**Ecology and Evolution**

Article

**Mixed-species group formation in response to multiple predators in the South African savannahs**

Franck Prugnolle1, Virginie Rougeron1, Lain E. Pardo2, Michael J. Somers3, Mike JS Peel5,6,7, Lourens H. Swanepoel8, Mark Keith9, Pierre-Cyril Renaud1, Lisa Nicvert10, Rob Davis2, Jan Venter1, 2, Hervé Fritz1

1IRL REHABS, Sustainability Research Unit (SRU), Nelson Mandela University, Madiba Drive, George, South Africa.

2Department of Conservation Management, Faculty of Science, Nelson Mandela University, George 6530, South Africa

3Mammal Research Institute, Centre for Invasion Biology, Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa

4Sustainability Research Unit, Faculty of Science, George Campus, Nelson Mandela University, George, South Africa

5ARC-Animal Production Institute, Rangeland Ecology Group, Nelspruit, South Africa

6School for Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, South Africa

7Applied Behavioural Ecology and Ecosystem Research Unit, University of South Africa, Florida, South Africa

8Department of Biological Sciences Faculty of Science, Engineering and Agriculture, University of Venda, Thoyoyandou 0950, South Africa

9Eugène Marais Chair of Wildlife Management, Mammal Research Institute Department of Zoology and Entomology , Faculty of Natural and Agricultural Sciences, University of Pretoria, Private Bag X20, Hatfield 0028, South Africa

10Université de Lyon, Université Lyon 1, CNRS, VetAgro Sup, Laboratoire de Biométrie et Biologie Evolutive, UMR5558, Villeurbanne, France

**Correspondance** : Dr Franck Prugnolle. Email: [prugnolle@gmail.com](mailto:prugnolle@gmail.com)

**Keywords :** anti-predator response ; polyspecific / interspecific associations; stress-gradient hypothesis; interspecific competition;

**Open research statement:** data and codes for statistical analyses are provided for peer review. The link to the data is:

<https://github.com/prugnolle498/MSG-formation-multiple-predators>

Upon acceptance, data and codes for statistical analyses will be provided via the CNRS Research Data repository: https://entrepot.recherche.data.gouv.fr/dataverse/cnrs

**Abstract**

The formation of mixed-species groups (MSGs) (also called heterospecific or polyspecific groups) is often considered an anti-predator strategy used in response to an increased predation risk. Recent studies performed in savanna ecosystems in Africa have demonstrated an increased formation of ungulate MSGs in higher predation risk areas. Nevertheless, these studies only considered one predator species (the lion *Panthera leo*) in evaluating the response to predation risk. Yet, species evolve in multi-predator systems that require prey to respond to multiple sources of risks. Although the importance of incorporating these multiple predators in studies is recognized, there is still little information on the behavioral strategies, including MSG formation, that prey can use to minimize predation risk from multiple predators. In the present study, we analyzed a large dataset of camera trap data (more than 160,000 observations) from five sites located in savanna biome in South Africa. We studied the likelihood of MSGs formation in response to the presence of different predators (lion, leopard (*Panthera pardus*), spotted hyena (*Crocuta crocuta*), and wild dog (*Lycaon pictus*)). We demonstrated that the probability of forming MSGs increased when *(i)* the photographic rate of the interacting species increased and *(ii)* the photographic rate of predators increased. Our results showed that lion and spotted hyena are the predator species most likely to trigger the formation of MSGs for different prey species. These results suggest a “hierarchy of fear” among predators in which both species are the most feared, even for prey species that either predator does not preferentially select. A large number of MSGs formed in the presence of these two predator species included Burchell’s zebras (*Equus quagga burchellii)*, in association with species that have complementary modes of predator detection (e.g. wildebeest (*Connochaetes taurinus*) and giraffe (*Giraffa camelopardalis*). Our study highlights the importance of incorporating multiple predators into MSG analyses to better understand ecosystem functioning and, community formation.

**Introduction**

The formation of groups containing individuals from several species has been observed in many different ecosystems and for several animal taxa (e.g. birds, fishes, mammals…). They are generally called poly/interspecific/heterospecific groups or mixed-species groups (MSGs). MSGs may vary in species composition, size, duration or frequency of formation (Goodale et al. 2020; Stensland, Angerbjörn, and Berggren 2003).

Why species form these polyspecific groups, or MSGs, has been an important question in ecology. Their formation has been attributed to three main causes (Goodale et al. 2020; Goodale, Beauchamp, and Ruxton 2017). First, they may be formed simply by chance. When two or more species share the same habitat or the same trophic resource, then there is a chance that the two species will be at the same place at the same time (e.g. Waser 1982; Syme, Kiszka and Parra 2023). Second, species may actively seek to form these groups because formation of these groups is beneficial for one or all species (Stensland, Angerbjörn, and Berggren 2003). One benefit can be linked to the access to resources, as one or the other species may be an indicator of the presence of a trophic resource or facilitate access to it (Stensland, Angerbjörn, and Berggren 2003). Increasing the number of individuals using the same resource at the same time and location may nevertheless increase competition. Such behavior is therefore expected to occur more frequently when resources are abundant (Stensland, Angerbjörn, and Berggren 2003; Beaudrot et al. 2020). Finally, species may benefit from aggregating with other species because this reduces their predation risk (Goodale et al. 2020; Goodale, Beauchamp, and Ruxton 2017). This can occur because the increased number of individuals dilutes the risk of predation, because the interacting species present better or complementary abilities to detect predators (e.g., heightened visual, acoustic or olfactory perception) or because it is more susceptible to being preyed on due to predator preferences or lower predator-avoidance abilities. Under this predator-driven hypothesis (called the predator-based stress gradient hypothesis), it is assumed that MSGs should be formed more frequently when the risk of predation is high (Beaudrot et al. 2020; Kiffner et al. 2022).

This predator-based stress gradient hypothesis has recently been tested on mammals in two geographically close African savannah sites in Tanzania (Serengeti National Park (Beaudrot et al. 2020) and the ﻿Manyara Ranch in the Tarangire ecosystem (Kiffner et al. 2022). Both studies demonstrated that MSGs of ungulates are more likely to occur in habitats where lion-associated predation risk is high, but less likely when the availability of food (plant biomass) declines. Although both studies demonstrated the importance of predation risk for the formation of MSGs, they present some limits. In the first study (Beaudrot et al. 2020), they considered all MSGs as a whole and did not consider each dyadic MSG (i.e. a group of two interacting species) independently. Yet, several studies have shown that species do not necessarily form interspecific groups for similar reasons, i.e. the benefits of mixed-group formation are not necessarily identical for each species or group of species (Goodale et al. 2020; Kiffner et al. 2022). In addition, by considering all MSGs together, it is not statistically possible to correct for the abundance of the interacting species and hence the probability of forming MSGs only by chance. This information is yet fundamental to determine whether the formation of these multi-specific groups is more important than expected by chance.

The second study (Kiffner et al. 2022) did analyze the formation of each dyadic MSG and indeed found that the response to predation was different between pairs of species (by comparing areas with and without lions, *Panthera leo*). They, thus, showed that in areas where lions were present, ﻿dyadic associations of ﻿Thomson’s gazelles (*Eudorcas thomsonii*) and eland (*Tragelaphus oryx*) with giraffes (*Giraffa camelopardalis*) were more pronounced than in areas without lions.

Another limitation of both studies is that they restricted the analysis of MSGs to certain ungulate families (Bovidae, Giraffidae and Equidae) while a non-negligible proportion of MSGs in savannah ecosystems may involve other families of ungulates or some non-ungulate species. For example, associations with primates (like baboons, *Papio spp*.), swids (e.g. common warthogs, *Phacocherus africanus*) and birds (e.g. ostriches, *Sthruthio spp.*) (e.g. Meise, Franks and Bro-Jørgensen 2020) are often observed but were disregarded in those studies.

Finally, in both studies, the only predation risk that was considered was that of lions. Neither study included other terrestrial predators such as spotted hyenas (*Crocuta crocuta*; hereafter hyena), wild dogs (*Lycaon pictus*) or leopards (*Panthera pardus*) that also consume large mammals (Hayward, 2006; Hayward et al., 2006; Hayward, O’Brien, Hofmeyr, & Kerley, 2006). Species evolve in multi-predator systems that require prey to respond to multiple sources of risks. Although the importance of incorporating these multiple predators in studies is recognized (Thaker et al. 2011; Creel et al. 2017; Owen-Smith 2019), there is still little information on the behavioral strategies, including MSG formation, that prey can use to minimize predation risk from multiple predators. Part of the formation of MSGs depends on the capacity of prey species to identify cues left by predators(visual, olfactory, . As these cues may vary from one predator to another and are more or less easy to identify, it is important to analyze independently the impact each predator may have on the formation of MSGs.

In this study, we aimed to understand to what extent multi-predator abundance (lion, leopard, hyena, wild dog) influenced the formation of MSGs (by considering both whole MSGs and each dyadic pair of species independently). For this, we used camera trap data across five reserves in South Africa from the *Snapshot Safari-South Africa project*, a multi-site long-term biodiversity monitoring project (Pardo et al. 2021). Our analysis considered ungulate and non-ungulate species involved in forming MSGs.

**Material and Methods**

**Study sites**

The *Snapshot Safari-South Africa project* includes the survey of 31 locations, of which 21 are permanent grids for long-term monitoring purposes (Pardo et al. 2021). We restricted our analyses to five sites (mainly private or national game reserves) from the *Snapshot Safari-South Africa* Project. These sites were chosen for two reasons: first, they were all located in the savannah biome and thus had similar mammal communities (Figure 1). Second, these sites had the highest sampling effort in this biome. The sites were: the Associated Private Natural Reserves (APNR including Balule Nature Reserve, Kapama, Klaserie, Timbavati, Thornybush and Umbabat Game Reserves), Pilanesberg National Park (PLN), Madikwe Game Reserve (MAD), Venetia Game Reserve (VEN) and Kruger National Park (KRU) (Figure 1).

**Camera trapping**

Camera traps were deployed in a 5 km2 cell size grid. Fifty six camera traps were deployed in APNR, 47 in Madikwe (MAD), 20 in Pilanesberg (PLN), 40 in Kruger (KRU) and 19 in Venetia (VEN). In Madikwe, we removed five camera traps from the dataset that were placed in front of waterholes. This was done as many species congregate at waterholes to consume water and tend to form MSGs. Because the dynamic and cause of formation of these MSGs may differ from MSGs performed in other areas, we decided to discard these cameras to avoid adding further complexities to the analysis. At each site, cameras were fixed to poles at 30-50 cm height, facing animal trails or any any location showing evidence of animal activity. Cameras were never directed towards human roads or areas where there was high human activity (Pardo et al. 2021). No bait was used. Cameras were programmed to take three pictures per activation (event) and the delay time between events was 1 minute. All cameras were Cuddeback® black and white flash (Cuddeback, Wisconsin, USA). Camera batteries and memory cards were changed regularly, approximately every two to three months. Classification of images was conducted with the help of citizen scientists through the online platform Zooniverse (see Pardo et al. (2021) and its supplementary material). ﻿We used data from 2016 to 2019 with total survey periods ranging from 515 days in MAD to 1163 in VEN (Table 1). The distribution of photographs with at least one identified animal by site and camera is provided in Supporting Figure S1.

**Testing of the effect of different predators on the formation of mixed-species groups**

***Selection of species***

We defined a mixed-species group (MSG) as a camera trap observation (picture) showing two distinct species. The pictures containing more than two (representing only a small proportion of detections) were not considered. The frequency of each observed mixed-species group (over the total amount of pictures presenting an MSG) in each site was determined. A network of the most frequent pairs observed within each park (representing more than 1% of the MSGs) is provided in Figure 1. Seven species were most frequently observed forming MSGs in these parks: impala *(Aepyceros melampus)*, Burchell’s zebra (*Equus quagga burchellii)*, giraffe (*Giraffa Camelopardalis)*, blue wildebeest (*Connochaetes taurinus*), kudus (*Tragelaphus sp.*), Chacma baboon (*Papio ursinus*) and common warthog *(Phacochoerus africanus)* (Figure 1). The following analyses were performed only on these species observations. All these species are present in all reserves (Table S1).

***Independence of events***

To ensure data independence, pictures taken at the same camera trap of the same species within a time period of 30 minutes were considered a single event. When groups of pictures included within this range contained only the focal species, then a single random picture was kept within this group. When groups of pictures contained both pictures with single (only the focal) and mixed-species (including the focal), then the first picture containing the mixed-group was retained.

***Data analysis***

To determine whether species tendency to form MSGs increased under higher predation risk in comparison to single-species groups, we used logistic general linear mixed models (GLMM) where the response variable was a binary variable with 0 representing single-species groups (of one of the seven focal species previously listed) and 1 representing MSGs including the focal species plus one of the other six species (interacting species) (Beaudrot et al. 2020). Single-species groups were defined as pictures where at least one individual of the focal species was observed and no other species was identified as being present. Groups involving the focal species were considered as MSGs when at least one individual of another species (interacting species) was observed on a picture containing the focal species. Again, pictures containing more than two species (that represented a minority of the MSGs – see Table S1) were discarded from the analysis.

Two categories of analyses were performed. First, for each focal species, we considered all MSGs without distinction of the interacting species (the response variable is hereafter referred as MSG\_all). Second, GLMMs were performed for each pair of MSG independently (MSG\_pair), including the focal species and any of the six other possible interacting species like impala (focal) – zebra (interacting) (MSG\_zebra), impala (focal) – giraffe (interacting) (MSG\_Giraffe), impala (focal) - wildebeest (interacting) (MSG\_wildebeest). An example of file, with impala as focal species, including the different response variables (MSG\_all and each of the dyadic MSG\_pair) is provided in the supplementary material (Table S2). GLMs were performed considering a binomial distribution and a logit link in R using the package lme4 (Bates et al. 2015). Wald tests were used to determine the significance of each explanatory variable.

Different explanatory variables were considered in each model (Table S2). As the probability of forming MSGs may vary from one reserve to another, we included the variable “reserve” as a random effect. The second explanatory variable was the season (dry or wet). This variable was taken as a proxy of food abundance for herbivores, with plant biomass being more prominent during the wet season than during the dry season (Kiffner et al. 2014). This variable was considered as an indirect way to account for the effect of foraging competition on the formation of MSGs, as increased resource availability in the wet season should reduce the level of competition for food between herbivores (Stensland, Angerbjörn, and Berggren 2003). On the contrary, when food is scarce, then foraging competition is high and this increase in competition for resources could counterbalance the benefits of forming MSGs against predators. Observation occurring from November to May were classified as “wet season” and all other observations were considered as “dry season” (Phakula 2016). The probability of forming a group with another species is directly related to the probability of meeting this species, which is directly correlated to both abundance and habitat sharing. As a third explanatory variable, we therefore also considered the photographic rate of each interacting species. We estimated the “photographic rate” as the number of independent observations of the species at a particular camera trap within each roll (a roll is the period separating battery replacement for each camera which usually extends to 2-3 months) (Sollmann 2018). Because sampling effort may vary between camera traps (due to different length of functioning of batteries within each roll), we divided this value by the number of days separating the first picture of a camera trap roll and the last day a picture had been taken. We acknowledge that this correction might not be perfect because the period between the first and the last pictures does not necessarily correspond to the time the camera was active but the camera used did not provide any metadata of this kind. The photographic rate can be interpreted as an index of activity, where the activity of a species at a site can increase because more individuals use that site and/or because individuals use that site more frequently (Sollmann 2018). This variable was considered in all models except the ones with MSG\_all as a response variable.

The last predictor variable used was “predator photographic rate”. We approximated it similarly to the interacting species photographic rate, namely by counting the number of observations of the different predator species (lion, leopard, hyena, wild dog) per camera trap and roll divided by the sampling effort. For both interacting and predator species, estimates were obtained after applying the 30 minutes-interval filter for picture independence.

Another variable that should be considered is the “camera Trap” effect, as our observations of single species versus mixed-group species were repeated several times at each camera (one repetition in our study is a roll). Consequently, these data are not independent, and a random “camera trap effect” should ideally be added to correct this pseudoreplication problem. However, we did not add this factor because there was a significant relationship between the Camera trap effect and the other predictor variables, in particular the predator photographic rates (Table S3).

As explanatory variables need to be orthogonal (i.e. non-collinear), we tested for potential collinearities between explanatory variables using the Variance Inflation Factor (VIF) (Dormann et al. 2013). The VIF estimates how much the variance of a regression coefficient is inflated due to multicollinearity in the model. When the VIF is close to 1 then the explanatory variable is not correlated to any of the other variables in the model. A VIF larger than 5 indicates that the model has problems estimating the coefficient and so it is advised to discard that variable. The VIF was estimated for each model and each explanatory variable using the R-package “car” (Fox and Weisberg 2019). All covariates had VIF values <5, indicating multicollinearity was not an issue in our data set (Dormann et al. 2013) (Table S4).

**Results**

The entire camera trap dataset comprised 163 144 pictures taken from march 2016 to November 2019. Out of those pictures, 37 690 represented independent pictures with only one of the seven focal species (impala, zebra, giraffe, kudu, wildebeest, warthog or baboon) and 630 corresponded to pictures with two distinct species (Table 2). Species grouping the most with other species, in proportion, were zebra followed by wildebeest, baboon, giraffe, warthog, kudu and finally impala. For predators, the number of pictures overall reserves varied from 419 for lions to 61 for wild dogs (Table 3).

Generalized linear models on MSG\_all for each focal species revealed that season had a significant effect on the formation of MSGs for three focal species out of the seven studied including zebra, giraffe and wildebeest, with more MSGs formed during the wet season for zebras and giraffe but opposite for wildebeest (Figure 2 and Table S5). Regarding predation, we mainly observed an effect of the “photographic rate” of lions and hyenas on the formation of MSG\_all. This effect was significant for three out of seven focal species for lions (zebra, giraffe and kudu) and five out of seven for hyenas (impala, zebra, wildebeest, kudu, baboon). For both predators, MSGs were more likely to occur when the number of predators detected by a camera trap during a roll increased.

We repeated GLM analyses by considering each species pair independently (MSG\_pair). These analyses allowed us to account for each interacting species photographic rate at each camera trap and roll. Results of the GLMs for each focal species and each MSG are provided in Figures 3 and 4 and Table S5. For most focal species, there was a significant positive effect of the photographic rate of the interacting species on the probability of forming an MSG. The more the interacting species were observed in an area during a roll, the higher was the probability of the focal species to group with these species. Regarding season, significant effects were only observed for impala, zebra and wildebeest as focal species. MSG formation increased during the wet season for MSG involving zebra and impala but, for all others involving wildebeest as focal or interacting species, the effect was reversed. Finally, regarding the presence of predators, most significant effects on the formation of MSGs were linked to lions and hyenas (Figure 4 and Table S5). In most cases, species tended to form MSGs when lions and hyenas were more present / active in an area. This effect concerned, as focal species, zebra, giraffe, wildebeest and kudu for lions and impala, zebra, giraffe, kudu and baboon for hyenas. Note that for lions, the effect was significant for the zebra-giraffe pair, whether they are considered as focal or interacting species. For hyenas, a similar observation could be made for impala-baboon as the pair of species.

**Discussion**

The reason species form MSGs is often considered to be associated with a reduction in predation risk or a trophic benefit. In the present study, we analyzed the factors that affected the likelihood of the formation of such polyspecific groups in some south African savannah ecosystems, by focusing on the most frequent pairs formed in our study sites and on the influence of multiple predators. Our study focused on the most frequent pairs of species observed within the study area and not on ungulates alone.

**Species photographic rates and MSGs**

The photographic rate of the interacting species influenced the formation of MSGs for most of the interspecific dyads. When the interacting species’ photographic rate increased so did the number of MSGs formed by the focal species with this species. This observation is expected as the probability of meeting another species increases with the abundance / activity rate of the species (Hutchinson and Waser 2007; Waser 1982), especially when species share the same habitat or the same trophic resources. Beyond this effect of abundance / activity rate of the interacting species, other factors predict part of the variance of the probability of forming MSGs. The abundance / activity of predators is, as hypothesized under “the stress gradient hypothesis”, one of them.

**Multiple-predator stress and MSGs**

The presence of predators had a significant positive effect on the formation of MSGs. Most effects were observed for lions and hyenas and were almost always in the same direction. As the predator photographic rate increased so did the probability of forming MSGs. Only two significant effects were found for other predators like leopards or wild dogs when considering each pair of MSG but these effects were opposites (i.e. a reduction of the formation of MSG in the presence of a predator).

Our study suggests that lions and hyenas would be more important in triggering the formation of MSGs than the other predators considered in the study, namely leopards and wild dogs. Our results suggest therefore that there might be a hierarchy of fear against different predators and that the response of prey species to their presence (here the formation of MSGs) depends on the predator species.

For lions, this confirms recent studies that showed that anti-predator reactions (e.g. run away, vigilance) were more important against lions than against other predators (leopard, hyena, wild dogs), even for prey species that were not preferred by lions (like impalas) (Palmer and Packer 2021; Epperly, Clinchy, Zanette, and McCleery 2021; Rigoudy et al. 2022).

Why do lions seem to be so scary?Several factors could explain this. First, lions are the largest and most dominant predators in most African landscapes (Creel et al. 2001). Indeed, lions generally dominate the large carnivore guild in biomass (Owen-Smith and Mills, 2008a) and consume a broad range of prey species. Second, lions are ambush predators (also known as sit-and-pursue predators). For such predators, territory and space use are generally more stable and predictable than for actively hunting and cursorial predators like hyenas or wild dogs. Given that cues (e.g., visual, acoustic and olfactory) from territorial predators provide more information about the possible presence of predators, and therefore of the immediate local predation risk (Lima and Bednekoff 1999), antipredator responses are often expected to be stronger in response to ambush predators than against cursorial predators (Schmitz 2005, Preisser et al. 2007).

The effect of hyenas on the formation of MSGs is more surprising. Although hyenas are important predators in African savannah ecosystems (Owen-smith and Mills 2008), the few studies that have experimentally studied the response of herbivores to their presence or to cues of their presence have noted fewer reactions against them than against other predators (like lions or leopards) (Epperly, Clinchy, Zanette, and McCeery 2021; Creel et al. 2019). Other studies nevertheless revealed some behavioral modifications of certain ungulates species when hyenas were present in the vicinity. Thus, Pays *et al.* (2021) demonstrated that impalas increased their vigilance when lions and hyenas were both present in the surroundings and that this effect was stronger than when lions were present alone. They did not obtain enough data to test the effect of hyenas alone but the fact that the largest increase of vigilance was observed when the two predators were present suggested a substantial effect of hyenas. In another study, Creel, Schuette and Christianson (2014) analyzed the response of different ungulate species to predation risk. They noted that changes in grouping behavior and proportion of adults foraging were stronger in the presence of hyenas than in the presence of lions, again suggesting that hyenas could be ranked higher in this hierarchy of fear. Comparative studies of anti-predator response against multiple predators, including hyenas remain nevertheless scarce and more studies should be done to understand the role of this predator in triggering anti-predator behavioral response.

**Direct or indirect predation risk assessment**

Why do prey species form MSGs more frequently under higher levels of predation risk? The underlying mechanisms that trigger the formation of these MSGs in savannah mammal communities are still poorly understood ﻿(Kuijper et al. 2014). In particular, how prey species assess or perceive predation risk caused by carnivores is unclear. This can either be indirectly through habitat characteristics or directly through the recognition of cues left by predators, or both. It has been recurrently shown that risky habitats (like habitats with low visibility) tend to be avoided or generate anti-predator behaviors from prey species (e.g. increased vigilance) (Thaker et al. 2011; Creel, Schuette, and Christianson 2014; Périquet et al. 2012). This is especially true when ambush predators like lions are present in the ecosystem. The formation of MSGs has also been shown to be, at least partly, influenced by habitat. ﻿Thus, in the Serengeti, Beaudrot *et al.* (2020) found that MSGs were more likely to occur in woodland habitat (where lions preferentially hunt) and closer to kopjes (rocky outcrops that lions use to scope their prey) but did not find a direct relationship between lion densities and the formation of MSGs. This is also in accordance with results from Creel, Schuette and Christianson (2014) who demonstrated that variation in prey group size was more affected by habitat type (which is a correlate of long-term predation risk) than directly by predator presence. In our study (but also in Kiffner *et al.* (2022)), the habitat was not characterized and we could not determine whether it played a role in the formation of the MSGs. Contrary to Beaudrot et al., (2020), we did find a positive relationship between the formation of MSGs and the abundance of predators. That the presence and abundance of predators are directly associated with the formation of MSGs suggests that, in our system, prey may be able to directly respond to cues (visual, acoustic or olfactory) left by predators. Obviously, one could also argue that the habitats where predators are the most present are also the habitats where the perceived risk is the highest for prey species (like woodland habitats) and both hypotheses could be true. Further studies will be needed to disentangle both possibilities.

**What species form MSGs in response to predators?**

In our study, we observed a higher probability of forming MSGs in the presence of predators (and after correcting for the abundance / activity of each species) for impala, zebra, giraffe, wildebeest, kudu and baboon as focal species and zebra, giraffe, wildebeest, baboon and warthogs as interacting species. The formation of MSGs and how species associate in heterospecific groups likely results from trade-offs between the probability of detecting a predator, the dilution benefit in case of attack of a predator and the competition for resources between species. According to Meise et al. (2020), who analyzed the factors influencing the formation of polyspecific groups in one African savanna ecosystem, species with low vigilance, high vulnerability to predation and high responsiveness to heterospecific alarm calls tend to be more prone to form MSGs than the other species.

In our study, zebras were included in five out of 10 species pairs showing more association in the presence of predators, either as focal or interacting species. Zebras show significantly higher rates of association with wildebeest, giraffe and kudu in the presence of predators. Zebras are not considered highly vulnerable to predation (except from lions) but are considered low-vigilant species, which may explain why they tend to associate with other species like wildebeest or giraffe in the presence of predators. Studies by Schmitt *et al.* (2014) and Schmitt, Stears and Shrader (2016) indeed indicate that, for zebras, the association to both species, which are also preferred lion prey species, tend to induce a reduction in their vigilance. This reduction of vigilance from zebra, in the presence of wildebeest, can be explained by two non-mutually exclusive hypotheses, according to Schmitt *et al.* (2014). First, they do so because wildebeest would present complementary predator detection sensory modes (like olfaction), and thus their presence could increase the ability to detect predators. Second, wildebeest could also be a preferred prey for lions, compared to zebras, and thus represent a good risk-dilution partner. On the contrary, the authors did not see a decrease in vigilance when zebras were forming mixed herds with impalas, a non-preferred prey for lions. The explanation of their association with giraffe is likely a result of the ability of the giraffe to detect large predators due to their height, their good eyesight and high levels of vigilance, given that giraffe are only rarely preyed upon by lions (Schmitt et al. 2014).

Another species association that increased in the presence of predators (especially hyenas) is the association between impalas and baboons. This association is frequently observed in savannah ecosystems (Kitchen et al. 2010; Davis and Ebersole 2015). From the analyses of Davis and Ebersole (2015), impalas tend to follow baboons (and not the opposite) because they facilitate access to resources they could not obtain without the indirect help of baboons (like flowers and fruit of the sausage tree) but also because baboons could detect predators more readily and help them reduce their vigilance and predation risk. This agrees with our observations. Thus, impalas responded to baboon alarm calls more efficiently than other ungulate species (Kitchen et al. 2010).

Beyond the anti-predator benefits, competition for resources likely plays a role in the probability of forming heterospecific groups. As Meise et al. (2020) observed, species that tend to have overlapping diets tend to avoid forming MSGs. The importance of this competition may nevertheless vary with the time of year and be reduced when resources are abundant. This reduction of competition should translate into higher probabilities of forming heterospecific groups even for species with overlapping diets. This may be the case in our study for dyads involving zebras and impalas. For these species, the probability of forming an MSG was higher in the wet season than during the dry season. Zebras and impalas may both feed on grass of the same height, which can result in higher levels of competition for food between them (Arsenault and Owen-Smith 2008). On the contrary, wildebeest may feed on different sizes of grass than zebras and impalas (Arsenault and Owen-Smith 2008). Competition for food should then be less of a problem between these species. The higher association of wildebeest with these two other species during the dry season should therefore be due to other factors. That wildebeests have a higher propensity to form MSGs with several other species during the dry season was also found by Kiffner *et al.* (2014), but no explanation was provided. Further research should be performed to understand why they tend to form more heterospecific mixed-herds during the dry season.

**Conclusion**

In conclusion, our study demonstrates that the formation of MSGs is more likely to occur in the presence of predators but that not all predators influence their formation. We observed that lion and hyena are the main species driving the formation of these heterospecific groups, thus suggesting that there is a hierarchy of fear for different predators and that the response of prey species to their presence is dependent on the predator species, thus confirming previous observations on other anti-predator behaviors (such as vigilance). Our study also demonstrates that the formation of certain MSGs under predator pressure is balanced by the competition between prey species for resources and that the likelihood of forming MSGs may increase when resources are abundant.

**Acknowledgements**

We thank all the Zooniverse volunteers who contribute classifications to Snapshot Safari, and the moderators who donate their time and expertise to our projects. We also thank sponsors of our work, including the Centre National de la Recherche Scientifique (CNRS), the South African National Biodiversity Institute (SANBI), Foundational Biodiversity Information Programme (FBIP), South African National Research Foundation (NRF), Fynbos Trust, Nelson Mandela University, Fairfields, Detroit Zoological Society, Zoo Miami, Cincinnati Zoo Angel Fund, Seneca Park Zoo, and The Living Desert. We thank the Minnesota Supercomputing Institute for providing resources for data storage and processing, among others. Finally, we thank all the people and institutions participating in Snapshot South Africa, including the reserve managers and owners for providing access and supporting this programme, National Parks Institutions of every country, students and volunteer groups helping to maintain the grids, and the governmental institutions overseeing these parks and reserves.

**Author contributions**

F. Prugnolle, V. Rougeron, L. Pardo, PC Renaud, R. Davis and H. Fritz conceived the project idea. F. Prugnolle analyzed the data with input from V. Rougeron, L. Pardo, L. Nicvert, P.C. Renaud, R. Davis and H. Fritz. M. J. Somers, M.J.S. Peel, L.H. Swanepoel, M. Keith, J. Vanter and H. Fritz developed the *Snapshot Safari project South Africa* and provided the camera trap data. F. Prugnolle led the writing of the article with input from all co-authors.

**References**

Arsenault, Randal, and Norman Owen-Smith. 2008. “Resource Partitioning by Grass Height among Grazing Ungulates Does Not Follow Body Size Relation.” *Oikos* 117 (11): 1711–17. https://doi.org/10.1111/j.1600-0706.2008.16575.x.

Bates, Douglas, Martin Mächler, Benjamin M. Bolker, and Steven C. Walker. 2015. “Fitting Linear Mixed-Effects Models Using Lme4.” *Journal of Statistical Software* 67 (1). https://doi.org/10.18637/jss.v067.i01.

Beaudrot, Lydia, Meredith S. Palmer, T. Michael Anderson, and Craig Packer. 2020. “Mixed-Species Groups of Serengeti Grazers: A Test of the Stress Gradient Hypothesis.” *Ecology* 101 (11): 1–11. https://doi.org/10.1002/ecy.3163.

Butts, Carter T. 2008. “Network: A Package for Managing Relational Data in R.” *Journal of Statistical Software* 24 (2): 1–36. https://doi.org/10.18637/jss.v024.i02.

Creel, Scott, Matt Becker, Egil Dröge, Jassiel M’soka, Wigganson Matandiko, Eli Rosenblatt, Thandiwe Mweetwa, et al. 2019. “What Explains Variation in the Strength of Behavioral Responses to Predation Risk? A Standardized Test with Large Carnivore and Ungulate Guilds in Three Ecosystems.” *Biological Conservation* 232 (February): 164–72. https://doi.org/10.1016/j.biocon.2019.02.012.

Creel, Scott, Egil Dröge, Jassiel M’soka, Daan Smit, Matt Becker, Dave Christianson, and Paul Schuette. 2017. “The Relationship between Direct Predation and Antipredator Responses: A Test with Multiple Predators and Multiple Prey.” *Ecology* 98 (8): 2081–92. https://doi.org/10.1002/ecy.1885.

Creel, Scott, Paul Schuette, and David Christianson. 2014. “Effects of Predation Risk on Group Size, Vigilance, and Foraging Behavior in an African Ungulate Community.” *Behavioral Ecology* 25 (4): 773–84. https://doi.org/10.1093/beheco/aru050.

Davis, Brooke. R., and J.J. Ebersole. 2015. “Note and Record.” *Afr. J. Ecol.* 54: 238–41.

Epperly, Haley K., Michael Clinchy, Liana Y. Zanette, and Robert A. McCleery. 2021. “Erratum: Publisher Correction: Fear of Large Carnivores Is Tied to Ungulate Habitat Use: Evidence from a Bifactorial Experiment (Scientific Reports (2021) 11 1 (12979)).” *Scientific Reports* 11 (1): 14303. https://doi.org/10.1038/s41598-021-93822-4.

Epperly, Haley K, Michael Clinchy, Liana Y Zanette, and Robert A. McCeery. 2021. “Fear of Large Carnivores Is Tied to Ungulate Habitat Use: Evidence from a Bifactorial Experiment.” *Scientific Reports* 11 (1): 12979. https://doi.org/10.1038/s41598-021-92469-5.

Fox, J., and S. Weisberg. 2019. “An R Companion to Applied Regression.” In , edited by CA Sage, Thousand Oaks, Third Edit.

Goodale, Eben, Guy Beauchamp, and Graeme D. Ruxton. 2017. *Mixed-Species Groups of Animals: Behavior, Community Structure, and Conservation*. *Mixed-Species Groups of Animals: Behavior, Community Structure, and Conservation*.

Goodale, Eben, Hari Sridhar, Kathryn E. Sieving, Priti Bangal, Gabriel J. Colorado Z., Damien R. Farine, Eckhard W. Heymann, et al. 2020. “Mixed Company: A Framework for Understanding the Composition and Organization of Mixed-Species Animal Groups.” *Biological Reviews* 95 (4): 889–910. https://doi.org/10.1111/brv.12591.

Hayward, M. W. 2006. “Prey Preferences of the Spotted Hyaena (Crocuta Crocuta) and Degree of Dietary Overlap with the Lion (Panthera Leo).” *Journal of Zoology* 270 (4): 606–14. https://doi.org/10.1111/j.1469-7998.2006.00183.x.

Hayward, M. W., P. Henschel, J. O’Brien, M. Hofmeyr, G. Balme, and G. I.H. Kerley. 2006. “Prey Preferences of the Leopard (Panthera Pardus).” *Journal of Zoology* 270 (2): 298–313. https://doi.org/10.1111/j.1469-7998.2006.00139.x.

Hayward, Matt W., John O’Brien, Markus Hofmeyr, and Graham I.H. Kerley. 2006. “Prey Preferences of the African Wild Dog Lycaon Pictus (Canidae: Carnivora): Ecological Requirements for Conservation.” *Journal of Mammalogy* 87 (6): 1122–31. https://doi.org/10.1644/05-MAMM-A-304R2.1.

Hutchinson, John M.C., and Peter M. Waser. 2007. “Use, Misuse and Extensions of ‘Ideal Gas’ Models of Animal Encounter.” *Biological Reviews* 82 (3): 335–59. https://doi.org/10.1111/j.1469-185X.2007.00014.x.

Kiffner, Christian, Diana M. Boyle, Kristen Denninger-Snyder, Bernard M. Kissui, Matthias Waltert, and Stefan Krause. 2022. “Refining the Stress Gradient Hypothesis for Mixed Species Groups of African Mammals.” *Scientific Reports* 12 (1): 1–13. https://doi.org/10.1038/s41598-022-22593-3.

Kiffner, Christian, John Kioko, Cecilia Leweri, and Stefan Krause. 2014. “Seasonal Patterns of Mixed Species Groups in Large East African Mammals.” *PLoS ONE* 9 (12): 1–22. https://doi.org/10.1371/journal.pone.0113446.

Kitchen, Dawn M., Thore J. Bergman, Dorothy L. Cheney, James R. Nicholson, and Robert M. Seyfarth. 2010. “Comparing Responses of Four Ungulate Species to Playbacks of Baboon Alarm Calls.” *Animal Cognition* 13 (6): 861–70. https://doi.org/10.1007/s10071-010-0334-9.

Kuijper, Dries P.J., Mart Verwijmeren, Marcin Churski, Adam Zbyryt, Krzysztof Schmidt, Bogumiła Jedrzejewska, and Chris Smit. 2014. “What Cues Do Ungulates Use to Assess Predation Risk in Dense Temperate Forests?” *PLoS ONE* 9 (1): 1–12. https://doi.org/10.1371/journal.pone.0084607.

Meise, Kristine, Daniel W. Franks, and Jakob Bro-Jørgensen. 2020. “Alarm Communication Networks as a Driver of Community Structure in African Savannah Herbivores.” *Ecology Letters* 23 (2): 293–304. https://doi.org/10.1111/ele.13432.

Owen-Smith, Norman. 2019. “Ramifying Effects of the Risk of Predation on African Multi-Predator, Multi-Prey Large-Mammal Assemblages and the Conservation Implications.” *Biological Conservation* 232 (February): 51–58. https://doi.org/10.1016/j.biocon.2019.01.027.

Owen-smith, Norman, and M G L Mills. 2008. “Predator – Prey Size Relationships in an African Large-Mammal Food Web,” 173–83. https://doi.org/10.1111/j.1365-2656.2007.01314.x.

Palmer, Meredith S., and Craig Packer. 2021. “Reactive Anti-Predator Behavioral Strategy Shaped by Predator Characteristics.” *PLoS ONE* 16 (8 August): 1–17. https://doi.org/10.1371/journal.pone.0256147.

Pardo, Lain E., Sara Bombaci, Sarah E. Huebner, Michael J. Somers, Herve Fritz, Colleen Downs, Abby Guthmann, et al. 2021. “Snapshot Safari: A Large-Scale Collaborative to Monitor Africa’s Remarkable Biodiversity.” *South African Journal of Science* 117 (1–2). https://doi.org/10.17159/SAJS.2021/8134.

Pays, Olivier, Pierrick Blanchard, Simon Chamaillé-Jammes, Marion Valeix, Andrew J. Loveridge, David W. Macdonald, Stéphanie Périquet, et al. 2021. “Disentangling the Roles of Bottom-up and Top-down Drivers in the Trade-off between Food Acquisition and Safety in Prey with Multiple Predators.” *Functional Ecology* 35 (2): 435–49. https://doi.org/10.1111/1365-2435.13710.

Périquet, S., L. Todd-Jones, M. Valeix, B. Stapelkamp, N. Elliot, M. Wijers, O. Pays, et al. 2012. “Influence of Immediate Predation Risk by Lions on the Vigilance of Prey of Different Body Size.” *Behavioral Ecology* 23 (5): 970–76. https://doi.org/10.1093/beheco/ars060.

Phakula, S. 2016. “Modelling Seasonal Rainfall Characteristics over South Africa.”

Rigoudy, Noa L.A., Michael Clinchy, Mike J.S. Peel, Sarah Huebner, Craig Packer, and Liana Y. Zanette. 2022. “Hierarchy of Fear: Experimentally Testing Ungulate Reactions to Lion, African Wild Dog and Cheetah.” *Behavioral Ecology* 33 (4): 789–97. https://doi.org/10.1093/beheco/arac041.

Schmitt, Melissa H., Keenan Stears, and Adrian M. Shrader. 2016. “Zebra Reduce Predation Risk in Mixed-Species Herds by Eavesdropping on Cues from Giraffe.” *Behavioral Ecology* 27 (4): 1073–77. https://doi.org/10.1093/beheco/arw015.

Schmitt, Melissa H., Keenan Stears, Christopher C. Wilmers, and Adrian M. Shrader. 2014. “Determining the Relative Importance of Dilution and Detection for Zebra Foraging in Mixed-Species Herds.” *Animal Behaviour* 96: 151–58. https://doi.org/10.1016/j.anbehav.2014.08.012.

Sollmann, Rahel. 2018. “A Gentle Introduction to Camera-Trap Data Analysis.” *African Journal of Ecology* 56 (4): 740–49. https://doi.org/10.1111/aje.12557.

Stensland, Eva, Anders Angerbjörn, and Per Berggren. 2003. “Mixed Species Groups in Mammals.” *Mammal Review* 33 (3–4): 205–23. https://doi.org/10.1046/j.1365-2907.2003.00022.x.

Syme, Jonathan, Jeremy J. Kiszka, and Guido J. Parra. 2023. “Habitat Partitioning, Co-Occurrence Patterns, and Mixed-Species Group Formation in Sympatric Delphinids.” *Scientific Reports* 13 (1): 1–12. https://doi.org/10.1038/s41598-023-30694-w.

Thaker, Maria, Abi T. Vanak, Cailey R. Owen, Monika B. Ogden, Sophie M. Niemann, and Rob Slotow. 2011. “Minimizing Predation Risk in a Landscape of Multiple Predators: Effects on the Spatial Distribution of African Ungulates.” *Ecology* 92 (2): 398–407. https://doi.org/10.1890/10-0126.1.

Waser, Peter M. 1982. “Primate Polyspecific Associations: Do They Occur by Chance?” *Animal Behaviour* 30 (1): 1–8. https://doi.org/10.1016/S0003-3472(82)80230-3.

**Figure Legends**

**Figure 1. Location of the study sites and main mixed-species groups (MSGs) observed within each park.** A. Location of the study sites on a map of south Africa displaying the range of the main ecological biomes. Each color corresponds to a different biome. Orange: savanna; Red: grassland; light brown: nama karoo; dark brown: succulent karoo; light green: fynbos; dark green: thicket. Vegetation map shapefile acquired from http://bgis.sanbi.org/vegmap/biomes.asp . B,C,D,E,F: Network of species interaction obtained within each site based on the frequency of MSGs. Nodes represent each species and the edges between species the frequency at which two are observed together. Only MSGs forming more than 1% of the observed MSGs are represented. B: APNR (Association of Private Natural Reserves); C: KRU (Kruger); D: VEN (Venetia); E: MAD (Madikwe); F: PLN (Pilanesberg). This matrix of frequencies was then used to build a network of interaction using the R-package Network (Butts 2008). Each node represents a species and the edges connecting the nodes the frequency of the MSGs among all MSGs. Only edges representing dyadic pairs representing more than 1% of the MSGs within each site were included in the networks.

**Figure 2.** **Results of the GLM tests performed to determine the effect of season and of the photographic rate of different predator species on the formation MSGs (response variable MSG\_all).** A. *P-values* obtained for each of the explanatory variable (season, lion, leopard, hyena, wild dog) for each focal species. The focal species is on the left of the panel and the explanatory variable is indicated on top of the panel. The size of the circle is correlated to the *p-value*. When the *p-value* is lower than 0.05, one star was put in place of the circle; two stars for *p-value*<0.01 and three when *p-value* <0.001. B. Values of the coefficients (+-95% confidence intervals (CI)) for the significant tests (*p-value*<0.05). Detailed results of GLMs are provided in supplementary Table S5.

**Figure 3. Results of the GLM tests regarding the effect of the photographic rate of the interacting species and of season (dry-wet) on the formation of each MSG (response variable MSG\_pair).** A.*P-values* obtained for the photographic rate of the different interacting species on the formation of each dyadic MSG. In each MSG, the focal species is on the left of the panel and the interacting species is indicated on top of the panel. The size of the circle is correlated to the *p-value*. When the *p-value* is lower than 0.05, one star was put in place of the circle; two stars for *p-value*<0.01 and three when *p-value* <0.001. B. values of the coefficients (+-95% confidence intervals (CI)) for the MSG for which a significant effect of the interacting species was found. Species names on the y-axis are indicated as focal species – interacting species. C. *P-values* obtained for the effect of season on the formation of each dyadic MSG (dry season is the reference season). In each MSG, the focal species is on the left of the panel and the interacting species is indicated on top of the panel. The size of the circle is correlated to the *p-value*. When the *p-value* is lower than 0.05, one star was put in place of the circle; two stars for *p-value*<0.01 and three when <0.001. D. Values of the coefficients (+-95% confidence intervals (CI)) for the MSG for which a significant effect of the interacting species was found. Species names on the y-axis are indicated as focal species – interacting species. Detailed results of GLMs are provided in supplementary Table S5.

**Figure 4.** **Results of the GLM tests performed to determine the effect of the photographic rate of different predator species on the formation of the different dyads.** A. *P-values* obtained for each of the predators (lion, leopard, spotted hyena, wild dog) and each interspecific dyad. In each dyad, the focal species is on the left of the table and the interacting species is indicated on top of the panel. The size of the circle is correlated to the *p-value*. When the *p-value* is lower than 0.05, one star was put in place of the circle; two stars for a *p-value*<0.01 and three for a *p-value*<0.001. Cells are highlighted in red when the effect of the predator on the formation of MSG is positive and in yellow when the effect is negative. B. Values of the coefficients (+-95% confidence intervals (CI)) for the pair of MSG for which a significant (*p-value*<0.05) predator effect was observed. Species names on the y-axis are indicated as focal species – interacting species. Detailed results of GLMs are provided in supplementary Table S5.

**Tables**

**Table 1**. **Information on camera trap data in each of the studied sites.** The number of camera traps per site, the date of the first picture taken in each site (DFP), the date of the last picture (DLP) as well as the number of days separating the first and last picture are provided.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sites | Number of CT | Date of first picture (DFP) | Date of last picture (DLP) | DLP-DFP (in days) |
| APNR | 56 | 28/06/2017 | 26/11/2019 | 881 |
| MAD | 47 | 11/06/2018 | 08/11/2019 | 515 |
| KRU | 20 | 10/04/2017 | 14/09/2019 | 887 |
| PLN | 40 | 07/10/2017 | 26/11/2019 | 780 |
| VEN | 19 | 01/03/2016 | 08/05/2019 | 1163 |

**Table 2. Number of independent pictures presenting single species and mixed species groups (MSGs) all over the studied sites**. Regarding the number of pictures showing MSGs, the values provided are given for each species as focal and interacting species. Note that the number of MSG may vary above and below the diagonal because of the independence criterium used to filter pictures and that select the first MSG occurring in one group of events (sometimes MSGs with two distinct interacting species may occur in one group of pictures). NA: not available. The proportion of pictures showing a MSG for each focal species is also provided.



**Table 3. Number of independent predator pictures obtained per reserve.** APNR: Associated Private Nature Reserve; KRU: Kruger National Park; MAD: Madikwe Game Reserve; PLN: Pilanesberg Game Reserve; VEN: Venetia Game Reserve.

