Final Report (Phase I)

(Carnivore Diet- All seasons)

Investigating Spatio-Temporal Variations in Herbivore and Carnivore Diet in Tadoba-Andhari Tiger Reserve, Maharashtra

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Co-Principal Investigators: Field Director, Deputy Director core and Deputy Director buffer, Tadoba Andhari Tiger Reserve, Maharashtra

Introduction:

Mammalian diet is crucial in understanding trophic interactions and their influence on ecosystem functioning. Several co-occurring species with similar habitat requirements might show high overlap in their niches leading to interspecific competition (Polis et al. 1989; Donadio & Buskirk 2006). Studying the width of the dietary niche of species can help us understand the degree of dietary specialization as a result of interspecific competitions and in response to environmental factors. The species with narrow dietary niches can be vulnerable to the effects of climate change, and invasive species amongst other environmental and anthropogenic pressures (Schure et al. 2021).

Here we present the results of diet analysis for three carnivores (tiger, leopard and dhole), with emphasis on spatial and temporal variation. Sampling was done during monsoon (Sept-Oct 2022), winter (Jan-Feb 2023) and summer (May-Jun 2023) seasons from core and buffer ranges of Tadoba Andhari Tiger Reserve, Maharashtra.

Methodology and Work Progress:

We obtained fecal samples collected by Tadoba Andhari Tiger Reserve field staff for diet analysis from monsoon season (September to October 2022), winter season (January to February 2023), and summer season (May to June 2023). The details of all the samples obtained can be found in Table 1. Post receiving the samples we extracted DNA using the QIAamp stool DNA mini kit (Qiagen). The extractions were carried out in batches of 23 samples with one negative control to check for potential contaminations. Genetic species identification was done for all the samples using species-specific mitochondrial DNA markers.

Only samples with confirmed species identity were taken ahead for creating the metabarcoding library and sequencing on next next-generation sequencing platform to understand the diet. After ascertainment of species identity, all DNA samples were subjected to PCR reactions using a universal vertebrate primer 12SV5 along with the blocking oligonucleotides (designed for this study) to amplify a 136bp sequence of the mitochondrial genome.

The amplified products were cleaned using Ampure beads and indexed using i5 and i7 indexes (self-designed for this study) to uniquely identify the samples. The indexed samples were purified again, quantified and pooled in equal concentrations. The resulting library was sequenced at the MiSeq platform.

The data generated was analysed to identify different species in the carnivore diet. We used a reference library (already constructed) of all probable species in the carnivore diet using the NCBI database. *Obitools* was used to analize the data, and assign a taxonomic identity to all the reads obtained for every sample.

Table 1: Season-wise details of all the samples obtained from TATR field team, species identification and sequencing.

S. No.	Species	Season	Samples obtained	Genetically identified	Labelled as different species	Taken for sequencing
1	Tiger	Monsoon	78	37	5	167
		Winter	107	67	5	
		Summer	74	45	8	
2	Leopard	Monsoon	32	12	8	92
		Winter	76	33	9	
		Summer	47	23	7	
3	Dhole	Monsoon	99	82	-	212
		Winter	84	62	-	
		Summer	77	68	-	

Results:

The sampling method and sequencing protocol we developed and used in this study were found to be robust and enabled us to generate good-quality data to analyse animal diet. In this section, we will describe species-wise results with respect to the area of sampling (core and buffer) and season (monsoon, winter and summer).

Tiger diet

- **Overall**

In total, we have found 16 species in tiger diet in TATR from 143 samples which worked after sequencing. The data for all these 143 samples will be presented in consideration of spatial and temporal variation in tiger diet in TATR. Sambar, chital and wild boar were found to be among the top three prey species. Our results show more than 90% of tiger diet is composed of wild prey. However, we did observe a shift in dietary patterns with space and time. We also obtained dhole DNA from some of the samples. We think this can be due to co-predation of the same kills by both species.

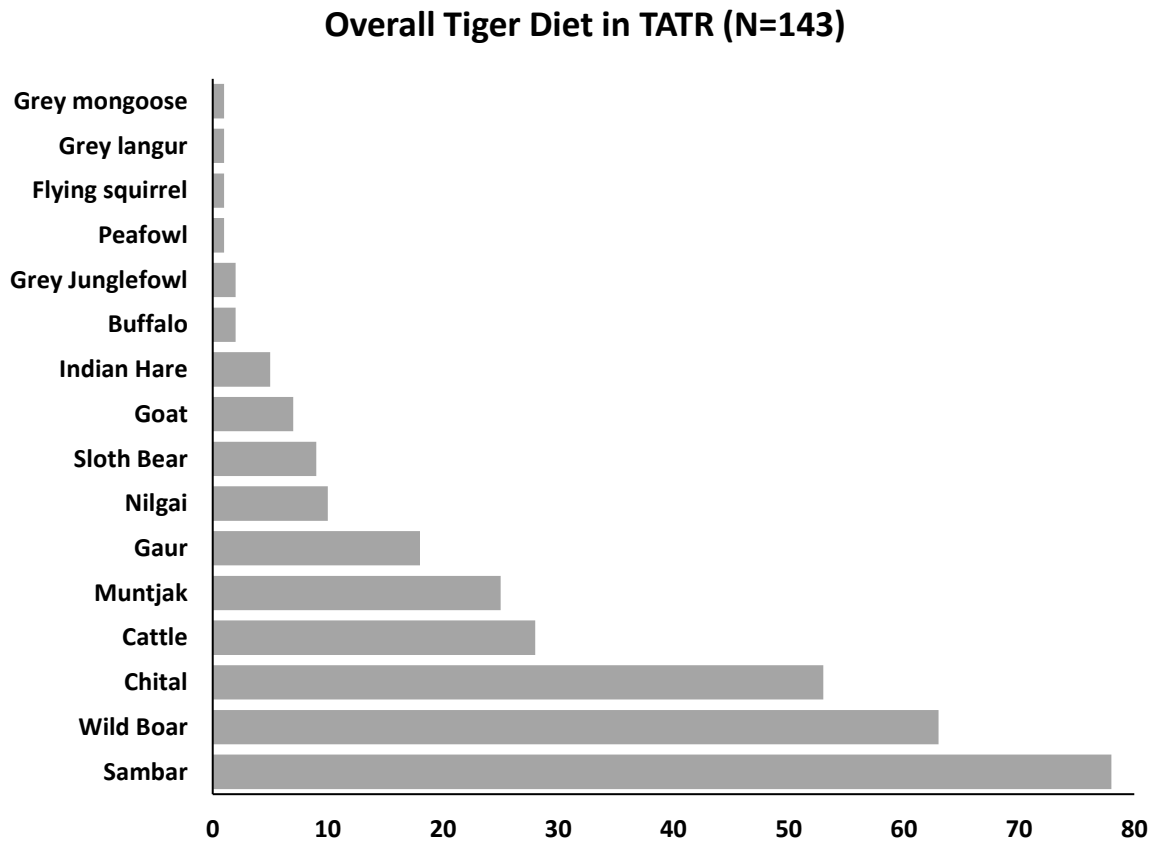


Figure 1: Overall dietary composition of tiger in Tadoba Andhari Tiger Reserve

- **Seasonal variation**

Overall, we did not observe a stark difference in the tiger's diet across the three seasons. We identified 12 prey species in both the monsoon and winter seasons and 10 species in summer, with the same top three prey species present in all seasons. In the monsoon, grey langur and jungle fowl were detected, while Indian peafowl and buffalo were found in winter. During summer, grey jungle fowl and grey mongoose were the unique species identified in tiger scat, along with other species common across seasons. However, gaur and Indian hare were absent in the scats collected during summer. We observed an increase in muntjac consumption during winter compared to other seasons. Additionally, the occurrence of nilgai and sloth bear in the diet increased in summer relative to other seasons.

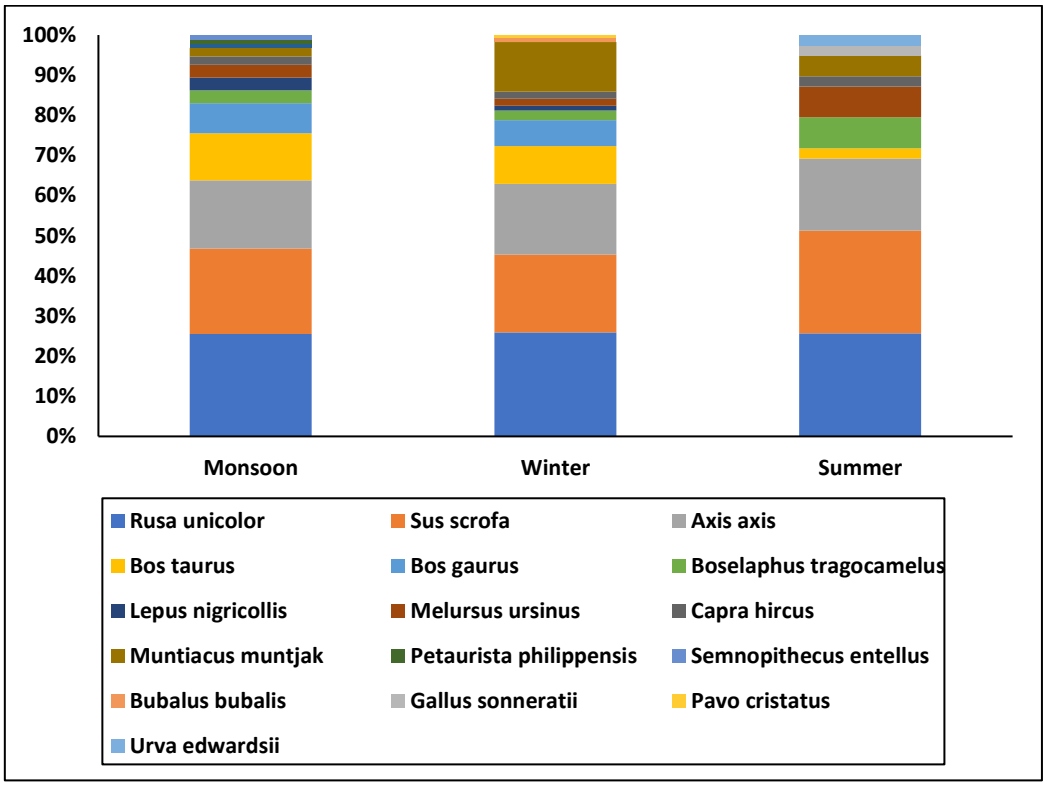
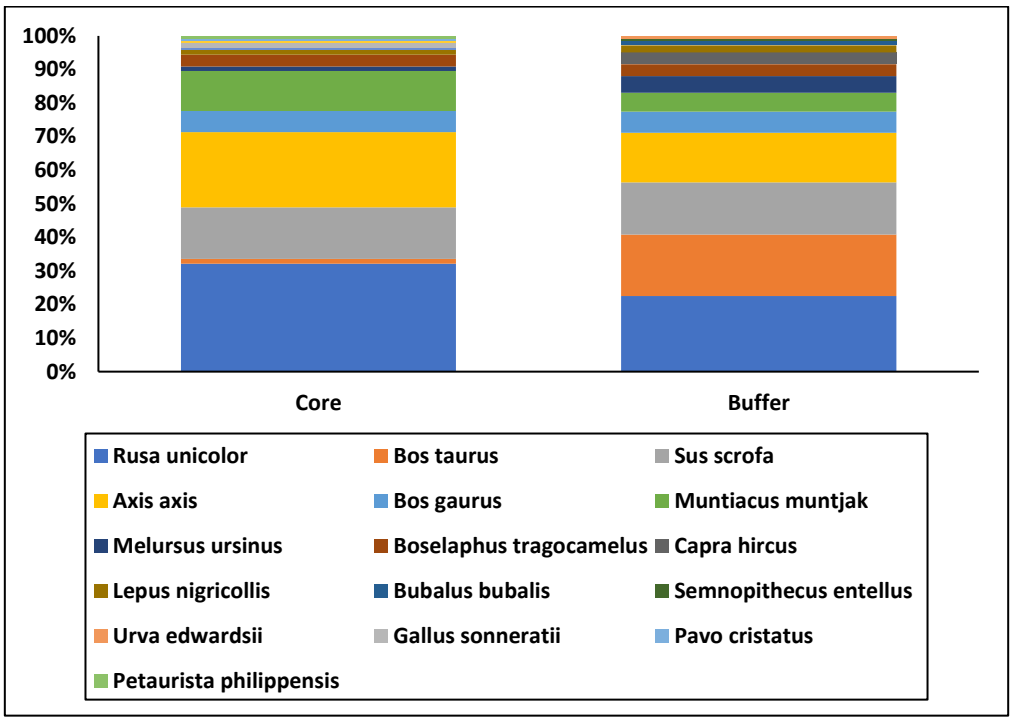


Figure 2: Tiger diet during monsoon, winter and summer seasons in Tadoba Andhari Tiger Reserve

- **Spatial variation**

We observe a substantial difference in tiger diet in the core and buffer areas of Tadoba Andhari Tiger Reserve, Maharashtra. In core, the majority of the diet was composed of wild prey including sambar, chital, barking deer and wild boar. We also obtained cattle DNA in some of the scats found in core areas, but it could be potentially due to individuals moving between core and buffer areas. In buffer areas, increased cattle presence and reduction of wild prey in diet were recorded.



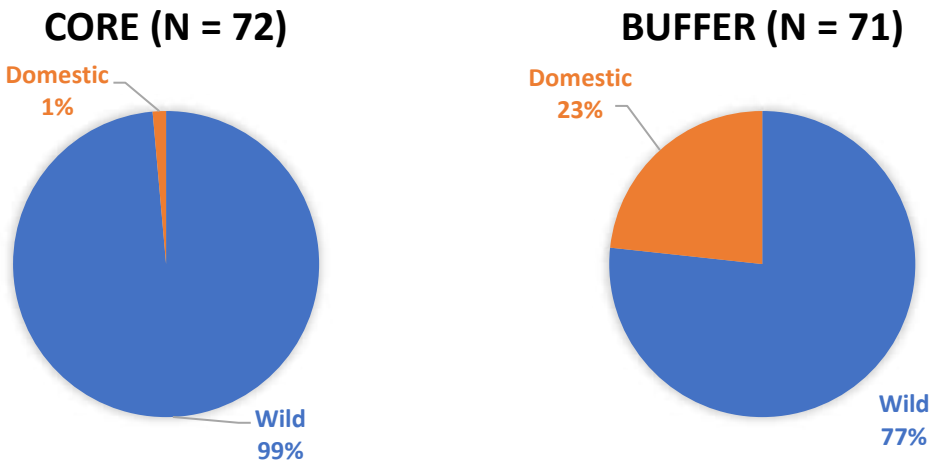


Figure 3: Tiger diet in core and buffer ranges of Tadoba Andhari Tiger Reserve

Leopard diet

- **Overall**

As expected, leopards exhibit a much wider dietary breadth as compared to tigers. We have found 26 different species in the leopard diet from 75 samples which worked post sequencing. Chital and wild boar were the primary prey in both core and buffer areas. In core, wild prey like chital, wild boar, sambar, muntjak and Indian hare were found among the top prey species. Interestingly, in buffer, the dietary composition was found to be higher and many domestic species were observed. We found chicken, village dogs, cows and buffalo among other wild and domestic species in the leopard diet in buffer areas.

In one sample, collected from the Chandrapur range, we found human DNA. This can be because of two reasons: 1) the actual human DNA in leopard scat, if there was a conflict incident reported during that time in that area, or 2) human contamination during the sample collection step. Genetically we cannot distinguish between these two events.

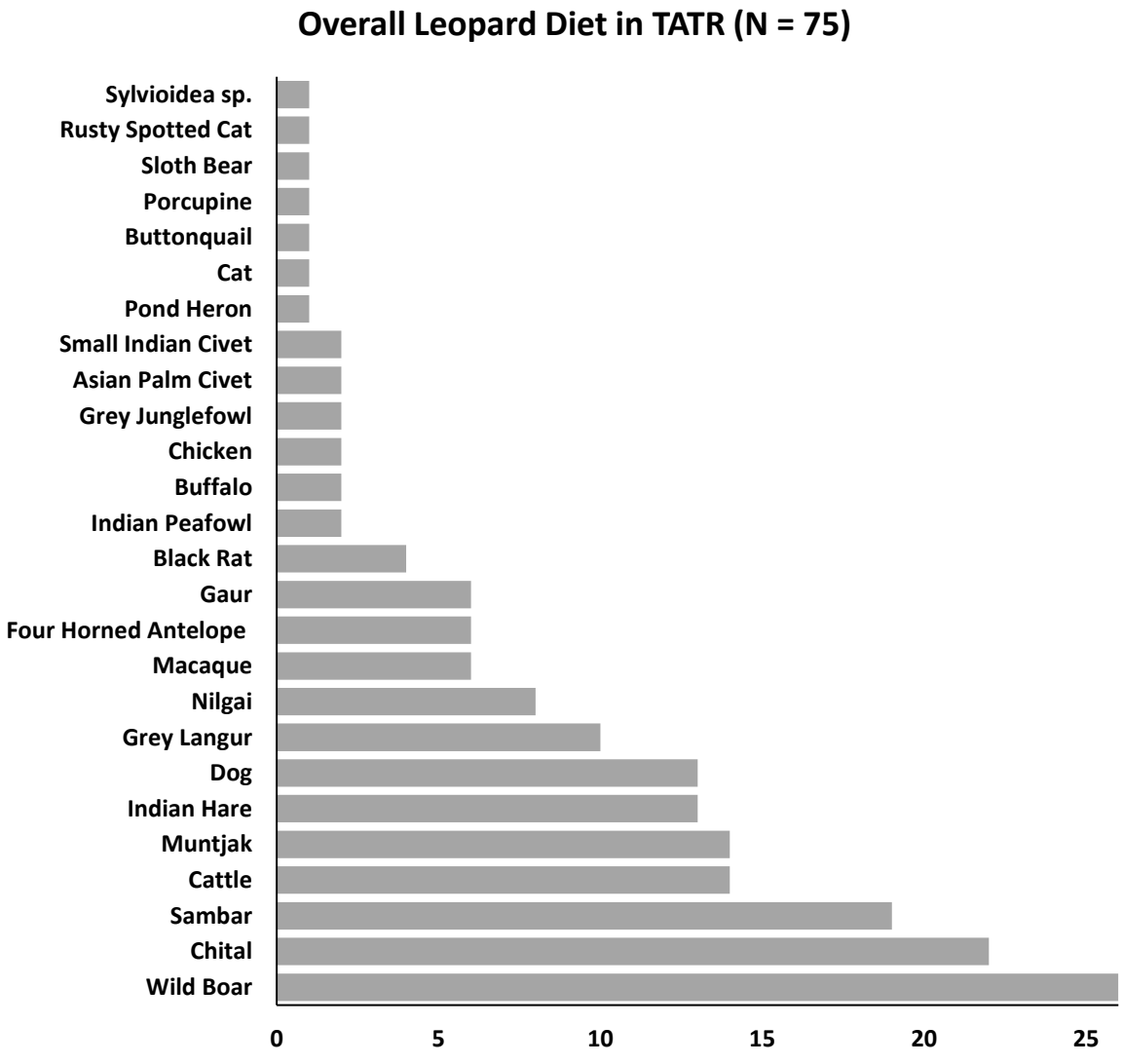


Figure 4: Overall leopard diet in Tadoba Andhari Tiger Reserve

- **Seasonal variation**

Overall, we observed a stark difference in the leopard's diet across the three seasons, particularly in summer compared to the other two seasons. The highest dietary diversity was recorded in winter, with 21 species, followed by the monsoon with 14 species, while the lowest diversity was observed in summer, with 12 species identified from leopard samples. During winter, species such as civets, porcupine, grey junglefowl, chicken, and buffalo were detected in the leopard's diet, but these were absent in other seasons. In the monsoon, unique bird species like Indian peafowl, buttonquail, and pond heron were identified, along with other species common across seasons. We also observed an increase in the consumption of cattle, langur, and nilgai during summer compared to the other seasons.

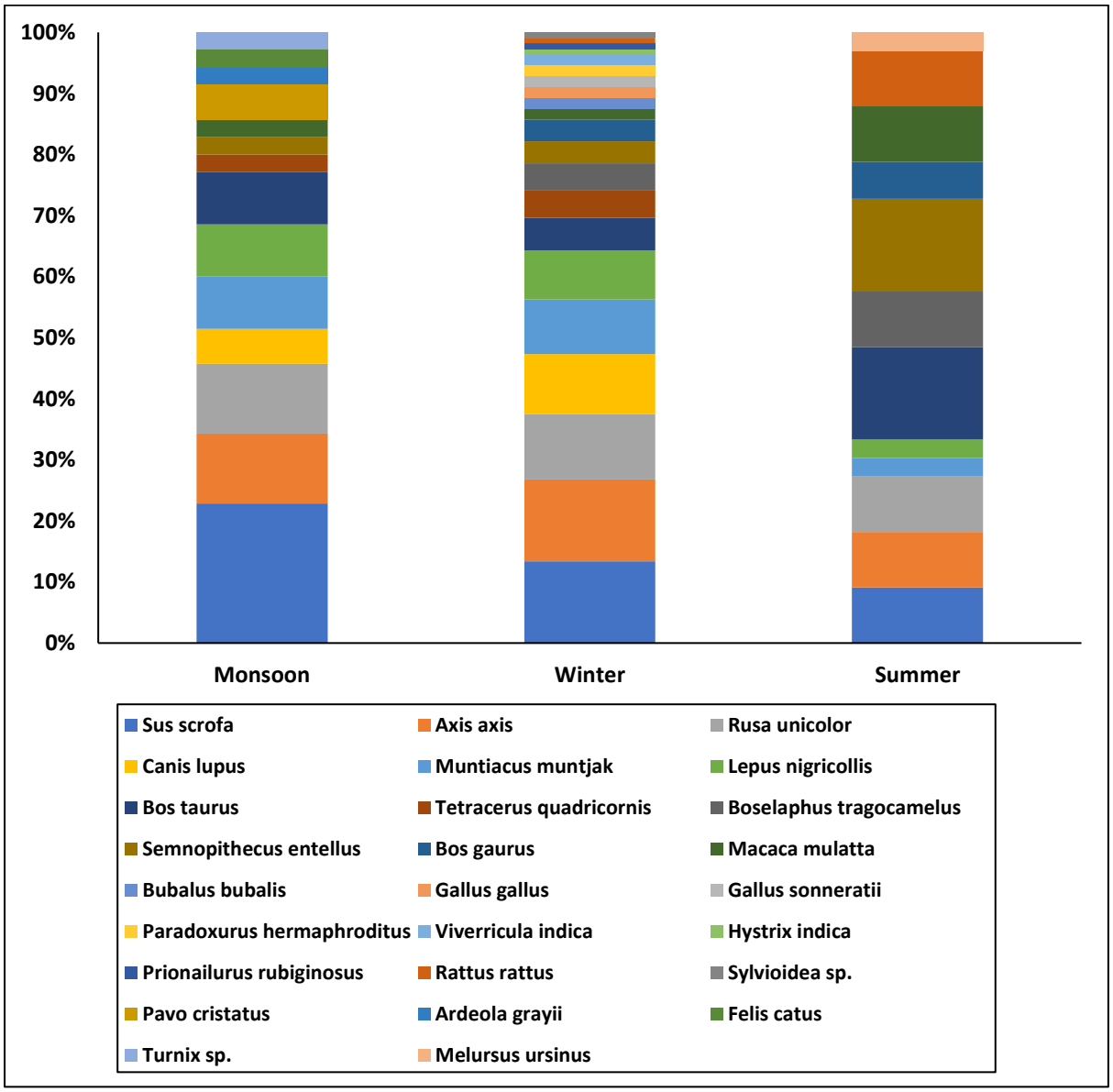


Figure 5: Leopard diet during monsoon, winter and summer seasons in Tadoba Andhari Tiger Reserve

- **Spatial variation**

We observed a substantial difference in leopard diet between core and buffer ranges of Tadoba Andhari Tiger Reserve. In core, the majority of the diet is composed of wild prey including sambar, chital, barking deer and wild boar. We also obtain cattle DNA in some of the scats found in core areas, but it is potentially due to individuals moving between core and buffer areas. In buffer areas, domestic prey presence and reduction of wild prey in the diet were recorded. We identified 19 species in the leopard diet in core areas as compared to 22 species in buffer ranges. We also obtained dhole DNA from 3 leopard scats, that can be attributed to interaction between two species on the kill sites and feeding on the same prey.

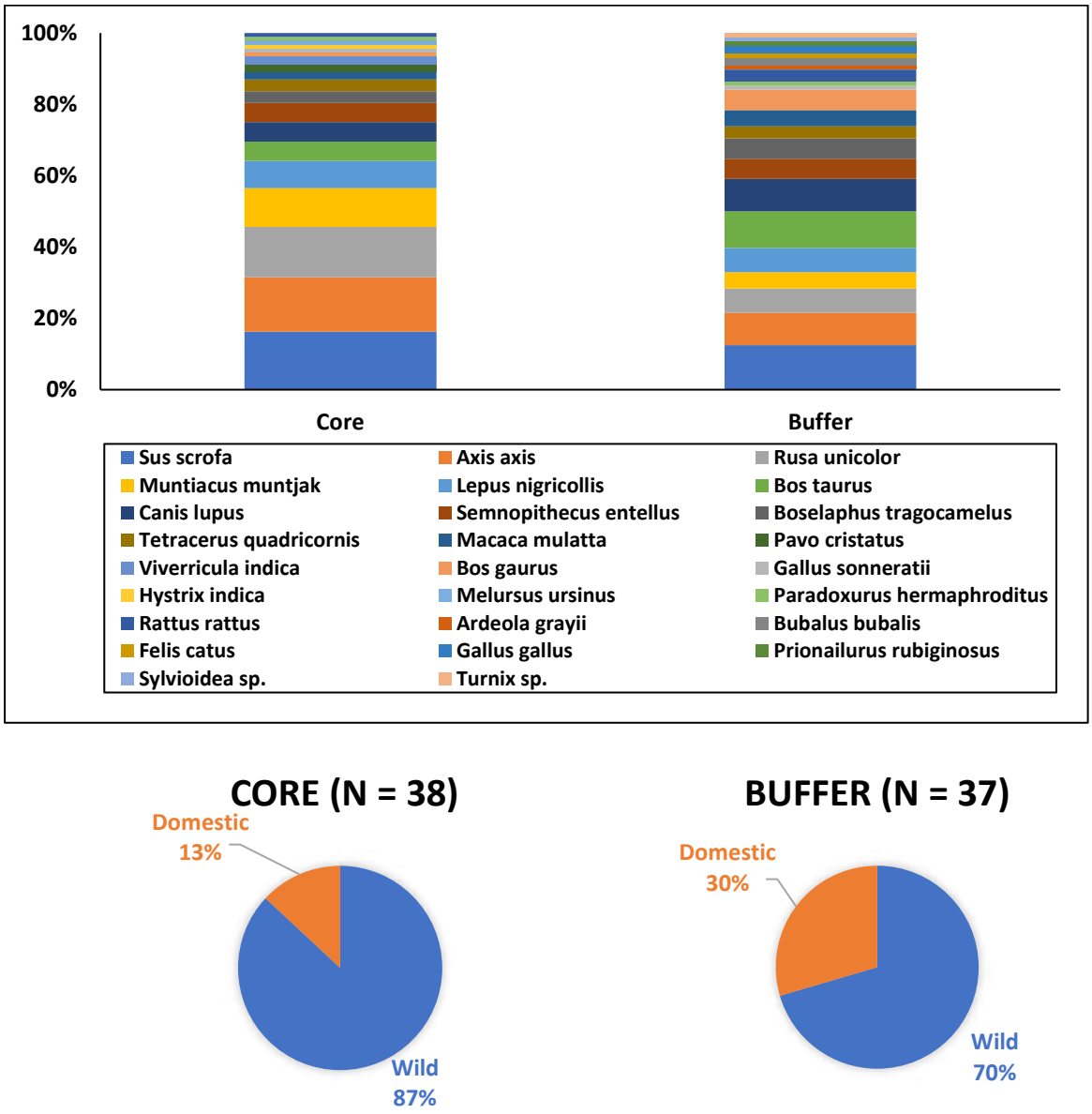


Figure 6: Leopard diet in core and buffer ranges of Tadoba Andhari Tiger Reserve. The red arrows indicate domestic species found in the leopard diet in buffer areas.

Dhole diet

- **Overall**

Using 179 dhole scat samples that successfully underwent sequencing, we identified 19 prey species in the dhole diet. Sambar, chital, barking deer, Indian hare, and wild boar were among the top five prey species. Our results indicate that wild prey constitutes more than 90% of the dhole's diet. However, we observed interesting shifts in dietary patterns across both space and time. Additionally, Panthera DNA (from leopards and tigers) was detected in some dhole samples, which we believe may result from co-predation or scavenging on tiger or leopard kills.

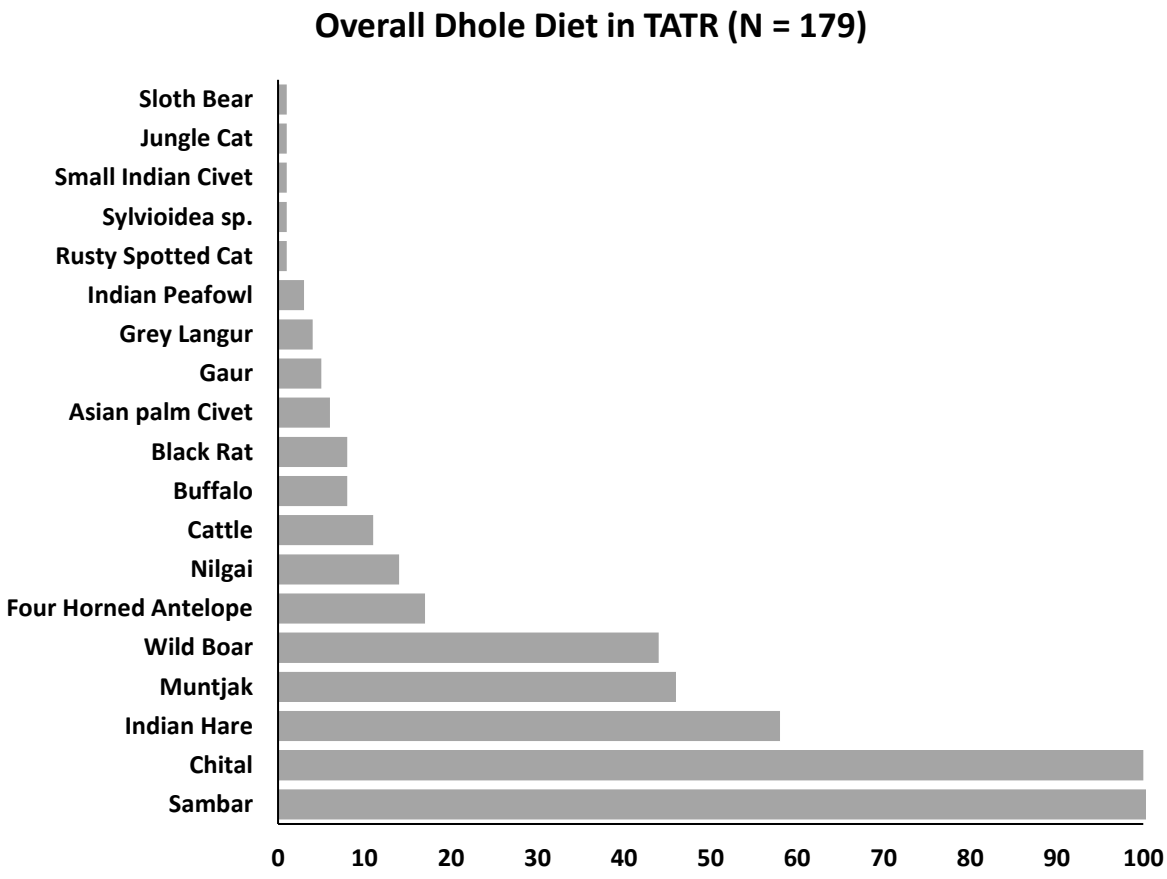


Figure 7: Dietary composition of dhole in Tadoba Andhari Tiger Reserve

- **Seasonal variation**

We observed stark differences in the dhole diet across seasons. In the monsoon, we identified 17 prey species, while winter and summer had 12 species each. During the monsoon, Indian hare was among the top three prey species, whereas in winter, the dhole diet primarily consisted of wild ungulates. In summer, sambar, chital, muntjac, and Indian hare were among the primary dietary species. Wild boar was also a dominant prey species during the monsoon, whereas in winter, sambar, chital, and barking deer were the primary prey. This variation is likely driven by prey availability rather than preference.

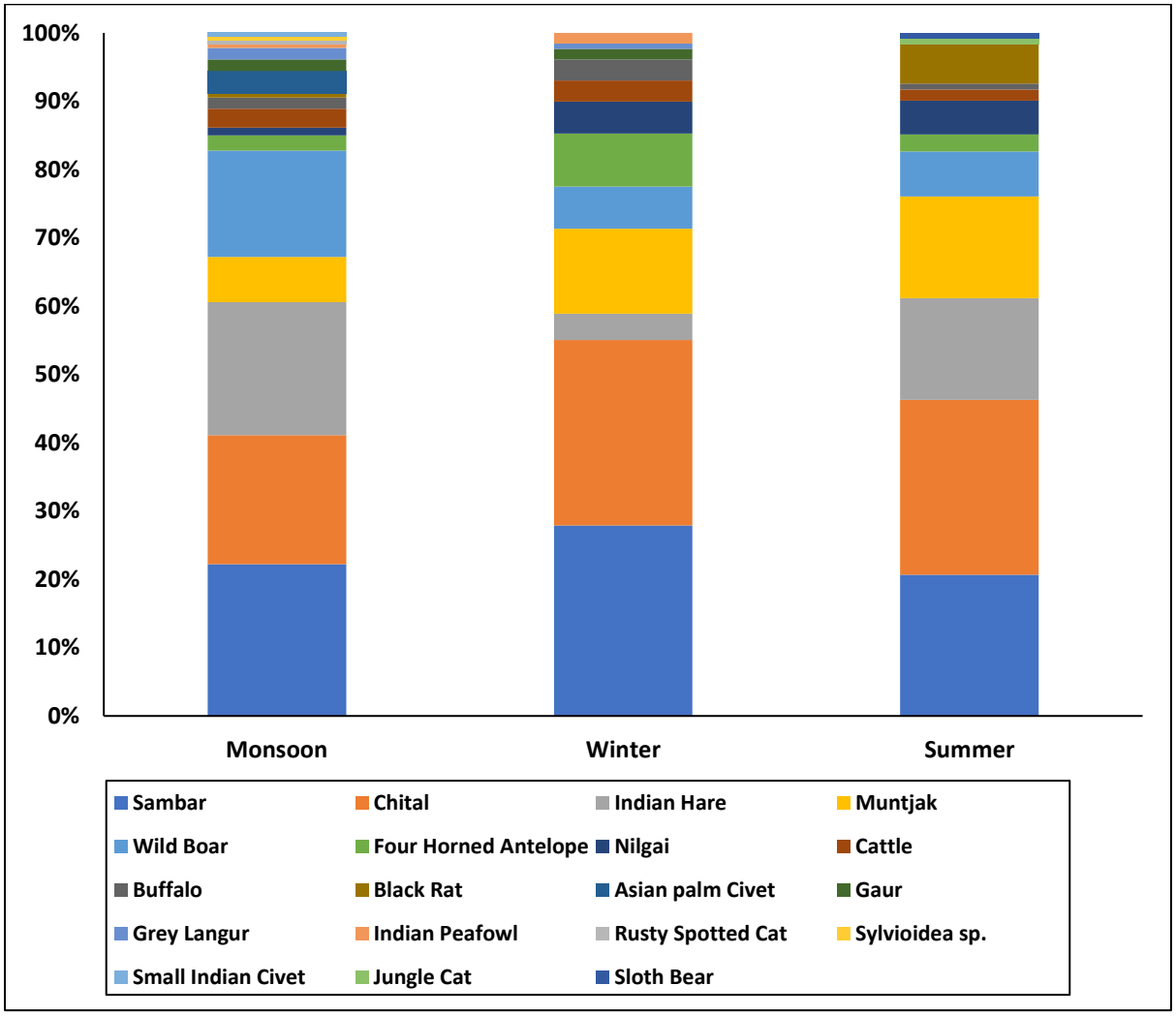
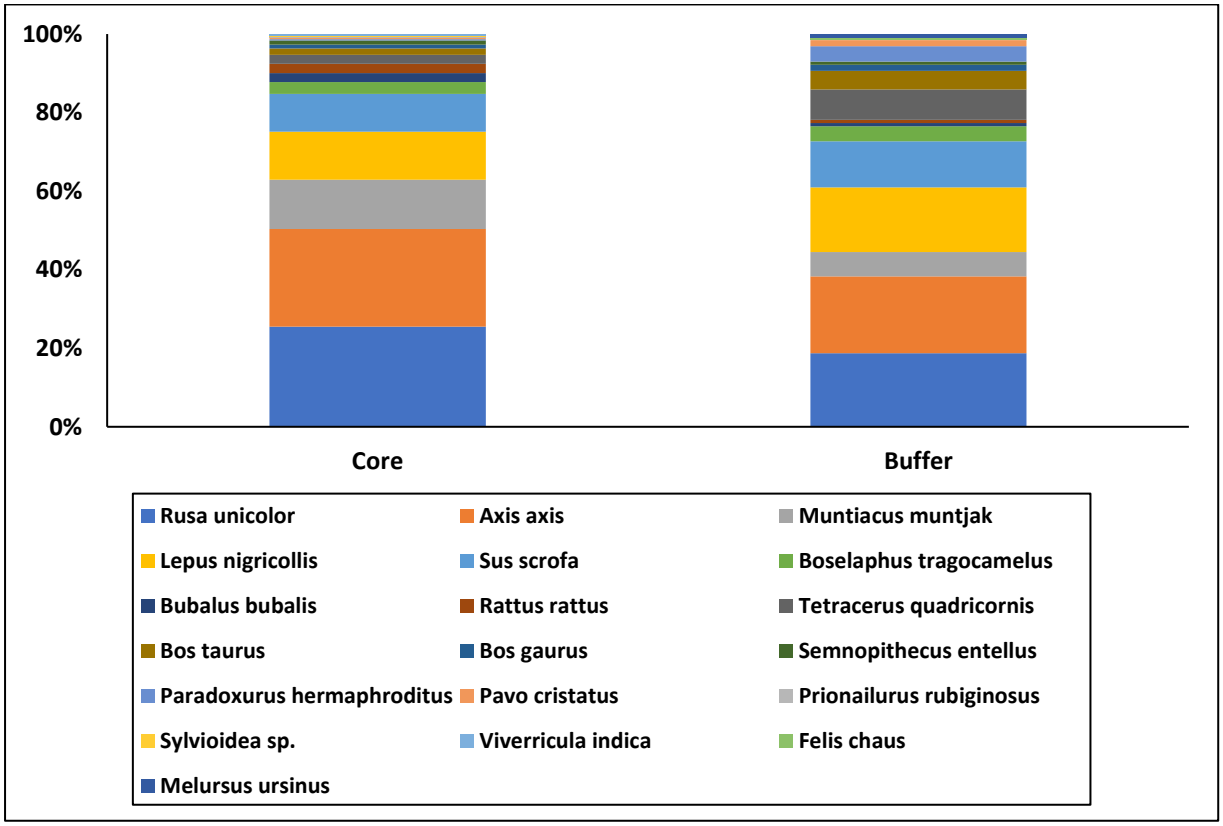


Figure 8: Dhole diet in monsoon, winter and summer seasons.

- **Spatial variation**

We observed a substantial difference in the dhole diet between core and buffer ranges. We identified 17 prey species in the core and 16 in the buffer. While wild prey dominated the dhole diet in both areas, the composition varied between them. Dholes were the only carnivore in our study that exhibited higher dietary diversity in the core compared to the buffer. In the core, the diet was primarily composed of wild prey, including sambar, chital, and barking deer. In contrast, although the buffer had slightly fewer prey species, its dietary composition was more uniform. Wild boar and Indian hare together accounted for about 30% of the dhole diet in the buffer, alongside other ungulate species.



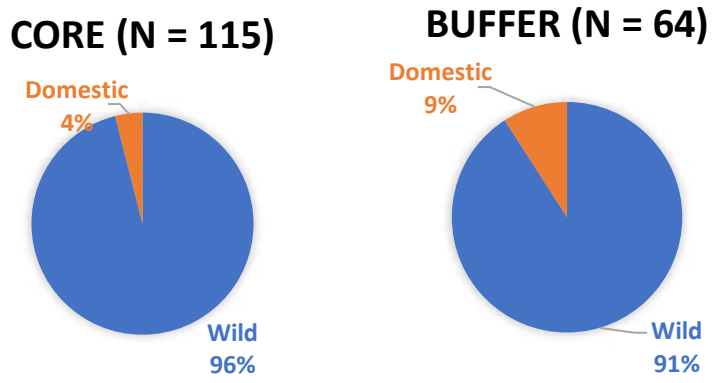


Figure 9: Dhole diet in core and buffer areas of Tadoba Andhari Tiger Reserve.

Table 2: Species recovered from tiger diet:

S.No.	Species	Found in no. of samples
1	Sambar (*Rusa unicolor*)	78
2	Wild Boar (*Sus scrofa*)	63
3	Chital (*Axis axis*)	53
4	Cattle/Cow (*Bos taurus*)	28
5	Indian Muntjac (*Muntiacus muntjak*)	25
6	Gaur (*Bos gaurus*)	18
7	Nilgai (*Boselaphus tragocamelus*)	10
8	Sloth Bear (*Melursus ursinus*)	9
9	Goat (*Capra hircus*)	7
10	Indian Hare (*Lepus nigricollis*)	5
11	Buffalo (*Bubalus bubalis*)	2
12	Grey Junglefowl (*Gallus sonneratii*)	2
13	Peafowl (*Pavo cristatus*)	1
14	Flying squirrel (*Petaurista philippensis*)	1
15	Grey langur (*Semnopithecus entellus*)	1
16	Grey mongoose (*Urva edwardsii*)	1

Table 3: Species recovered from leopard diet:

S.No.	Species	Found in no. of samples
1	Wild Boar (*Sus scrofa*)	26
2	Chital (*Axis axis*)	22
3	Sambar (*Rusa unicolor*)	19
4	Cattle/Cow (*Bos taurus*)	14
5	Indian Muntjac (*Muntiacus muntjak*)	14
6	Indian Hare (*Lepus nigricollis*)	13
7	Dog (*Canis lupus*)	13
8	Grey langur (*Semnopithecus entellus*)	10
9	Nilgai (*Boselaphus tragocamelus*)	8
10	Macaque (*Macaca mulatta*)	6
11	Four-horned Antelope (*Tetracerus quadricornis*)	6
12	Gaur (*Bos gaurus*)	6
13	Black Rat (*Rattus rattus*)	4
14	Indian Peafowl (*Pavo cristatus*)	2
15	Buffalo (*Bubalus bubalis*)	2
16	Chicken (*Gallus gallus*)	2
17	Grey Junglefowl (*Gallus sonneratii*)	2
18	Asian Palm Civet (*Paradoxurus hermaphroditus*)	2
19	Small Indian Civet (*Viverricula indica*)	2
20	Pond Heron (*Ardeola grayii*)	1
21	Cat (*Felis catus*)	1
22	Buttonquail (*Turnix sp.*)	1
23	Porcupine (*Hystrix indica*)	1
24	Sloth Bear (*Melursus ursinus*)	1
25	Rusty-spotted Cat (*Prionailurus rubiginosus*)	1
26	Sylvioidea *sp.*	1

Table 4: Species recovered from dhole diet:

S.No.	Species	Found in no. of samples
1	Sambar (*Rusa unicolor*)	101
2	Chital (*Axis axis*)	100
3	Indian Hare (*Lepus nigricollis*)	58
4	Indian Muntjac (*Muntiacus muntjak*)	46
5	Wild Boar (*Sus scrofa*)	44
6	Four-horned Antelope (*Tetracerus quadricornis*)	17
7	Nilgai (*Boselaphus tragocamelus*	14
8	Cattle/Cow (*Bos taurus*)	11
9	Buffalo (*Bubalus bubalis*)	8
10	Black Rat (*Rattus rattus*)	8
11	Asian Palm Civet (*Paradoxurus hermaphroditus*)	6
12	Gaur (*Bos gaurus*)	5
13	Grey langur (*Semnopithecus entellus*)	4
14	Indian Peafowl (*Pavo cristatus*)	3
15	Rusty-spotted Cat (*Prionailurus rubiginosus*)	1
16	Sylvioidea *sp.*	1
17	Small Indian Civet (*Viverricula indica*)	1
18	Jungle Cat (*Felis chaus*)	1
19	Sloth Bear (*Melursus ursinus*)	1

The way forward:

We have already expanded the geographical scope of the project for Phase II. Currently, we are generating data from samples collected in the forest divisions of Chandrapur district. Additionally, we are developing DNA barcodes for plant species, which will eventually be used to analyze herbivore diets. We have also extracted and genetically identified species from all herbivore samples received from TATR staff across all seasons. The preliminary results will be submitted along with this report.

Preliminary Report (Phase I)

(Herbivore Diet- All seasons)

Investigating Spatio-Temporal Variations in Herbivore and Carnivore Diet in Tadoba-Andhari Tiger Reserve, Maharashtra

Principal Investigators: Prof. Uma Ramakrishnan and Dr. Abhinav Tyagi, National Centre for Biological Sciences (NCBS), Bangalore

Co-Principal Investigators: Field Director, Deputy Director core and Deputy Director buffer, Tadoba Andhari Tiger Reserve, Maharashtra

Introduction:

Mammalian diet is crucial in understanding trophic interactions and their influence on ecosystem functioning. Several co-occurring species with similar habitat requirements might show high overlap in their niches leading to interspecific competition (Polis et al. 1989; Donadio & Buskirk 2006). Studying the width of the dietary niche of species can help us understand the degree of dietary specialization as a result of interspecific competitions and in response to environmental factors. The species with narrow dietary niches can be vulnerable to the effects of climate change, and invasive species amongst other environmental and anthropogenic pressures (Schure et al. 2021).

Here we present our preliminary results of diet analysis for three herbivore species (gaur, sambar, chital), with emphasis on spatial and temporal variation. Sampling was done during monsoon (Sept-Oct 2022), winter (Jan-Feb 2023) and summer (May-Jun 2023) seasons from core and buffer ranges of Tadoba Andhari Tiger Reserve, Maharashtra.

Methodology and Work Progress:

We obtained fecal samples collected by Tadoba Andhari Tiger Reserve field staff for diet analysis from monsoon season (September to October 2022), winter season (January to February 2023), and summer season (May to June 2023). The details of all the samples obtained can be found in Table 1. Post receiving the samples we extracted DNA using the QIAamp stool DNA mini kit (Qiagen). The extractions were carried out in batches of 23 samples with one negative control to check for potential contaminations. Genetic species identification was done for all the samples using species-specific mitochondrial DNA markers we developed (Tyagi et al., 2024).

Only samples with confirmed species identity were taken ahead for creating the metabarcoding library and sequencing on next next-generation sequencing platform to understand the diet. After ascertainment of species identity, all DNA samples were subjected to PCR reactions using four chloroplast markers (designed for this study) to amplify DNA sequences ranging from 12 -290bp of the chloroplast genome.

The amplified products were cleaned using Ampure beads and indexed using i5 and i7 indexes (self-designed for this study) to uniquely identify the samples. The indexed samples were purified again, quantified and pooled in equal concentrations. The resulting library was sequenced at the MiSeq platform.

The data generated was analysed to identify different species in the herbivore diet. We used a reference library (constructed for this study) based on all plant species data available on global databases like the NCBI database. *Obitools* was used to analize the data, and assign a taxonomic identity to all the reads obtained for every sample.

Table 1: Season-wise details of all the samples obtained from TATR field team, species identification and sequencing.

S. No.	Species	Season	Samples obtained	Genetically identified	Labelled as different species	Taken for sequencing
1	Gaur	Monsoon	62	48	-	214
		Winter	119	95	-	
		Summer	91	71	-	
2	Sambar	Monsoon	70	20	-	98
		Winter	107	39	5	
		Summer	76	34	-	
3	Chital	Monsoon	77	60	-	208
		Winter	99	78	20	
		Summer	77	50	-	

Results:

The sampling method and sequencing protocol we developed and used in this study were found to be robust and enabled us to generate good-quality data to analyse animal diet. In this section, we will describe species-wise results with respect to the area of sampling (core and buffer) and season (monsoon, winter and summer).

Gaur diet

In total, we identified plant species from 75 plant families in the gaur diet in TATR from 214 samples that yielded results after sequencing. The data from these 214 samples will be presented with consideration of spatial and temporal variation in the gaur diet in TATR. Our results show that Rubiaceae, Combretaceae, Phyllanthaceae, and Fabaceae are among the top plant families found in the gaur diet. The data presented here is based on a single marker; we are currently analyzing data from three additional markers used to generate dietary information and gain a deeper understanding of herbivore diets. However, we observed a stark shift in dietary patterns across space and time, which correlates with plant phenology and species availability in different seasons.

Table 2: Season-wise details of all the samples sequenced post extraction, species identification and quality checking along with the number of reads obtained.

Gaur	Monsoon (Core)	Monsoon (Buffer)	Winter (Core)	Winter (Buffer)	Summer (Core)	Summer (Buffer)
No. of Sample	34	14	52	43	39	32
Read count	712459	347750	1211386	967576	972200	968611

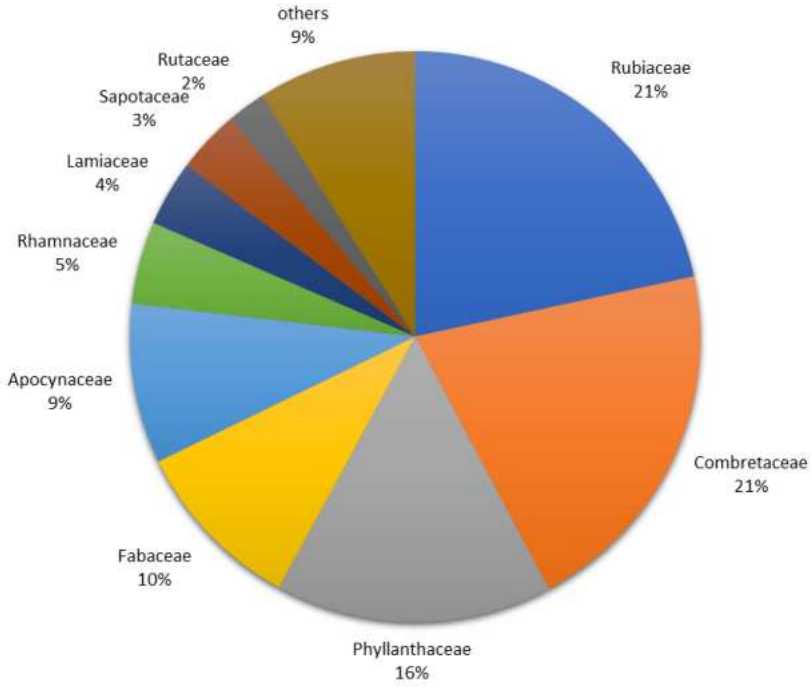


Figure 1: Overall dietary composition of gaur in Tadoba Andhari Tiger Reserve

- **Spatial and seasonal variation**

Overall, we observed a stark difference in the gaur diet across the three seasons. The highest dietary diversity was identified during the monsoon season, followed by winter and summer. This could be attributed to the availability of plant species in different seasons across the landscape. During the monsoon, the diet was found to be evenly distributed among a few plant families, including Combretaceae, Fabaceae, and Lamiaceae (the proportion of reads was highest, with no significant difference between core and buffer zones). In winter, the gaur diet was dominated by Phyllanthaceae, particularly in buffer zones compared to core areas, followed by Rubiaceae, Combretaceae, and Apocynaceae. During summer, dietary diversity was lowest, with the diet primarily composed of two families: Combretaceae and Rubiaceae. The proportion of Fabaceae declined post-monsoon, likely due to the reduced availability of legumes.

We also observed substantial differences in the gaur diet between the core and buffer areas of Tadoba Andhari Tiger Reserve, Maharashtra. For example, the proportion of Rhamnaceae was consistently higher in buffer areas compared to core areas across all seasons.

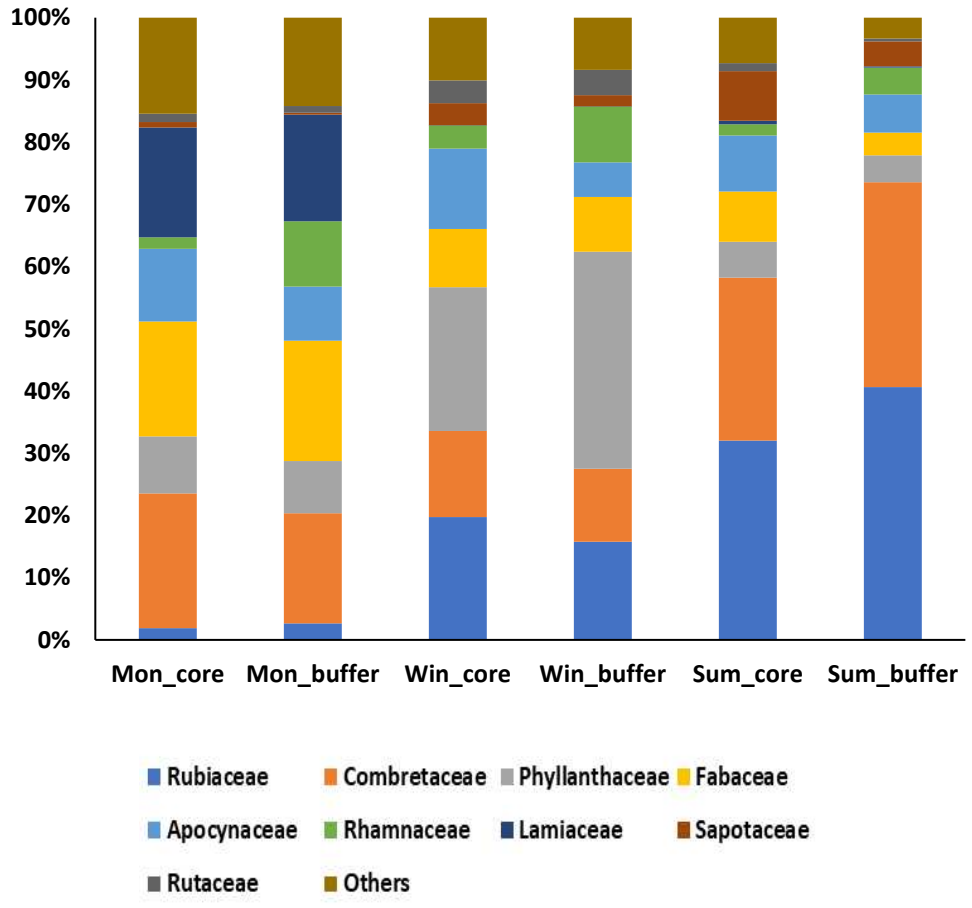


Figure 2: Gaur diet in different season and core and buffer in Tadoba Andhari Tiger Reserve

Sambar diet

In total, we identified plant species from 56 plant families in the sambar diet in Tadoba Andhari Tiger Reserve (TATR) from 93 samples that yielded results after sequencing. The data from these samples will be analyzed with consideration of spatial and temporal variations in the sambar diet in TATR. Our results show that Rubiaceae, Combretaceae, Phyllanthaceae, Apocynaceae, Sapotaceae, and Fabaceae are among the most prevalent plant families in the sambar diet.

The data presented here is based on a single marker; however, we are currently analyzing data from three additional markers to further refine dietary insights and enhance our understanding of herbivore diets. Notably, we observed a stark shift in dietary patterns across space and time, which appears to correlate with plant phenology and species availability in different seasons.

Table 3: Season-wise details of all the samples sequenced post extraction, species identification and quality checking along with the number of reads obtained.

Sambar	Monsoon (Core)	Monsoon (Buffer)	Winter (Core)	Winter (Buffer)	Summer (Core)	Summer (Buffer)
No. of Sample	28	11	16	18	16	4
Read count	1244751	559112	756698	1006395	665216	201863

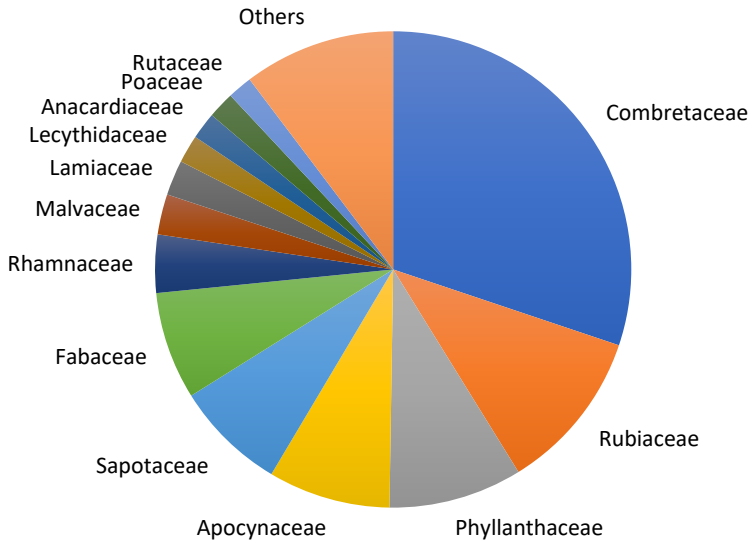


Figure 3: Overall dietary composition of sambar in Tadoba Andhari Tiger Reserve

- **Spatial and seasonal variation**

Overall, we observed a stark difference in the sambar diet across the three seasons. The highest dietary diversity was recorded during the monsoon season, followed by winter and summer. This variation could be attributed to the seasonal availability of plant species across the landscape.

During the monsoon, the diet was evenly distributed among a few plant families, including Combretaceae, Fabaceae, and Phyllanthaceae, with a significant difference between the core and buffer zones. In winter, the sambar diet was dominated by Combretaceae, followed by Rubiaceae and Fabaceae. During summer, dietary diversity was lowest, with the diet primarily composed of Combretaceae, Rubiaceae, Phyllanthaceae, Apocynaceae, and Sapotaceae.

We also observed substantial differences in the sambar diet between the core and buffer areas of Tadoba Andhari Tiger Reserve, Maharashtra. For example, the proportion of Apocynaceae and Rhamnaceae was consistently higher in buffer areas compared to core areas across all seasons.

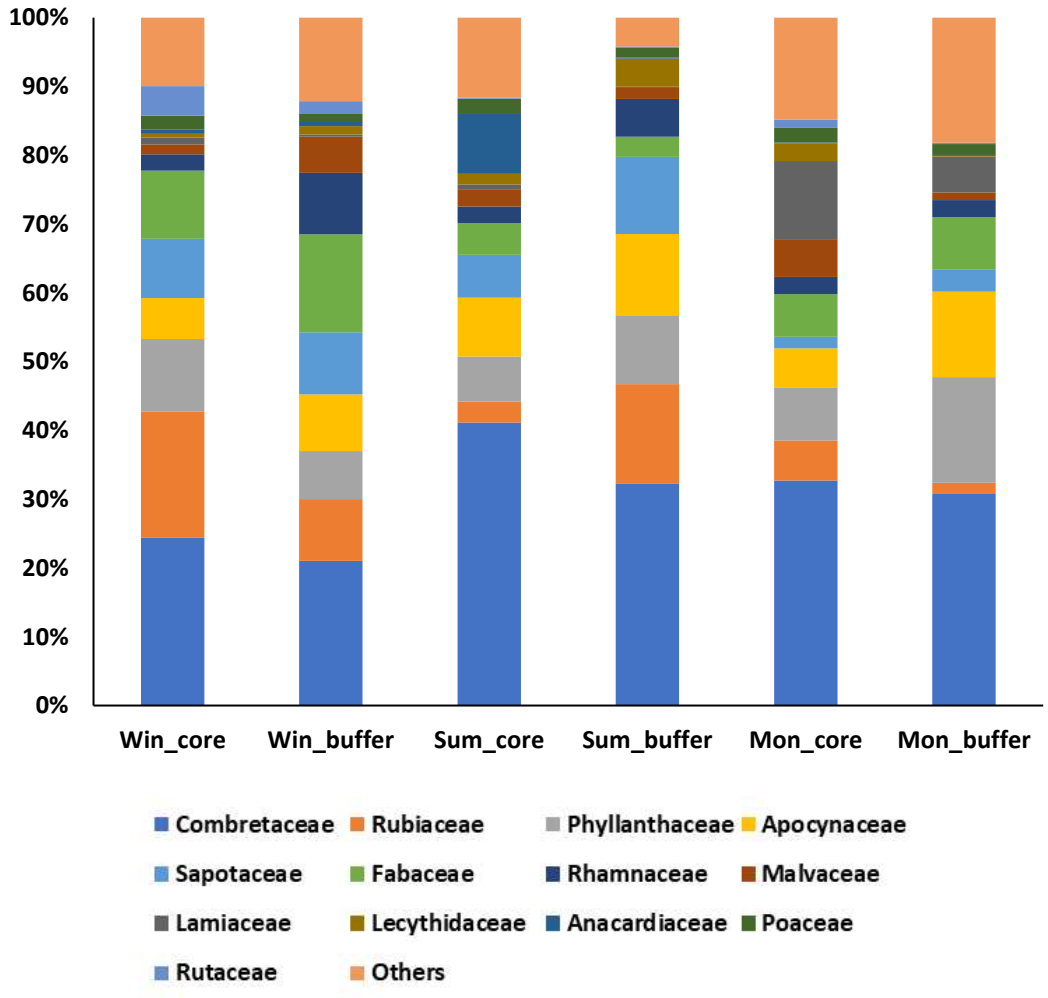


Figure 4: Sambar diet in different season and core and buffer in Tadoba Andhari Tiger Reserve

Chital diet

In total, we identified plant species from 88 plant families in the chital diet in Tadoba Andhari Tiger Reserve (TATR) from 206 samples that yielded results after sequencing. The data from these samples will be analyzed with consideration of spatial and temporal variations in the chital diet in TATR. Our results show that Rubiaceae, Combretaceae, Fabaceae, Apocynaceae, and Sapotaceae are among the most prevalent plant families in the chital diet.

The data presented here is based on a single marker; however, we are currently analyzing data from three additional markers to further refine dietary insights and enhance our understanding of herbivore diets. Notably, we observed a stark shift in dietary patterns across space and time, which appears to correlate with plant phenology and species availability in different seasons.

Table 4: Season-wise details of all the samples sequenced post extraction, species identification and quality checking along with the number of reads obtained.

Sambar	Monsoon (Core)	Monsoon (Buffer)	Winter (Core)	Winter (Buffer)	Summer (Core)	Summer (Buffer)
No. of Sample	38	21	44	54	29	20
Read count	1794188	915858	2257554	2714648	1734017	1115219

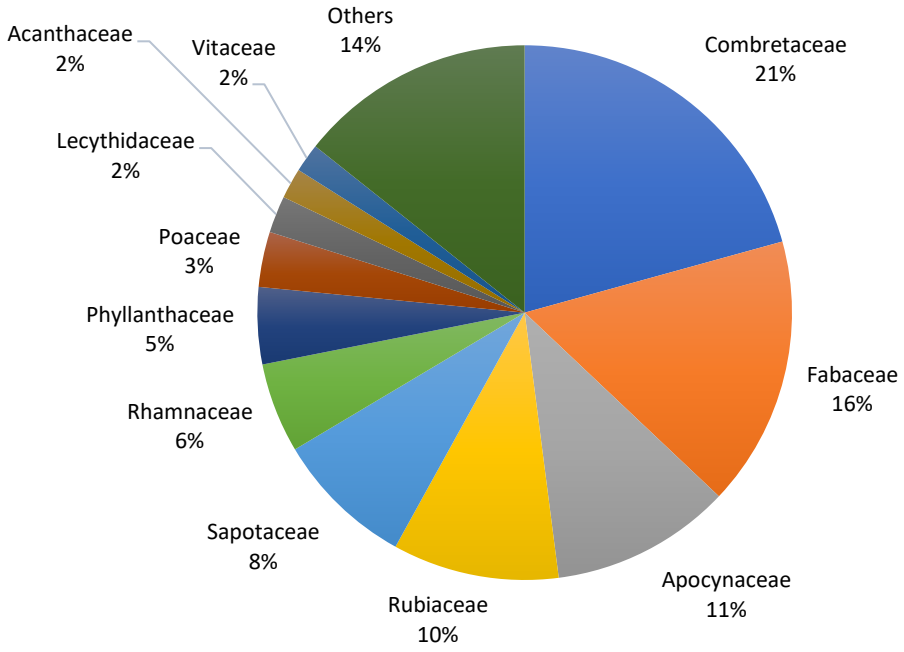


Figure 5: Overall dietary composition of chital in Tadoba Andhari Tiger Reserve

- **Spatial and seasonal variation**

Overall, we observed a stark difference in the chital diet across the three seasons. The highest dietary diversity was recorded during the monsoon season, followed by winter and summer. This variation could be attributed to the seasonal availability of plant species across the landscape.

During the monsoon, the diet was mainly dominated by two families namely Combretaceae, and Fabaceae with a significant difference between the core and buffer zones. In winter, the chital diet was dominated by Combretaceae, Fabaceae, Apocynaceae, Rubiaceae and followed by Sapotaceae. During summer, dietary diversity was lowest, with the diet primarily composed of Combretaceae, Apocynaceae, and Sapotaceae. We also observed substantial differences in the chital diet between the core and buffer areas of Tadoba Andhari Tiger Reserve, Maharashtra.

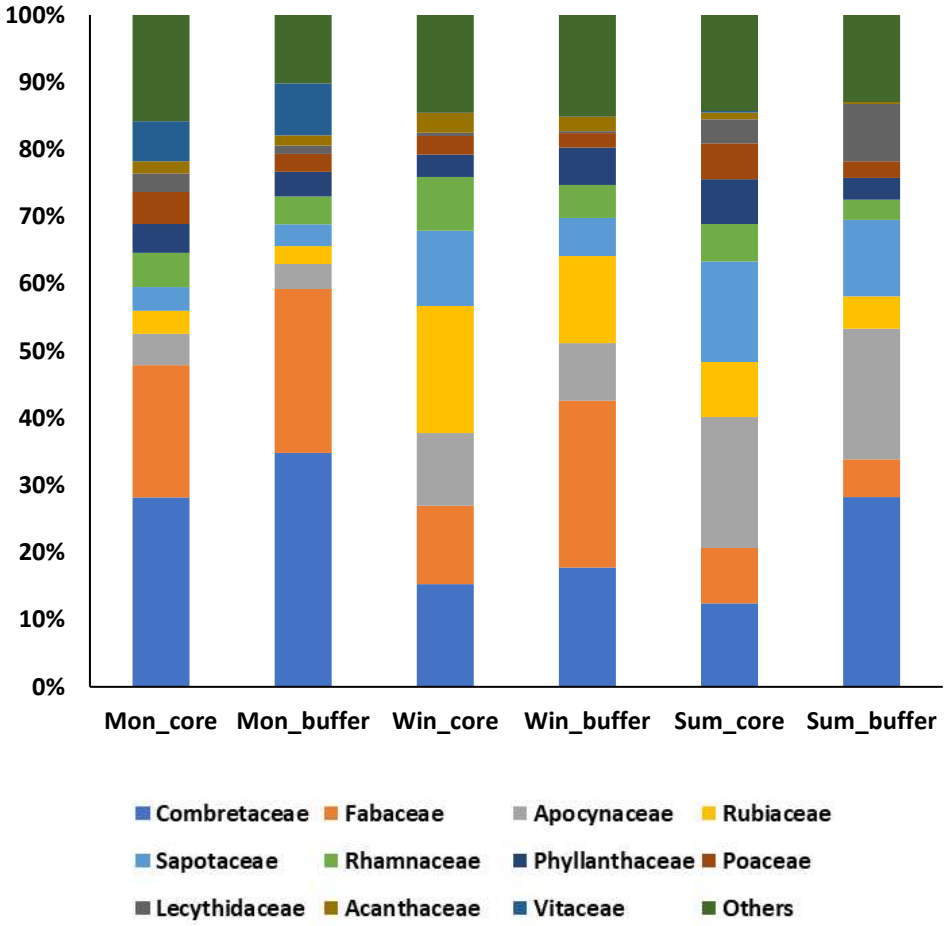


Figure 6: Chital diet in different season and core and buffer in Tadoba Andhari Tiger Reserve

Way forward:

We are currently analyzing the herbivore diet using three additional markers that we developed for this study to achieve a finer resolution at the genus and species level. We are also generating DNA barcodes for plant species, which will eventually be used to better understand the herbivore diet.

As we have already initiated Phase II of this project, we have received samples from the territorial divisions of Chandrapur district and have begun working on carnivore samples from outside protected areas. These samples, collected during the winter season, were provided by ground staff of Bramhapuri and Central Chanda divisions. We will be conducting sequencing for metabarcoding soon.