. Biogeogr. 39: 2007–2020.

Spotswood, E. N. et al. 2012b. How safe is mist netting? Evaluating the risk of injury and mortality to birds. – Methods Ecol. Evol. 3: 29–38.

Teixido, A. L. et al. 2022. Anthropogenic impacts on plant–animal mutualisms: a global synthesis for pollination and seed dispersal. – Biol. Conserv. 266: 109461.

Torroba Balmori, P. et al. 2013. Semillas de frutos carnosos del norte ibérico: guía de identificación. – Ediciones Univ. de Valladolid.

Uriarte, M. et al. 2011. Disentangling the drivers of reduced long-distance seed dispersal by birds in an experimentally fragmented landscape. – Ecology 92: 924–937.

Vitorino, B. D. et al. 2022. Influence of sampling methods on the description of a Neotropical seed dispersal network. – Acta Oecol. 114: 103805.

Wang, B. C. and Smith, T. B. 2002. Closing the seed dispersal loop. – Trends Ecol. Evol. 17: 379–386.