CHAPTER 3

MATERIALS AND METHODS

Due to the variation in size of impala in the Limpopo Province, four farms at different locations within the province were identified to obtain impala samples from. Two farms, Ndzalama and Selati, were identified in the lowveld area near Tzaneen. While the other two, Mara Research Station and Messina Nature Reserve, were in the northern parts of the Limpopo Province, near Louis Trichardt.

3.1 Description of the study areas

South Africa has one of the richest floras in the world. The vegetation can be divided into seven biomes and 68 vegetation types. Each biome is characterised by its own ecological capacity. The biomes are: Thicket, grassland, succulent karoo, forest, nama-karoo, savanna and fynbos. These biomes are again divided into 13 ecological regions (Bothma, 1989). The savanna biome is the largest biome in southern Africa. It is well developed over the lowveld and Kalahari region of South Africa. This biome is characterised by relatively high summer rainfall, and high mean temperatures. The savanna is characterised by a grassy ground layer and a distinct upper layer. Where the upper layer is near the ground the vegetation may be referred to as shrubveld; where it is dense it is known as woodland, and the intermediate stages are locally known as bushveld. Savanna provides the best regions for game ranch management, since the large diversity of vegetation can support a large variety of browsers and grazers (Bothma, 1989).

3.1.1 Savanna

This biome is sub divided into five veld types, namely valley bushveld, Kalahari bushveld, sour mixed bushveld, sweet bushveld and lowveld. The Selati Game Reserve and Ndzalama are situated in the mixed and lowveld veld type. The biome is characterised by species such as red bush willow (*Combretum apiculatum*) and knob thorn (*Acacia nigrescens*).

A dense grass stratum occurs and consists of rooigras (*Themeda triandra*), guinea grass (*Panicum maximum*), bushveld signal grass (*Urochloa mosambicensis*) and finger grass. The most abundant tree species are mopane (*Colophospermum mopane*), red bush willow and sickle bush (*Dichrostachys cinerea*)

The average rainfall is 235 - 1 000 mm per annum, and frost may occur for 120 days per year. Almost every major geological and soil type occurs within the biome.

3.1.2 Mixed lowveld Bushveld

The locality of this vegetation type is flat undulating landscapes between 350 - 500 m above sea level, in Limpopo and includes Mara Research Station. The annual rainfall varies from 450 - 600 mm and temperatures vary between -4 °C to 45°C, with an average of 22°C.

The soil is characterised by sandy soils in the uplands and clayey soils with high sodium content in the bottomlands. The geology is granite and gneiss with numerous dolerite intrusions (Low & Rebelo, 1998).

The vegetation type can be described as dense bush on the uplands, open tree savanna in the bottomlands, and dense riverine woodland on riverbanks. The tree layer is characterised by red bush willow, sickle bush, silver cluster leaf (*Terminalia sericea*) and knob thorn. The grass layer consists of guinea grass, finger grass and bushveld signal grass. This vegetation type is ideal for game farming, ecotourism and cultivation of subtropical fruit.

3.1.3 Mopane Bushveld

This veld type is located on the undulating landscapes from the Kruger National Park to the Soutpansberg in Limpopo, and includes the Messina Experimental Farm and Nature Reserve.

There is an annual summer rainfall of between 250 to 500 mm, with temperatures varying between 1.5 °C and 42.5°C, with and average of 22 °C.

This mopane veld occurs on loamy sand and clayey soils in the undulating granitic landscape of the northern Kruger National Park and also on sandstone shale from north of the Soutpansberg in the Limpopo River Valley.

The vegetation is dominated with a fairly dense growth of mopane and mixtures of mopane and red bush willow, associated with knob thorn, and umbrella thorn. The shrub layer is moderately well developed and individuals of wild raisin bush.

The herbaceous layer includes grasses such as common nine-awn grass (*Enneapogon cenchriodes*), guinea grass and finger grass.

3.2 Study Areas

3.2.1 Ndzalama

Ndzalama Wildlife Reserve and Pieterskamp are part of Thiergarten, a 10 000 ha game ranch, situated in the Limpopo Province of South Africa, 80 km to the west of the Kruger National Park. The road divides Thiergarten from Letsitele to Eiland, with Ndzalama encompassing 8000 ha and Pieterskamp approximately 2000 ha, on either side of the road. The Shangaan people, who first explored the northeastern Lowveld, gave this area its name, which means "sacred rock". Both sections are privately owned, and part of a much larger business.

3.2.1.1 *Climate*

Ndzalama wildlife reserve and the Vorster farm have a distinctive wet and dry season. The wet season lasts from December till April with a peak in January and February. Figure 3.1 represents the rainfall on Ndzalama and Figure 3.2 represents the rainfall on the Vorster's Farm. The average rainfall is approximately 450 mm. The average minimum temperature is 15.3 °C (Tzaneen/ Grenshoek pol, weather station no. 0679106 3), (Figure 3.3) and the maximum temperature is 25.4°C (Figure 3.4).

Figure 3.1: Ndzalama - Average monthly rainfall

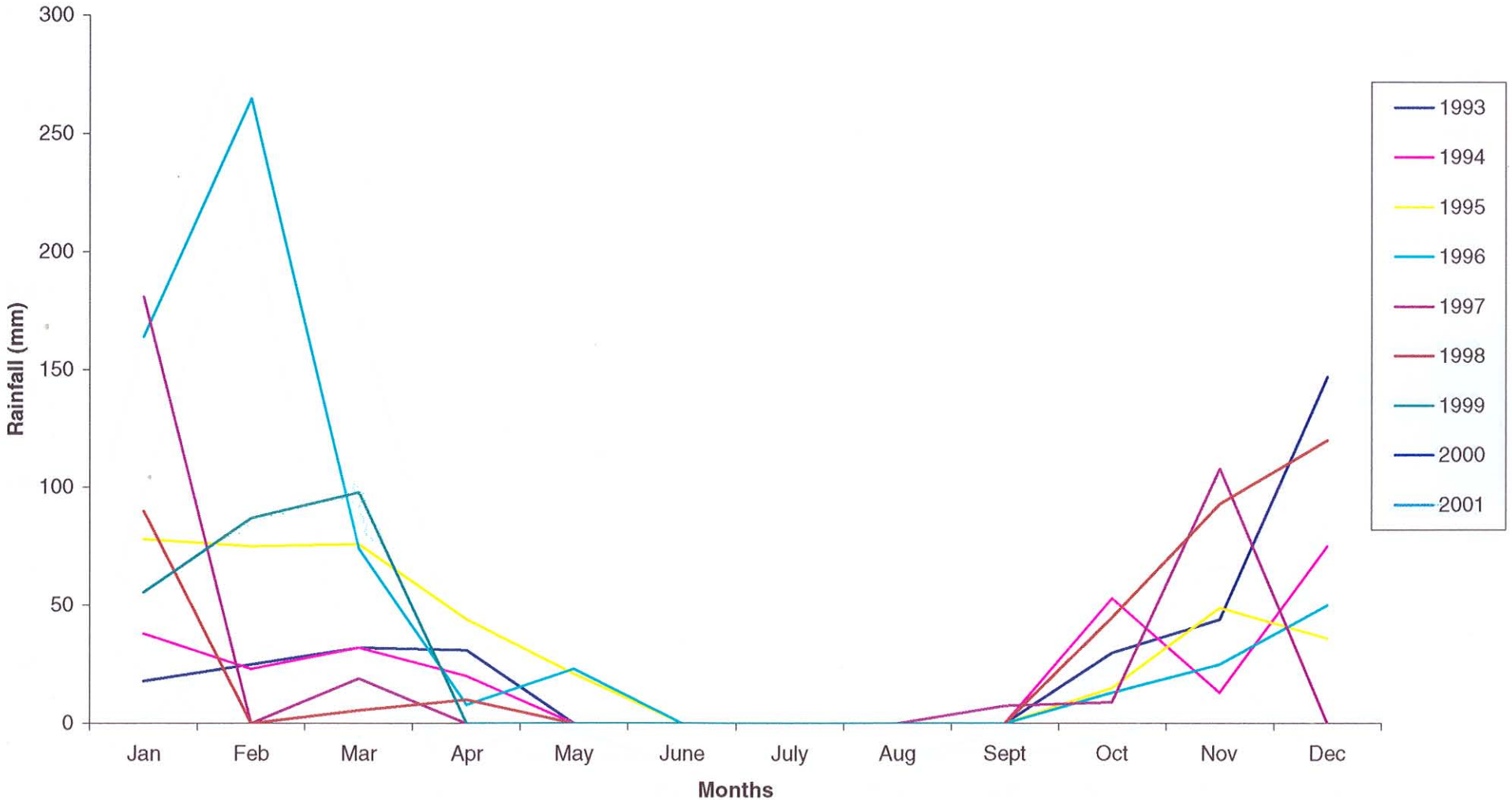


Figure 3.2: Thiergarten - average monthly rainfall

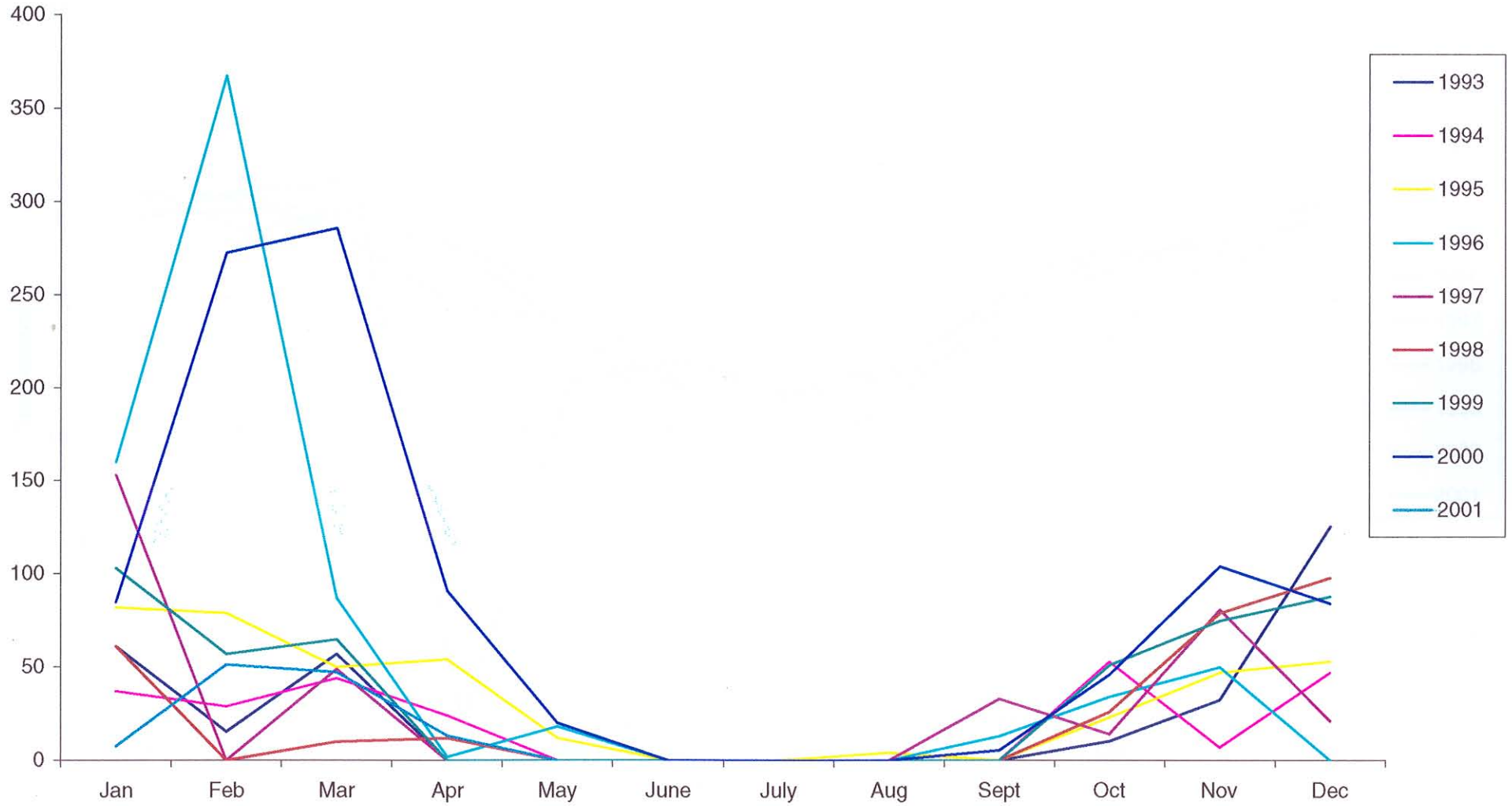


Figure 3.3: Ndzalama - Average monthly minimum temperatures

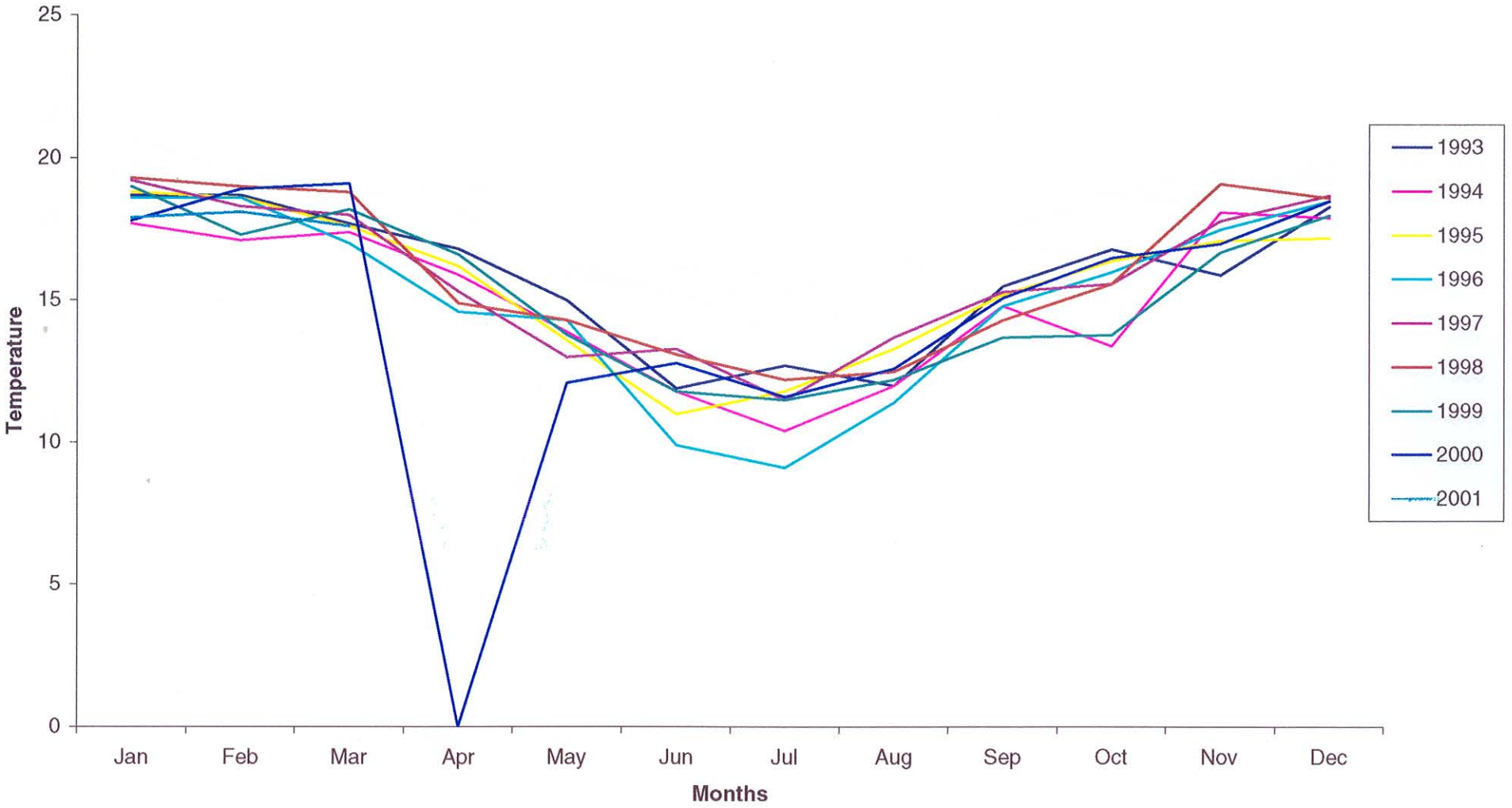
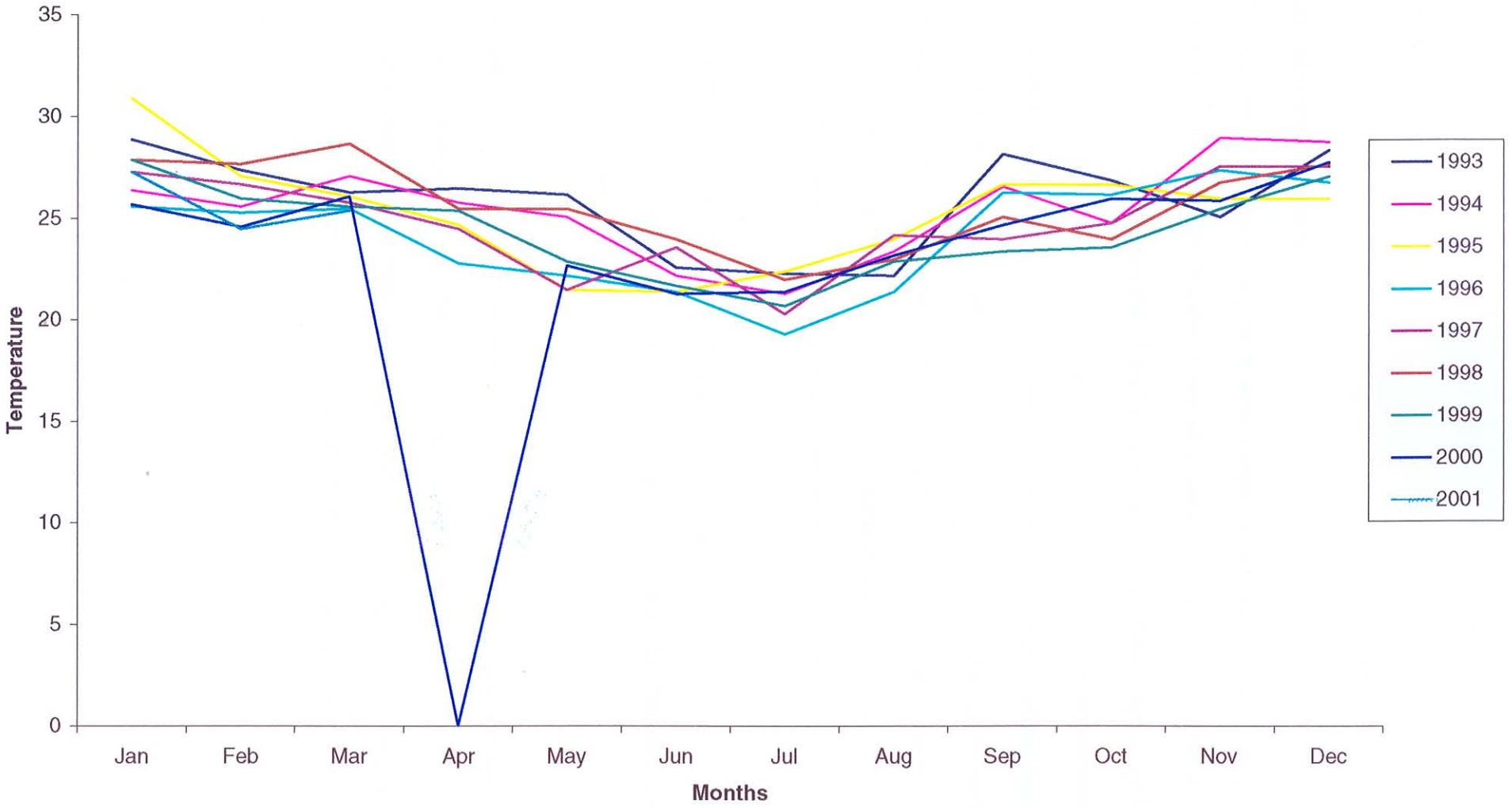


Figure 3.4: Ndzalama - average monthly maximum temperatures



3.2.1.2 *Animal species present*

Today, the reserves are a sanctuary to a wide variety of wildlife, including impala, giraffe, blue wildebeest, kudu, lion (*Panthera leo*), elephant (*Loxodonta africana*), white rhino (*Cerathotherium simum*), leopard (*Panthera pardus*), eland (*Taurotragus oryx*), bushbuck (*Tragelaphus scriptus*), nyala (*Tragelaphus angasii*), warthog (*Phacochoerus aethiopicus*), cheetah (*acinonyx jubatus*), tsessebe (*Damaliscus lunatus*), klipspringer (*Oreotragus oreotragus*), mountain reedbuck (*Redunca fulvorufula*), duiker (*Sylvicapra grimmia*), reedbuck (*Rednca arundinum*), sable antelope (*Hippotragus niger*), steenbok (*Raphicerus campestris*), zebra (*Equus burchellii*), hartebeest, waterbuck (*Kobus ellipsiprymnus*), caracal (*Felis caracal*) and hippopotamus (*Hippopotamus amphibious*).

The reserve partakes in the Sable breeding project and has a number of lions in a fenced camp. Due to the size, intensive management is required to maintain the optimum ecological capacity.

Although Ndzalama is only 30 km from Selati, it has a larger water surface and has a number of artificial dams, which fill in the summer months. The infestation of external parasites is compounded by this increase in water and dense grass cover. The small pepper tick (*Rhipicephalus appendiculatus*) is of great concern on the reserve.

3.2.1.3 *Vegetation*

The vegetation is typical of the mixed lowveld vegetation type, with an abundance of mopane, red bush willow and knob thorn. A dense grass stratum occurs and consists of *Themeda triandra* , guinea grass, bushveld signal grass and finger grass.

There are a number of lodges and other facilities present on the reserve.

3.2.2 Selati Game Reserve

The Selati game reserve is perhaps one of the largest private conservancy's. The reserve comprises of 27 372 hectares of unspoiled lowveld wilderness located in the north eastern Limpopo bushveld within the triangle formed by the tarred roads joining Phalaborwa, Mica and Gravelotte, known as the Selati Triangle. Originally the land was used for cattle farming with several low-key hunting and tourist orientated operations. The area within the reserve is best known for its Sable antelope herds. There are high deposits of ore in the area of Phalaborwa. This gives rise to the unique topography with varied and interesting geological formations manifesting themselves throughout the area. The reserve also boasts the cycad (*Encephlartos dyerianos*), which does not occur naturally anywhere else in the world.

The reserve is a joint ownership of seven landowners, with a goal "*to conserve and enhance the bio-diversity of the ecosystem and to realise its full economic potential on a sustainable basis*".

From the initial meetings in 1992 till now the participants are working on a well managed reserve with no internal fencing and significant commercial activity.

3.2.2.1 Climate

The Selati game reserve has an altitude of approximately 400 – 778 m. The average rainfall is approximately 500 mm per annum (Tzaneen/ Grenshoek pol, weather station no. 0679106 3) (Figure 3.5), with a ten- year cycle. The rainfall season is from December till April, with the peak being in February. The average minimum temperature is 15.6 °C (Figure 3.6), with the coldest month being June. The average maximum temperature is 25.4 °C (Figure 3.7) with the hottest month being January.

3.2.2.2 Animal species present

There are 22 species of large animals to be found on the reserve: white rhinoceros, eland, kudu, bushbuck, elephant, blue wildebeest, nyala, leopard, warthog, cheetah, tsessebe, klipspringer, mountain reedbuck, duiker, reedbuck, sable antelope, steenbok, zebra,

Figure 3.5: Selati average monthly rainfall

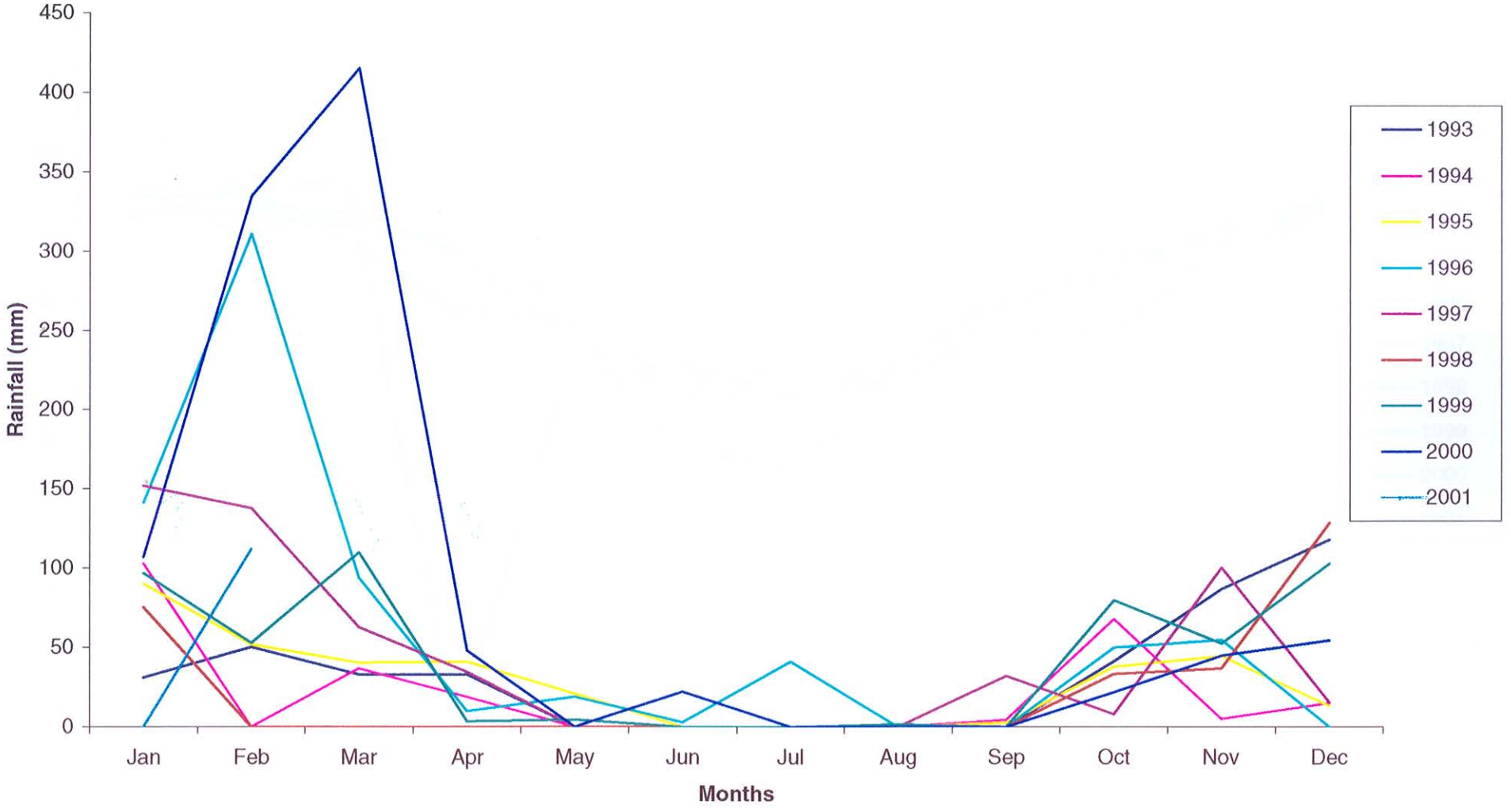


Figure 3.6: Selati average monthly minimum temperature

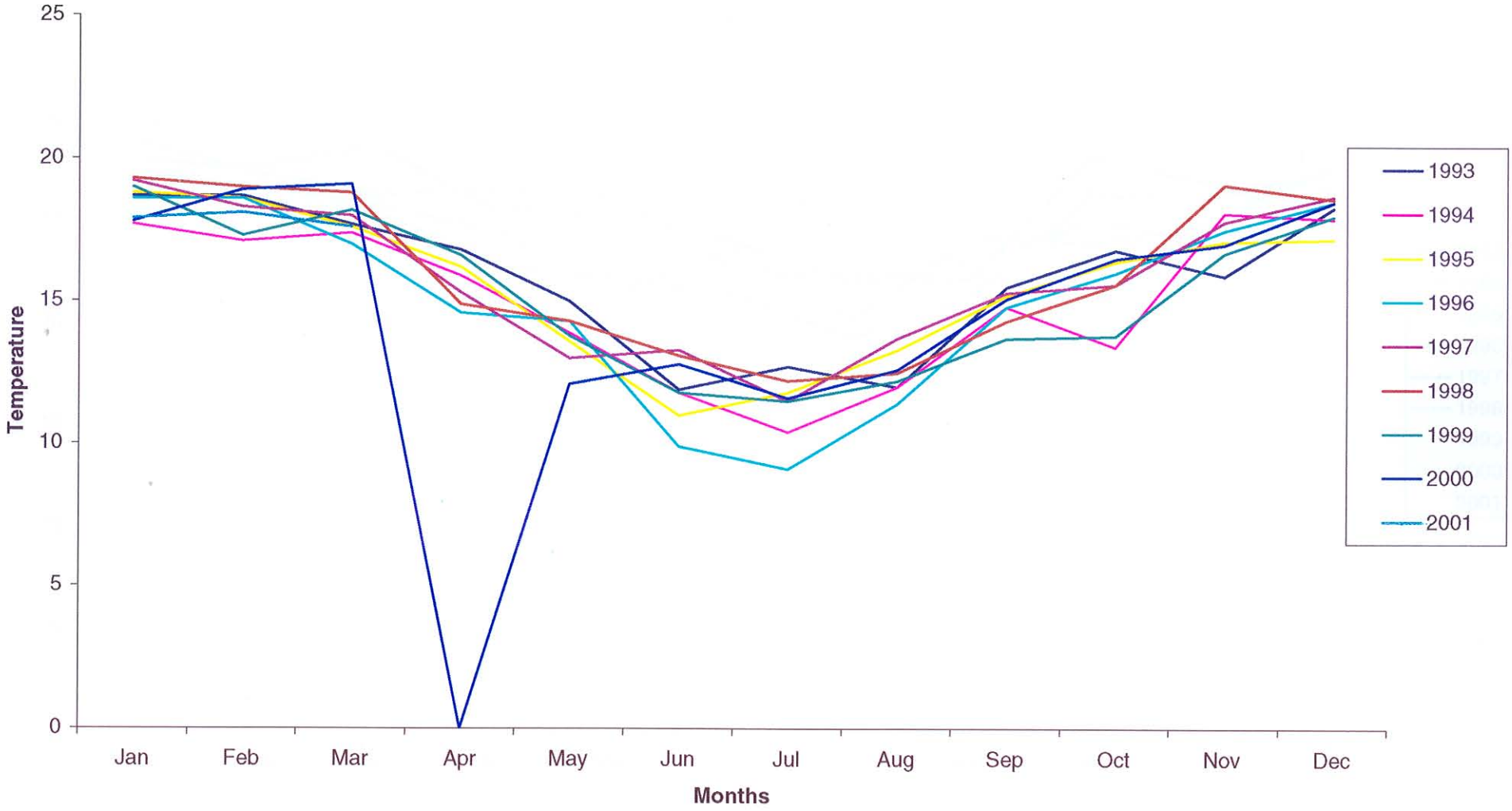
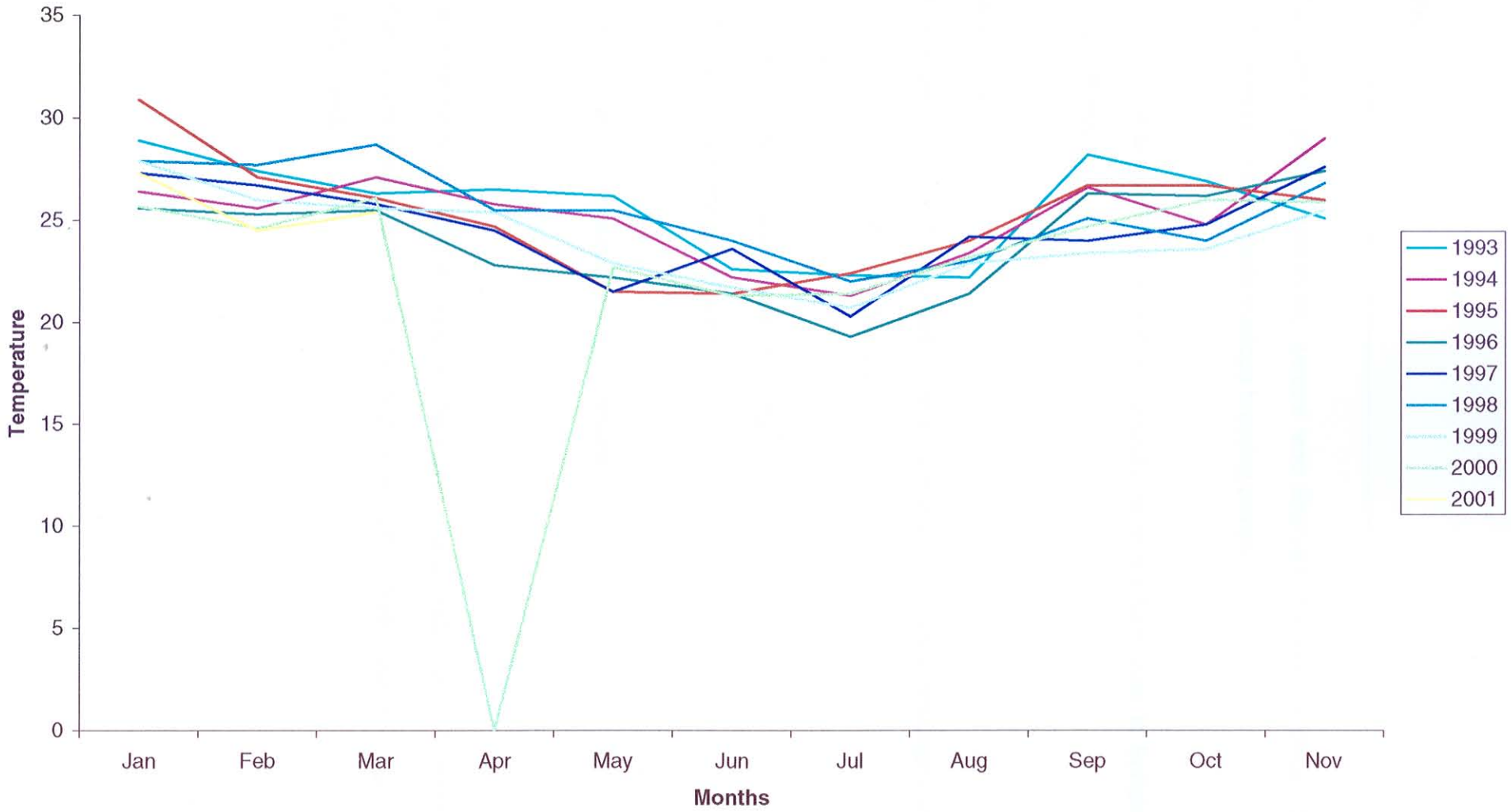


Figure 3.7: Selati - Average monthly maximum temperautre



impala, hartebeest, giraffe, waterbuck, caracal and Sharpe's grysbok (*Raphicerus sharpei*). The reserve also has sable antelope breeding camps.

Cattle farming is still present on the northern boundaries of the farm, next to Buffalo Ranch and BVB Ranch.

3.2.2.3 Vegetation

This reserve is characteristic of the mixed lowveld bushveld. The varied topography of the area encompasses six different veld types:

a. *Combretum* veld
b. Mixed *combretum* and marula on quartz
c. Mixed mopane and *combretum*
d. Mopane
e. Mixed mopane, *combretum* and cederwood
f. *Terminalia*

There is an abundance of mopane trees, red bush willow and knob thorn. A dense grass stratum occurs and consists of *Themeda triandra*, guinea grass, bushveld signal grass and finger grass.

3.2.3 Mara Research Station

Mara is a government run research station, belonging to the Department of Agriculture, Limpopo province situated approximately 30 km from Louis Trichardt. It covers 10 000 hectares of sweet bushveld. Mara is an experimental farm for cattle with a small compliment of wildlife present.

3.2.3.1 *Climate*

Mara research station has an altitude of 961 m. The average rainfall is 452 mm (Mara pol, weather station no. 0722099 1) (Figure 3.8). The average minimum temperature is 12.8 °C (Figure 3.9) with the coldest month being June. The average maximum temperature is 27.5 °C (Figure 3.10), with the hottest months being December. On average the daily maximum temperature exceeds 20°C for 337 days of the year.

3.2.3.2 *Animal species present*

Mara is an experimental farm with only naturally occurring wildlife present. Wildlife species present include: civet (*Civettictis civetta*), steenbuck, kudu, impala, warthog, duiker, waterbuck, caracal, black backed jackal, cheetah and leopard.

Figure 3.8: Mara average monthly rainfall

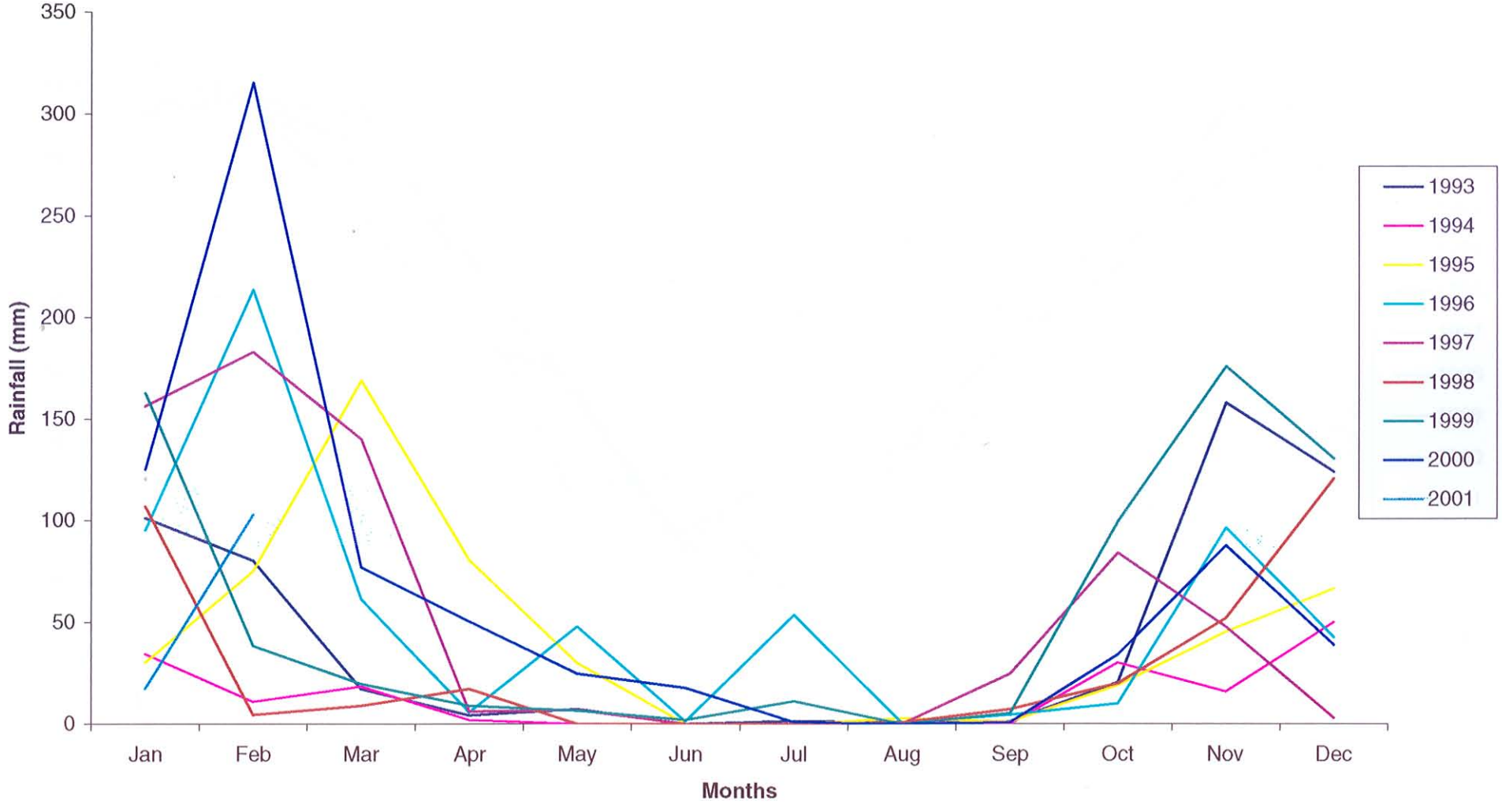


Figure 3.9: Mara - average monthly minimum temperatures

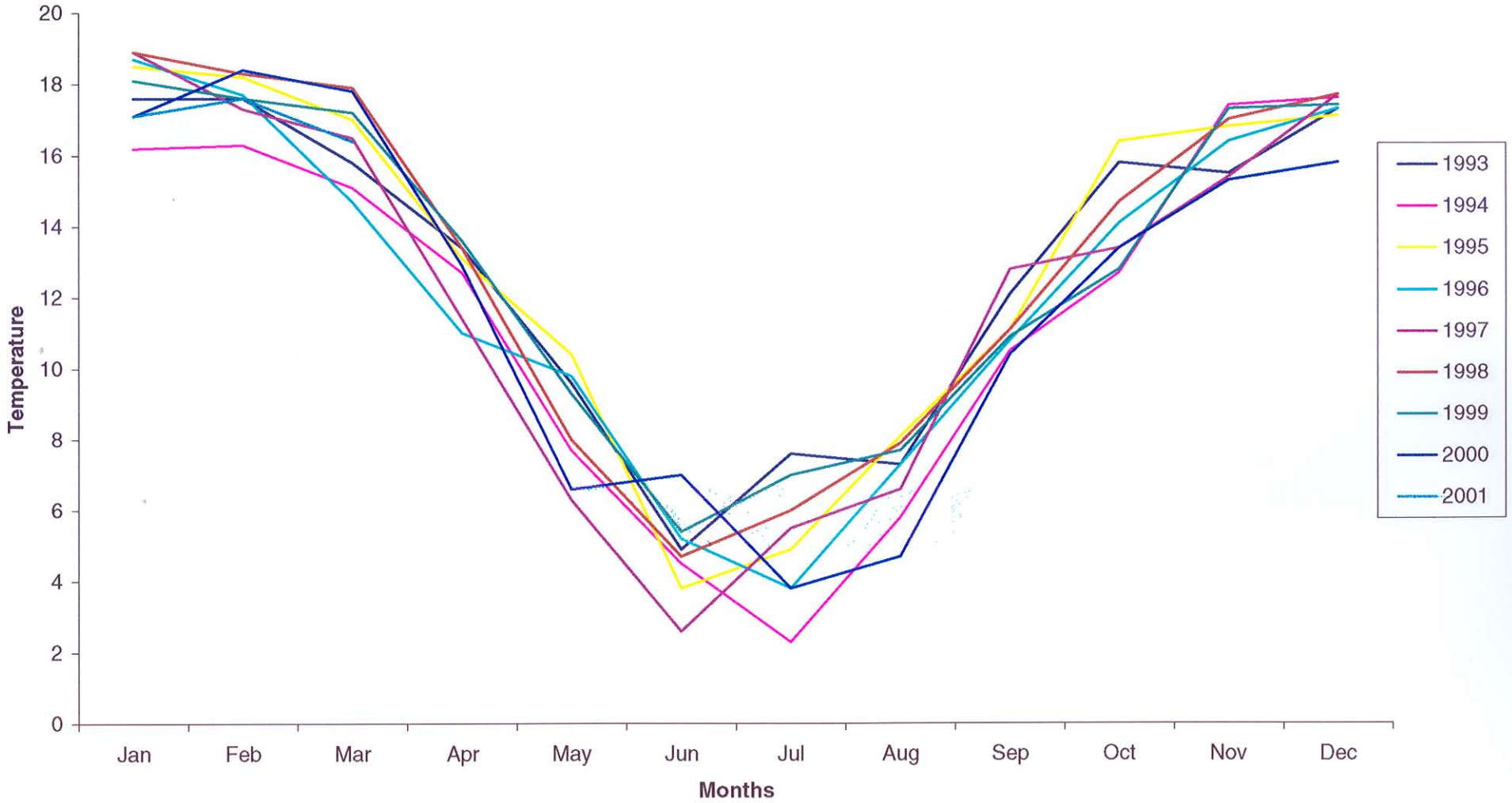
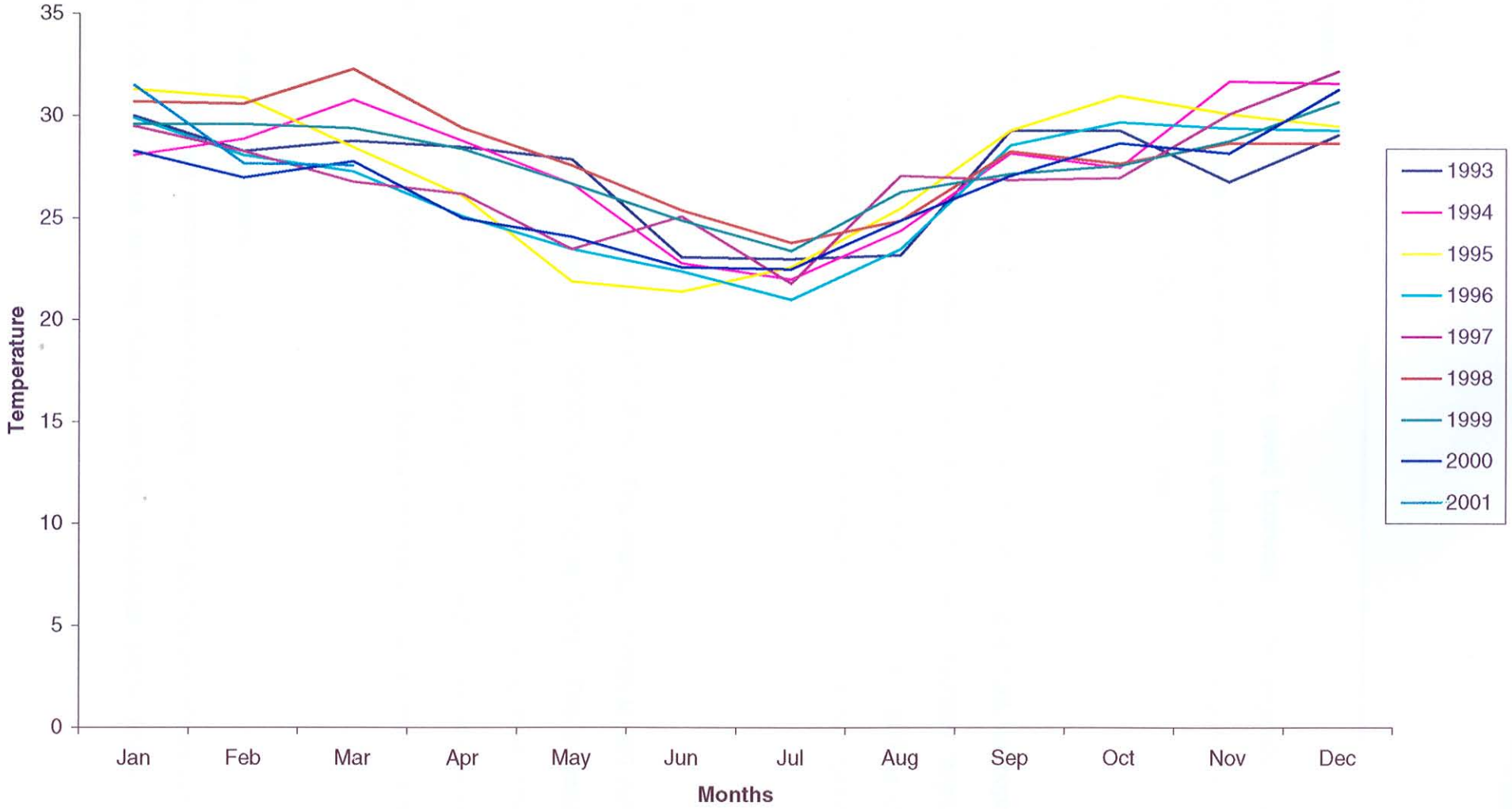


Figure 3.10: Mara - average monthly maximum temperature



3.2.3.3 *Vegetation*

The vegetation here is characteristic of the sweet bushveld. The vegetation is dominated with silver cluster leaf, wild raisin bush and umbrella Thorn. The grass layer is dominated with guinea grass and bushveld signal grass.

3.2.4 Messina

Messina is a nature reserve, which is run by the Department of Agriculture, Limpopo Province. It is located on the northern side of the Soutpansberg mountain range, approximately 100 km from Louis Trichardt. The nature reserve spans 7 500 ha of mopane veld, with only game present on the reserve. During the hunting season, game is hunted either for biltong or for trophies.

3.2.4.1 *Climate*

Messina Nature Reserve has an altitude of 780 m. The average rainfall is 350 mm (Messina / Macuville weather station no. 0809706 X) (Figure 3.11). The average minimum temperature is 16.0 °C (Figure 3.12) with the coldest month being June. The average maximum temperature is 30.1 °C (Figure 3.13), with the hottest months being December. On average the daily maximum temperature exceeds 22°C for most of the year.

3.2.4.2 *Animal species present*

Messina has only naturally occurring wildlife present. Wildlife species present include: civet, steenbuck, kudu, impala, warthog, duiker, waterbuck, wildebeest, zebra, giraffe,

Figure 3.11: Messina average monthly rainfall

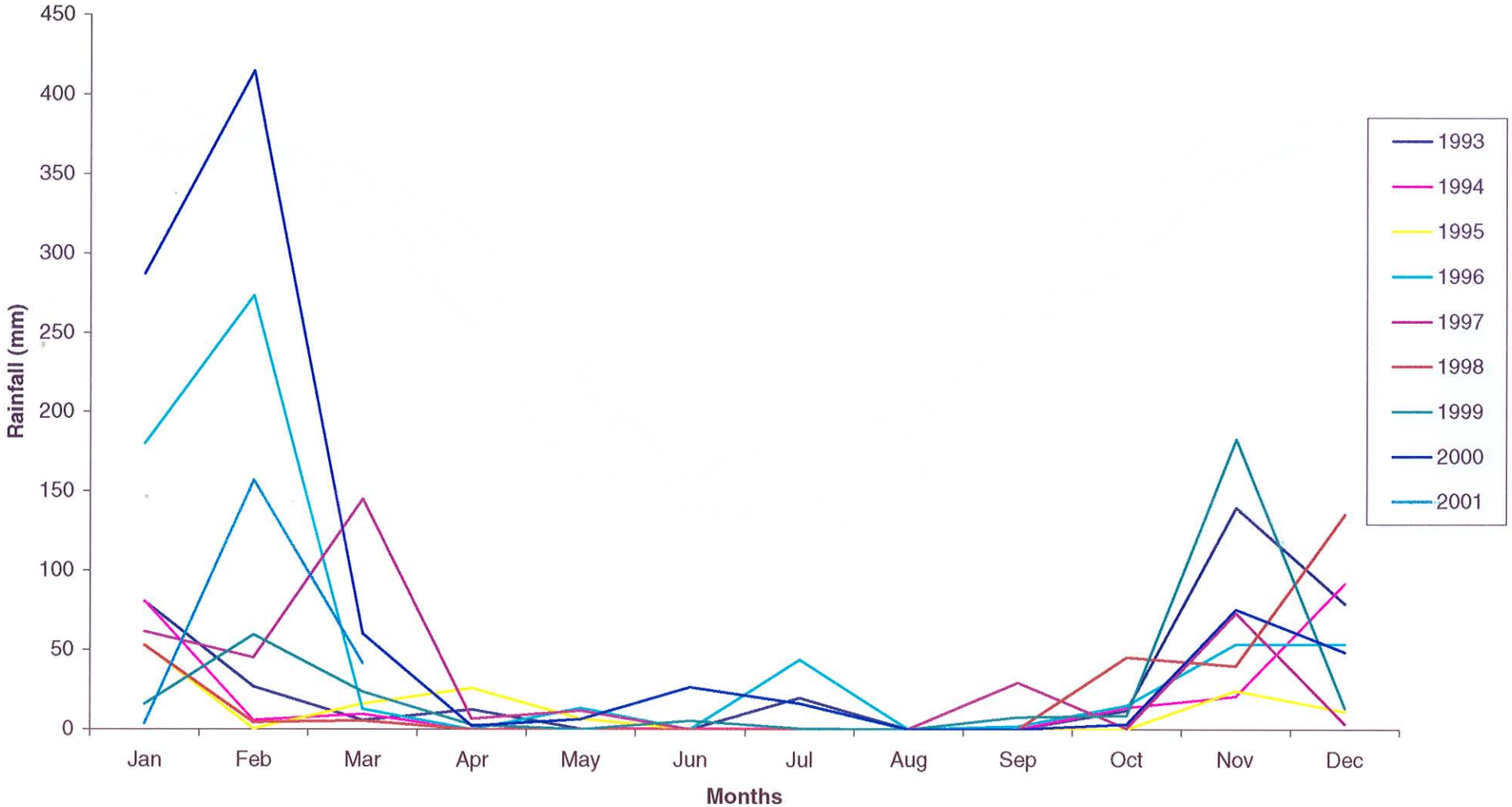


Figure 3.12: Messina - average monthly minimum temperatures

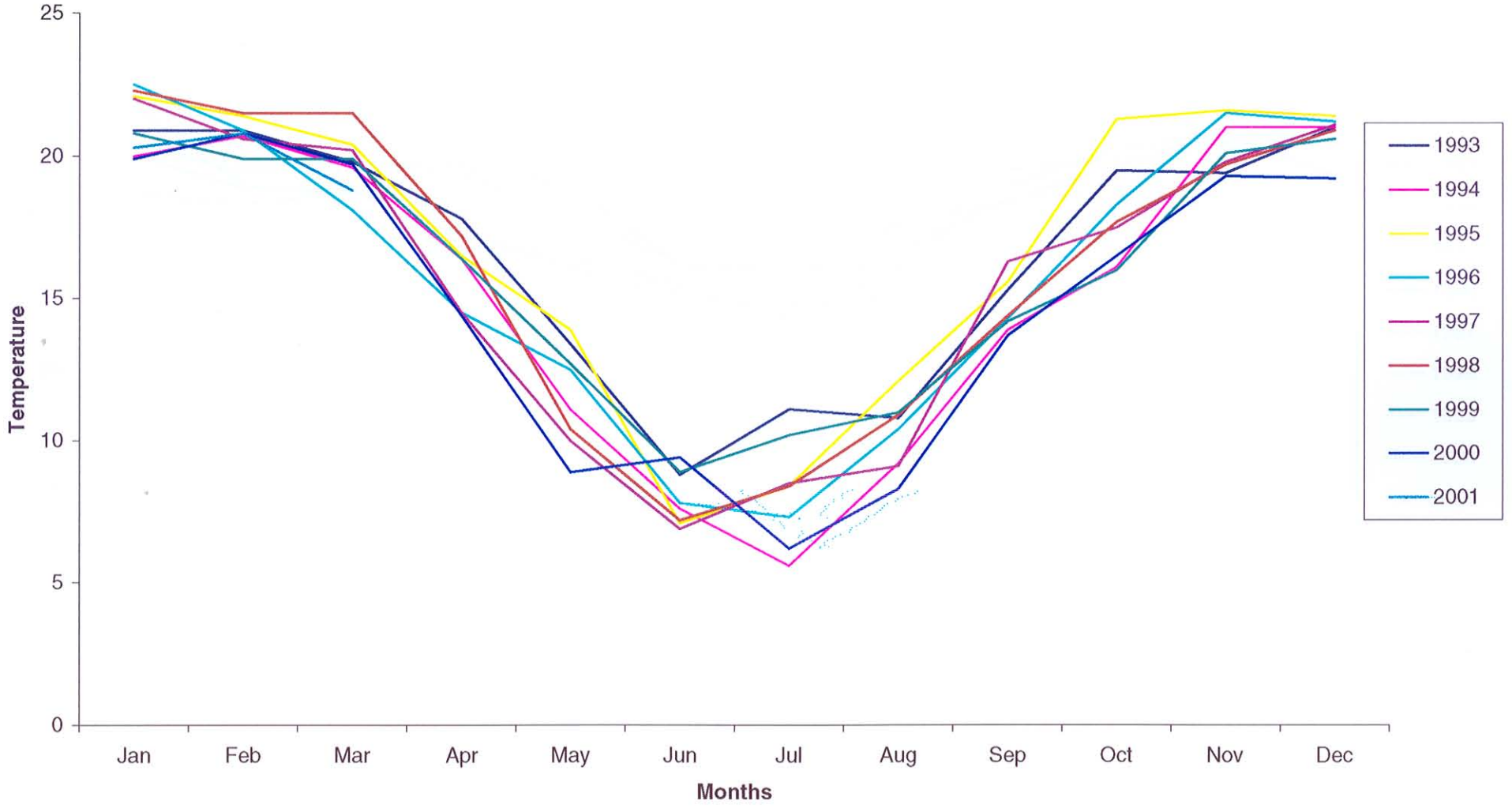
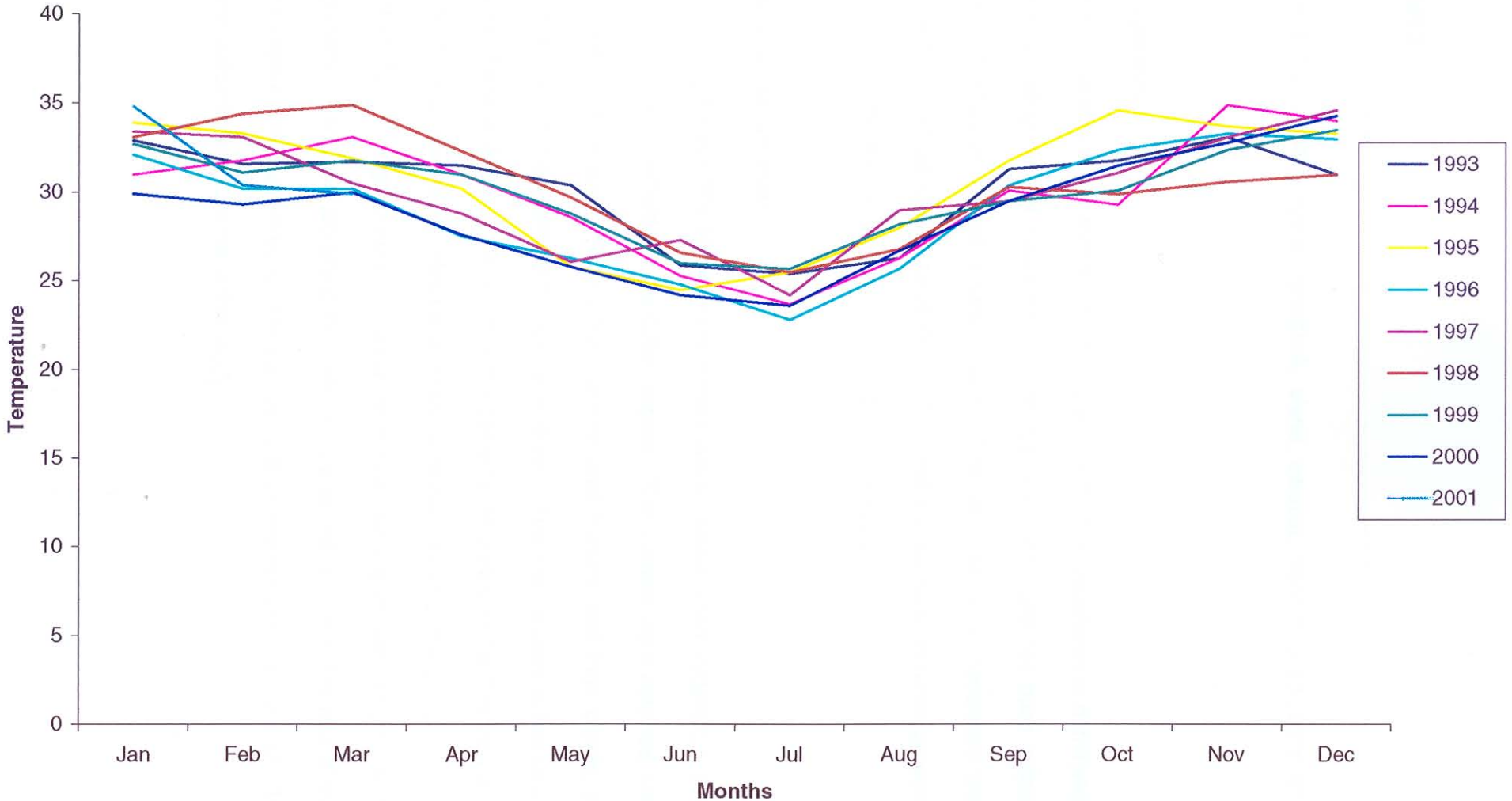


Figure 3.13: Messina - average monthly maximum temperatures



leopard, wild dog, spotted hyena, gemsbok, eland, caracal, black backed jackal and cheetah.

3.2.4.3 *Vegetation*

The vegetation here is characteristic of the mopane veld. The vegetation is dominated with a fairly dense growth of mopane and mixtures of mopane and red bush willow, associated with knob thorn and umbrella thorn. The shrub layer is moderately well developed and individuals of wild raisin bush. The herbaceous layer includes grasses such as common nine-awn grass, guinea grass and finger grass

3.3 Experimental animals

This study required many different samples: impala livers, impala blood, vegetation, soil, water and linear measurements of the culled impala. The animals were obtained with the help of different persons, ranging from professional hunters and their clients, to assistant managers or managers of the game reserves. The impala were culled using either a .223 rifle or a .375 rifle. At each of the game farms visited in the Tzaneen and Louis Trichardt area, impala samples were taken randomly. Most of the samples were collected from April through to August, during the normal hunting season. Night culling was the preferred method of obtaining the samples, so as not to disturb the rest of the herd. The impala was numbered on the ear using a permanent pen. A total of 114 impala were culled for the purpose of this study.

3.4 Blood and Liver samples

The aim of the experiment was to identify the mineral status through the Cu, manganese (Mn) and Se concentrations in the livers of the impala. A genetic analysis was done on the blood to determine if there was any evidence of inbreeding.

The blood was taken immediately after the animal was culled. This was done by making an incision into the jugular vein. The blood was placed in a test tube with heparin or EDTA and tilted thoroughly, so to avoid clotting. The test tubes were labelled with the number of the impala and the area in which it was shot. The blood samples were placed on ice till returning to the lodge.

Back at the lodge, plasma was prepared by centrifugation of the heparinised blood. This, however, proved to be problematic, as it was often a couple of hours before returning to the lodge.

The liver samples were preserved in buffered formalin. The formalin was prepared at the University of Pretoria prior to departing to the game reserves. The buffered formalin was prepared using sodium orthophosphate, sodium hydroxide pellets and glucose dissolved in analytical grade formalin. The solution was then made up to five litres using distilled water. Liver samples were taken on return to the lodge, the impala were disembowelled and a 6 x 6 cm block (150 g) sample was taken from the centre of the liver. This sample was then placed in a sample bottle containing 200 ml buffered analytical grade formalin.

The bottle was labelled with the number and area where the impala was culled. This sample in formalin was then kept in the refrigerator. Whilst travelling back from the game farms the samples were kept on ice until they could be placed in the cool room at the University of Pretoria.

3.5 Faecal Samples

The aim of collecting these samples was to determine the mineral concentration of the faeces. Nitrogen and ash was also determined. The minerals analysed, include Cu, Mn, P, Zn, Ca and Mg.

Faecal samples were collected every second month from commencement of the project, from impala on each of the farms and analysed.

Wrench *et al.* (1996)'s suggestion for the collection of faeces of free ranging animals was followed for the collection of the impala faeces:

- Faecal samples were collected from free ranging animals
- Samples up to a day old, showing no signs of dung beetle activity were collected The samples were kept in brown paper bags in a ventilated room at room temperature, to prevent fungal growth.

Whilst travelling back from the game farms the samples were kept cool until they could be placed in an oven at the University of Pretoria to dry.

3.6 Vegetation, soil and water samples

Soil samples were taken from each of the farms to determine the soil fertility, soil type and soil acidity on each of the farms. Samples were taken by digging a hole 500 mm deep and then taking hand sample of the soil.

A sample of the graze and the browse was taken from each of the farms. These samples were taken to determine the mineral concentration of the graze and browse. Nitrogen and ash were also determined. The minerals that were analysed included Cu, Mn, P, Zn, Ca and Mg.

The condition of the veld was determined for each of the seasons and records of the rainfall and distribution were collected. The following information was determined:

Veld management:

- Type of veld in which the impala move in
- Average rainfall and distribution
- The presence of licks
- Sources of drinking water

Samples were taken from the veld in areas where the impala were seen to be grazing. Grass samples included the roots, which were included in the analyses of the grass samples. Excess soil was shaken off the grass samples. The samples were placed in brown paper bags. The grass sample was then sun dried and kept in a cool place until leaving the game farms.

Samples were taken from the trees and shrubs that were utilised by the impala. Leaves, pods and branches were taken. These were placed in a brown paper bag in the field. At the lodge the samples were sun dried and placed in a labelled brown paper bag and stored in a cool place until transferred to the University of Pretoria.

A water analysis was done by the Department of Soil and Climate during November 1998. Samples were collected in accordance with prescribed methods for water quality. Samples were collected from many subterranean sources, predominantly from the point of intake by the game. Natural earth dams were also included (Meyer, 1999).

3.7 Laboratory analysis

Liver, vegetation and faecal samples were oven dried at 60 °C, to a constant weight at the University of Pretoria. The livers were left in the oven for 48 hours while the vegetation and the faeces were left in the oven for 24 hours, until the samples were at a constant weight . All the samples were then ground to fine particles for subsequent laboratory analysis.

3.7.1 Dry matter analysis

This was done on all the samples. The DM concentration was determined using the AOAC (1984) method. One gram of the sample was weighed into porcelain crucibles and then placed in an oven at 100 °C overnight. Thereafter, the crucibles were put in a desiccator for 30 minutes to cool before being weighed.

To determine the dry mass of the sample the following equations are used:

1	Mass of empty, dry and clean crucible	14.5192 g	
2	Mass of crucible and air dried sample	15.7286 g	
3	Mass of crucible and dry sample	15.6042 g	
4	Mass of air-dried sample	1.2094 g	(2 –1)
5	Mass of over dried sample	1.0850 g	(3 –1)

Percentage of dry material $\frac{1.0850\ g}{1.2094\ g} \times \frac{100}{1} = 89.71\ \%$

3.7.2 Ash Determination

A crucible with a dry sample for DM determination was placed in a cold incinerating oven. The sample was incinerated at 600 °C for four hours. The residue was then left to cool down for at least two hours before being placing it in a desiccator to cool for another half an hour. The crucible and ash were then weighed and the value recorded.

To determine the ash of the sample the following equations are used:

1	Mass of crucible	14.5192 g
2	Mass of crucible of dried sample	15.6042 g
3	Mass of air dried sample	1.2094 g
4	Mass of crucible and ash	14.6000 g
5	Mass of ash	0.0808 g

Percentage of ash in sample: $\frac{0.0808\ g}{1.2094\ g} \times \frac{100}{1} = 6.68\ \%$

3.7.3 Determination of crude protein

The Macro-kjeldahl method (AOAC, 1984) was used to determine the protein concentration of the faecal and vegetation samples. One gram of the sample was placed into a digestion flask (250 ml) and placed on the bloc digester and thereafter the sample was distilled with a Tecor kjeltec system model 1026 (Distillation Unit, manual part No: 1000 2790, T8806, Prabin & Co, Klippan.

The N and CP were calculated as follows:

% N = {sample titration – blank titration (factor) / sample mass (g)} x 100

% N was corrected for DM and reported as g N/kg DM

% CP = % N x 6.25

3.7.4 Determination of minerals

These analyses were done on the liver, faeces and vegetation. However, not all the minerals were analysed on the liver, as discussed earlier. Minerals analysed include, Cu, Mn, Mg, Zn, Ca and P. The wet ashing method for digestion was used for the samples.

A gram of each sample, in duplicate, was digested in a block digester at 230 °C. The concentrations of Ca, Mg ad Zn were determined using the Perkin Elmer 2380 Atomic Absorbtion Spectrophotometer pp Ay II. The minerals Mn and Cu were determined using the Varian Atomic Absorbtion Spectrophotometer 50 (1997). The concentration of P was determined from calibration curve using the Auto Analyser.

The concentration of the macrominerals, Ca, P and Mg were expressed as g/kg DM and those of the trace minerals, Zn, Cu and Mn, were expressed as mg/kg DM.

Selenium analyses were only done on the blood and liver samples. One gram of sample was used for the determination of Se. This sample was digested on a block digester with a temperature-timed programmer. The concentration of Se was determined on the same spectrophotometer that was used for Ca and Mg except that a continuous flow hydride vapour generator was attached. An internal laboratory standard liver sample with known concentration was included in the analysis. The readings of all the samples were done on the spectrophotometer and expressed as mg/kg. This value was converted to ng/g by using the following equation:

$$\text{Se ng/g} = \frac{\text{Reading (mg/kg)} \times \text{Dilution factor}}{\text{Mass}}$$

3.7.5 Determination of Neutral Detergent Fibre

This analysis was done on the vegetation samples using the Dosi Fiber and Fibertec system (Robertson & Van Soest, 1981). The samples were air dried and milled to pass through a sieve with circular openings 1 mm in diameter.

Exactly 1 gram samples were weighed into the sintered glass crucibles (porosity 2) and placed on the hot extraction unit of the system. A neutral detergent solution (NDS) was added into the crucible and allowed to boil for one hour. The solution was then washed out with hot distilled water. The residues in the crucibles were dried at 100 °C overnight, cooled the following day a desiccator for 30 minutes.

After cooling the samples were weighed and placed in a muffle furnace to be ashed at 600 °C for 3 hours. The furnace was allowed to cool to at least 250 °C. The crucibles were then removed and placed in a desiccator and cooled for 30 minutes and then weighed. The following equation was used to determine the NDF:

- RCD = residue in crucible after drying

  RCA = residue in crucible after ashing

  $$\%NDF = \frac{RCD - RCA}{\text{original sample mass}} \times 100$$

  Corrected for dry matter content of the sample

3.7.6 Determination of Acid Detergent Fibre

The ADF was determined according to the method of Goering & Van Soest (1970) using the Tecator fibretec system. The samples were air dried and milled to pass through a sieve with circular opening 1 mm in diameter (Goering & van Soerst, 1970). One gram of sample was weighed. The analysis is exactly the same as the NDF analysis except that the acid detergent solution (ADS) was used.

- RCD = residue in crucible after drying

  RCA = residue in crucible after ashing

  $$\%NDF = \frac{RCD - RCA}{\text{original sample mass}} \times 100$$

  Corrected for DM content of the sample.

3.8 Linear measurements

All measurements were taken using a flexible roller measuring tape, using the metric scale. Linear measurements were taken from the impala that were culled. The animals were hung upside down, from their hindlegs. The measurements were taken on the right side of the carcass. The following parameters were collected for comparison:

- Body length – the length was taken from the atlas- joint along the neck and the curve of the body on the back bone till the tip of the tail.
- Shoulder height – this was measured at the right foreleg of the animal. The measurement was taken from the tip of the hoof to the most protruding part of the scapula. These measurements were used as an indicator of growth and size.
- Horn length – the length of the impala horn is measured along the front curve, from the base to the tip of the horn (Bothma, 1989).
- Horn circumference – this measurement is taken at the base of both horns as close as possible to the head, at right angle to the axis.
- Testicular circumference – the testis were pulled down and the measurement was taken at the widest part of the testis.
- Further measurements were done on the bone structure of the animal. These included measuring the metatarsus and the metacarpus

The data obtained for the impala in the Kruger National Park (KNP) was kindly provided by Dr. V de Vos, Head of Research at the KNP, for comparative purposes.

See figure 3.14 for detailed measurements. Abbreviations used are:

Bp – Breadth of the proximal end of the metatarsus

Gl – Greatest length

Sd – Smallest breadth of diameter

Li – Lateral length of the outer side

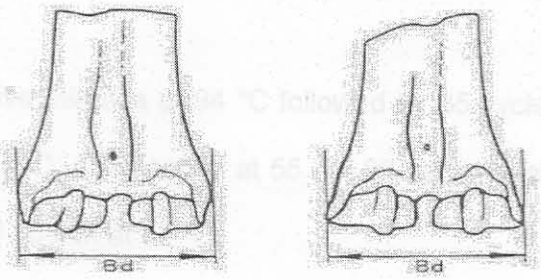
Gli – Greatest length of the lateral part

Bd – Greatest breadth

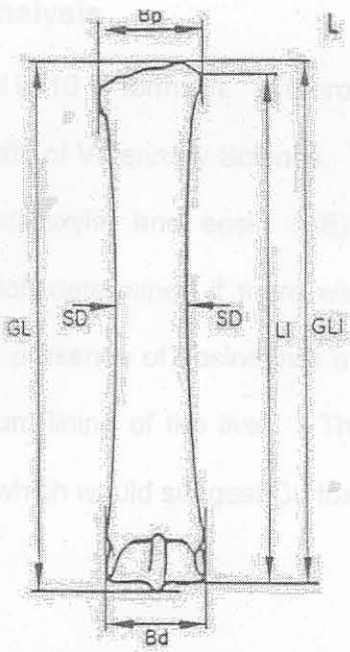
3.9 Genetic Analysis

To determine the genetic variation of the impala populations, either a hair or blood sample was taken from the culled impala. The blood samples were kept in EDTA tubes and stored at -70°C at the University of Pretoria. The DNA extraction was done using the puregene DNA – isolation kit (Gentra systems, Minneapolis). Primers were used as standards for comparison of the impala DNA. A primer is a synthetic oligonucleotide – also referred to as a marker. The markers used for the impala are mostly from the Bovine, as this genome map have a large number of microsatellite (microsats) markers useful for diversity studies. Microsats, are short tandem repeats found in the non-coding region of the DNA, highly polymorphic and, therefore, often used to distinguish between individuals, parentage verifications and biodiversity. Eight microsats were used for these samples

Figure 3.14: Schematic representation of the metatarsus and metacarpus measurements



Metatarsi – dorsal view



Metacarpus – dorsal view

PCR – Polymorphic :

- Add 1 x 10 mM buffer, $MgCl_2$, 200uM dNTP's 4 pmol of each primer, 02, units Taq Polymerase (Promega) and 50 ng of DNA. The final volume equals 15 ul.
- Samples are then placed into Thermal controller (Perkin Elmer) with the following program:
    - Five minutes at 94 °C followed by 35 cycles consisting of 30 seconds at 94 °C, 45 seconds at 55 °C, 90 seconds at 72 °C and an extension step of 10 min at 72 °C.
- PCR products are then analysed on an automated DNA sequencer (ABI 373 A).

3.10. Histopathology analysis

Liver samples were collected in 10 % formalin. A thorough histological examination was done on the liver at the Faculty of Veterinary Science. The samples were sectioned and stained routinely with haematoxylin and eosin (HE) for examination under a light microscope. The examination determined if there were any abnormalities in the bile ducts, epithelial hyperplasia, presence of eosinophils and lymphocytes in the ducts and any changes to the epithelium lining of the liver. The livers were examined also for signs of a haemolytic crisis, which would suggest Cu toxicity.

3.11 Statistical analysis

Data was analyses by the analysis of variance procedure of the General Linear Models (GLM) program (SAS, 1985), with measurements, concentration of minerals and age, area and gender as variables. To determine statistical differences in mineral concentrations classified according to soil, vegetation and faeces the Bonferroni (Dunn) *t* test was used in the case of empty cells. Means and standard errors were calculated for all parameters. A multi-factorial analysis of variation (ANOVA) was used to analyse the effect of area and environment on liver mineral concentrations for the impala.