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Full Length Article

Feeding Ecology of Four Freshwater Turtle Species in Pothwar Plateau and its Potential Implication in Turtle Farming in Pakistan

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Abstract

Turtles are being used in different countries for meat and eggs. This increasing demand of turtles in market has led people to start turtle farming. Farming practices are usually kept as secret by farmers due to competition. This study was conducted to observe the diet composition of freshwater turtle species inhabiting the Pothwar Plateau by using stomach contents flushing method, so that it may help the turtle farmers for better production. Stomach contents samples (n=410) of four different turtle species were collected from the study area and fixed in 70% ethanol till final analysis. During analysis, each sample was segregated into different categories including animal matter (insects and other invertebrates), plant matter (like vegetation including algae), and other materials. Contents were identified up to the lowest possible taxa. Analysis of stomach contents of freshwater turtles revealed five different kinds of prey items including insects, earthworms, snails, plants, and algae, in addition to soil particles and some unidentified material. The turtles were found omnivorous in their feeding behavior, but highly opportunistic, feeding on any available prey item in the environment. Mean percent volume (% V) of prey items recovered from stomach contents included insects (26.3%), earthworms (19.9%), snails (16.1%), plants (10.2%), algae (11.4%), soil particles (7.5%) and unidentified material (8.4%).

Keywords: Feeding behavior; Foraging ecology; Food habits; Turtle farming

Introduction

Meat of wild animals is an important source of animal protein and source of earning easy money for many communities across the globe, especially in tropical countries (Abernethy *et al.,* 2013). Meat of wild animals is consumed due to its taste, medicinal values and in some areas due to economics. Turtles are one of the most commonly used wild animals for this purpose. There are about 350 species of freshwater turtles and tortoises in the world. Many countries of the world like USA, Thailand, China, Hong Kong, Cuba, Japan and Australia use turtle meat for human consumption (Aureggi *et al.,* 1999; Thorbjarnarson *et al.,* 2000). Turtle eggs are also popular in many countries of the globe, some communities use it for their aphrodisiac properties while other underprivileged communities use it due to economics after illegal harvesting (Lagueux, 1991; Campbell, 1998). Apart from meat and eggs, skin of turtle is also being used for production of fashion accessories like belts, bags etc. (Thorbjarnarson *et al.,* 2000). Even blood, oil and penis of turtle is being used in different parts of world for its aphrodisiac properties and treatment of various diseases, especially in Chinese ethno-medicine (Miller, 1989; Thorbjarnarson *et al.,* 2000). In addition to their use for feed and medicine there is a big market demand of turtles for pet supplies.

Demand of turtles in china is very high, where turtles are being used for food, production of medicine and fashion accessories. This high demand of freshwater turtles is met by commercial turtle farming. A small scale survey of only about 700 turtle farms in china showed that farmer are doing trade of more than US$750 million/year (Haitao *et al.,* 2008). In Bangladesh export of turtles of worth over US$ 8.5 million was recorded during 1996-1997 (Rashid and Khan, 2000). At present demand of chines market is met by supplies from Malaysia, Indonesia, Bangladesh and Vietnam (Cheung and Dudgeon, 2006). So, commercial production of turtles and export to different countries especially china may generate a good source of income for poor rural communities of Pakistan. Commercial turtle farming has already emerged as a multi billon US dollar market in china.

Other than this commercial demand of turtles, they are also very important for environment. Though their role is not visible like role of birds and large mammals but, turtles play a vital role in the ecosystem by feeding on a variety of aquatic and semi-aquatic animals, viz. insects, snails, dead animals and fragments of dead bodies and are helpful in

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reducing the water pollution (Hossain and Sarker, 1995). Turtles play an important role in agriculture biodiversity by spreading the seeds of plants and preserve the environment by acting as scavengers (Rao, 1985).

Keeping in view the above mentioned importance of freshwater turtles, start of freshwater turtle farming in Pakistan will potentially play a significant role in poverty alleviation due to high demand for export. Most of the commercial turtle farmers keep the information of farming protocol a secret due to increased competition (Haitao *et al.*, 2008). This study on food habitat will be helpful for potential farmers to manage their farms in more effective and natural way. This information on natural diet of freshwater turtles will also be helpful for veterinary practitioners who routinely treat the pet turtles.

Materials and Methods

Study Area

Current study was conducted in the Pothwar Plateau located at 33º30"0' N and 73°0" 0' E. The Plateau comprises of four districts namely; Jhelum, Chakwal, Rawalpindi and Attock (Fig. 1). The Pothwar Plateau is bounded in the east by Jhelum River, in the west by Indus River, in the north by Kala Chitta Range and Margallah Hills and in the south by the Salt Range. The approximate area of the Plateau is approximately 2.2 million hectares. Dominant habitat type in the area is dry sub-tropical, semi-evergreen scrub forest (Roberts, 1991). The climate of the area is sub-humid, sub-tropical continental type. There are two distinct rainy seasons; the summer season or the monsoon rains start by about mid July and last until the mid of September. Winter rains begin in January and persist up to the beginning of March. The maximum temperature is 45^{o}C during summer but drops below 0^{o}C during winter (Awan *et al.*, 2004).

Stomach Contents Flushing

Investigation of food habits of the freshwater turtles inhabiting the study area were carried out by stomach contents flushing (Legler, 1977). Briefly, freshwater turtles belonging to four different species Viz. Indian flap shell turtle (*Lissemys punctata*), Indian soft shell turtle (*Nilssonia gangetica*), Brown river turtle (*Pangshura smithii*) and Indian roofed turtle (*Pangshura tecta*) were live captured using cast net at selected sampling sites at day time. After the capture, a "Naso–gastric" tube attached with a 50 mL plastic syringe was introduced through the esophagus into the stomach of the turtle. The neck muscles of the turtles were partially anesthetized by injecting anesthesia [(30 mg/kg b.w. (Zoletil 50; Vivric)]. In order to induce the regurgitation of food items, approximately 100-200 mL tap water was delivered into the stomach.

Water with stomach contents was then drained into a sieve. The food items were picked up with forceps and fixed in 70% ethanol. Then the stomach contents were directly washed and segregated with tap water from the gauze. Each sample was segregated into different categories including animal matter (insects and other invertebrates), plant matter like vegetation (including algae), and other materials. Each item was identified to the lowest possible order level (Fig. 2).

Collection of Reference Plant Samples

Analysis of stomach content samples by micro-histological techniques is the most commonly used method for determining the botanical composition of herbivore diet (Alipayo *et al*., 1992). For this purpose, a reference collection of plants was carried out from the same study area simultaneously along with the stomach contents flushing samples. The collected plant samples were identified and used for preparation of reference slides for comparison.

Seasonal Variation in Diet Composition

For investigating seasonal variation in the diet composition of each freshwater turtle species, the samples were pooled season-wise including summer, winter and rainy seasons. In addition, pray species richness (S), Diversity Index (H') of prey species and Evenness index (E) refers to how close in numbers each species in an environment is, which was calculated from the data on seasonal variation in the diet composition of each turtle species as follows:

Prey species richness index (S): It was calculated by taking into account the total numbers of plant and animal prey species consumed by each turtle species in a specific season.

S= number of prey species present per sample

Diversity index (H'): Diversity index (H') of prey species was calculated by Shannon's index:

$$H'=-[Pi\times \log Pi]$$

Where Pi is the proportion (n/N) of individuals of particular prey species (n) divided by total number of prey species (N).

Evenness index (E): Evenness index was calculated using Pielou's formula:

$$E= H'/\text{Log of } S$$

Where, H' representing Shannon's index and S the prey species richness index.

Results

Percent Frequency (% F) and Percent Volume (% V) Occurrence of Prey Items

A total of five food items were identified in the stomach contents of the freshwater turtle species captured from the

Pothwar Plateau. Frequency (n) of prey items recovered included insect body parts 83.9% (n=344), earthworms 73.9% (n=303), snails 58.2% (n=239), plant parts 41.2% (n=169) and algae 25.6% (n=105), soil particles 38.5% (n=158) and unidentified material 20% (n=82) (Table 1). The percent volume (% V) occurrence of food items in stomach contents of all four freshwater turtles studied showed (Table 2) insects body parts in highest quantity, followed by earthworms and snails, while plants parts and algae were also represented in reasonable volume.

Statistical analysis (Two-way Analysis of Variance, ANOVA) showed significant ($p < 0.05$) difference between consumption of prey items consumed by four turtle species, similarly consumption of prey items was significantly ($p < 0.05$) different to each other. The significance was calculated by using Box plot comparison (Fig. 3).

Seasonal Variation in Food Consumption of Four Freshwater Turtle Species

Food habits of the four freshwater turtle species were found variable during three different seasons of the year including winter, summer and rainy seasons (Table 3). Analysis of samples (n=50) for each of the three seasons (winter, summer, and rainy season) of stomach contents of *L. punctate* showed higher consumed food item was insects (25.5±3.3, 29±2.1 and 30.2±2.0) in every season. In *N. gangetica* results showed that highest consumption of food item was insects (22.4±1.5, 24.3±1.1 and 31.2±0.8) and minimum was algae (8.9±0.9, 8.9±0.4 and 9.8±1.1). Similarly, during three seasons stomach samples of *P. smithii* were analyzed. The highest consumed food items was plant material and lowest was algae. For *P. tecta* the highest consumption of food items during different season was plant material (Table 3). Mean consumption of food items in winter, summer and rainy season was statically compared by using two ways ANOVA. The consumption of prey items of three seasons was found to be significant ($p < 0.05$) in *P. tecta* while in rest of three species viz. *L. punctata, N. gangetica* and *P. smithii* it was non-significant ($p > 0.05$).

Prey Species Richness (S), Diversity Index (H') and Evenness Index (E)

The diet composition of the four freshwater turtle species varied in three different seasons of the year, and this data was used to compute prey species richness (S), diversity Index (H') and evenness Index (E) of each turtle species studied. Prey species Richness (S) for *L. punctata* was found maximum (14) in summer season, while least (09) in winter. Diversity Index (H') of prey species was high during summer and rainy season (2.6) but lowest least during winter season (2.2). The Evenness Index (E) of the prey species of *L. punctata* was also high during summer and rainy seasons (0.6) as compared to winter season (0.4). Detailed results for other species have been presented in Table 4.

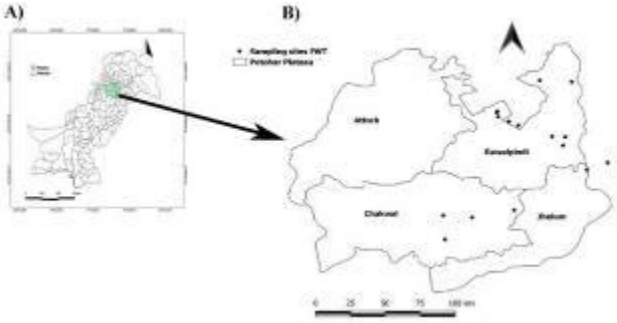

Fig. 1: Map of Pakistan showing location of Pothwar Plateau and study area

Fig. 2: Photographs taken during the stomach contents flushing procedure, (A) Passing Naso-gastric tube into the turtle's stomach, (B) filling the turtle's stomach with water, (C) showing stomach contents being forced out and (D) snails recovered from stomach contents

Insects Orders Consumed by Freshwater Turtles

During current study, percent volume consumption of major insect orders by different turtle species from the study area revealed five different insect orders. The consumed insect orders included Hymenoptera (29.5±9.5), Coleoptera (28.5±7.8), Diptera (23.5±7.8), Homoptera (18.2±7.3) and Odonata (19.7± 4.3) (Fig. 3). The Indian flap shell turtle consumed Hymenoptera and Coleoptera in equal percentage (40%) followed by Diptera (36%), Homoptera (25%) and Odonata (22%). The Indian soft shell turtle, The Brown river turtle and The Indian roofed turtle consumed Hymenoptera (50%), Coleoptera (21%) and Coleoptera (10.0%) in highest percentage, respectively.

Table 1: Percent frequency (% F) of the prey items recovered from the stomach contents of freshwater turtles collected from selected study sites.

Prey item	*Lissemys punctata* (n=150)	*Nilssonia gangetica* (n=100)	*Pangshura smithii* (n=110)	*Pangshura tecta* (n=50)	Mean % (N=410)
Insects parts	93.3(140)	85 (85)	71.8 (79)	80 (40)	83.9 (344)
Earthworm	87.3 (131)	72 (72)	60.9 (67)	66 (33)	73.9 (303)
Snails	65.3 (98)	66 (66)	45.4 (50)	50 (25)	58.2 (239)
Plant parts	40 .6(61)	20 (20)	59.0 (65)	46 (23)	41.2 (169)
Algae(*Oscillatoria acuminate*)	20 (30)	20 (20)	31.8 (35)	40 (20)	25.6 (105)
Soil particles	32.6 (49)	33 (33)	48.1 (53)	46 (23)	38.5 (158)
Unidentified Material	25.3 (38)	15 (15)	17.2 (19)	20 (10)	20 (82)

Table 2: percent volume (% V) occurrence of the prey items recovered from the stomach contents of freshwater turtles collected from the study area

Prey items	*Lissemys punctata* (n=150)	*Nilssonia gangetica* (n=100)	*Pangshura smithii* (n=110)	*Pangshura tecta* (n=50)	Mean % (N=410)
Insects parts (%)	36.2	30.4	22.4	16.6	26.3
Earthworm (%)	30.8	25.8	12.6	9.8	19.9
Snails (%)	20.9	19.7	12.7	11.6	16.1
Plant parts(%)	9.1	8.6	12.5	10.7	10.2
Algae (*Oscillatoria acuminate*) (%)	10.2	10.1	15.7	9.8	11.4
Soil particles (%)	8.2	7.9	7.4	5.6	7.5
Unidentified material (%)	9.9	8.8	8.5	6.7	8.4

Table 3: Seasonal variation in consumption of different food items by four turtle species in the Pothwar Plateau

Species	Seasons	Insects	Earthworms	Snails	Plant material	Algae	Soil particles	Unidentified material
Lissemys punctata	Winter	25.5±3.3	22.8± 1.4	19.7±1.5	11.1±1.2	7.8± 1.4	6.5±1.4	6.6±1.0
	Summer	29±2.1	23±2.8	19.8± 1.3	9.9± 2.0	9.8±2.0	5±0.8	3.5±0.4
	Rainy	30.2±2.0	24±1.9	17.3±1.3	9.2±0.5	7.8±0.3	5.2±0.3	6.3±0.5
Nilssonia gangetica	Winter	22.4±1.5	20.7±0.6	18.7±0.5	12.7±0.7	8.9±0.9	9.9±0.7	6.7±0.3
	Summer	24.3±1.1	20.9±0.5	17.6±1.7	11.7±0.3	8.9±0.4	9.9±0.2	6.7±0.4
	Rainy	31.2±0.8	21.4±1.2	15.4±1.6	13.4±0.5	9.8±1.1	3.9±0.9	4.9±1.1
Pangshura smithii	Winter	19.8±0.1	29.5±0.3	17.6 ±3.8	12.6±0.4	7.8±0.6	6.4±0.4	6.3±0.4
	Summer	20.9±0.5	32.9±0.5	12.5±0.6	12.6±1.4	8.8±1.4	5.0±1.2	7.7±0.9
	Rainy	12.6±1.6	12.3±1.6	26.8±3.7	27.2±2.2	8.8±0.8	8.8±0.8	4.0±0.3
Pangshura tecta	Winter	19.8±0.1	12.6±0.4	17.6 ±3.8	29.5±0.3	7.8±0.6	6.3±0.4	6.4±0.4
	Summer	12.6±1.4	32.9±0.5	12.5±0.6	20.9±0.5	8.8±1.4	7.7±0.9	5.0±1.2
	Rainy	27±2.2	12.3±1.6	26.5±3.7	12.6±1.6	7.8±0.8	9.0±0.8	4.8±0.3

Table 4: Prey species richness (S), diversity Index (H') and evenness index (E) calculated during three different seasons of the year for each of the four turtle species in the Pothwar Plateau

	Seasons			
Turtle Species		Winter	Summer	Rainy
Lissemys punctata	Prey species richness (S)	09	14	13
	Diversity index (H')	2.2	2.6	2.6
	Evenness index (E)	0.4	0.6	0.7
Nilssonia gangetica	Prey species richness (S)	10	11	12
	Diversity index (H')	2.3	2.4	2.5
	Evenness index (E)	0.8	0.7	0.8
Pangshura smithii	Prey species richness (S)	13	12	12
	Diversity index (H')	2.6	2.5	2.5
	Evenness index (E)	0.7	0.9	0.7
Pangshura tecta	Prey species richness (S)	12	11	12
	Diversity index (H')	2.5	2.3	2.5
	Evenness index (E)	0.8	0.5	0.9

Detailed results have been presented in (Table 5). Insect orders and turtle species were compared through ANOVA, using General Linear Model (GML SPSS-17). Analysis showed a significant difference ($F = 25.2$ df =3 and $p < 0.05$) between turtle species consumption of different insect orders, and non-significant difference among various insects orders consumed ($F = 0.29$ df = 6 and $p = 0.87$).

Table 5: Percent volume (% V) of insects orders recovered from the stomach contents of freshwater turtle species (N=410) collected from selected study sites

Species	Hymenoptera	Coleoptera	Diptera	Homoptera	Odontata	Mean± S.E
L. punctata	40	40	36	25	22	32.6± 3.8
N.gangetica	50	43	38	34	30	39± 3.5
P. smithii	20	21	12	14	18	17± 1.7
P. tecta	8	10	8	0	9	7± 1.8
Mean± S.E	29.5± 9.5	28.5± 7.8	23.5± 7.8	18.2± 7.3	19.7± 4.3	

Table 6: Percent volume (% V) of plant species recovered from the stomach contents of freshwater turtle species (N=410) collected from the study area

Species	*Artemisia scoparia*	*Parthenium hysterophorus*	*Withania somnifera*	*Cynodon dactylon*	*Carthamus oxycantha*	Mean ± S.E
L. punctata	10.2	11.3	7.8	9.8	6.9	9.2 ± 1.0
N.gangetica	3.2	2.7	0	1.5	3.0	2.1 ± 0.8
P. smithii	9.6	10.9	10.7	8.2	8.6	9.6 ± 0.5
P. tecta	4.0	6.2	5.6	3.9	11.3	6.2 ± 1.4
Mean ± S.E	8.0 ± 2.3	6.3 ±2.3	5.4 ± 1.8	6.6 ± 1.7	7.4 ± 1.7	

Plant Species Recorded from Turtles Species Stomach

For determination of the botanical composition of herbivore diet, micro-histological technique was applied. Analysis of 410 stomach flushed samples of four different freshwater turtle species of the study area revealed that they consumed five different plant species during the study period; redstem wormwood (*Artemisia scoparia),* carrot grass (*Parthenium hysterophorus),* ashwagandha *(Withania somnifera),* couch grass *(Cynodon dactylon)* and wild safflower (*Carthamus oxycantha)* (Fig. 4).

The Indian flap shell turtle (*Lissemys punctata)* consumed *Parthenium hysterophorus* in highest percent volume (11.3%). For Indian soft shell turtle (*N. gangetica*), plant material comprised of only 2.1 ± 0.8%, on average (Table 6). The plant species consumed in highest percentage volume was *Artemisia scoparia* (3.2%), the other three plant species were consumed in minor percentages, while *Withania somnifera* was not in the diet of this turtle species**.** In stomach contents of Brown river turtle contribution of plant material was 8.2-10.9%, while in Indian roofed turtle this quantity was 6.2±1.4%.

Discussion

Food is a primary link between an animal and its environment. Chelonians play significant role in maintaining a healthy ecosystem and are considered as indicators of healthy aquatic ecosystem. They are the natural scavengers of aquatic ecosystem, thereby, cleaning water from dead organic detritus. Turtles feed on a variety of aquatic and semi-aquatic organisms' viz. worms, insects, snails, slow moving aquatic crabs, dead animals and fragments of dead bodies, thus lowering the water pollution. Diets of freshwater turtles often reflect the availability of food resources in the environment also an opportunistic carnivore that obtains its food from a wide variety of sources like plankton, nekton, benthic macro-organisms, carrion, and terrestrial organisms that fall upon the water.

There is little literature available on the feeding ecology of freshwater turtle species in Pakistan. In the current study, we applied stomach contents flushing method to investigate the diet composition of the freshwater turtle species in the Pothwar Plateau. Since the turtles are very shy, direct observations for feeding habits are very rare. On the basis of analysis of stomach contents, the most preferred food items of freshwater turtles in the study area were found insects, earthworm, plants, snails and algae. Earlier on, Vijaya (1981) had reported similar findings that the freshwater turtles feed on earthworms and tadpoles. Lima *et al*. (1997) concluded that turtles are omnivorous and feed in a strategic way on a greater quantity of benthonic macro-invertebrates; insects, snails and crustaceans.

Among all stomach contents samples of the freshwater turtles analysed, insect body remains constituted highest percent frequency (% F) of occurrence 83.9% (344), whereas the minimum % F was recorded for algae 25.6% (105). Kennett and Tory (1996) agreed that most favorable food items of turtles are aquatic insects (87.5%) and less favorable were mollusca and gastropoda (8.8%).

Results of the current study have indicated freshwater turtle species as omnivores because diverse types of food items were recovered from the stomach contents of the specimens captured. Stomach contents analysis revealed, on average insects body parts 26.3%, earthworm 19.9%, snails 16.1%, algae 11.4% and plants 10.2%. Auffenberg and Khan (1981) showed that turtles prey on living organisms, scavenge on organic detritus from the pond bottom and household garbage. One earlier published study (Bury, 1986) reported that freshwater turtles feed by volume occurrence of prey items, insects (45.1%), vegetation (0.8%), algae (24%), crustacean (18.2%) and amphibian (11.9%).

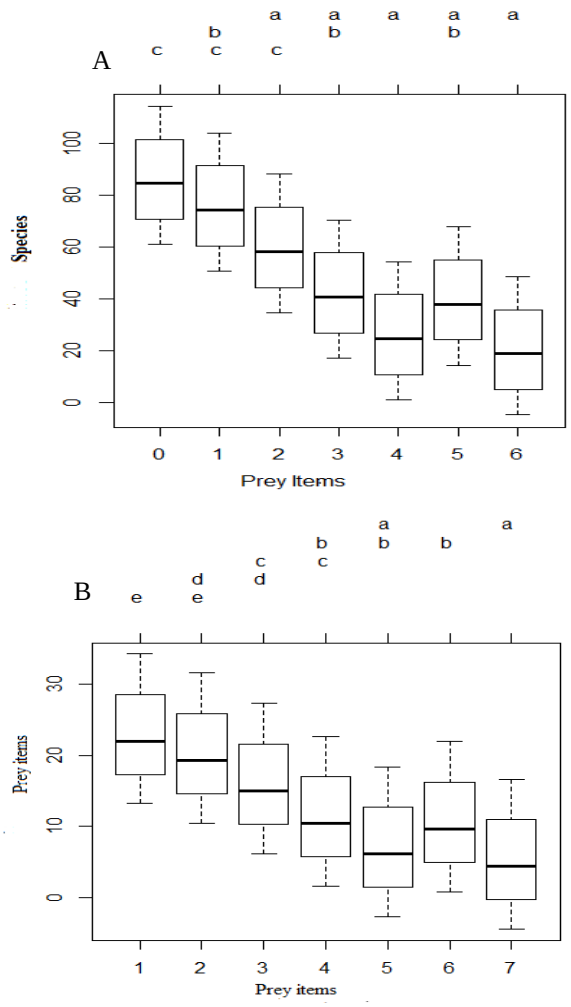

Fig. 3: Two-way Analysis of Variance (ANOVA) showing Box plot giving comparison that (A) prey items consumption was significantly different among four freshwater turtle species and (B) different prey items significantly differ from each other

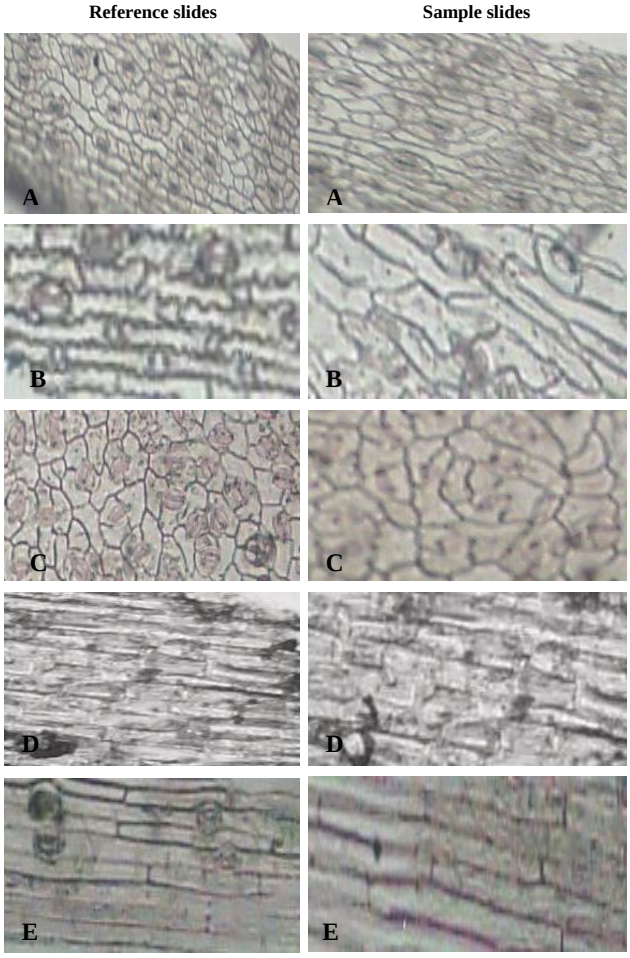

Fig. 4: Photographs of reference and sample slides plant species prepared from recovered plant materials from the stomach contents of reshwater turtle species from the study area (A) *Artemisia scoparia (B) Parthenium hysterophorus (C) Withania somnifera* (D) *Cynodon dactylon* (E) *Carthamus oxycantha*

The average consumption of food items in turtle species varied during different seasons, viz. winter, summer and rainy, in the study area. During rainy season maximum food items were consumed by different turtle species, followed by in summer season, but in winter season quantity of food items consumption was found lower as compared to other seasons. Luiselli *et al.* (2004) studied seasonal feeding habits of four freshwater turtle species and found significant difference in the frequency of consumption of prey items that were consumed more in the wet season but less in dry season by four species. The inter-seasonal differences in frequency of food items consumption was mainly due to the availability and preference of food items.

Five different insect orders consumed by the four freshwater turtle species in the current study included Hymenoptera, Coleoptera, Diptera, Homoptera and Odonata. The most preferred insect order for turtle species during study period was hymenoptera whereas the least preferred was homoptera. Caputo and Vogt (2008) had reported that Testudines (Chelidae) feed on 14 different insect orders; Trichoptera, Diptera, Coleoptera, Chironomidae, Psychodidae, Hymenoptera, Blattodea, Hemiptera, Orthoptera, Psocoptera, Ephemeroptera, Plecoptera, Entognath and Arachnida. Similarly, Novelli *et al.* (2013) reported that turtles consume 16 categories of prey items, belonging to 16 orders and 70 families of Insects, including 3 families of Diplopoda, 2 of Crustacea, and 13 families of Arachnida. Yamashita (1990) identified several orders of insects in feces of turtle species including Coleoptera, Isoptera, Hemiptera and Neuroptera.

Results of the current study have also indicated that freshwater turtle species of the study area are opportunistic omnivores that nconsume diversity of plant and animal items. Plant material recovered from the stomach contents

showed that freshwater turtles consumed five different plant species; wormwood (*Artemisia scoparia*), carrot grass (*Parthenium hysterophorus*), ashwagandha (*Withania somnifera),* couch grass (*Cynodon dactylon*) and wild safflower *Carthamus oxycantha*. In a similar study Brock (1988) had investigated that plant material is important component of turtle's diet. Similar findings related to foraging strategy among Emydidae species, were recorded by Caputo and Vogt (2008) who concluded that turtles feed on any available prey item in their habitat.

Conclusion

Most preferable food items of family Chelidae included different stages of plant material (seed, fruit, and leaves) including seven different plant families; Arecaceae (seed) Rubiaceae seed, Leguminosae fruit, Rodophyta, Batrachospermum, Guttiferae fruit and Rodophyta.

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