1 To Group or not to group: Group size dynamics and intestinal parasites in Indian peafowl
2 populations

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Abstract

Animals can form groups for various reasons that include safety from predators, breeding and benefits of allo-parental care. However, there are also costs associated with living in a group such as competition for food and/or mates with other members of the group, higher chances of disease transmission, etc. In the current study we explored the links between group size dynamics and intestinal parasites of Indian Peafowl (*Pavo cristatus*) in the context of seasons and food provisioning. Data for group size was collected across three seasons- Pre-monsoon, Monsoon and Post-monsoon at three field sites- Morachi Chincholi, Nashik and Rajasthan. Individual and group sightings of peafowl were noted down along with group size and composition (no. of males, females, adults, juveniles, sub-adults). Faecal samples were collected from food provision and non-provision areas across three seasons- Pre-monsoon, Monsoon and Post-monsoon at same field sites. Parasite load in the samples was quantified using microscopic examination. Group size was significantly higher in Pre-Monsoon season as compared to Monsoon and Post-Monsoon seasons. While, monsoon and post-monsoon seasons had higher parasite prevalence and load as compared to pre-monsoon season. Interestingly, parasite load was significantly greater at food provision sites as compared to non-provision sites while parasite prevalence was comparable. Aggregation of individuals at the food provision sites may influence the parasite transmission and group dynamics in Indian Peafowl.

Key words: Indian Peafowl, Group size, Parasites, Season, Fission-fusion and Food provisioning

Introduction

Animals can form groups for various reasons that include safety from predators (Powell, 1974; Pulliam, 1973; Silk, 2007), finding food (Clark & Mangel, 1986; Silk, 2007), breeding (Silk, 2007), and benefits of allo-parental care (Wittemeyer et al., 2005). However, there are also costs associated with living in a group such as competition for food (Krause & Ruxton, 2002) and/or mates (Silk 2007) with other members of the group, higher chances of disease transmission (Hochberg 1991), more negative interactions such as harassment (Clutton-Brock & Parker, 1995), aggression (Krause & Ruxton, 2002), and becoming more conspicuous to predators (Cresswell, 1994; Krause & Ruxton, 2002). Social groups form when the benefits of being in a group outweigh the costs (Sutton, 2019). These cost and benefits can also be dynamic depending on environmental factors such as resource availability (Sutton, 2019; Wittemeyer et al., 2005), predation risk (Cresswell, 1994; Krause & Ruxton, 2002) and can ultimately influence the decision to be part of the group or not.

In many group-living species, groups can merge (fusion) or split (fission) as they move through the environment (Couzin, 2006). Fusion-fission societies are thought to be good at adjusting to rapid changes in local environment and balancing the cost-benefits of grouping by changing the group sizes (Aureli et al., 2008). Fusion-fission societies have been mostly reported in long-lived and cognitively complex organisms such as dolphins (Smith et al., 2016), giraffe (Sutton, 2019), chimpanzees (Lehmann & Boesch, 2004), Orangutans (van Schaik, 1999) and elephants (Fishlock et al., 2013; Nandini et al., 2017). These type of grouping structures have also been reported in bats (Popa-Lisseanu, 2008; Kashima et al., 2013), guppies (Croft et al., 2004) and avian systems (Silk, 2014). In shorebirds and wildfowl, lower amount of food resources and changes in predation risk during tidal cycles influenced their grouping decisions and consequently, fission-fusion dynamics (Beauchamp, 2010; Inger et al., 2006; Fleischer, 1983). In turn, fission-fusion dynamics are also known to

influence endo-parasite transmission and susceptibility in brown-spider monkey and gorillas (Rimbach et al., 2015; Caillaud et al., 2006). Flocking behaviour affected *Plasmodium* and *Haemoproteus* infections in Afro-tropical birds (Lutz et al., 2015). Parasite infections were also reported to change with respect to season in backyard poultry (Bhatt et al., 2014) and communal goats of Zimbabwe (Pandey et al., 1994).

Indian peafowl (*Pavo cristatus*) is a species that has co-habited human dominated landscapes for centuries in its native geographical range. This avian species is native to Indian subcontinent and has been introduced in many parts of the world relatively recently. Although, it's native habitat is undergrowth in open forest and woodlands near a waterbody, it is also known to occur near farmlands, villages and increasingly becoming common in urban and semi-urban areas (Burton & Burton, 2002). Social organization of feral Indian Peafowl has been studied in context of mating behaviour (Rand et al., 1984), but it is not known if their grouping behaviour changes across space and time. Previous study by Paranjpe and Dange (2019, in press) showed that food provisioning changes feeding behaviours of Indian Peafowl. Provisioning is known to affect parasite infections in racoons (Gompper & Wright, 2005) and elk populations (Cross et al., 2007). Supplementary food available at bird feeders is known to have far reaching consequences on avian ecology in terms of increasing survival during overwintering, enhanced breeding success, changing sex-ratios of offspring in smaller avian species, range expansion of species (Robb et al., 2008). It is not known if provisioning can affect grouping behaviour and parasite infections in Indian Peafowl populations. Therefore in this study, we tried to explore the links between the intestinal parasite infections and group dynamics in Indian Peafowl populations in context of seasonal gradients and food provisioning (Fig 1). The objectives of this study were two-fold:

- 1) To study the effects of season on grouping behaviour and parasite infections in Indian Peafowl.
- 2) To understand the effect(s) of food provisioning on parasite infections in Indian Peafowl populations.

Materials and methods

Study sites:

Study was conducted at following field sites: Morachi Chincholi, Nashik and Rajasthan (villages on the periphery of Ranthambhore Tiger Reserve) from 2016 to 2019. (Table1). Selection of study areas was based on several criteria such as their closeness to human habitation, accessibility to the field sites throughout the year and history of peafowl populations in the area. Areas located outside protected areas and close to human habitation were given preference since food provisioning is likely to happen in these areas and studying the effects of food provisioning on grouping and parasite infections was one of objectives of the study.

According to our estimate, ~30-35 Kg food grains are offered to peafowl per day in the village and surrounding areas of Morachi Chincholi, while ~15Kg grains are offered per day around homes and temple premises in the villages in Rajasthan included in this study. In both these study areas, grains are offered at designated places everyday throughout the year. Thus, Indian peafowl population in Morachi Chincholi has access to diet rich in carbohydrates (cereals) and proteins (pulses) throughout the year (Paranjpe and Dange, 2019, in press). As much as 71% of their diet consisted of food provisioned indirectly in the form of crops or directly as grains offered in the village surroundings. Villages on the periphery of Ranthambhore Tiger Reserve (RTR), Rajasthan offer relatively less variety of cereals and very few pulses, yet the diet of peafowl in Rajasthan has up to 61% of food provisioned by

humans versus 39% natural food. Peafowl population in Nashik, on the other hand has access to less reliable and less varied food offered by humans (~ 3-5 Kg per day), as a result only about 40% of their diet consists of provisioned food (Paranjpe and Dange, 2019, in press).

Group size

Populations of Indian peafowl were studied throughout the year using direct field observations during morning (6 am to 10 am) and evening (4 pm to 7 pm) at selected field sites. Individual and group sightings of peafowl were noted down along with date, time of day, habitat, group size and composition (no. of males, females, adults, juveniles, sub-adults). Seasons were noted as Monsoon, pre-monsoon and post-monsoon. Arrival of Monsoon rains marks a remarkable transition of seasons on Indian subcontinent. Monsoon season is associated with greater availability of water, food and sowing of kharif crops. Monsoon season for Morachi Chincholi and Nashik is June to September while for Rajasthan it is July-September as monsoon rains start in Rajasthan later than the other two sites. Post monsoon season is October- January, while pre-monsoon season is February to May for Morachi Chincholi and Nashik, February to June for Rajasthan.

Detection of intestinal parasites from faecal samples

Fresh faecal samples were collected from the study areas using clean forceps. The appearance and consistency of the sample was noted down along with the GPS co-ordinates of the location where it was collected. The sample was divided roughly in two equal parts - one part in pre-weighted sample bottle containing 5ml saline and another part in pre-weighted empty bottle. The bottles were weighed again to estimate the weight of faecal sample. Faecal samples of peafowl were subjected to two methods of analysis for estimating parasitic load and diversity.

Method 1: Samples collected in physiological saline (0.83 %-0.9 %) was further processed with 2 % Potassium dichromate ($K_2Cr_2O_7$) to enhance sporulation of cyst forming parasites. Then parasites were isolated using Sheather's Sugar Flootation method (Duszynski & Wilber, 1997).

Method 2: Sample was fixed using 10% formalin and no sporulation agent was used. Further the sample was washed with tap water, centrifuged at 2000 rpm for 5 minutes and re-suspended in 2.79M Zinc Sulfate hepta-hydrate ($ZnSO_4$). The sample was again centrifuged at 2000 rpm for 5minutes (Watve & Sukumar, 1992).

Samples processed in $K_2Cr_2O_7$ enhance the sporulation of cyst forming parasites while the samples fixed in formalin might show both cyst and non-cyst forming parasites. 75-80 micro-litre of processed sample thus obtained using both the methods were subjected to microscopy for quantification and photo-documentation. In the beginning, the parasites were screened in the fields ranging from 11 to 20, to see if, number of fields affect the counts of parasites. As there was no correlation between number of fields sampled and parasite load (Pearson's correlation, $r=0.0837$, $p=0.1925$), 20 fields were screened for each sample thereafter. Parasite load was quantified by sampling 20 fields under the microscope for each method respectively. Parasites were identified at the broader levels- group, family, class and phylum wherever possible using references of identified parasites in literature (Atkinson et al., 2008; Jaiswal et al., 2013). Photos with uncertain identity were not included in the analysis.

Statistical Analysis

All data were analysed using statistical software STATISTICATM version 13.2 (Dell Inc. 2016). Group sizes were compared across habitats, field sites (Morachi Chincholi, Nashik, Rajasthan), time of day (morning, afternoon, evening), and seasons (pre-monsoon, monsoon and post-monsoon) using non-parametric tests as the group size data did not follow normal distribution. Parasite prevalence was calculated as (presence or absence of parasites/total no. of samples screened*100) and was not different across methods ($K_2Cr_2O_7=74\%$ and $ZnSO_4=75\%$, $N=185$, Table 2). Hence, for calculating parasite prevalence, presence/ absence data of parasites detected using both methods was pooled and prevalence was compared across field sites (Morachi Chincholi, Nashik and Rajasthan), season (Pre-Monsoon, Monsoon and Post-Monsoon) and provisioning status (presence/absence of feeding site). Parasite load was calculated as count of parasites in 20 fields for both the methods respectively. As parasite load was not different across methods (Wilcoxon Matched Pair tests, $p=0.74$, $N=185$), it was also pooled for both the methods and calculated by using following formula:

$$\text{Parasite load} = \frac{\text{Total counts of parasite } (K_2Cr_2O_7 + ZnSO_4)}{\text{Total no. of fields scanned for both the methods}}$$

$$= \text{Counts/field}$$

Further, the parasite load was compared across field sites; season and provisioning. Parasite types were identified till family level and categorized into following groups: Eimeriidae, Nematode, Cestode, Trematode and Helminth.

Results

Group size

Group size of Indian peafowl ranged from 1-14 individuals (665 groups observed). 170 out of 665 groups observed (25.56%) comprised of 3 or more individuals. 99 groups contained just two individuals (14.88%), while 396 single individuals (59.54%) were observed during the study period. 57 out of 99 pairs observed (57.57%) had adult males as part of the pair. Out of 396 single individuals, 314 individuals were adult males (79.29%), 54 females (13.63%), 26 sub-adult males (6.5%) and two juveniles (0.5 %). Thus, adult males are mostly seen to live on their own or in pairs while, females, sub adult males and juveniles mostly live in groups of two or more individuals.

Group size was found to be similar across our field sites (Median test, Chi Square=1.992640, df=2, p=0.3692). Time of the day (morning or evening) did not influence the size or composition of the group (Mann-Whitney U test, p=0.08), which indicates that the groups were not momentary assemblages of individuals for the purpose of feeding or movement but relatively long-term associations of at least few days/ months. The group size changed significantly with season (Median test, Chi square=11.4459, df=2, p=0.0024). Multiple pairwise comparisons showed that pre-monsoon (N=314) groups were significantly greater in size compared to groups during monsoon season (N=238) ($p = 0.0024$), while group sizes in post-monsoon season (N=68) were comparable with pre-monsoon and monsoon seasons ($p=0.7796$) (Fig.2).

Intestinal Parasites

Parasite prevalence and load (counts/field) of Indian Peafowl changed according to the season. Parasite load (counts/field) ranged from 0 to 25.54. In pre-monsoon season parasite prevalence was the least (64%) in all study populations, which increased to 91 % prevalence in Monsoon season and 92 % in Post-monsoon season (Table 3). Similar seasonal trend was

seen for parasite load (Fig 3), where parasite load was significantly lower in pre-Monsoon (N=97) season compared to Monsoon (N=81) season (Median test, Chi Square=16.54, df=2, p=0.0003). Multiple comparison tests showed comparable parasite load between post-monsoon (N=66), monsoon (N=81) and pre-monsoon (N=97) seasons (p=1.0000). Parasite prevalence in samples collected at provisioned site (85%) was comparable with samples collected at non-provisioned sites (78%) (Table 4) However, parasite load was significantly higher in faecal samples collected near the food provision sites (N=113) compared to non-provision sites (N=131) (Mann-Whitney U test, $p = 0.004$) (Fig.4). Parasite prevalence (Table 5) and load was not different at field sites-Morachi Chincholi (N=116), Rajasthan (N=65) and Nashik (N=63) (Median test, Chi Square=2.556, df=2, p=0.2786).

The $K_2Cr_2O_7$ method was better for detecting and identifying parasites belonging to family Eimeridae based on the structure of cysts compared to $ZnSO_4$ method. Nematodes were detected with higher percentage in samples processed using $ZnSO_4$ compared to $K_2Cr_2O_7$. The percentages of various parasite types--Eimeridae, Nematode, Cestode, Trematode and Helminth, found in both the methods were comparable across seasons-Pre-monsoon, Monsoon and Post-Monsoon. Similarly, the percentages were also comparable at provisioned and non -provisioned sites.

Discussion

Effect of season on group size and intestinal parasites of Indian Peafowl

Peafowls showed larger group size in Pre-monsoon season as compared to Monsoon and Post-monsoon seasons. Pre-monsoon season consists of dry hot summers at all three field sites. During the summers as vegetation dries up, peafowls can be easily spotted at large distances by their predators/intruders. In Morachi Chincholi and Nashik, feral dogs are often their predators while caracals as well as feral dogs, tigers are said to be the predators of Indian peafowl in Rajasthan (anecdotal). Remaining in larger groups in Pre-monsoon season may, thus, provide safety to them from predators/ intruders or simply reduce the risk of predation due to dilution effect (Foster and Treherne 1981; Creswell, 1994). In another study in Redshanks, the probability of attack by raptors was lesser in individuals staying in larger flocks as compared to individuals staying in smaller flocks (Cresswell, 1994). Group size dynamics was driven by tidal cycles in Light Bellied Geese where their flock size increased with the predation risk (Inger et al., 2006). When the monsoon commences crops and the vegetation grow tall. The vegetation reduces visibility and provides more hiding avenues for peafowls to escape from predation till January-February (post-monsoon). This may reduce the risks of not being part of group and may lead to smaller group size of peafowls in monsoon and post-monsoon seasons.

Parasite prevalence and load in Indian Peafowl was significantly higher in Monsoon and Post-monsoon seasons as compared to Pre-monsoon season. Lower parasite prevalence and load in Pre-Monsoon season may be an indicator of lower transmission as compared to Monsoon and Post-Monsoon seasons. In backyard poultry, the prevalence of endoparasites was highest in the monsoon season (83%) in North Indian region of India (Bhat et al., 2014). Nematode burden was the least in dry season and highest in the rainy seasons in communal land goats of Zimbabwe (Pandey, 1994). Results in the present study indicate association

between season and parasite load and prevalence. Lower group sizes in Monsoon and Post-monsoon season when parasite prevalence/load is higher, might be a strategy to control parasite transmission. Peafowls could get benefits in terms of lower parasite transmission and reduced risk of predation when they were seen in larger groups during Pre-Monsoon season. Control of parasite transmission may be important in determining the integrity of social group composition and regulation of group size (Freeland, 1979). This temporal pattern of fission-fusion of groups in Indian peafowl may indicate the strategies developed to tackle predation risk and parasite transmission. Beyond a simple dependence on group size, however, recent work in the field of network epidemiology has shown that infectious disease spread largely depends on the organization of infection-spreading interactions between individuals (Sah et al., 2017). Group composition/ organization in Indian peafowl populations consisted of adult males, who were seen to live on their own or in pairs while, females, sub adult males and juveniles were seen in groups of two or more individuals. But we were not able to study the network epidemiology in those groups, as we did not have the individual identity of individuals within a group.

Effect of provisioning on intestinal parasites of Indian Peafowl

Samples found at food provisioned sites had significantly higher parasite load than non-provisioned sites. Higher parasite load at provisioning sites may be due to aggregation of groups at provisioning sites and the increased risk of transmission of parasites. Such trend was observed in racoon populations, where the resource distribution was altered through experimental manipulation to enhance aggregation of individuals. This resulted in significant increase of prevalence (up to 54%) of the parasitic nematode *Baylisascaris procyonis* in the experimental population (Gompper & Wright, 2005). Similarly, the brucellosis infection was highly correlated with the duration and aggregation of individuals at the feeding grounds in elk populations (Cross et al., 2007). Nutritional quality of provisioned food may also affect

the parasite load in the host. For example, supplemental feeding of rock iguanas by tourists in the Bahamas with carbohydrate-rich foods such as cereals and grapes was associated with altered nutritional status and increased hookworm burdens (Knapp, 2013). On the contrary, in lace monitor, supplementary feeding in form of accidental urban waste, improved nutrition and lowered intensity of pathogen *Haemogregarina varanicola*. (Jessop et al., 2012). In the current study, aggregation of groups of Indian Peafowl at the provisioned site may have affected their parasite prevalence and load.

Conclusions

Peafowls could get benefits in terms of lower parasite transmission during pre-Monsoon season, hence could be seen in larger groups. This also reduced risk of predation due to dilution effect. Monsoon and post-monsoon seasons showed increased parasite load and prevalence which increased the chances of getting infections hence the peafowl chose to be in smaller groups or stay solitary. This temporal pattern of fission-fusion of groups in Indian peafowl may indicate the strategies developed to tackle predation risk and parasite transmission. Interestingly, parasite load was significantly greater at food provision sites as compared to non-provision sites while parasite prevalence was comparable. Aggregation of individuals at the food provision sites may also influence the parasite transmission and group dynamics in Indian Peafowl.

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Declarations

Authors contributions- DAP conceived the study, designed methodology, collected data and helped in manuscript preparation. PD designed the methodology, collected data, analysed the data and prepared the manuscript. PM collected and analysed the data.

Conflict of interest- Authors declare no conflict of interest.

Permissions- No live animals/ samples were handled during this study. The study was conducted outside protected areas. Therefore, the work did not require special permissions.

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Tables

Table 1: Brief description of study areas

Field sites	GPS co-ordinates and elevation	Provisioning of food grains (in kg)
Morachi Chincholi	18° 48' 26.9" N, 74° 09' 29.3" E, elevation 641m	~30-35
Villages in the vicinity of Ranthambhore Tiger Reserve		
1)Govindpura	26° 04.826" N, 76° 48.097" E, elevation 227m	
2)Bangarda Kalan	26°03'57.78"N, 76°42'35.76"E, elevation 225m	~15
3) Kuttalpara	26° 03' 58.5" N, 76° 26' 13.4" E, elevation 261m	
4) Shyampura	26 01' 31.6" N, 76 23' 20.5" E elevation 265m	
5)Sanwata-Kalakhora	26°06'41.46"N, 76°38'33.81"E, elevation 267m	
Nashik	20° 01'11.0"N, 73° 48'04.0"E, elevation 404m	~ 3-5

Table 2: Comparison of parasite prevalence (in %) between two methods

Methods	Sample size	Parasite prevalence in%
K ₂ Cr ₂ O ₇	185	74
ZnSO ₄	185	75

Table 3: Comparison of parasite prevalence (in %) across seasons

Season	Sample size	Parasite prevalence in%
Monsoon	81	91
PreMonsoon	97	64
PostMonsoon	66	92

Table 4: Comparison of parasite prevalence (in %) across provisioned and non-

provisioned sites

Provisioning	Sample size	Parasite prevalence in%
Non-Provisioned sites	131	77
Provisioned sites	113	84

Table 5: Comparison of parasite prevalence (in %) across field sites

Field site	Sample size	Parasite prevalence in%
Morachi Chinchili	116	74
Nashik	63	87
Rajasthan	65	86

Figure Legends

Fig1. Conceptual framework of the study

Fig 2: Group size of Indian Peafowl across seasons

Fig 3: Parasite load of Indian Peafowl across season

Fig 4: Parasite load at provisioned and non-provisioned sites

Figures

Fig1. Conceptual framework of the study

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