CHAPTER 3

MATERIALS AND METHODS

Three agroecosystems namely Paddy, Pigeon pea and Castor the community structure of spiders were studied. The choice of these crops was based on the differential input of insecticides on these crops, varied plant architecture, differential farm management techniques required for these three crops. For the present work field studies as well as laboratory studies were carried out. Field studies were carried out on 3 crops namely Paddy, Pigeon pea and Castor which represent cereal, pulses and oilseed crops respectively. The study included the following parameters:

Field Study

1. Sampling of spiders in various crop and habitats
2. Identification of Spiders
3. Seasonal Dynamics of spiders and pests
4. Species Diversity of Spiders in Various crops
5. Factors affecting the Spider assemblages in the crops.
6. Diversity between the different crops
7. Habitat specific spiders

Laboratory Study

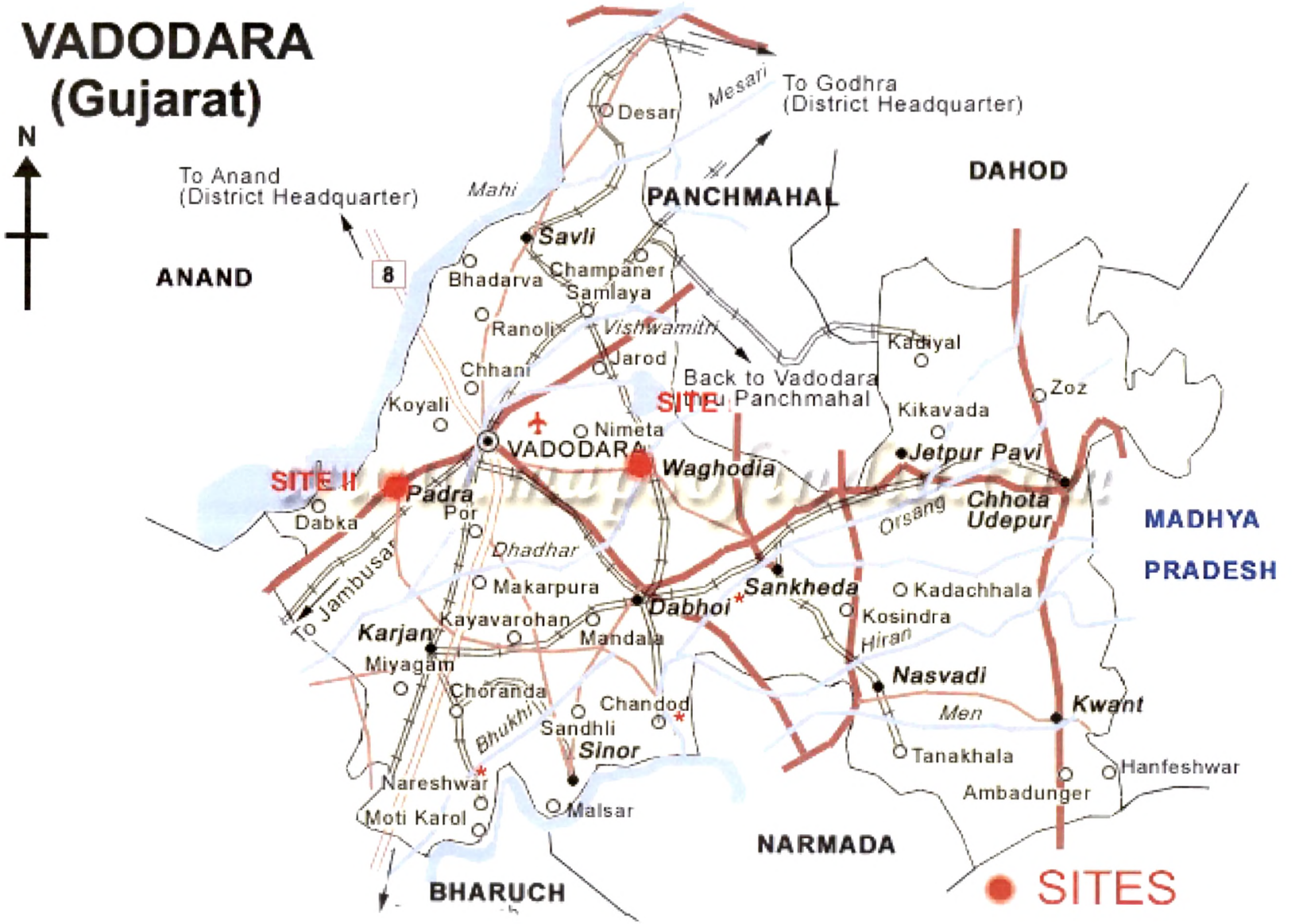
1. Feeding Potential of Spiders to different Insect pests.
2. Feeding ecology: Numerical response and Functional response

PLATE I

STUDY SITES, SITE I AND SITE II LOCATED 20 KM, SOUTH WEST AND EAST OF VADODARA CITY RESPECTIVELY

SITE I - CONVENTIONAL PADDY FIELD

SITE II - ORGANIC PADDY FEILD


VADODARA
(Gujarat)
N
To Anand
(District Headquarter)
ANAND
8
To Godhra
(District Headquarter)
DAHOD
PANCHMAHAL
Mahi
Mesari
Desar
Savli
Champaner
Bhadarva
Samlaya
Ranoli
Vishwamitri
Jarod
Chhani
Koyali
Nimeta
VADODARA
SITE
Back to Vadodara
Thru Panchmahal
Kadiyal
Zoz
Kikavada
Jetpur Pavi
Waghodia
SITE II
Padra
Por
Dabka
Dhadhar
Orsang
Chhota
Udepur
MADHYA
PRADESH
To Jambusar
Makarpura
Sankheda
Dabhoi
Kadachhala
Kosindra
Kayavarohan
Mandala
Karjan
Hiran
Miyagam
Nasvadi
Choranda
Chandod
Bhukhi
Men
Kwant
Sandhli
Sinor
Tanakhala
Hanfeshwar
Nareshwar
Ambadunger
Malsar
Moti Karol
NARMADA
BHARUCH
SITES

Chapter 3

STUDY SITE

The spiders were sampled from paddy fields of two different management practices – Conventional and Organic. The two sites are located approximately 20 kilometers to South (Padra) named hereafter as **Site I** and towards west (Timbi) named here after as **Site II** from the Vadodara city. The duration of the sampling period was between (2002-03 to 2004-05). In the **Site II,** the Conventional fields were sprayed with insecticide (Organophospharous) twice during the cropping season at the time of pest outbreak. In Site I, Organic fields of paddy was identified that did not use synthetic chemicals for Fertilizer, weed, Insect, or for pathogen management.

Sampling of Spiders in Pigeonpea and Castor were done in the **Site II.** The duration of the study was the same as that of the paddy crop. Pigeonpea crop received a regular calendar based spray of chemical after the onset of flowering season at an interval of 15 days while the pesticide input in castor was not there. For all the three crops the data regarding the sampling year 2002-03 was not used for the data analysis. The data for the year 2003-04 is hereafter called as **Phase I** and that of the year 2004-05 is called as **Phase II** for all the three crops.

Chapter 3

FIELD STUDY

PADDY

Crop Phenology: Rice cultivation is thought to have originated in Northeast Thailand , early 9000 years ago, from then on rice has been a staple food for more than half of the worlds population. It is a unique agroecosystem requiring standing water throughout the growing season. The diversity of the insects and spiders are higher than several natural ecosystems. In the rice fields' spiders play a potential role in managing insect pests (Way and Heong, 1994, Kenmore et al, 1984). In India the paddy growing regions are restricted to the Gangetic basin, Godavari – Krishna basin, Cavery delta and other pockets in the India. In these areas paddy is grown in three seasons per year; however in the other areas paddy is grown only once a year. Several types of insect pests are seen attacking paddy. Paddy is cultivated in the tropics and it requires standing water throughout the cropping season, which varies from 4 -6 months depending on the variety.

Sampling Protocol: The following sampling techniques are used for sampling spiders in paddy crop, Quadrat count (1sq meter), Hill count (Way and Heong, 1994), Net sweeps, Vacuum sampler (Samu et al, 1999) and pitfall traps. We found Hill count sampling to be the best sampling technique on the basis of the suitability of sampling the aerial and ground habitat equally (Heong et al, 1991) and also on the basis of time and money. In hill count sampling 10 hills are taken as one sampling unit and each hill is thoroughly searched as well as ground

strata for spiders as well as pests. 10 such sampling units constituted one observation. Weekly observations were taken from the date of transplanting till the harvesting of the crop. Sampling was done 1 meter inside the field margin to negate the edge effect. Spiders collected during the sampling were taken to the laboratory were preserved in 70% ethanol for their taxonomic identification. Manual collection was also made to know their presence in field margins. The vertical stratification of spiders in the paddy was observed, as it would give precise idea about the resource partitioning by spiders.

PIGEON PEA

Crop Phenology: Pigeonpea (*Cajanus cajan*) is an important pulse crop in the semi arid tropical and subtropical areas of the world. Asia accounts for 90% of the world production. Pigeonpea is the 3rd most important pulse crop and India is among the largest producer (Nene & Sheila, 1990). In the vegetarian regions of South Asia, it is an important source of dietary protein. The nitrogen fixing ability of Pigeonpea makes it an important component in sustainable cropping system and farmer recognize the ability of Pigeonpea to replenish the soil when planted after cereal crop (Kelly et al, 1996). There are more than 200 species of insect pests feeding on Pigeonpea; but only a few of them cause significant and considerable damage to crop (Lateef and reed; 1983). The pests are categorized into Flowering-pod feeding lepidopterans, Pod sucking hemipterans and seed feeding dipterans. The Pigeonpea crop can tolerate the early season losses of flower and pods by producing more flowers and it is only those pests that are

continuously present or present during the later stages of crop cycle are economically important. Of these pests focus was on *Clavigralla horrens and Clavigralla gibbosa*, which attack the Pigeonpea during the end of the cropping season and cause huge economic loss. In Gujarat Pigeonpea cultivation starts after the monsoon sets in (June – July) and crop is harvested in the month of February – March. Pigeon pea crop is grouped under pulses and has the flexibility of being irrigated once in two weeks. The distance between two rows is about 2 meter. The Castor bean crop is an annual crop; it is grown as early crop and as late season crop. The time of sowing the seeds is dependent on the monsoon rains. Normally the crop is grown about one week after the onset of rains, however if the rains are excessive leading to water logging in the field then the crop is postponed and it is seen when the monsoon is normal the castor bean crop is sown during late August and the Crop is Harvested in the month of January. When the Monsoon is not normal late sowing of the crops are done in the month of November and the crops are harvested in the month of March. The Overall period of the crop ranges for a period of 120 days to 150 days. For study purpose cropping stages can be divided into four stages namely the Vegetative stage which starts from the time of sprouting till about 60 days prior to the appearance of flowers, flowering stage, Fruiting stage and mature fruiting stage, the duration of each stage taking an average of 30 days.

Sampling protocol: The possible sampling techniques for spiders in pigeon pea are pitfall sampling, quadrat sampling, visual count, vacuum sampling and

inverted umbrella method. We took quadrat sampling as it is more efficient in sampling aerial and ground arachnofauna and is used for sampling most of the pulse crops (Alderweireldt, 1994). 10 quadrats were sampled at weekly intervals from the start of the cropping season till the post harvest. The spiders were collected in different vials and then were grouped into morphospecies and were finally identified under sterozoom microscope (Leica MZ16A). Observation was also made in the field on prey feeding by spiders on the field.

CASTOR

Crop Phenology: Castor requires a moderately high temperature 20-27°C with low humidity throughout the growing season. It grows best in areas where there are clear warm sunny days. Prolonged cloudy weather with high temperature at the time of flowering results in poor seed set. High temperature above 41°C at flowering time even for a short period results in blasting of flowers and poor seed set. The plant is considered to be very resistant to drought but even then about 80-1000 mm evenly distributed rainfall in required for optimum growth. Heavy rainfall at flowering reduces the yield. The cropping season extends from September to January when the sowing takes place and the harvesting season is in the months of December to March in the early and late sowing castor respectively. The duration of the crop extends between 145 to 205 days depending on the variety of the castor grown. In Gujarat two varieties of castor is grown namely GCH –3, J-44, and GAUC –1. The cropping pattern of castor is varied. It is either grown as a trap crop along the field margins of Cotton crop or

is intercropped with Cotton or Pigeon pea in western and southern India or is Monoculture of Castor as found in Arid regions of India. The distance between two plants in a row is about 40 – 45 cms and the distance between two rows is about 1.5m. When castor is intercropped with Cotton or Pigeon pea the distance between the adjacent crops get reduced to 30 cms. The right time of planting castor varies from 20th June to 5th July. It is advisable to sow the seed as soon as the monsoon breaks in second fortnight of June. Sowing after 20th July gives poor yields in Kharif season. Sowing time varies slightly in different states as given below:

State	Sowing time
Uttar Pradesh	June-July
Gujarat	August-Sept.

Under irrigated conditions for dwarf varieties, a row to row distance of 60 cm and rainfed conditions 90 cm has been found optimum for good plant growth. Plant to plant distance should be 45 cm. The seeds may be sown at 8 cm depth behind the plough or maize planter at the rate of 18-20 kg seed per hectare. The castor crop matures between 145-280 days after planting depending upon the variety. Harvesting is done when capsules turn yellowish. However, all the spikes do not mature at the same time. The central spike on main rachis matures first and thereafter the spikes on the side branches start maturing. Therefore, usually two to three pickings may be needed for harvesting the entire crop. The spikes should be dried in the sun for four to five days and then threshed. It is essential to dry the seeds completely before storage.

Sampling Protocol: The castor crop is planted at a distance of about 1 meter. So quadrat sampling is not a viable sampling technique, so we used manual search of 15 leaves of individual plant along with search for spider on the ground level. This constituted one sampling unit. 15 such sampling units were taken at weekly intervals from the date of sowing of the crop till the harvesting of the fruit. The spiders were manually searched and were brought to the laboratory for identification

LABORATORY STUDY

The laboratory study included the Container studies, functional and numerical response of the dominant spiders found in the 3 agroecosystems; in this the spiders were brought to the laboratory in plastic vials and were kept alive by feeding them natural arthropod prey. The spiders were than sorted into two groups for the functional response in which the prey was given in two different densities namely 5 and 15 to know how the spiders interact (eat) the prey when available at low and high densities.

Culturing of Insect Pests

For the purpose of testing the feeding potential, functional response, numerical response, prey preference and the prey quality for the spider; different insect pests were cultured in the laboratory. The Lepidopterous pest spodoptera litura was cultured in the laboratory on natural and artificial diet. We have standardized the culturing technique.

Clavigralla horrens, which is an occasional pest in pigeon pea, the egg mass of calvigrella horrens on the leaf and on the pods is 12.4. The eggs are laid in clusters on the pods and on the either side of leaves. The juveniles that hatch from the eggs are slow moving and they move to the young pods of the crop and suck the sap. They are usually found in gregarious condition on the pods. The subadults are usually found in groups of 3-4 on the young pods and on the tender shoots. The egg masses were brought to the laboratory from the field and were reared on the fresh Pigeonpea pods. We also standardized the culturing of this Hemipteran pest by giving sugar solution in cotton; the adult survival ratio was about 50%.

Spodoptera litura: the eggs were collected from the field and were brought to the laboratory. When the eggs hatched the larvae were transferred to castor leaves till the 5th instar. The 5th instar were later transferred to bowl containing moist soil where it under went pupation. The adults emerging from the pupae were transferred to the insect rearing cages and were provided with the adult food and castor leaves were made available for oviposition.

Feeding potential of Spiders

The feeding potential of the Oxyopidae was done on the 3rd instar of *Spodoptera litura*. The spiders from each species 15 in number were collected from the field and were sorted out into three categorized namely Adult Males (AM), Adult Females (AF) and Juveniles (JU). From each Category 6 individuals were taken

for the experiment. The field collected spiders were brought to the laboratory and were maintained at 26-29 C, 65-70% RH and 12:12 light and dark period in polypropylene containers of 15 cm in diameter and 28cms in height.

The collected spiders were fed with ad- libitum diet of hoppers collected from the field. After that the spiders were starved for 7 days (week) prior to the start of the experiment. The experiment started with the introduction of 3 third instar larvae into the container and a single spider in each container and observations were taken every 24 hours till one week. The spiders were measured prior and after the experiment to know the body weight gained by them during the trial. During the observation the dead larvae were taken out and the alive ones were transferred to the food and a fresh stock of larvae were added to each container. The control of the experiment consisted of 3 third instar larvae only without any predator to know the natural mortality. The experiment was repeated 6 times.

Feeding Ecology of Spiders on Clavigralla horrens

Clavigralla is a hemipteran pest of pigeonpea and it feeds on the sap of the pod. *Oxyopes shweta*, (Lynx spiders) were tested for their feeding potential to insect pests. All of these are generalist predators and they are observed in the field to be feeding on the nymphs of *Clavigralla*. The leaves having Clavigralla eggs were brought to the laboratory for rearing and also the spiders (*Oxyopes shweta)* were collected and brought to the laboratory. The spiders were housed in individual containers, 4cm in diameter and 6 cm in height. The spiders were

starved for a week prior to the start of the experiment. The spiders collected from the fields were grouped into morphospecies and one of the individual was sacrificed to confirm the taxonomic identification. The spiders of a single morphospecies were grouped into male, female and subadults depending on the presence of sexual characters. The prey was grouped into 2 groups namely Adults and Nymphs. We grouped the spiders of one species on the basis of sex and the type of prey given. Five preys were released in each replication and the amount of feeding was noted for every 24 hours; fresh ones replaced the dead prey.

Adult Male and females of *Oxyopes shweta* (Tikader) were used in the experiments. *Spodoptera litura* (3rd instar) and *Clavigralla horrens* (adult and juveniles) were used as prey items. The spiders were kept in container of dimension 9cm in diameter and 6 cm in height. 10 prey items of a single type were introduced into the containers and the experiment started. Observations were taken after 24 hours. Prior to the start of the experiment the spiders were starved for 7 days. A single set consisted of 6-8 such sets were kept for each type of prey used and to the sex of the predator. The observations were taken as eaten, killed but not eaten and alive. For this setup a parallel set of control were kept to see whether there was any natural mortality.

Functional Response of Spiders

Functional response is the change in the behavior of a predator in response to different prey densities. To test the functional response of spiders against

different insect pests; the spiders were collected from the field and were well fed and then were starved for a week prior to the start of the experiment. The plant twigs (4-7 leaves) were cut from the field and placed in a plastic vial and brought to the laboratory prior to the start of the experiment. The larvae at a particular density were released and one spider per microcosm was introduced. Following which observations were taken covered by a bigger container of 19 cm in diameter and 28 cm height. The insect pests were released in each of the plant twig at various densities Viz. 3, 5, 10, and 15. One spider per twig was released and the observation was taken every 3 hours and then the experiment was terminated. The experiment was started with standardization of the protocol; then the original experiment was started. The experiment was done in the laboratory where the Temperature was 26-27 C; RH 65-70%; 12:12 hours of light and dark. The observation included the Handling time, attacking time and number of prey eaten. There were 3 replications for each prey density. The control was kept for each prey density in which the predator was absent.

Numerical Response of Spiders

The numerical response is the behavior of the predator when a number of predators are present at constant prey density. The experiment consisted of the plant twig planted in a beaker and covered with a container. Each sets had 1, 2, 3, 5, 7 predators in each setup.6 replication of each was carried out. The duration of the experiment was 24 hours.

Adult male and Females of *Oxyopes shweta* (Tikader) were starved prior to the experimentation. For this experiment the spiders were kept at varying densities in a single test arena against 15 prey (*Spodoptera litura* (3rd instar) \ *Clavigralla horrens* (Juvenile). This study was undertaken to know about the intraguild predation in *Oxyopes shweta* and to know about their predation responses in competitive environment (Intraspecific competition). The procedure is same as given for functional response.

Prey Preference studies

The experimental protocol was similar to the above. The 2nd and 3rd instar larvae were given to the spiders and 6 replications were made. The prey preference of *Oxyopes shweta* (Tikader) to *Spodoptera litura* and *Clavigralla horrens* was tested in the laboratory as per the procedure of Li and Jackson (1996).

Alternate day experiments

In this half the number of predators were given prey 1 and the other half were given Prey 2. On the next day this trend was reversed. The experiment started when the prey and the spider were introduced in the arena and the test ended when the spider took the prey or 60 minutes elapsed which ever came first.

Simultaneous Prey test

In this both the prey were introduced into the arena and the spider was introduced into the arena. The test ended when the spider took the prey or 60 min elapsed which ever came first. In both the tests two set of conditions were tested.

(A) **Satiated Predator**: in satiated condition the spider was fed ad-libitum 7 days prior to the experiment.

(B) **Hungry Predator**: in this condition the spider was fed ad-libitum 14 days prior to the start of the experiment.

Data analysis

Spearman's Correlation Coefficient was used for comparing the impact of aboitic factors like Temperature, Relative Humidity, Rainfall, Soil Temperature etc. on the population density of spiders. For quantifying the various aspects of community structure of the spiders in Rice ,Pigeonpea and Castor agroecosystems like the seasonal abundance , diversity and species richness and population dynamics etc . Alpha diversity indices were used. It is a known that no single formula can measure of diversity of fauna correctly so I calculated 6 diversity indices in my study. These formulae and the sensitivity of each of the diversity indices are given below.

The purpose of determining diversity by numerical index is rather to provide a means of comparison between less clear cut communities than compare two communities where the species diversity and proportional abundance are amply visible to naked eye. Diversity is of theoretical interest and it is related to the stability of the environment, Maturity, Productivity, Predation, Spatial heterogeneity and Evolutionary time. We find that no diversity index has pre-eminent advent and that different authors have plausibly proposed different

indices. Different indices measures different aspects of the portion of abundance between species.

1. Simpson Index: (D)

One method for comparing diversity of species is Simpson's Diversity Index. Simpson's Diversity takes into account the number of species present, as well as the abundance of each species. Simpson's Index (D) measures the probability that two individuals randomly selected from a sample will belong to the same species (or some category other than species). There are two versions of the formula for calculating D. Simpson's Index of Diversity 1 – D. The value of this index also ranges between 0 and 1, but now, the greater the value, the greater the sample diversity. This makes more sense. In this case, the index represents the probability that two individuals randomly selected from a sample will belong to different species.

Simpson's Reciprocal Index 1 / D: The value of this index starts with 1 as the lowest possible figure. This figure would represent a community containing only one species (The higher the value, the greater the diversity). The maximum value is the number of species in the sample. Simpson index is sensitive to abundance of only more plentiful species with sample and is regarded as a measure of dominance concentration.

$$D = \frac{\sum n(n-1)}{N(N-1)}$$

For the purpose of community description we should express measures of diversity on a uniform scale that is it the reciprocal Simpson index has to be used (1/D) and (Not 1-D).

2. Shannon – Weiner (Shannon –Weaver Index)

This presents the Shannon-Wiener (also incorrectly known as Shannon-Weaver) diversity index for each sample. A simple plot of the way the index changes between samples is displayed by clicking on the Graph tab on the output window. The function was originally devised to determine the amount of information in a code or signal, and is defined as:

$$H = -\sum_{i=1}^{S} p_i \log_e p$$

where pi = the proportion of individuals in the ith species. Species Diversity & Richness calculates the index using the natural logarithm.

In terms of species abundance:

$$H = \log_e N - \frac{1}{N}\sum_{i=1}^{\infty} (p_i \log_e p_i) n_i$$

Where ni = the number of species with i individuals. The information measure is nits for base e and bits per individual for base 2 logarithms. This ever-popular index is really not as good as its popularity would imply. The value of the Shannon-Wiener Index usually lies between 1.5 and 3.5 for ecological data and rarely exceeds 4.0.

3. Species Number: It is strongly affected by presence of rare species. Thus Whittaker 1965 determines that a combination of Diversity concentration and Species rarity have to be used in combination for the determination of Diversity of any community.

4. Margalef d:

It states that if the evenness of the community is high meaning that if proportional abundance of individual species is nearly the same then, the value of the Margalef d is also high and vice versa.

$$d = S\text{-}1 / \ln N$$

S = total number of species, N = total proportional abundance of the species

5. Species Evenness:

Species Evenness or Equitability Index: it is denoted by **"E"**, is the relative distribution of individuals among the species present in a community. Evenness contrasts with dominance, and is maximized when all species have the same number of individuals. A way to compare evenness would be to prepare a pie-

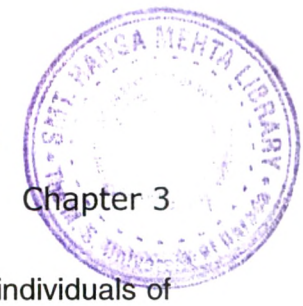


chart of each species found in an area in relation to the number of individuals of each species present. A statistical method for comparing species evenness would include calculating the % of each group represented in the entire sample.

Calculations:

Evenness can be calculated by summing all of the calculations for the number of individuals in species #1 divided by the total number of species in your sample.

a) **First** calculate Relative abundance "Pi" = ni/N

ni = number of individuals in species i

N = total number of individuals in all species

b) **Next**, calculate the Shannon-Wiener index "H"

This index is determined by both the number of species and the even distribution of individuals among those species (relative dominance). It indicates the degree of uncertainty of predicting the species of a given individual picked at random from the community. In other words, if the diversity is high, the chance of correctly predicting the species of the next individual picked at random. H (the uncertainty of predicting the species) will range from 0 for a community with a single species, to over 7 for a very diverse community.

H = - sum (Pi ln[Pi])

Pi (relative abundance) = ni/N

6. Rank Abundance :

One way to visually assess diversity is to create a "rank/abundance plot". To do this, the species are listed along the x-axis from most abundant to least abundant and the y-axis is set to indicate proportional abundance in log scale. Rank/abundance curves can be categorized as following one of three general patterns, known as the broken stick, log normal and geometric series. Some generalizations can be made about communities containing each type of diversity pattern.

7. Fisher Alpha (α)

The following values are for Fisher's alpha diversity index (Fisher et al.1943) given the number of individuals (N), number of species (S) and number of individuals minus the number of species (N-S) in a sample. Fisher's alpha is usually computed for S and N, but N-S was used to emphasize values for which the relationship was least linear (S approximately equal to N). Values are given for S from 1 to 50 and N-S from 1 to 10. Fisher's Alpha is a mathematical model used to measure diversity, and is defined by the formula: $S = ax \ln(1 + n/a)$, where S is the number if species, n is the number of individuals, and a is alpha.

8. Renyi Diversity Ordering

The Diversity Ordering was calculated using the software programme Pisces Conservation Software. Both variants of Simpson's index are based on *D = sum*

p_i^2. Choice simpson returns *1-D* and invsimpson returns *1/D*. Shannon and Simpson indices are both special cases of Rényi diversity

$$H.a = 1/(1-a) \log \text{sum } (p^a)$$

where *a* is a scale parameter, and According to the theory of diversity ordering, one community can be regarded as more diverse than another only if its Rényi diversities are all higher.

10 Feeding Ecology studies and Prey Preference tests

For the feeding ecology studies **Student t test** was used to measure the significant differences among the parameters if any.

In Prey Preference tests, In the Alternate day tests, only those test pairs in which spider took one prey but not the other provided evidence of preference and data was analysed using **McNemar Test**. McNemar test is a variation of the Chi Square test used to determine the direction and the extent of change in pairs of repeated measures. Yates Correction was also used for continuity when the degree of freedom is 1.

In the Simultaneous Presentation tests, a series of tests in which one type of prey was consistently taken provided the evidence of preference and **Chi square goodness of fit test** were used for the data analysis.