Impact of host sex and age on the diversity of endoparasites and structure of individual-based host-parasite networks in nyalas (*Tragelaphus angasii* Angas) from three game reserves in KwaZulu-Natal province, South Africa

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Helminth communities of nyalas

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Abstract

In recent years, numerous studies have examined the effect of host sex and age on the structure of parasite communities in several host taxa under various environmental conditions and in different geographic regions. However, the influence of such factors on the structure of host-parasite networks has received less attention and remarkably few studies have been carried out on large terrestrial mammals. In this study, we investigated the effects of host age and sex on the parasite infra- and component communities of nyalas (*Tragelaphus angasii*) and on the structure of individual-based nyala-endoparasite networks. We also aimed to evaluate to what extent these effects vary spatially and if they are mediated by conservation management. Based on a data set of internal macroparasites of 74 nyalas from three game reserves in KwaZulu-Natal province, we found that host age strongly influenced parasite community structure as well as the structure of parasite-nyala networks, whereas host sex played a minor role. However, the effects of both host sex and age were mediated by environmental conditions and thus led to different patterns at the three localities. Our findings highlight that host-parasite communities from different localities should not be pooled when conducting host-parasite network and community studies as this may bias results and mask patterns that are typical for a given locality.

Keywords Helminths • Host-parasite interaction • Infracommunities • Nestedness • Similarity • Traits

Key findings

- Helminth parasite communities of nyalas are moderately species rich and mainly composed of directly transmitted parasites.
- Host age is more important than host sex in determining community and network structure.
- Environmental conditions influence helminth transmission and cause local variation of infection patterns.
- Data from different sites should not be pooled for host-parasite network analyses as this could mask locality specific trends.

Introduction

Parasites have the potential to seriously impact host survival, fecundity, and population dynamics, and consequently play an important role in regulating wildlife populations (Tompkins et al. 2002; Citterio et al. 2006; Huang et al., 2014; Esser et al. 2016). Recognition of the importance of understanding host-parasite interactions for successful conservation management has led to several studies in recent years (Pilosof et al. 2015; Hoberg et al. 2008). Amongst other topics, these studies investigated the effect of host sex and age on infection patterns in various host and parasite taxa under various environmental conditions and in different geographic regions (Arneberg 2002; Calvete et al. 2003; Ruiz-Fons et al. 2013; Amundson 2016; Chaisiri et al. 2017; Krasnov et al. 2022).

Historically, several extensive parasite surveys have been conducted to investigate the composition of parasite assemblages in southern African ruminants, including data on parasite abundance, prevalence and seasonality (see Junker et al. 2015 for a review). However, studies that elucidate the structure and dynamics within these ecological communities are still scant. Fellis et al. (2003) and Negovetich et al. (2006) examined the parasite communities of greater kudus (*Tragelaphus strepsiceros*) and impalas (*Aepyceros melampus*), respectively, at two localities in southern Africa. With an emphasis on the influence of host sex and age, they investigated patterns of association, nestedness and co-occurrence in these hosts. In both kudus and impalas, host demographics, i.e. variation in social and feeding behaviour between the different age-sex classes, were important factors in determining the organization of the helminth communities (Fellis et al. 2003; Negovetich et al. 2006). Furthermore, kudus harboured significantly nested parasite assemblages, and biogeography contributed significantly to variation in parasite community structure (Fellis et al. 2003).

To establish if similar patterns of helminth community organization can be found in other large ruminants that form part of host species assemblages in South African conservation areas, we examined a data set produced during parasite surveys of nyalas (*Tragelaphus angasii*) in three game reserves in KwaZulu-Natal province, South Africa, during 1983 to 1984 (Boomker et al. 1991). Most parasites infect more than one host species (Walker et al. 2017; Spickett et al. 2019; Horak et al. 2021) and to fully understand transmission of parasites in multi-host systems, it is important to identify the underlying factors that enable parasite transmission on the level of host populations as well as host individuals (Pilosof et al. 2015; Walker et al. 2017). Nyalas are common in many of the South African conservation areas where their habitat may overlap with other tragelaphine species, especially bushbuck (*Tragelaphus scriptus*) and kudus, but also with impalas and other ungulates (Skinner and Chimimba 2005). In addition, as intermediate mixed feeders (Skinner and Chimimba 2005), they are likely to be exposed to the infective stages of parasites of grazers and browsers alike as seen in impalas (Negovetich et al. 2006). Both traits make nyalas good candidates for parasite sharing or parasite switching (Huang et al.

2014). Perceiving what drives parasite transmission patterns in nyalas, could contribute to a better understanding of epidemiological processes in complex multi-host communities.

In contrast to multiple studies of the effects of host sex and age on parasite diversity and community composition, the influence of these factors on the structure of host-parasite networks has received less attention. Nevertheless, ecological networks have been used to elucidate patterns and processes in a number of species interactions (Campião and Dáttilo 2020; Cardoso et al. 2021; Runghen et al. 2021), including those between hosts and their parasites. Starting from pioneering studies on fish-endoparasite networks (Poulin and Guégan 2000; Bellay et al. 2015), the structure of host-parasite networks has been investigated for rodents (Chaisiri et al. 2017; Rynkiewicz et al. 2019), amphibians (Campião and Dáttilo 2020; D'Bastiani et al. 2020), reptiles (Benítez-Malvido et al. 2019) and even cetaceans (Bellay et al. 2020). Although the number of studies on the structure of host-parasite networks is consistently growing, variation in the structure of these networks (e.g., nestedness) in relation to host sex or age is poorly understood. To the best of our knowledge only a single study compared nestedness in host-parasite networks between male and female hosts of different ages and found that nested patterns were more pronounced in parasite infracommunities of older than in younger pilot whales (Bellay et al. 2020).

In the present study, we therefore aimed to investigate the effects of age and sex on (a) the composition of endoparasite communities harboured by individual nyalas and (b) individual-based nyala-endoparasite networks. We also aimed at understanding whether these effects vary spatially and how conservation management may mediate these effects. First, we asked whether parasite infracommunities in terms of parasite counts and species richness differed between young and adult male and female nyalas taken from different reserves. Secondly, we determined whether species composition of parasite infracommunities differed between nyalas of different sex and age from different reserves. Thirdly, we estimated the non-random structural patterns in individual-based nyala-endoparasite networks for young and adult male and female nyalas from different reserves. Finally, we tested for the associations between sex and age of individual nyalas and their importance in nyala-endoparasite networks (i.e., their roles in parasite sharing and transmission).

Materials and methods

Host species

Nyalas are non-territorial, medium-sized antelopes, with females (54.9–68.1 kg) being distinctly smaller than males (92.5–126.5 kg). They are gregarious and while typically found in small groups of 2–3, herd size can reach 30 animals of mixed sexes at preferred feeding sites. However, these associations are not long lasting. Family or female groups, comprising one or several females with their offspring, are the most stable groupings. Males are predominantly solitary or form transient groupings of 2–3 individuals. In South Africa, nyalas occur naturally in the eastern parts of the Limpopo and Mpumalanga provinces as well as in the eastern parts of KwaZulu-Natal province. They are strongly associated with thickets, which serve as cover, but can otherwise use a wide variety of habitats, such as dry savanna woodland, floodplains, riverine woodlands or dry forest. Nyalas are intermediate mixed feeders and their diet generally varies in response to seasonal changes. They graze extensively when the grass is soft and green after good rains and mostly browse on several plant species, including their leaves, twigs, flowers, and fruits, during the dry season (Skinner and Chimimba 2005).

Study sites

The Ndumo Game Reserve (Ndumo) in the far north of KwaZulu-Natal province encompasses 11,898 ha and is situated between latitude 26° 49’ 55’’ S and 26° 56’ 10’’ S and between longitude 32° 10’ 50’’ E and 32° 21’ 05’’ E. In addition to the Usuthu river, which forms its northern boundary, and the Phongolo river flowing through it, Ndumo is characterized by a number of pans, extensive wetlands and reedbeds. Altitude ranges from approximately 18–170 m.a.s.l., with a mean of ca. 30 m.a.s.l. Because much of Ndumo is low lying, flat land, these areas are often inundated with water when the Usuthu and Phongolo rivers are in flood. Ndumo is part of the summer rainfall region, and the heaviest rains usually fall in the middle to late summer (December to March), with lighter rain in early summer (October to November). The mean annual precipitation ranges between 500–750 mm. Winters are dry, with mild to warm temperatures and no frost, whereas summer temperatures often exceed 40°C. Despite its official proclamation in 1924, human habitation and subsistence agriculture continued in Ndumo until possibly as late as 1969, with significant impact on the vegetation and wildlife population. During this time, the wildlife population was small and comprised mainly bushbuck, red duiker (*Cephalophus natalensis*), hippopotamus (*Hippopotamus amphibius*) and crocodiles (*Crocodylus niloticus*). Fencing of Ndumo and conservation management efforts subsequently led to an increase in numbers and diversity. The vegetation within Ndumo is very varied. Important vegetation units are Floodplain communities (29% of the reserve), Bushland and thicket (35%), *Acacia tortilis* woodland (11%) and Mixed *Acacia nigrescens* wooded grasslands (5%). Approximately 4% of the surface area is covered by open water, pans, and rivers (4%) (Mucina and Rutherford 2006; Ezemvelo KZN Wildlife 2009).

The iMfolozi Game Reserve (iMfolozi) was originally proclaimed in 1895. Together with the Hluhluwe Game Reserve, proclaimed the same year and the Corridor Reserve, proclaimed in 1989, it presently forms the Hluhluwe-iMfolozi Park. With a size of 47,753 ha, iMfolozi is the largest of the three sections and stretches from latitude 27° 59’ 55’’ S to 28° 26’ 00’’ S and from longitude 31° 41’ 40’’ E to 32° 09’ 10’’ E. Situated at the junction of the Mozambican coastal plains and the foothills of the KwaZulu-Natal interior, altitude ranges from 130–580 m.a.s.l. As part of the summer rainfall region, most rain falls between October and March. Mean annual precipitation ranges from 650 - 985 mm within the Hluhluwe-iMfolozi Park. Summers are hot and humid, and winters are generally frost-free and mild. Annual temperatures range from ca. 13– 35°C. The vegetation is mostly Zululand Sourveld and Zululand Lowveld, with the remainder comprising grassveld, scarp forest, vleis, wetlands and riverine forests. Since a large percentage of the park comprises savanna, Hluhluwe-iMfolozi Park plays an important role in the conservation of the fauna of the southern extremity of the savanna biome (Boomker et al. 1991; Mucina and Rutherford 2006; Ezemvelo KZN Wildlife 2011).

The Mkhuze Game Reserve (Mkhuze) is also situated in the Maputaland region of northern KwaZulu-Natal, at the southwestern extent of the Mozambican coastal plains as well as the eastern slopes and southern foothills of the Lebombo Mountains. Proclaimed in 1912, it is approximately 40,000 ha in size and extends from latitude 27° 35’ 00’’ S to 27° 44’ 00’’ S and from longitude 32° 08’ 35’’ E to 32° 25’ 15’’ E, and altitude ranges from 130–300 m.a.s.l. Much of its area falls within the Zululand Lowveld vegetation zone and is characterized by broad-leafed woodland interspersed with grasslands and wooded grasslands. Rain falls mainly in summer and the mean annual precipitation ranges from 500–750 mm. Winters are mild and generally frost-free, whereas summers are warm to hot, with mean monthly temperatures ranging from 19°C in July and 33°C in January (Mucina and Rutherford 2006; Hunter et al. 2007; Balme et al. 2010; Govender 2010).

In both iMfolozi and Mkhuze, game populations had been severely impacted by uncontrolled hunting activities in the 19th and 20th century as well as by wildlife eradication programs launched in the early 20th century in an attempt to control trypanosomosis (nagana) in cattle. Removal of wildlife was thought to eliminate the maintenance hosts for the tsetse fly vector. Since then, concerted conservation efforts and re-introduction programs have helped to restore the fauna at both localities. iMfolozi is currently home to some 15 species of wildlife (Boomker et al. 1991; Ezemvelo KZN Wildlife 2011), and in 1990, Mkhuze's wildlife population comprised 14 species of ungulates ranging in size from suni to zebra (Hunter et al. 2007; Balme et al. 2010; Govender 2010).

Host and parasite collection

To regulate wildlife populations, culling formed part of management processes in KwaZulu-Natal game reserves in the 1980s. This presented an opportunity to obtain data on the parasite fauna of various species of wildlife, and from 1983 to 1984, 74 nyalas were made available for parasite surveys (Boomker et al. 1991). In Ndumo, 14 nyalas (4 adult and 2 young males, 4 adult and 4 young females) were examined during April 1983 to May 1984; in iMfolozi, 40 nyalas (13 adult and 4 young males, 14 adult and 8 young females, as well as one unsexed adult) were examined during March 1983 to April 1984, and in Mkhuze, 20 nyalas (6 adult and 6 young males, 5 adult and 3 young females) were examined during March 1983 to May 1984. Animals were shot throughout the year, at roughly 2- to 3-month intervals. Calves, juveniles and subadults were classified as young animals, older animals as adults.

Parasites were collected as described by Boomker et al. (1989). Briefly, the carcass was opened, the abdominal cavity screened for parasites, and the heart, lungs, liver and gastrointestinal tract removed. All organs were examined separately. The gastrointestinal tract was divided into rumen and reticulum, abomasum, small intestine, and large intestine. Aliquots were taken from the ingesta of each of these sections as well as from the lungs and liver, and parasites were collected from the aliquots. Presence or absence of *Taenia* sp. metacestodes was determined by examining incisions of the masseter, triceps and ilio-psoas muscles.

All parasites were identified and counted, and where applicable, aliquot counts were converted into total counts. Parasites were identified using relevant keys and taxonomic works. The reader is referred to Boomker (1991) for taxonomic literature used, as well as to Eduardo (1983) for Paramphistominae, and Sachs et al. (1973) as well as Sambon (1922) for *Linguatula* spp. Within the Strongylida, it is often difficult to distinguish between congeneric females. Consequently, in host individuals where only females of a given genus were present, identification remained at genus level. In cases where males were also present, congeneric females were allocated to the respective species in proportion to the number of male representatives. To date, three species of rumen flukes, *Calicophoron microbothrium*, *Calicophoron calicophorum* and *Cotylophoron cotylophorum*, all belonging to the family Paramphistominae, have been recorded from nyalas (Pfukenyi and Mukaratirwa 2018). These trematodes are morphologically very similar and time constraints did not allow for their identification to species level. However, they also share very similar life cycle characteristics, using freshwater snails of the genus *Bulinus* as intermediate hosts (Le Roux 1930; Pfukenyi and Mukaratirwa 2018), and we have grouped them here as paramphistomines.

Data analysis: Parasite counts and species richness

To test whether parasite counts and species richness differ in dependence of a nyala's sex and age as well as the reserve in which the nyala was sampled, we applied Generalized Linear Mixed Models (GLMM). For the analyses of parasite counts, we selected the most prevalent species. These were the nematodes *Ostertagia harrisi*, *Cooperia rotundispiculum* and *Paracooperia horaki* as well as paramphistomine trematodes (Supplementary Table S1). Prior to running the various models, we tested for the distribution that best fitted the data separately for each of the above-mentioned parasite taxa (hereafter referred to as parasite species) as well as species richness. This was done using the package "fitdistrplus" (Delignette-Muller and Dutang 2015) implemented in the R statistical environment (R Core Team 2021). First, we fitted three discrete distributions (negative binomial, geometric and Poisson) and then selected the best, based on the Akaike Information Criterion (AIK). This was done using maximum likelihood estimation as a fitting method. The best-fitted distributions were negative binomial for parasite count data and Poisson for species richness data. Subsequently, we ran GLMM separately for each parasite species and species richness using the R package "glmmTMB" (Brooks et al. 2017) with options "family=nbinom1" for parasite counts and "family=genpois" for species richness and year of sampling as a random effect. For each parasite species or species richness, we first fitted the model that included all main effects and all interactions. Thereafter, we fitted models with all possible combinations of explanatory variables and interactions between them and selected the best models based on AIC corrected for small sample size (ΔAICc) using function "model.sel" of the R package "MuMin" (Barton 2020). Among all models, we selected those with ΔAICc equal to or smaller than 2 and averaged these models using function "model.avg".

Data analysis: Parasite community composition and similarity

To explore differences in parasite community composition between adult and young male and female nyalas from different reserves we applied non-parametric Permutational Multivariate Analysis of Variance using Distance Matrices (PERMANOVA; McArdle and Anderson 2001) implemented in function "adonis2" of the R package vegan (Oksanen et al. 2020), using the Bray-Curtis dissimilarity and 1000 permutations. To visualize differences in parasite community composition of nyalas in dependence of their sex, age and the game reserve in which they were sampled, we used Non-metric Multidimensional Scaling (NMDS), a non-constrained ordination method (Minchin 1987) with the function "metaMDS" from the "vegan" package. We used the prevalence of each parasite species in each host category (adult males, adult females, young males and young females from each reserve) as a measure of parasite abundance in the input data because (a) parasite distribution across host individuals is aggregated and (b) mean abundance or counts cannot be compared between parasites belonging to different species, families and phyla.

To investigate patterns of functional (dis-)similarity in parasite species composition among nyalas belonging to different sex/age cohorts in different reserves, we used a recently developed ordination approach (Double Similarity Principal Component Analysis, DSPCA) (Pavoine 2019). DSPCA allows us to place species and communities in the same ordination space and to identify species that drive similarity patterns in accordance with their traits. DSPCA considers a matrix of similarities between species based on their traits, while eigen-decomposes this matrix producing a series of independent axes, and places species in the resultant space according to their similarities. The matrix of species by communities is then used to place communities in the centers of the species they contain and to distribute them in the above-mentioned space so that the communities are positioned in the ordination space according to the coordinates of the species that comprise these communities in this space. Subsequently, new axes (principal components) that best describe the similarities between communities based on their species composition are produced and the coordinates of species and communities are projected on these principal components. The latter are orthogonal and reflect (from the first to the last) the ever-decreasing portion of similarities and, concomitantly, the ever-increasing portion of dissimilarity between communities. In the final ordination space of DSPCA, the coordinates of communities and species are bounded between -1 and 1 with species and communities being displayed by arrows, starting from the origin. The more acute the angle between the arrows of any two communities, the higher the similarity between them. A point (and arrow) of a community is directed towards the center of its species. An arrow of a species is directed towards the communities in which it occurs, and the length of the arrow depends on how well this species represents the composition of each community.

We built a community matrix with the nyalas of different sex/age cohorts and sampled in different reserves, as rows and parasite species as columns, with entries being prevalence of a given parasite in a given cohort from a given reserve and parasite trait matrices with parasite species as rows and traits as columns separately for each trait. These traits were (a) life cycle (direct or indirect; two binary variables; see Supplementary Table S2); (b) preferred site of infection within the host (11 binary variables; see Supplementary Table S2) and (c) transmission mode (ingestion, vector-borne, percutaneous invasion; three binary variables; see Supplementary Table S2). Subsequently, we calculated the functional (for each trait separately) similarity between parasite species found in nyalas of different sex/age cohorts from different reserves using the SOchiai index of similarity developed by Pavoine and Ricotta (2014). This was done using the functions “dsimTree” and “dsimFun”, respectively, of the R package “adiv” (Pavoine 2020). Finally, we ran the DCPCA for each trait using the function “dspca” of the package “adiv”.

Data analysis: network structure and indices for individual nyalas

To make our results comparable with the results of earlier studies on the effect of host sex and age on the structure of host parasite networks, we followed the approach of Bellay et al. (2020), and partly Chaisiri et al. (2017). Individual-based nyala-parasite networks were analyzed separately for each reserve, because nyalas in the three reserves obviously belonged to different populations. Each interaction network was represented by a binary matrix with individual nyalas in rows and parasites in columns. For each reserve, we constructed five interaction matrices, namely a matrix with all nyalas, a matrix with female nyalas only, a matrix with male nyalas only, a matrix with adult nyalas only and a matrix with young nyalas only. We did not use matrices for each sex/age cohort because these matrices were too small for reasonable analyses.

To estimate the non-random structural patterns in these networks, we calculated their nestedness, that is a pattern in which the resources (in our case, nyala individuals) exploited by more specialized consumers (in our case parasites) form a proper subset of those used by more generalist consumers (Bascompte et al. 2003, Joppa et al. 2010). Nestedness was calculated as the NODF index (Nestedness based on Overlap and Decreasing Fill; Almeida-Neto et al. 2008) that varies from 0 (not nested, i.e. random network) to 100 (perfect nestedness). We calculated NODF using the function “nestednodf” implemented in the R package “vegan” and tested for its significance using the function “oecosimu” of “vegan” with null model “r1” [preserves row (host individuals) frequencies and uses column marginal frequencies as probabilities of selecting species (parasites)] and 1000 permutations.

We estimated the importance of nyala individuals in each network considered for all nyalas in each reserve using three indices, namely index of individual nyala specialization (d'), individual host strength (IHS) and centrality (C). Individual nyala (host) specialization (d') describes the deviation from a neutral configuration of associations (Blüthgen al. 2006, Blüthgen 2010). In other words, it compares the frequency distribution of interactions with a null distribution when interactions between host individuals and parasites are proportional to their observed total frequencies and may vary from 0 (no specialization) to 1 (complete specialization). IHS is the species-strength index of Bascompte et al. (2006) for hosts, calculated as the sum of the dependencies of each parasite on each individual nyala. d' and IHS were calculated using function "specieslevel" of the R package "bipartite" (Dormann 2011, Dormann et al. 2008). The centrality metric allows one to estimate the role of each individual host in parasite sharing with other host individuals. A higher centrality of a host individual indicates its greater connection with other hosts and, thus, high level of parasite transmission in the network (Morand et al. 2014; Pilosof et al. 2015). To calculate centrality, we first projected each bipartite network to a unipartite network using function "projecting_tm" of the R package "tnet" (Opsahl 2009). Thereafter, we transformed the unipartite network into the network graph object using function "tnet_igraph" of the "tnet" and then calculated centrality as eigenvector centrality using function "evcent" of the R package "igraph" (Csardi and Nepusz 2006).

To test for the associations between the nyalas' sex/age and each of the above three indices, we performed GLMs with the nyalas' sex and age as explanatory variables and each index as a response variable. Because d' and centrality vary between 0 and 1, we applied the option "family=quasibinomial" for these indices in GLMs.

Results

The helminths collected from 74 nyalas in the KwaZulu-Natal game reserves belonged to 19 species in three phyla, representing the four main groups of internal macroparasites in antelopes, namely nematodes, cestodes, trematodes and pentastomes (Supplementary Tables S1 and S2). Nematodes were the most diverse group and comprised members of the Strongylida (n = 11) with direct life cycles as well as members of the Spirurida (n = 3) with indirect life cycles. Trematodes were represented by paramphistomine rumen flukes and the blood fluke *Schistosoma mattheei*. Of the two cestodes present, nyalas are definitive hosts for *Thysaniezia* sp., but act as intermediate hosts for *Taenia* sp., and for the pentastome *Linguatula* sp., whose larval stages were found in the viscera and blood vessels. Of the 74 nyalas examined, 71 (96%) were infected with one or more parasite species, whereas two calves and one juvenile at iMfolozi did not harbour any parasites. Overall species richness at the three localities combined ranged from 0–7 with a mean of 3.45±0.17. Two helminth species were common, with *O. harrisi* having the highest overall prevalence (93.2%), followed by *P. horaki* (51.4%). Helminths with an intermediate prevalence, i.e. infecting less than 50% but more than 10% of the hosts, were *C. rotundispiculum* (45.9%), paramphistomines (37.8%), *Linguatula* sp. (33.8%) and *Haemonchus vegliai* as well as *Setaria* sp. with a prevalence of 18.9% each. The remaining species were rare with a prevalence of less than 10%. The four most abundant species, with the highest overall counts at each of the three localities, were *O. harrisi*, followed by *C. rotundispiculum*, paramphistomines and *P. horaki*, however, in Ndumo, *C. rotundispiculum* was more abundant than *O. harrisi*. Species richness and prevalence of individual species varied between the localities, with nyala populations at iMfolozi harbouring the most diverse parasite communities, comprising 16 species, followed by those at Mkhuze with 11 and those at Ndumo with 10 species. Five species, *Gongylonema* sp., *Oesophagostomum* sp., *S. mattheei*, *Taenia* sp. and *Linguatula* sp., where only present in iMfolozi, whereas *Impalaia tuberculata* was restricted to Mkhuze, and *Thysaniezia* sp. was only collected from nyalas in Ndumo. With the exception of *Linguatula* sp., these parasites infected single or very few hosts at the locality in which they occurred (Supplementary Table S1, Fig. 1).

Fig. 1. Map of South Africa, indicating the three sampling sites in KwaZulu-Natal province. ▲ Ndumo Game Reserve; ♦ Mkhuze Game Reserve; ■ iMfolozi Game Reserve

Employing GLMM we investigated the effect of the nyalas' sex, age and geographic origin on parasite numbers. The only difference between male and female hosts was seen in *O. harrisi*, with females harbouring fewer nematodes (Table 1, Fig. 2). Considering the age cohorts, the numbers of *C. rotundispiculum* differed between adult and young nyalas (Table 1, Fig. 2). Counts were higher in young nyalas, and in addition, this parasite was more numerous in young nyalas from Mkhuze and Ndumo than in those from iMfolozi (Fig. 2; note significant coefficient of an interaction term in Table 1). All nyalas within each reserve, harboured similar numbers of *P. horaki*, but these numbers differed between reserves, being lower in iMfolozi than in the other two reserves (Table 1, Fig. 2). The nyalas' age and the reserve from which they were taken, independently affected the numbers of paramphistomines. In all reserves, young antelopes were infected less than adults, while adult nyalas from Mkhuze were more infected than those from Ndumo and iMfolozi (Table 1, Fig. 2). Our model selection procedure returned a single model for parasite species richness, with no effect of a nyala's age or geographic origin, and only a marginally significant effect of sex (coefficient=0.14±0.08, z=1.80, p=0.07). Given that the reference level for the sex factor in the model was "female" and the positive sign of the coefficient, this suggested a tendency of males harbouring richer parasite infracommunities than females.

Table 1 Averaged coefficients of the best generalized linear mixed-effects models with the negative binomial distribution of the effect of a nyala's sex (SX), age (A), game reserve (GR) from which a nyala was taken and their interactions on the numbers of the most prevalent parasite taxa. Random factor in all models was year of sampling. Reference levels of independent variables were female for sex, adult for age and Mkhuze for game reserve. Only significant coefficients are shown

Parasite	Explanatory variable	Coefficient±S.E.	*z*	*p*
Ostertagia harrisi	SX (males)	0.60±0.24	2.43	0.01
Cooperia rotundispiculum	A (young)	1.67±0.81	2.05	0.04
	A (young) x GR (iMfolozi)	-2.14±0.95	2.21	0.03
Paracooperia horaki	GR (iMfolozi)	-0.78±0.36	2.08	0.04
Paramphistomines	A (young)	-1.48±0.66	-2.23	0.02
	A (young) x GR (iMfolozi)	-1.73±0.46	-3.72	<0.001

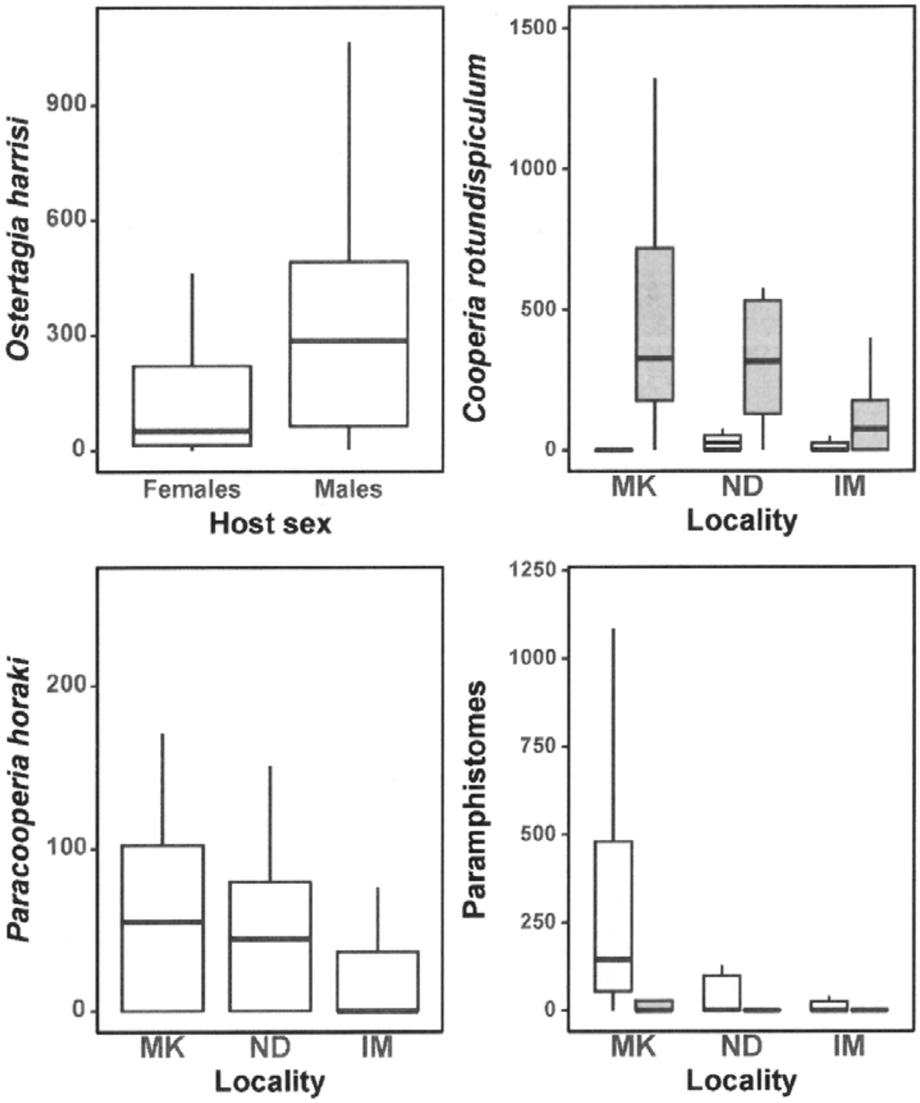


Fig. 2 Median (horizontal line), lower and upper quartiles (b) and maximal and minimal numbers of parasites in adult (white boxes) and young (grey boxes) male and female nyalas from three game reserves (MK – Mkhuze, ND – Ndumo, IM – iMfolozi). Outliers are not shown

Application of PERMANOVA demonstrated that parasite species composition in infracommunities differed between young and adult nyalas as well as between nyalas from different reserves (Table 2; see visualization using NMDS in Fig. 3). Moreover, there was a significant effect of interaction between the nyalas' age and the identity of a reserve (Table 2). Specifically, the difference between adult and young nyalas was more pronounced in Ndumo and iMfolozi than in Mkhuze (Fig. 3).

Table 2 Results of PERMANOVA of species composition in parasite communities harboured by adult and young male and female nyalas from three game reserves

Factor	DF	Sum of Squares	R^2	*F*	*p*
Age	1	1.14	0.12	10.89	>0.001
Sex	1	0.10	0.01	0.95	0.45
Game Reserve	2	1.34	0.14	6.41	>0.001
Age x Sex	1	0.05	0.01	0.51	0.70
Age x Game Reserve	2	0.70	0.07	3.36	>0.001
Sex x Game Reserve	2	0.06	0.01	0.27	0.92
Age x Sex x Game Reserve	2	0.35	0.04	1.65	0.12
Residual	58	6.06	0.62		
Total	69	9.79	1.00		

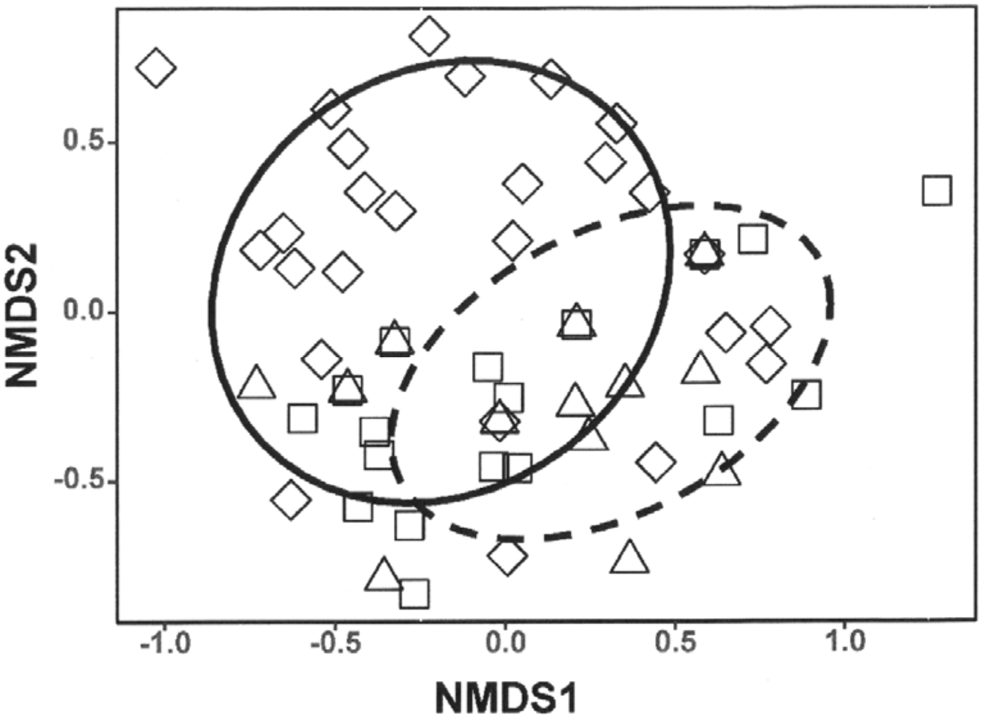


Fig. 3 Biplots of Non-Metric Multidimensional Scaling of species composition of parasite infracommunities harboured by adult (solid ellipse) and juvenile (dashed ellipse) nyalas in Mkhuze (squares), Ndumo (triangles) and iMfolozi (diamonds)

DSPCA demonstrated high functional similarities among parasite communities of adult and young male and female nyalas from different reserves. Average pairwise similarity among communities ranged from 0.76 for species composition when species were considered maximally dissimilar to 0.98 for transmission mode-based similarity (Table 3). Furthermore, each DSPCA produced only one eigenvalue >1 (Table 3). As a result, parasite communities of all sex/age cohorts from all reserves had similar scores on the PC1 for all DSPCA (Figs. 4-5). Nevertheless, the distribution of communities along PC2 suggested some degree of dissimilarity among them. In particular, when DSPCA was based solely on species composition, communities harboured by young antelopes were positioned at positive values of PC2, whereas communities harboured by adult antelopes were characterized by negative

PC2 scores (Fig. 4A). The distribution of parasite species and parasite communities along PC2 of DSPCA based on the preferred site of infection of a parasite within the host allowed us to distinguish between communities of nyalas from Mkhuze and Ndumo (positive scores on PC2 except those from young females from Mkhuze and young males from Ndumo) and those from iMfolozi (negative scores on PC) (Fig. 4C). Distribution of parasite species along PC2 (Fig. 4D) suggested that the former were mainly composed of parasites residing in the rumen, abdominal cavity and heart (Supplementary Table S2), whereas the latter comprised parasites of the small intestine and oesophagus. When DSPCA considered similarity among parasites in terms of life cycle strategy, communities containing many species with indirect life cycles, were mainly characteristic for adult nyalas and those with many species with direct life cycles were mainly seen in young nyalas (Fig. 5A, B). Analogously, when DSPCA was based on similarity among parasites in terms of transmission mode, parasites transmitted via percutaneous invasion and vectors were represented mainly in communities harboured by adult nyalas, whereas parasites transmitted via the trophic route were almost equally represented in parasite communities of nyalas independently of their sex, age or geographic origin (Fig. 5C, D; note values of scores on PC2).

Table 3 Mean similarity (MS) in parasite communities of nyalas and eigenvalues produced by Double Similarity Principal Component Analysis (DSPCA) for trait similarity in these communities. N_t: total number of eigenvalues, N_1: number of eigenvalues > 1

Trait	MS	N_t	N_1	1st eigenvalue	Last eigenvalue
Life cycle strategy	0.93	2	1	11.33	0.66
Preferred site in host	0.87	10	1	10.64	0.001
Transmission mode	0.98	3	1	11.78	0.03

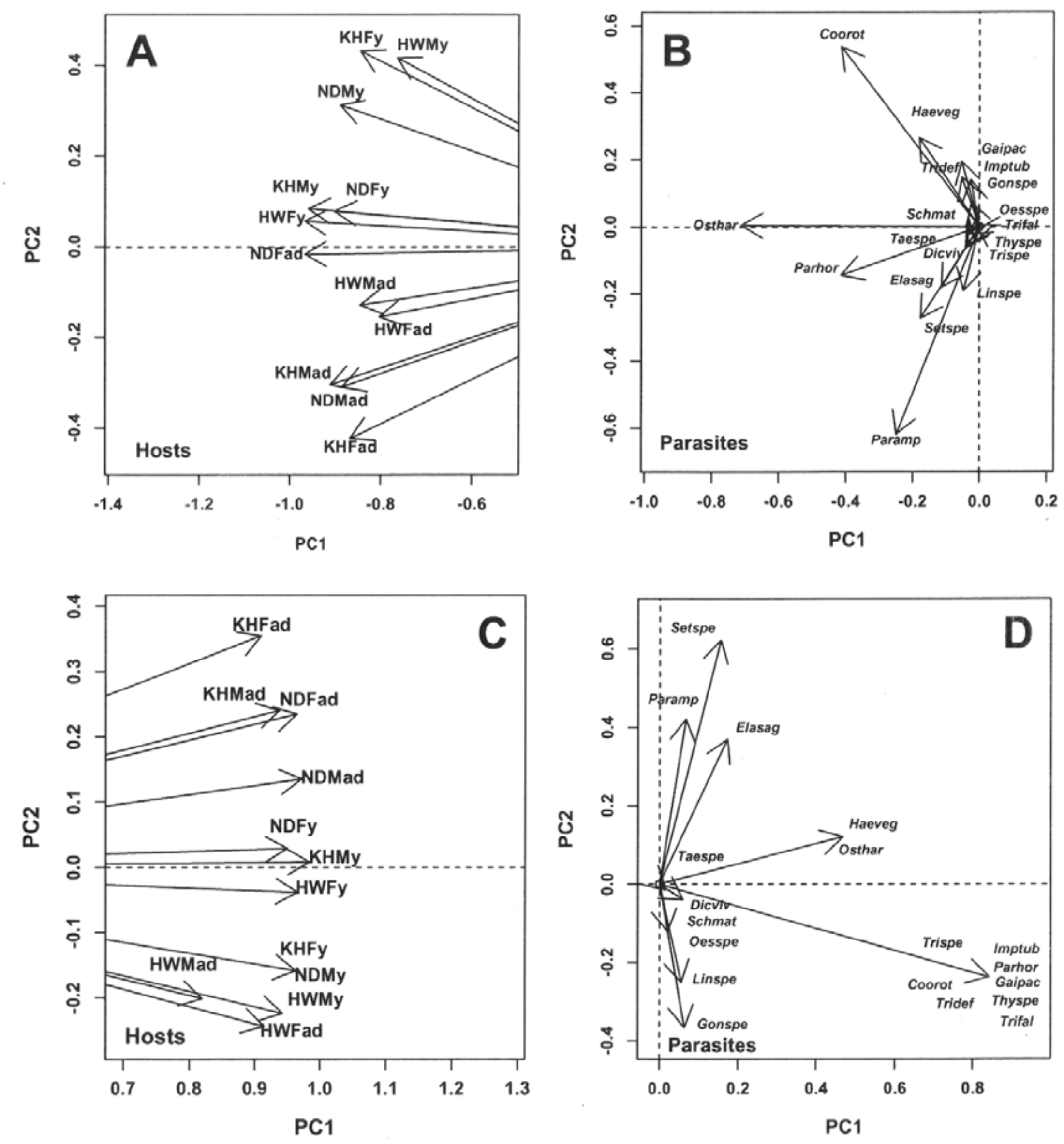


Fig. 4 Results of DSPCA applied to parasite communities in adult (ad in community label) and young (y in community label) male (M in community label) and female (F in community label) nyalas from Mkhuze (KH in community label), Ndumo (ND in community label) and iMfolozi (HW in community label) game reserves, based on parasite prevalence in sex/age cohorts from different reserves either considering parasites as maximally dissimilar (A, B) or based on similarities among parasites in preferred location within a host's body (C, D; see text for details). Hosts: host scores on principal components PC1 and PC2; Parasites: parasites scores on principal components PC1 and PC2. See Supplementary Table S2 for parasite species names

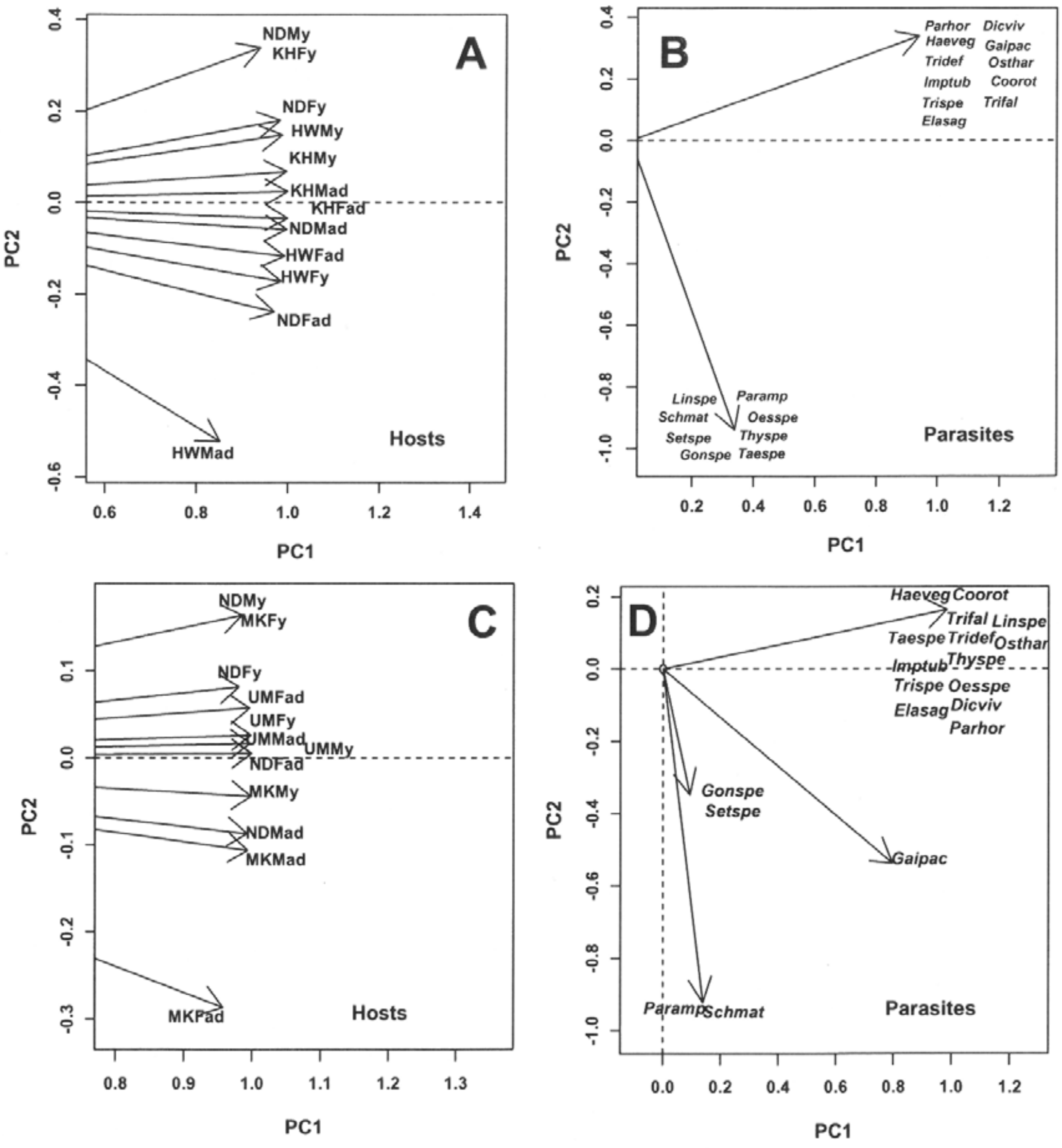
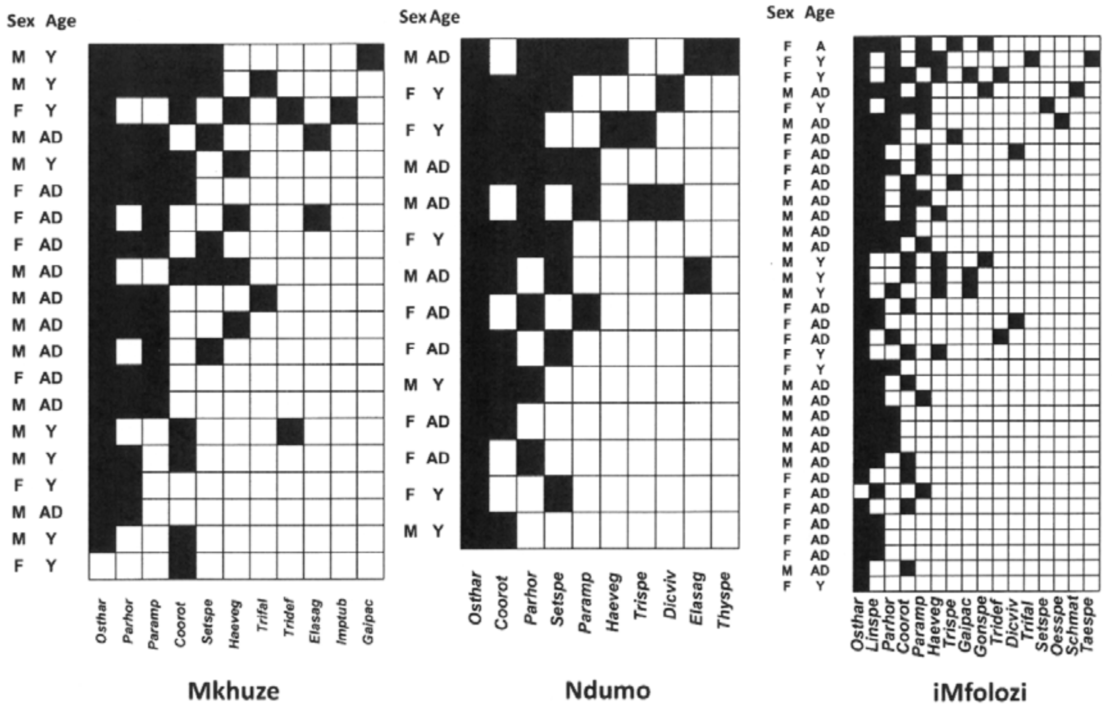


Fig. 5 Results of DSPCA applied to parasite communities in adult (ad in community label) and young (y in community label) male (M in community label) and female (F in community label) nyalas from Mkhuze (KH in community label), Ndumo (ND in community label) and iMfolozi (HW in community label) game reserves based on parasite prevalence in sex/age cohorts from different reserves and based on similarities among parasites either in their life cycle (direct versus indirect; A, B) or transmission mode (C, D; see text for details). Hosts: host scores on principal components PC1 and PC2; Parasites: parasites scores on principal components PC1 and PC2. See Supplementary Table S2 for parasite species names

The various nyala-parasite networks in the three game reserves are visualized in Fig. 6. These networks were significantly nested, with the exception of a network for females in Mkhuze and a network for males in Ndumo (Table 4). Summaries of GLM for the effects of a nyala's sex and age in three game reserves are presented in Table 5. In Mkhuze, values of centrality were higher in adult than young females, while no differences were found between adult and young males (Fig. 7). In Ndumo, IHS was affected by the interaction between sex and age, with values of this index being higher in young females than in adult females and higher in adult males than in young males (Fig. 7). There was no effect of sex or age on *d'* and IHS in Mkhuze and on *d'* and centrality in Ndumo. In iMfolozi, values of both d' and IHS were higher in young than adult antelopes, independently of their sex, whereas values of centrality were higher in male than female nyalas independently of their age.

Table 5 Summary of generalized linear models of the effect of a nyala's sex and age and their interaction on individual nyala specialization (*d'*), individual host strength (IHS) and centrality (C) in three game reserves (see text for explanations). Models for *d'* and C were with quasibinomial distribution and logit link. Models for IHS were with gaussian distribution and log link. Reference levels of independent variables were female for sex and adult for age. Only significant coefficients are shown

Reserve	Dependent variable	Explanatory variable	Coefficient±S.E.	*z*	*p*
Mkhuze	C	sex x age	-1. 49±0.63	-2.34	0.03
Ndumo	IHS	sex x age	-1.14±0.47	-2.41	0.03
iMfolozi	*d'*	age	0.95±0.36	2.66	0.01
	IHS	age	0.41±0.19	2.19	0.03
	C	sex	0.80±0.27	2.89	0.03



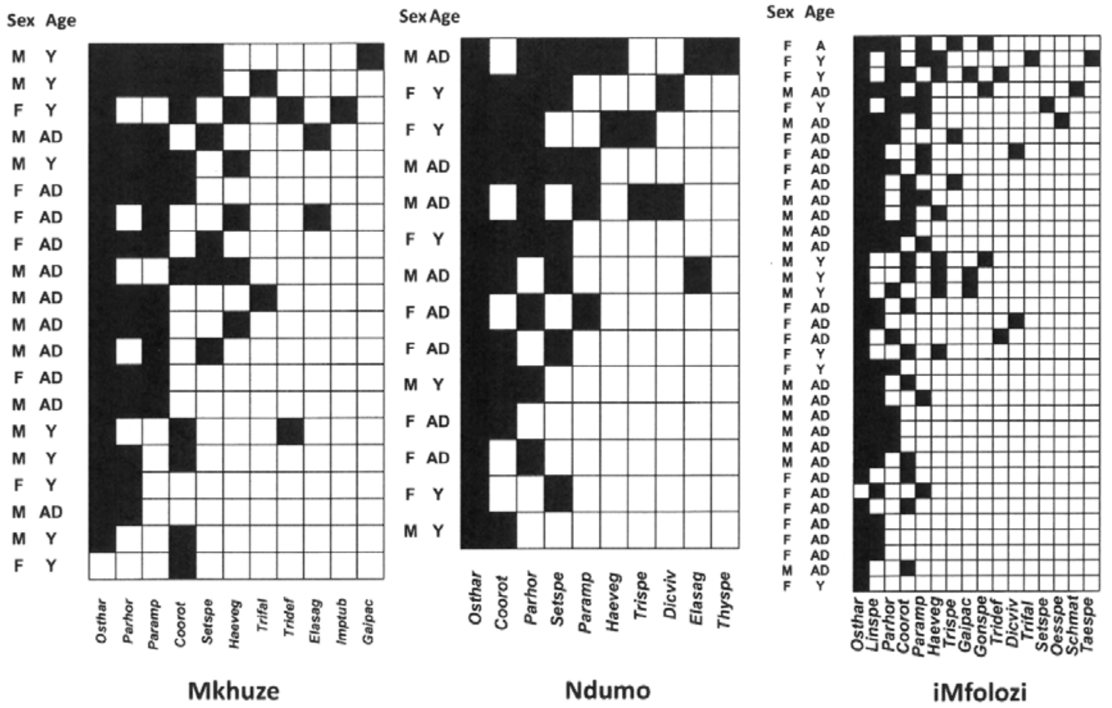


Fig. 6 Nested [i.e., sorted by row/(column sums)] network matrices of individual nyala–parasite associations in three game reserves based on presence/absence data. M and F – male and female nyalas, respectively; AD and Y – adult and young nyalas, respectively. See Supplementary Table S2 for parasite species names

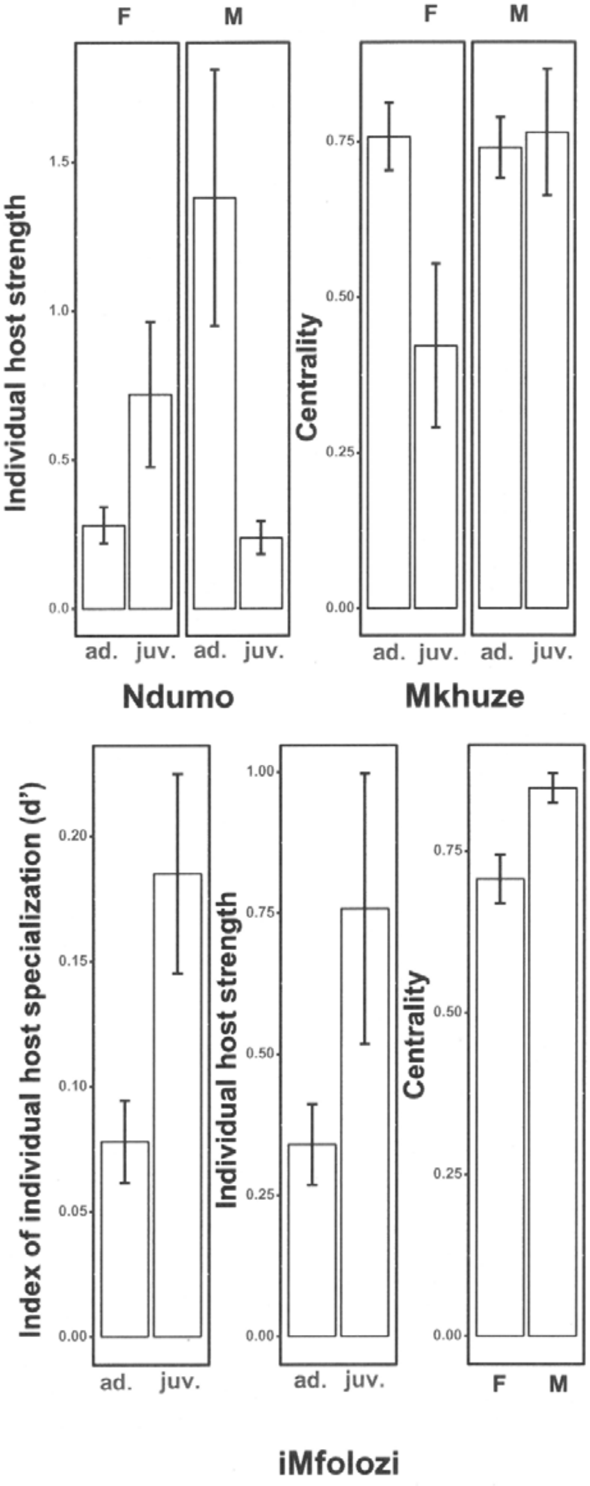


Fig. 7 Mean (±SE) values of individual nyala specialization (*d'*) and centrality in adult and young male (M) and female (F) nyalas from Mkhuze, Ndumo and iMfolozi

Discussion

The parasite communities of nyalas were moderately species rich and largely dominated by nematodes with direct life cycles. Six species were shared between the three localities, while others occurred at one or two localities only. *Linguatula* sp. and *Taenia* sp. occurred in iMfolozi, but were absent in Mkhuze and Ndumo. Parasites can only exist where their hosts exist (Poulin 1997; Harris and Dunn 2010), and both *Linguatula* spp. and *Taenia* spp. depend on large predators as definitive hosts (Verster 1969; Shamsi et al. 2020). Lions are a definitive host, and although present at iMfolozi (https://news.cision.com/ethos-marketing/r/the-pride-of-mkhuze---lions-return-to-mkhuze-game-reserve-in-kwazulu-natal--south-africa,c9534082), were absent from Ndumo and Mkhuze at the time of sampling (Ezemvelo KZN Wildlife 2009, 2011).

Adults supported larger numbers of paramphistomines than young nyalas. Higher parasite burdens in older animals have often been associated with an accumulation of parasites during the host's lifetime or different dispersal patterns leading to higher probabilities of parasite encounters (Calvete et al. 2003; Fellis et al. 2003; Negovetich et al. 2006; Nielsen et al. 2020). In addition, adult nyalas in Mkhuze hosted higher burdens than adults in Ndumo and iMfolozi. Paramphistomines recorded from nyalas use freshwater snails of the genus *Bulinus* as intermediate hosts (Le Roux 1930; Pfukenyi and Mukaratirwa 2018). Transmission success is influenced by host (snail) density (Calvete et al. 2004), which might be mitigated by environmental conditions that influence snail survival or by the presence of competitive species at the three localities; the invasive mollusc *Physa acuta* negatively affects the distribution of *Bulinus africanus* in the Phongolo River System (du Preez et al. 2016).

The influence of host sex, age and locality on abundance differed between the four most prevalent helminth species. The only effect of host sex on parasite numbers was seen with *O. harrisi*, where female nyalas had lower burdens than males. Host sex-related differences in the abundance of helminth parasites are often attributed to behavioural and/or physiological differences between males and females (Ruiz-Fons et al. 2013; Isomursu et al. 2006; Amundson et al. 2016). Higher parasitism in males has been associated with larger body size, higher vagility and decreased immune competency because of higher testosterone levels (Zuk and McKean 1996; Arneberg 2002; Moore and Wilson 2002; Decristophoris et al. 2007; Wirsing et al. 2007). Sexual dimorphism is distinct in nyalas, with males being larger than females (Skinner and Chimimba 2005). Larger males offer a larger habitat for colonization by parasites (Gallivan and Horak 1997) and likely ingest larger quantities of food to maintain higher body mass, thus increasing the possibility of exposure to parasites. In fact, male nyalas in 24 matched pairs of male and female animals from the three reserves, harboured significantly more *Rhipicephalus appendiculatus* male and female ticks ($p \leq 0.002$ and ≤ 0.005), biting lice ($p \leq 0.001$) and hippoboscid flies ($p \leq 0.001$) than females (Horak et al. 1995).

Higher infection rates of abomasal nematodes have also been recorded in male alpine ibex (*Capra ibex*) than in females (Martínez-Guijosa et al. 2015). Ferrari et al. (2010) modelled the host-parasite interaction of an alpine ibex population using increasing levels of sexual segregation, and found that the pattern of habitat use that was most common in nature, i.e. females preferring more segregated sites, reduced parasite impact on the entire ibex population. Similarly, the partial social segregation seen in nyalas, where females and their offspring form the most typical groupings (Skinner and Chimimba 2005), might minimize overall infection rates with *O. harrisi*.

Cooperia rotundispiculum was more abundant in young nyalas then in adults, while young animals in Mkhuze and Ndumo harboured higher burdens than young animals in iMfolozi. Acquired host resistance to infection with Strongylida in older animals is well documented (Reinecke 1983; Pletcher et al. 1988; Balic et al. 2000), and may have contributed towards the lower infection levels in adult nyalas. Dietary differences and a yet poorly developed immune system in young animals are widely accepted as resulting in lower resistance and higher worm burdens (Fellis et al. 2003; Sitko and Heneberg 2015).

The fact that nyalas will graze whenever palatable grass is available might explain the lower burdens of *C. rotundispiculum* in young nyalas in iMfolozi when compared to Mkhuze and Ndumo. Environmental conditions in iMfolozi that reduced the availability of grazing in favour of browse, or decreased the survival of free-living infective stages, could have lessened the exposure of young nyalas to infective larvae. Humidity and temperature are important environmental factors for the survival of the free-living stages of a number of parasites, with different thresholds for different species (Beveridge et al. 1989; O'Connor et al. 2006). Environmental factors could also have limited the numbers of *P. horaki* in nyalas in iMfolozi when compared to those in Mkhuze and Ndumo.

Contrary to several reports on host sex bias in parasite species richness in vertebrate hosts (Kiffner et al. 2014; Martínez-Guijosa et al. 2015), host sex had a weak influence on helminth parasite species richness in nyalas, with males tending to harbour slightly richer parasite infracommunities. Typical factors associated with males encountering a wider variety of parasites and being more susceptible to infection are their larger body size, higher vagility and larger home ranges, as well as the effect of immunosuppressive hormones (Folstad and Karter 1992; Martínez-Guijosa et al. 2015; Altizer et al. 2006). While sexual dimorphism in nyalas is pronounced, they are not territorial antelope and home ranges between and within sexes often overlap (Skinner and Chimimba 2005), raising the likelihood for the exchange of parasites while sharing the same habitat at preferred feeding sites or water sources.

Species and functional (in terms of parasite traits) composition of infracommunities differed mainly between ages and reserves, in that we observed significant differences between young and adult nyalas, between the different localities as well as an interaction between age and locality. More diverse feeding habits are often associated with richer helminth communities (Santoro et al. 2012). Young animals that experimentally consume a wide variety of vegetation (Frost 1981), and whose immune systems are still naïve, will likely ingest parasite species that do not form part of the typical species complement seen in older hosts (Negovetich et al. 2006). In addition, the acquisition of immunity in older animals may eventually prevent colonization with certain parasites (Nielsen et al. 2020).

Habitat and environmental characteristics are important factors in structuring the composition of parasite communities (Calvete et al. 2004; Chaisiri et al. 2017; Bellay et al. 2020). In each reserve, historical events had a major impact on its wildlife. Human settlement and livestock farming persisted in Ndumo as recently as 1969 (Ezemvelo KZN Wildlife 2009), and is likely reflected in the presence, albeit at low prevalence and intensity, of *Thysaniezia* sp., common cestodes in particularly older sheep and cattle (Reinecke 1983).

Wildlife eradication programs launched in the early 20th century to control nagana severely affected both Mkhuze and iMfolozi, whereas at Ndumo, concerted conservation efforts and wildlife re-introduction programs helped restore some of the original fauna (Boomker et al., 1991; Govender 2010; Ezemvelo KZN Wildlife 2011). Variance in the abundance and presence of wildlife species that overlap the home range of nyalas might provide different opportunities for parasite sharing in the three reserves (Huang et al. 2014).

Intermediate host diversity could also shape parasite communities at local level (Calvete et al. 2004). Tabanid flies are vectors of *Elaeophora* spp. (Boomker et al. 1989;

Baldacchino et al. 2014), and Esterhuizen (2006) recorded variations in the species diversity and composition of populations of tabanids in two conservation areas in Kwa-Zulu Natal. The absence of *E. sagitta* in iMfolozi may thus reflect the absence of the preferred vector fly species.

Infrapopulation differences between adult and young males were more pronounced in Ndumo and iMfolozi than in Mkhuze. The majority of parasites infecting nyalas in the three reserves are monoxenous nematodes. Their migration and survival are directly influenced by temperature, humidity and vegetation cover and modulated by species specific tolerances to environmental challenges (Heckler and Borges 2016). Consequently, disparities in the species composition of nyala infracommunities between the sites may stem from differences in climate and vegetation.

Parasite communities in young nyalas in Mkhuze were more like those of adult animals in the same reserve when compared to those in Ndumo and iMfolozi. Dissimilarity in parasite communities increases with spatial isolation (Pilosof et al. 2015; Bellay et al. 2020), while animals that share diets and habitats are often exposed to the same parasites (Holmes and Podesta 1968; Negovetich et al. 2006). While it is not possible to confirm, higher host densities in Mkhuze might have led to increased mingling of social groupings of nyalas, resulting in a more uniform species composition of their parasite communities.

Functional traits of hosts and parasites influence host-parasite interactions (Cardoso et al. 2021; Llopis-Blenguer et al. 2019, Dallas et al. 2019). In the present study, the functional similarity of parasite communities was generally high, irrespective of the age, sex or geographic origin of the nyalas. Nevertheless, some disparities between host populations could be found with respect to a given parasite trait.

Nyalas from Mkhuze and Ndumo mostly harboured parasites whose predilection site is the rumen, abdominal cavity or heart, indicating that in the presence of suitable vectors and intermediate hosts, niches other than the gastrointestinal tract can be colonised by a number of parasite species. Failure to reach a required threshold value in the host population density for successful parasite transmission (Cardoso et al. 2021) could be the reason for the lower abundance of paramphistomines in iMfolozi when compared to Ndumo and Mkhuze.

Infracommunities of adult nyalas contained many parasites with indirect life cycles, whereas those of young nyalas comprised predominantly parasites with direct life cycles. Adult hosts have lived longer and roam further, increasing their chances of random exposure to a variety of parasites (Chaisiri et al. 2017; Nielsen et al. 2020), including species that are either transmitted percutaneously or through vector activity. In contrast, infections with directly transmitted helminths contaminating the nyalas' habitat are readily ingested by young nyalas and are likely to colonise this segment of the population first. In addition, acquired immunity to many strongylids could further reduce the role of adults in maintaining directly transmitted parasites (Fellis et al. 2003). Interestingly, parasite species in which transmission depends on ingestion of infective stages were equally represented in adult and young nyalas. It is important to note that this group is diverse and comprises direct life cycle as well as indirect life cycle parasites.

Like in previous studies on the parasite communities of vertebrates (Johnson and Hoverman 2012; Lima et al. 2012; Benítez-Malvido et al. 2019; Rynkiewicz et al. 2019), nyala infracommunities were significantly nested, and the rarer parasite species tended to occur in host individuals that were also parasitized by more common species. Network structure varied between sexes and reserves. Furthermore, the role of a given parasite species in these networks changed with age, sex and reserve.

At Mkhuze, values of centrality were higher in adult than in young females, indicating that adult females contribute more to parasite sharing and transmission. This may be linked to older animals accumulating more varied and numerous parasites throughout their lifetime

(Bellay et al. 2020; Nielsen et al. 2020). Parasite species whose predilection site is the rumen, abdominal cavity or heart played an important role in infracommunities at Mkhuze. Notably, these parasites have indirect life cycles, a trait associated mostly with adult nyalas at all localities. Invading new hosts is likely more challenging for parasites with complex life cycles (Chaisiri et al. 2017), which therefore might more likely be found in older hosts.

In Ndumo, network variation was seen with respect to host strength, i.e. the sum of dependencies of each parasite species on each individual host (Bellay et al. 2020). IHS was higher in young than adult females, but higher in adult than young males. Species composition of parasite communities differed significantly between localities as well as between age groups. Thus, host age would have likely affected host strength differently at the three sites. In young nyala females, individual differences in the exposure to parasites which were either linked to feeding preference or immune competence, could lead to more pronounced differences in infracommunities when compared to older females. The greater importance of old males to the parasite community might be related to body size. In anuran-parasite networks, host body size was positively associated with individual host strength, with larger animals offering more space and more niches for interaction with a larger variety of parasites (Campião and Dáttilo 2020).

Interestingly, in iMfolozi, d' and IHS values were higher in younger than in adult hosts, while host sex played no role, whereas centrality reached higher values in males than females, but host age was of no consequence. Environmental changes could either lead to an increase or decrease in parasite abundance depending on the species (Krasnov and Poulin 2010). Since realized interactions within a network result from contact between parasites and hosts (Campião and Dáttilo 2020), which in turn is population density dependent, the observed patterns may stem from local variation in host density or environmental conditions.

Specialization was higher in young nyalas in iMfolozi than in adults, who were infected with a wider array of parasites. Rynkiewicz et al. (2015) expected parasite communities to follow a succession pattern and the more opportunistic composition of infracommunities seen in adult hosts, may reflect random acquisition of parasites over time.

Higher centrality of males when compared to females could reflect their more varied social interactions, enabling them to link parasite communities between the two sexes.

In conclusion, the structure of parasite communities of nyalas as well as the structure of parasite-nyala interaction networks were mainly driven by age and to a much lesser degree sex. The effects of host sex and age as drivers of community and network structure were mediated by the environment and thus were manifested differently at different localities. It is therefore important to note that pooling of host-parasite communities from different localities when investigating community and network structure should be avoided as it may lead to biased results and mask locality-specific patterns.

Author's contribution KJ and BRK conceived the study. JB and IGH collected the material and identified the parasites. BRK analysed data. KJ and BRK drafted the manuscript. All authors participated in finalizing the manuscript.

Data availability All data included are from previously published work. The datasets used and/or analysed are available from the corresponding author upon reasonable request.

Code availability We used standard codes of the respective R packages.

Declarations

Ethics approval All applicable institutional, national and international guidelines for the care and use of animals were followed.

Conflicts of interest The authors declare there are no conflicts of interest.

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