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SEED MORPHOMETRIC STUDIES IN THREE MEDICINALLY IMPORTANT ORCHID SPECIES OF GENUS *MALAXIS* FROM SHIMLA DISTRICT (H.P.)

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ABSTRACT

Seed morphometry and scanning electron microscopic studies on three terrestrial and medicinally important orchid species namely *Malaxis acuminata, Malaxis cylindrostachya* and *Malaxis muscifera* of Shimla district (Himachal Pradesh) were made. In presently investigated species, the seeds were elliptical in shape but different in size and volume, embryo size and volume and other characters of testa cells. Variations in seed length/width ratio, seed volume/embryo volume ratio and per cent air space existed amongst different species. Data on seed volume showed that higher seed volume is the result of both greater length and width. Presence of similar testa cell wall ornamentation pattern in *M. cylindrostachya* and *M. muscifera* pointed towards their closer affinity. Species distribution is highly influenced by seed volume/embryo volume ratio and the per cent air space present. Seed volume/embryo volume ratio and per cent air space were minimal in the least distributed species *M. muscifera* and suggesting thereby that the present data might have contributed to its endangered status.

Keywords: *Malaxis,* Seed morphometry, Seed length/width, testa cells.

INTRODUCTION

The orchid seeds vary considerably in number (20-40 to 80,000-40,00,000) per capsule, size (length ranged from 0.05-6 mm, width ranged from 0.01-0.9 mm), and weight (0.31-24 mg) (Arditti and Ghani, 2000). Clifford and Smith (1969) first time stressed the taxonomic value of orchid seeds and identified five basic seed shapes (cucumber, filamentous, pear, sac and spindle). Since then seed morphology has been used to elucidate systematic and phylogenetic relationships in orchids (Senghas, *et al.*, 1974; Barthlott, 1976; Arditti *et al.*, 1979, 1980, Barthlott and Ziegler, 1980, 1981; Khurana *et al.*, 1985; Prutsch *et al.*, 2000; Gamarra *et al.*, 2007). Seed shape, morphology, colour and fine details are also variable at generic and specific level (Arditti *et al.*, 1979). Variations in seed and embryo characters, and ratios between their volumes were successfully used to distinguish genus (Gamarra *et al.*, 2007) and species (Wildhaber, 1972; Hass, 1977; Barthlott and Ziegler 1980, 1981; Augustine *et al.*, 2001). Variations have also been noted in number, size, shape, and wall thickening pattern of testa cells (Molvray and Kores, 1995), and seed surface characters have been identified as a unique feature for a specific taxon (Swamy *et al.*, 2004).

Although the Orchid Wealth of India has been estimated at 1,229 species under 184 genera (Karthikeyan, 2000), there are few and far between reports available on orchid seed morphometry in this country and only a few taxa of orchids have been investigated in this regard so far (Khurana, *et al.*, 1985; Garg *et al.*, 1992; Vij *et al.*, 1992; Rani *et al.*, 1993; Augustine *et al.*, 2001; Swamy *et al.*, 2004). The genus *Malaxis* is represented by 3 species in Himachal Pradesh, namely *Malaxis acuminata* (D. Don), *Malaxis cylindrostachya* (Lindl.) Ktze. and *Malaxis muscifera* (Lindl.) Ktze., (Deva and Naithani, 1986). All these species are therapeutically important and locally used for curing a number of ailments; pseudobulbs of *Malaxis acuminata* and *M. muscifera* are well known for their medicinal value in Ayurvedic system of medicine and are commercially known as Jeevak. However, preparation from pseudobulbs of *M. cylindrostachya* is considered as tonic (Jalal *et al.*, 2008). The natural populations of these three species have considerably decreased due to their habitat destruction and illegal collections for medicinal purposes. *M. muscifera* has recently been assessed as endangered species (cf. Chauhan *et al.*, 2008). This study deals with the morphological characteristics of seeds, namely size, shape and volume of seed and embryo; fine structure and ornamentation of seed coat; and structure of testa cells and micropyler pores of these species under the genus *Malaxis*.

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MATERIAL AND METHODS

Mature seeds of three orchid species, namely, *Malaxis acuminata* (D.Don) (Fig.1A-B), *Malaxis cylindrostachya* (Lindl.) Ktze. and *M. muscifera* (Lindl.) Ktze were collected from naturally dehiscing mature capsules ('pods') from different locations of Shimla hills in Himachal Pradesh (Table 1). Seeds were examined both under light microscope and Scanning Electron Microscope (SEM). The characters observed included colour, shape, size and volume of seeds and embryos; shape, arrangements, and wall pattern of their testa cells; seed volume and embryo volume ratio and per cent air space present in seeds. For light microscopic examination, seeds were rinsed in water for 15-20 minutes and fixed in FAA (1:1:18 of formalin: acetic acid: 50% alcohol); subsequently seeds were suspended in absolute alcohol and mounted in euparal following Vij *et al.* (1992). Seed width and length were measured under light microscope with micrometer at the longest and widest axis of seed. Seed volumes were calculated using the following formula (cf. Arditti *et al.*, 1980).

Seed volume = $2[(W/2)^2 (½ L) (1.047)]$ where, W= Width; L= Length; 1.047= $\pi/3$

Orchid embryos were elliptical in cross section and, therefore, their volumes were calculated by using the formula $4/3\ \pi ab^2$ where a= ½ of length, b=½ of width.

The measurements were based on average values of at least five readings. Per cent air space and degree of truncation of seeds were determined following Arditti *et al.* (1979). Per cent air space was measured by using the following formula:

Per cent air space= Seed volume – Embryo volume/ Seed volume x 100.

Seeds with length/width (L/W) ratio of upto 6 were taken as truncated and those with a ratio of more than 6 as elongated.

Fine structure of seed coat (testa), and shape and size of micropyler pore were studied with the help of a Scanning Electron Microscope. For SEM studies, seed suspensions (in absolute alcohol) were mounted on aluminium/ bronze stubs and dried in a critical point dryer for 10-15 minutes and stored in a calcium chloride desiccator until use. The stubs containing critically dried samples were subsequently gold coated, observed and photographed under JSM-SEM-6100.

Table 1: Locality, Altitude and Phenology of the Orchid Species under Present investigation.

Species	Locality and Altitude		Time of Flowering	Time of Fruiting
	Collected from	Altitude (in meters)		
Malaxis acuminata (D.Don)	Tara devi, Shimla District	1851	July-August	August-September
Malaxis cylindrostachya (Lindl.) Ktze.	Fagu, Shimla District	2,440	July-August	August-September
Malaxis muscifera (Lindl.) Ktze.	Fagu, Shimla District	2,440	July-August	August-September

RESULTS AND DISCUSSION

Present study revealed that seed morphology of the species belonging to the genus *Malaxis* was nearly consistent with some variations; some differences existed in their seed size and volume, embryo size and volume, ornamentation pattern of testa cell walls, and per cent of air space present in the seed (Tables, 2-3). In nature, it is difficult to find out the color of an individual orchid seed as orchid seeds are microscopic in size, and the colour could be discerned only when they are either in group in the capsule or are released in millions from the dehiscing mature capsules. In presently investigated orchid species, colour of seeds when observed in their group was found to be in shades of white, ranging from cream white in *M. acuminata* to greenish white in *M. cylindrostachya* and *M. muscifera* (Table 2). Colour of seeds in six species under the genus *Neotinea* was in shades of brown by Gamarra *et al.* (2007). Garg *et al.* (1992) also reported darker seed colour in two different species of *Habenaria*. Further, a wide range of variability in seed colour in 10 different genera of orchids was reported by Swamy *et al.* (2004).

Table 2: Shape, Colour and Measurement data of Orchid Seeds and Size and Wall Patterns of their Testa cells.

Species	Seed						Testa cells		
	Shape	Colour	Length (µm)	Width (µm)	Volume (mm × 10^{-3})	L/W	Average Size (length in µm × width in µm)	Thickening pattern on Longitudinal and Transverse walls	Surface walls
Malaxis acuminata	Elliptical	Cream white	573.75	195.5	5.74	2.93	48.75 ×22.5	Thick, smooth	Transverse cross bands
Malaxis cylindrostachya	Elliptical	Greenish	408 white	124.61	1.658	3.27	35×20	Thick, ribbed	-
Malaxis muscifera	Elliptical	Greenish white	408	73.61	0.579	5.54	38.35×18.35	Thick, ribbed	-

Table 3: Shape, Colour and Measurement data of Embryo, Seed volume/ Embryo Volume Ratio and Per cent Air Space.

Species	Embryo						Seed Volume /Embryo Volume	Per cent Air space
	Shape	Colour	Length (µm)	Width (µm)	Volume (mm × 10^{-3})	L/W		
Malaxis acuminata	Oval	Golden Brown	148.75	89.25	0.620	1.67	9.26	89.2
Malaxis cylindrostachya	Oval	Light Brown	136	85	0.514	1.6	3.22	68.99
Malaxis muscifera	Oval	Brown	107.61	62.39	0.219	1.72	2.64	62.12

However, only a few reports are available commenting on colour of orchid seeds and almost no taxonomic importance has been attributed to this character. Since similar seed colour has been found in species under same genus, more studies are required to comment on their taxonomic importance. All the presently studied species of *Malaxis* exhibited uniformity in their seed shape; elliptical shape was observed as their basic shape though with slight variations. *Malaxis acuminata* seeds were elliptic with acute to slightly clavate ends (Fig. 1C-F) and they were nearly elliptical with wide, blunt and globose ends in *M. cylindrostachya* (Fig. 2 A-E). Short, classically elliptical seeds with blunt ends were observed in *M. muscifera* (Fig. 3 A-E). Molvray and Kores (1995) identified some variations in seed shape (filiform to fusiform, clavate to ellipsoidal and winged) in the subfamily Spiranthoideae and argued that this character can also be used as an additional taxonomic marker for species identification in some orchid genera. Intra-generic uniformity of seed shape has been reported earlier in *Aerides* (Vij *et al.*, 1992; Swamy *et al.*,2004), *Coelogyne, Dendrobium, Epipactis,* and *Habenaria* (Vij *et al.*,

1992) and in *Neotinea* (Gamarra *et al.*, 2007). Present finding of generic uniformity of seed shape further strengthened the use of this parameter as an important taxonomic marker for identification of different taxa in the family Orchidaceae. Seed size is one of the most striking features of the family Orchidaceae and variability do exists within and /or between the sub-families, in genera and even within species. Seeds of *M.* Seed Morphometric Studies In Orchids *acuminata* were longest and widest, and their average volume ($5.741 mm \times 10^{-3}$) was largest among these three species (Table 2). Volume of *M. muscifera* seeds was smallest ($0.579 mm \times 10^{-3}$). Although the average seed length of *M. cylindrostachya* and *M. muscifera* were same, the lesser seed volume in *M. muscifera* was the result of a lesser width of the seeds. That greater seed volume is a result of a greater width rather than length was also indicated earlier by Augustine *et al.* (2001) and Swamy *et al.* (2004). Some orchid genera (*Epipactis, Habenaria* and *Spiranthes*) showed relative uniformity in seed size while inter-specific variability in seed size observed in *Coelogyne, Cymbidium, Goodyera* and *Dendrobium* was also significant (Vij *et al.*, 1992). Inter-specific variability in seed size in the above and presently investigated species hints at its taxonomic importance suggesting thereby that there have been several cycles of gain and/ or loss of seed size during species diversification. Seeds of all the presently investigated species were truncated and maximum length/width ratio was found in *Malaxis muscifera* (5.54) followed by *M. cylindrostachya* (3.27) and *M. acuminata* (2.93). Augustine *et al.* (2001) also observed wide variations in length/width in different species under the genus *Bulbophyllum*. Arditti *et al.* (1979) considered the relative degree of truncation as taxonomic marker and Vij *et al.* (1992) stressed the utility of this parameter in species and generic identification. Presently, seeds of *M. muscifera* were highly truncated than other two species, utility of length/ width ratio in identifying species under the genus *Malaxis* has been suggested.

Under light microscope seed coat of *M. acuminata* and *M. muscifera* were nearly transparent with visible embryo located near the centre (Figs. 1, 3). Seed coat of *M cylindrostachya* was less transparent with embryo hardly seen through seed coat (Fig. 2). In presently studied species, mature testa cells were transparent and arranged longitudinally. Average numbers of testa cells in the longest and widest axis of the seeds of *M. acuminata* were 20 and 10, followed by *M. cylindrostachya* (14 and 8) and *M. muscifera* (10 and 7). The longest testa cell was found in *M. acuminata* (48.75 µm) and shortest in *M. cylindrostachya* (35 µm). The widest testa cell was observed in *M. acuminata* (22.5 µm) and the narrowest in *M. muscifera* (10.35 µm) (Table 2). In presently studied species, testa cells arranged in the median region were usually elongated and nearly rectangular whereas these were short, polygonal and nearly isodiametric towards apical and micropyler region. A direct positive correlation was found between seed size and testa cell size. Vij *et al.* (1992), however, reported the presence of shorter testa cells in relatively large seeded genera; these authors further mentioned that this relationship may not be universal. Presence of quadrilateral testa cells in presently investigated species is in accord to similar earlier findings in most of the terrestrial species studied by Vij *et al.* (1992).Testa cell wall was variously lignified in all the species under study and cell wall ornamentation was observed to be quite different between *M. acuminata* and other two investigated species. *Malaxis acuminata* seeds were characterized by the presence of transverse and oblique cross bands on surface walls of testa cells, which may be providing mechanical strength to testa cells, as the longitudinal and transverse walls were observed to be thin and smooth (Fig.1). Testa cell wall ornamentation was identical in *M. cylindrostachya* and *M. muscifera* and absence of cross band thickenings on surface walls was compensated by the presence of thick, ornamented, ribbed longitudinal and transverse

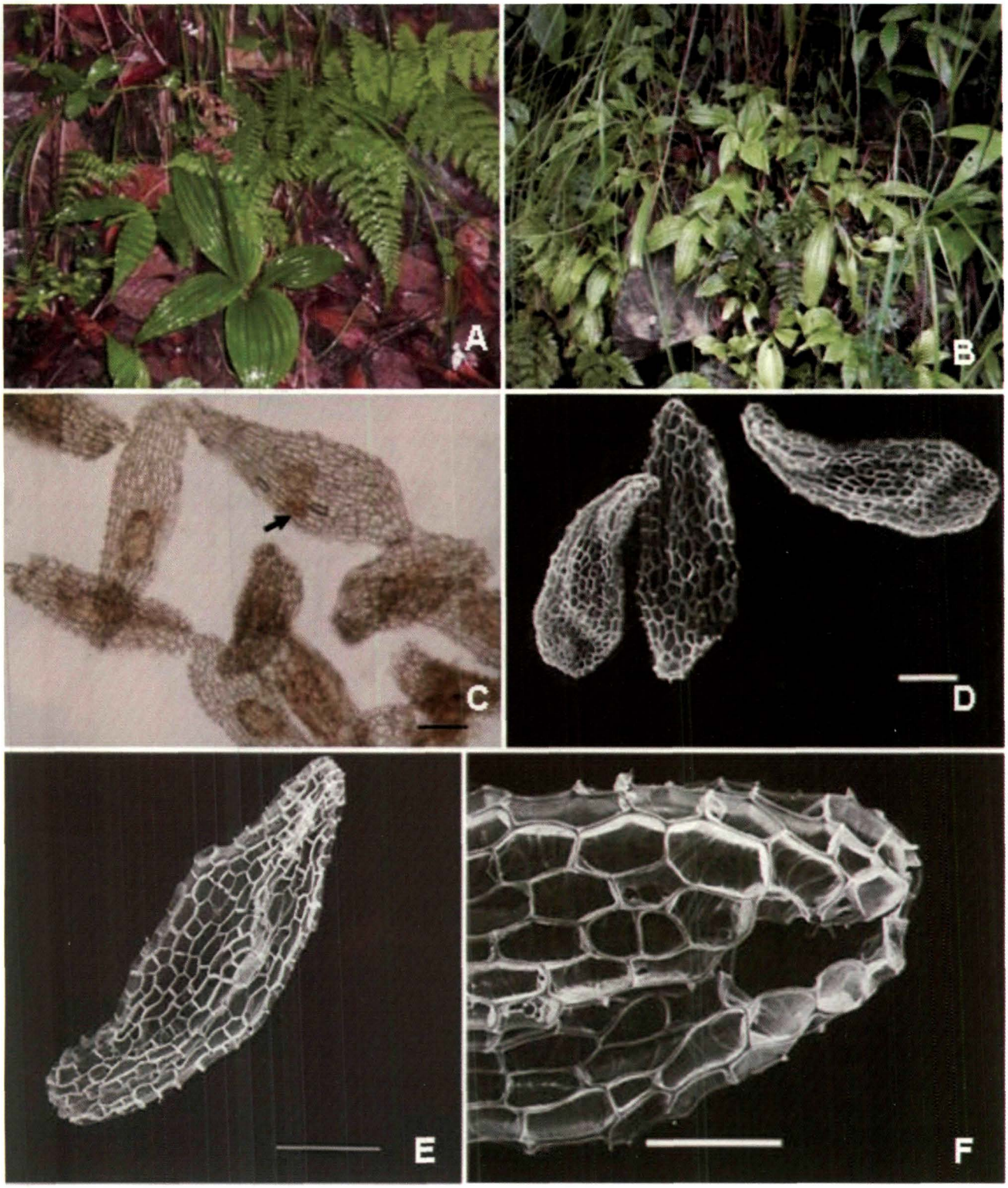

Fig. 1: Investigated plants and seed morphology in *Malaxis acuminata* : A-B, plants growing in their natural habitat : A, on moist forest floor, B, on stone crevices; C, light microscope photograph showing seed shape and arrow indicates seed with more than one embryo (bar = 100µm); D-F: SEM photographs, D, Seed shape (bar = 100 µm); E, single seed under high magnification (bar = 100 µm); F, micropyler pore and part of testa showing wall thickening pattern (bar = 50 µm).

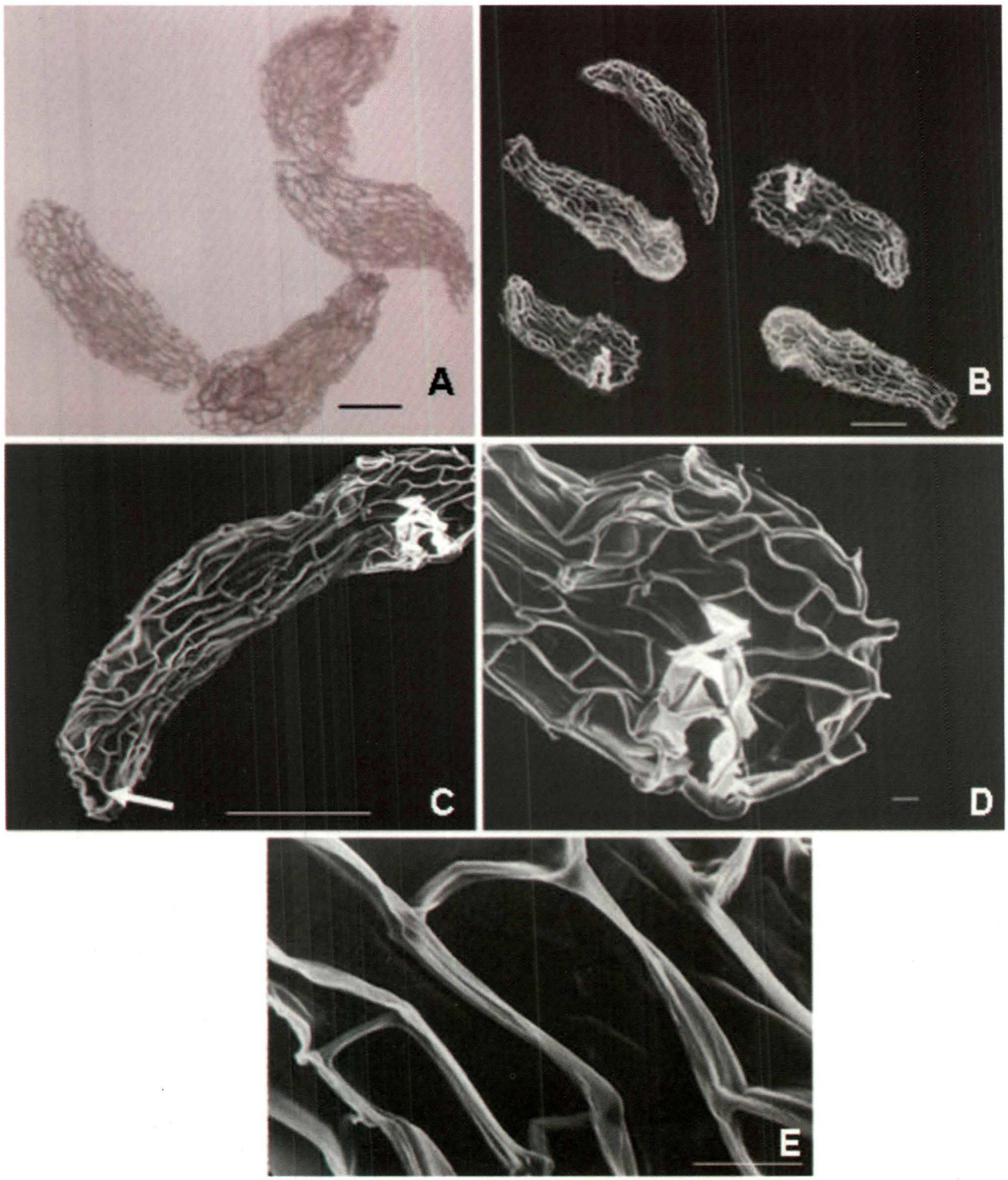

Fig. 2: Seed morphology in *Malaxis cylindrostachya* : A, seed shape under light microscope (bar = 100 µm); B-E: SEM photographs : B, seed shape (bar = 100 µm); C, a seed showing longitudinally arranged testa cells and arrow indicates micropyler pore (bar = 100 µm); D, apical region (bar = 10 µm); E, part of testa showing cell wall thickening pattern (bar =10 µm).

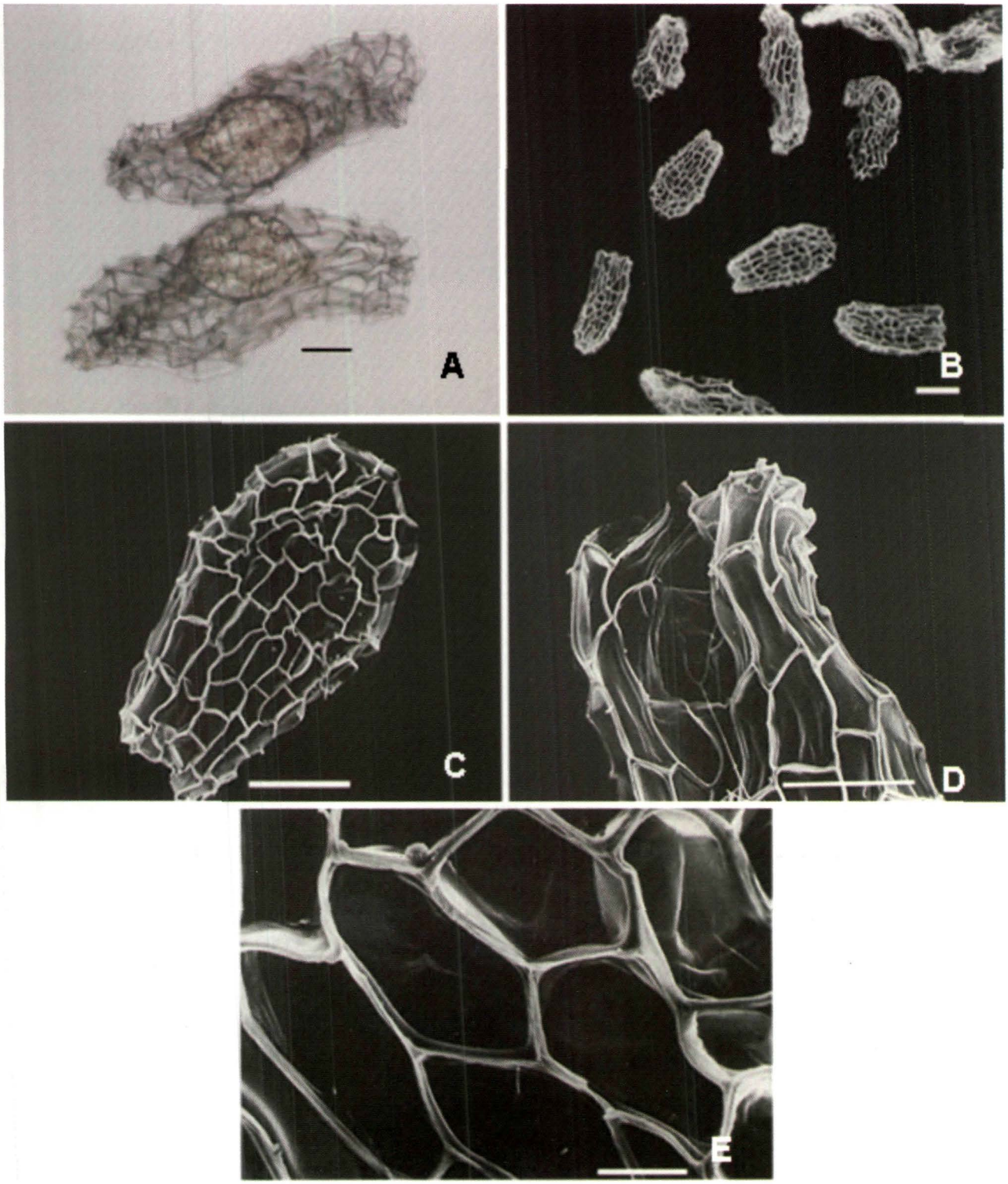

Fig. 3: Seed morphology in *Malaxis muscifera* : A, seed shape under light microscope (bar =100 µm), B-E SEM photographs : B, seed shape (bar = 100 µm); C, a seed under high magnification (bar = 50 µm); D, micropyler pore (bar = 50 µm); E, part of testa showing cell wall thickening pattern (bar = 10 µm)

walls in these two species (Figs.2, 3). Cross band thickenings were reported to be restricted in monandrous orchids by earlier authors (Senghas *et al.*, 1974; Barthlott, 1976; Arditti *et al.*, 1979) and presence of transverse cross bands were also reported earlier in members of tribe Malaxideae (Vij *et al.*, 1992). Presently studied *Malaxis cylindrostachya* and *M. muscifera* are closely related species according to floral morphology and absence of any significant differences in their seed coat characters further confirmed their closer affinity. Testa cell wall thickening has been considered as an adaptive and advanced feature, as the cell wall ornamentations were more prominent in epiphytic taxa than in terrestrial ones (Vij *et al.*, 1992; Swamy *et al.*, 2004). *Malaxis acuminata* is a terrestrial species that is occasionally found to grow as lithophyte (Fig. 1A-B) and lithophytic habitat has been generally considered as intermediate between primitive terrestrial and advanced epiphytic form. Therefore, development of profuse cell wall thickening pattern in presently investigated *M. acuminata* may be considered as an adaptive features and is of phylogenetic importance. In the presently studied species, the micropyler pores were nearly circular to polygonal and made up of 12-16 shorter polygonal testa cells. According to Vij *et al.* (1992), size, shape, and arrangement of testa cells surrounding the micropyler pore at maturity is a constant character for a species. Presently, seeds were mostly mono-embryonate but polyembryony was also observed in *M. acuminata* where 10% of the seeds contained more than one embryo (Fig.1). Colour of embryos in the presently studied species ranged from golden brown to light brown and embryos were oval in shape. Variation has also been observed with reference to length, width and L/W ratio of embryo (Table 3). Maximum embryo volume was found in *M. acuminata* and minimum in *M. muscifera* (Table 3). Length/width ratio was observed to be maximum in *Malaxis muscifera* (1.72) followed by *M. acuminata* (1.67) and *M. cylindrostachya* (1.6). Seed volume to embryo volume ratio was quite high in *M. acuminata* (9.26) followed by *M. cylindrostachya* (3.22) and *M. muscifera* (2.64). Highest percentage of air space was also noticed in *M. acuminata* (89.2) and lowest in *M. muscifera* (62.12) (Table 3). Although uniformity in embryo size within a genus has been previously reported (Healey *et al.*, 1980; Augustine *et al.* 2001), considerable variation can exist in the orchid seed and embryo volume at inter-specific level. Arditti *et al.* (1980), however, reported large variations in seed and embryo volumes and per cent air space among different species under same genera and even among different populations of the same species. Present seed measurement data in *M. muscifera* was slightly different from the previously published measurement data by Vij *et al.* (1992). In the present investigation, the seeds of *M. muscifera* seeds were narrower and less voluminous, but their shape and embryo measurement data were nearly similar in the species. Apparently seed shape and embryo size were more conservative in nature and utility of this parameter as taxonomic marker can not be denied. Being small and light in weight, orchid seeds are adapted for wind dispersal and because of the presence of large internal air space, they are buoyant in both air and water. Seeds with higher ratio of seed volume/embryo volume possess larger air space and are expected to be more buoyant than those with a lower ratio of seed volume/embryo volume. Arditti and Ghani (2000) suggested that seed volume and per cent free air space present inside the seed were important factors that determined the floatation time in orchids and a direct correlation between seed buoyancy and distribution pattern in orchids has been reported by Swamy *et al.* (2004). Instead of having the smallest seed volume, restricted distribution of *M. muscifera* within the Himalayan region of Indian sub-continent, may be attributed to the presence of minimum air space (62.12%) in seeds and lowest ratio of seed volume/ embryo volume (2.64) in this species (Table 3). However, the highest seed volume/embryo volume ratio (9.26) and presence of maximum air space (89.2%) in *M. acuminata* seeds justified their wide distribution throughout the tropical and subtropical Asia to North Australia. In *M. cylindrostachya*, air spaces present in seeds and seed volume/embryo volume ratio were slightly larger than *M. muscifera* (Table 3), and this species shows a far better distribution pattern. Further, confinement of *M. muscifera* may be associated to its habitat specificity. Hence, in addition to the collection pressures on its natural populations for medicinal purposes, seed characters and habitat specificity of *M. muscifera* seems to have further added to the misery of its ever shrinking populations, leading to its endangered status.

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NATURAL BREEDING OF WALKING CATFISH, *CLARIAS BATRACHUS* THROUGH ENVIRONMENTAL MANIPULATION

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ABSTRACT

An effort has been made to understand the natural breeding of *Clarias batrachus* under pond conditions hitherto not reported earlier in this state. The spawning season of *C. batrachus* is coincidental to south-west monsoon and the availability of flowing or good quantum of water in a standing position, hence, the experiments on the above catfish were conducted during the south-west monsoon (breeding) season from May-August. For the natural breeding of *C. batrachus* in the experimental ponds, some aquatic conditions were manipulated similar to that provided in the natural environment. During the month of August, the temperature ranged between 26-29°C which is most suitable for the breeding of the experimental fish and the rain water was also available for 2-3 times. The fish successfully breeds under natural environment producing a large number of larvae, however, the survival rate of fry has been found to be very low.

Keywords: *C. batrachus*, fry, manipulation, south-west monsoon season, breeding

INTRODUCTION

Asian countries have achieved great success in culture fisheries during the last five decades. Within the culture fisheries, the major contributor has been the freshwater aquaculture. In the freshwater sector, Indian major carps are considered to be the best cultivable species, but culture of catfishes has received increased interest in recent years, due to their high market price and hardy nature. Government of India has reviewed the research and development in freshwater aquaculture and suggests that catfish culture should be taken as a national priority in Indian aquacultural practices.

Among the catfishes, the air-breathing species *Clarias batrachus* (walking catfish/desi magur) is a popular culturable fish in Asian countries. It has good prospectus for developing domestic trade in India. It has many advantages over other species. Its flesh has medicinal and therapeutic values as it has iron, copper, vitamin B_{12}, and unsaturated fatty acids in sufficient quantities. Due to this hardy nature and tolerance to adverse ecological conditions it can be cultured in small and shallow ponds with high stocking density. It is an important part of the diet for children and lactating mothers and also prescribed as diet for the convalescent of the patients.

C. batrachus attains sexual maturity within the first year of life cycle, normally breeds from April to August and grows up to maximum total fish length of 35 cm and 250 g in weight (Choudhury, 1981). It breeds in shallow marginal waters of ponds, ditches, natural depression and in undated paddy fields during summer monsoon and rainy seasons (Ahmed *et al.*, 1985).

Barathakur, (2000) reported that the fish migrates to shallower region of the adjoining paddy fields, make small pits along the margin, congregate in pairs and spawn with the onset of south-west monsoon. The male guard the young ones for a few days, however, the female moves out of the pits. Keeping all these points in mind the aim of the present study was to make possible natural breeding of *C. batrachus* under subtropical conditions of Hisar, Haryana, India, which was a challenge.

MATERIALS AND METHODS

During the present study healthy brooders were procured from the Sultan Singh fish farm, Nilokheri, Haryana, India and the experiments were conducted in earthen ponds with area about 0.01ha and 1.5m depth at the fish farm of Department of Zoology and Aquaculture, CCS Haryana Agricultural University, Hisar, Haryana, India (Plate 1). Earthen ponds with area were selected for the natural breeding.

Pre-breeding management

To provide natural environment for natural breeding of the fishes, a trench was dug out in the middle of the

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pond having depth and width of 50 cm and 75 cm respectively (Plate 2).

Several pits (1.5 diameter x 2.3 depth) were dug out at the bottom of the experimental pond on both sides of the middle trench. In these, earthen pots were placed for providing nesting site to the brood fishes (Plate 3). The experimental ponds were manured with organic fertilizer (cow dung and poultry) as per the standard practices. After 14-15 days of manuring, paddy were planted in the pond bottom including aquatic weeds so as to provide nesting material to the spawners as this fish exhibits parental care during the breeding period.

Selection of brood male and female fish

Mature male and female brood fishes were selected on the basis of genital papilla. The males were selected on the basis of pointed and reddish genital papilla, while females by a round and reddish papilla, softness of abdomen and uniform size of intra-ovarian oocytes (Plate 4).

Release of brooders in experimental breeding ponds

After the pre-breeding management, the breeding ponds were filled with water, male and female brood fishes were released in the ponds in the ratio1:1 respectively (Plate 5). Before release of the brooders in the ponds, individual weight and length of brood fish was taken as shown in Table-1. Fecundity was also calculated as given in the standard formula (Garg *et al.*,2001). After the release of brooders in the pond, established showering system at the breeding pond was used every day with the help of tube well water, for the initiation of courtship behavior before spawning.

After an interval of 5 days of the release of the brooders, water level of the pond was periodically reduced (Plate 6) to check the breeding status and the earthen pots were checked after every 5th day, whether, the nests were formed by female brood fish or not. The fry were collected through netting on 15 day, with the help of fishermen (Plate 7). The fry were transferred to the laboratory for further rearing and management.

Analysis of pond water for physico-chemical parameters

During the course of present investigations, water samples were collected periodically and the physico-chemical parameters like temperature, dissolved oxygen (DO), total alkalinity, electrical conductivity, total hardness and pH were analyzed at an interval of 15 days except, temperature which was recorded daily and DO, which was recorded at a week interval in the laboratory. The physico-chemical analysis was carried out, following standard methods (APHA, 1998).

RESULTS AND DISCUSSIONS

Under natural conditions fishes get mature and breed during the breeding season producing larvae in large numbers. However, under controlled conditions and in static pond water most of the cultivable fishes attain maturity but do not breed on their own (Kapur, 1978).

The sexual maturity status of male and female brood fish were also keenly observed. Out of 15 pairs of brooders, 5 pairs were selected, which were ready for breeding. Observations were also recorded to judge the real status of the maturity of the selected pairs. In case of fully mature female brood fish, a chain of mature ova came out from the belly of the fish as soon as the belly was slightly pressed with the help of thumb, However, in case of fully mature male brood fish a flush of milt came out as soon as the belly was slightly pressed with the help of thumb. This test shows whether the female/male brood fish is fully matured or partially riped (Table-1).

Spawning in the fishes is precipitated by a consortium of environmental factors which prevail during the monsoon season (May-August). In standing waters, fishes develop the roe but do not spawn. Under these conditions, the gonads undergo normal growth and development, but the final events of oocyte maturation, ovulation and spermiation do not occur (Donaldson and Hunter, 1983). Once ovarian recrudescence is initiated, new oocytes are appeared and yolk accumulation continues. Eggs attain maturity and need a trigger for final maturation and spawning (Nagahama, 1987). So, the oocytes, which are mature at a particular time, will spawn upon induction of environmental factor(s) under natural conditions or by hormonal factors in captivity.

Environmental control of reproductive activity of captive fish is feasible but, with few exceptions, is currently impractical for most species. Therefore, chemical methods of manipulating reproductive activity are used in fish production operations worldwide. Most research on the control of reproduction in fishes has focused on female physiology because ovarian development and maturation are easily disturbed by environmental stresses (Patino, 1997).

Various physicochemical characters such as, meteorological conditions, depth, free carbon dioxide, dissolved oxygen, pH, carbonates and chloride contents

Plate 1: *Fish farm at CCS HAU, Hisar where the earthen ponds were used for natural breeding of Magur,* C. batrachus.

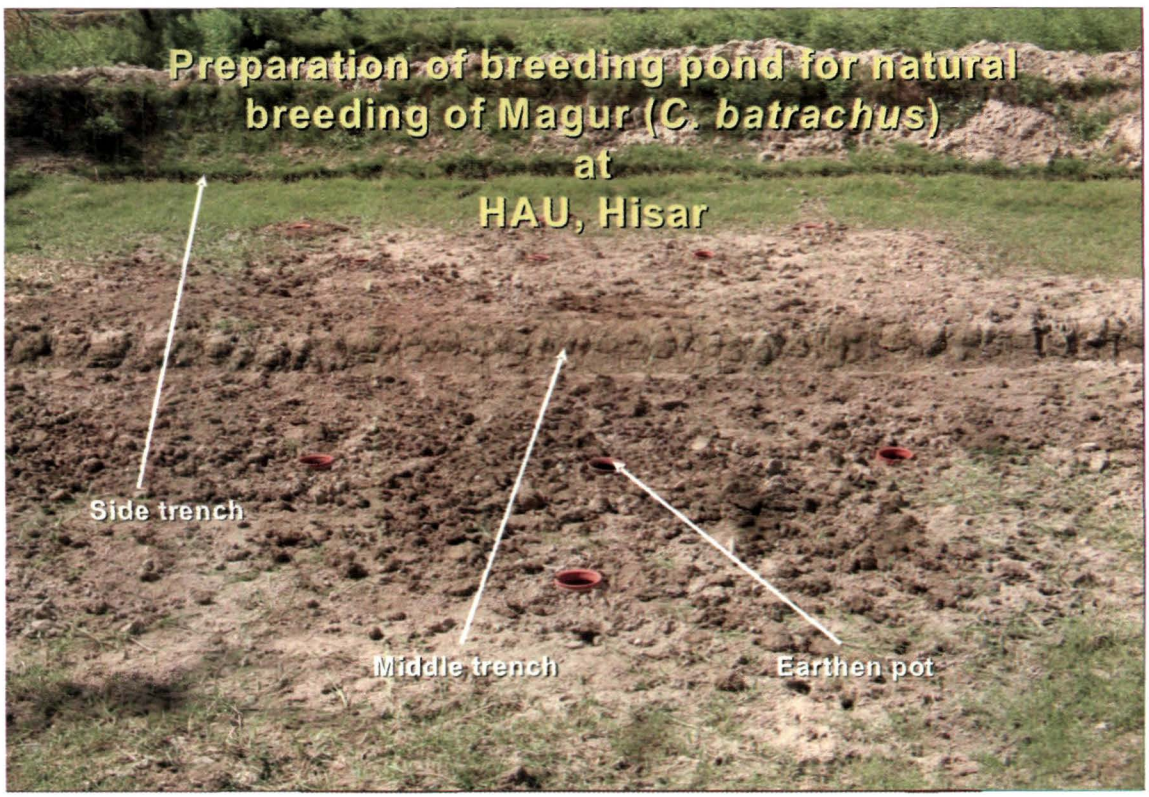

Plate 2: Preparation of breeding pond for natural breeding of *C. batrach*

Plate 3: Close view of earthen pot in a breeding tank used as the nesting site

Plate 4: Selection of brood female (A) and male (B) of catfish, *C. batrachus*

Plate 5: Release of brooders of magur, *C. batrachus* in the breeding pond for natural breeding

Plate 6: Reducing water level in the breeding pond to check the fry production in magur, *C. batrachus*

Plate 7: Fry of magur, *C. batrachus* during netting

also affect the spawning of fishes, under natural condition (Kapur, 1978). The monsoon season is characterized by relatively low water temperature, for natural breeding. The natural breeding of *C. batrachus,* in the present studies took place, with the water temperature ranged between, 26^{0}-29ºC, pH 7.6-7.9 and DO ranged between 7.1-7.6 mg/l (Table-2) as is evidenced from the present studies.

A combination of suitable aquatic environmental factors under the natural conditions is responsible for the proper gonadotropic stimulation and spawning in the ponds. Artificial raining was provided in the breeding tank to decrease the level of water temperature, and for the initiation of spawning of the brood fishes in the experimental tanks, under natural conditions with the showering of water. The present observations are supported by Pati *et al.* (2004).

The reproduction and reproductive behavior in fishes are usually regulated by the external aquatic environmental factors these triggers the internal mechanism in the body of the fish. The weight of female brood fishes ranged between 500-800g and the weight of the male brood fishes between 400-600g. For the trigger of the spawning of the experimental brood fishes, under natural conditions nesting site was also provided in the form of earthen pots and the nesting material in the form of paddy plantation, along with aquatic weeds in the breeding ponds, as this fish exhibits parental care, therefore, forms the nest with the help of nesting material.

After laying the eggs in the nest by the female, the male take over the responsibility to guard the nest and the eggs. Due to the provision of the nesting condition artificially in the experimental breeding tanks, the experiment became successful and the brood fish spawned under constituted artificial conditions at the fish farm.

During the studies it was observed that the recovery rate of fry from the experimental pond was very poor and low, which ranged between 1-2% of the total percentages of hatching of the eggs (Table-3). This study recommended that there is a further need to study the survival and growth of the spawn/fry of this fish with the help of feeding trials with various supplementary feed. In the present study, various environmental factors have been observed that influence the reproductive cycle of

Table 1: Length and weight the male and female brooders of magur, *Clarias batrachus* during the natural breeding season (August, 2007)

Breeding pairs used (No.)	Length of female brood fish (cm)	Weight of female brood fish (g)	Sexual maturity Status of female brooder	Length of male brood fish (cm)	Weight of male brood fish (g)	Sexual maturity status of male brooder
1	26.00	725.00	+	22.00	450.00	+
2	20.00	560.00	++	20.00	380.00	++
3	24.35	600.00	++	20.55	550.00	++
4	25.09	650.00	+	21.30	500.00	++
5	26.00	700.00	++	22.50	410.00	++

(++) Fully mature

(+) Mature (partially riped)

Table 2: Physiochemical parameters of breeding pond water during natural breeding during the month of August, 2007 at Hisar.

Sr. No.	Parameters	Range
1	Temperature(°C)	26-29
2	Dissolved oxygen (mg/l)	7.1-7.6
3	ph	7.6-7.9
4	Alkalinity (mg/l)	250-290
5	Total hardness (mg/l)	200-225
6	Free CO_2 (ppm)	1-2
7	Electrical conductivity (micro mhos/cm)	0.48-0.51

Table 3: Expected weight of the ovary and fecundity of the female brood fish magur, *Clarias batrachus*, during the natural breeding (August, 2007)

Breeding pairs used (No.)	Weight of female brood fish (g)	*Expected weight of the Ovary (g)	**Expected fecundity) (no. of eggs)	Total fry obtained after 15 days (No.)	Advance fry survival after 20 days (No.)
1	725.00	53.16	18,233	1678	1569
2	560.00	41.06	14,085	1405	1569
3	600.00	43.99	15,091	1491	1236
4	650.00	47.66	16,349	1545	1201
5	700.00	41.33	17,607	1594	1398

* Based on the body wt. of female brood fish

** Based on the weight of ovary of the female brood fish

the experimental fish such as- water temperature, photoperiod, rain intensity and a status of substrate, aquatic plants, gravel and the presence of the rocks and gravel strata on the bottom of the breeding aquatic environment of the fish. Thakur, (1991) and Barathakur, (2000), have also observed similar type of environment conditions, which influence the breeding of the *C. batrachus*.

CONCLUSIONS

In the Haryana state, natural breeding of *C. batrachus* was not recorded earlier. The present studies with the manipulation of environmental conditions, natural breeding in this fish has been made possible. The recovery of the fry was very poor because of the poor survival of the spawn/seed of this fish. Therefore, there is a need to stress more on the survival and growth including feeding of the earliest stages of fry of this fish to get the better results. Hence, efforts should be made to improve management practices for the survival and growth of spawn/seed with the advance management practices.

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ICHTHYOFAUNAL COMPOSITION AND CONSERVATION STATUS OF FIVE NORTH-INDIAN STATES (HARYANA, HIMACHAL PRADESH, PUNJAB, RAJASTHAN AND UTTARAKHAND)

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ABSTRACT

The fish fauna of five states, *viz.*, Haryana, Himachal Pradesh, Punjab, Rajasthan and Uttarakhand (also known as Uttaranchal) has been described. A total number of 196 fish species inhabits the freshwaters of these states. The area under report supports 15 exotic fish species, introduced in the past for various purposes and now considered to be the part of native fish species of these states. Out of 196 fish species, the occurrence of 37 fish species is doubtful, because they are endemic to other areas. Majority of the reported fish species are included in one or the other IUCN conservation categories, hence, major part of fish faunae of these states under report needs conservation measures. On the basis of Jaccard's Similarity Index (JSI), it is opined that these neighbouring states shows high value of JSI. Out of 196 fish species, 29 occur in all the five states.

Keywords: Ichthyofaunal, north-India, conservation status, similarity index.

INTRODUCTION

The fish fauna of Indian sub-continent is well documented (Hamilton, 1822; Day, 1875-1878; Misra 1962; Talwar and Jhingran, 1991; Jayaram, 2010). During Vedic and pre-vedic periods, some reliable information on fish fauna and some aspects of bionomics of commercial species are ascribed in the sculptures (Agarwal, 2006). After the publication of Day's fish fauna, several attempts have been made to study the fish fauna of geographical and administrative units and large area of the Asian sub-continent (Chaudhuri, 1919; Misra, 1962; Saxena, 1968; Johal and Dhillon, 1981; Sharma and Johal, 1982, 1984; Sen and Dey, 1984; Srivastava, 1966; Tandon and Gupta, 1975; Lipton, 1983-84; Dutta and Malhotra,1984; Kottelat, 1985; Johal *et al.*, 1989, 2001; Pethiyagoda, 1991; Kottellat and Whitten, 1996; Rainboth, 1996; Mirza, 2003; Mirza and Ahmed, 2004; Nelson, 2006). The Indian sub-continent which includes the present day India, Pakistan, Afghanistan, Sri Lanka, Bangladesh and Myanmar supports 2500 fish species (both marine and freshwater) and constitutes 8.93% of the world's fish diversity. Jayaram (2010) listed 1,042 freshwater fish species inhabiting the various freshwater bodies of Indian sub-continent. The above data was based on the various publications some of which are not accessible today.

During the last four decades, extensive ichthyofaunal surveys have not been undertaken because most of the fishery workers concentrated on the pisciculture practices and the field work got the back seat. However, some workers such as Kar *et al.* (2006) and Sreekantha *et al.* (2007) does provide some information about north-east India and Western Ghats respectively but due to changed priorities for fisheries research, urbanization, water management practices, pollution, introduction of more than 23 exotic fishes (Welcomme, 1988), construction of dams and barrages, drastic change in the original ichthyofaunal composition, reduction in zoogeographical range and decline/disappearance of native/endemic fish species (Johal and Tandon, 1983) have been observed widespread in the recent past.

It has been observed that majority of the publications on the Indian fish fauna published earlier lacked one of the major aspect concerning the conservation status of the reported fish species as per IUCN criterion (1994).

Geographically, present day India is divided into three main regions, *viz.*, the Himalayas, the Gangetic Plains and the Peninsular India. These three regions have distinct fish fauna and some of the fish species are endemic (region wise), however, unplanned transplantations and exotic introductions are responsible for drastic shift in the regional species composition.

The fish faunae of Gangetic Plains and Peninsular India are well documented to some extent (Hamilton, 1822; Hora, 1937, 1941; Menon, 1974), but there were many gaps in the Himalayan and too some extent in the Aravalli's Range too because of difficult and inaccessible

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terrain. Although, the first scientific document on the Indian fish fauna is on the Ganges River (Hamilton, 1822; Day, 1875-78). In the subsequent years, the fish fauna of the north Indian States (Punjab, Haryana, Himachal Pradesh, Rajasthan and Uttarakhand) have not been surveyed periodically for long. Hence, the information on the conservation status of fish species inhabiting the waters of these states are not available, therefore, till now conservation strategies for the endangered and vulnerable species have not been suggested. It is considered appropriate that the scientific information given in the present communication shall be helpful to evaluate the exact status of taxonomically valid reported fish species, hitherto, not reported earlier.

MATERIAL AND METHODS

To prepare this document on the fish diversity of five states, *viz.*, Haryana, Himachal Pradesh, Punjab, Rajasthan and Uttarakhand (earlier called as 'Uttaranchal') of northern India, the works of Datta Gupta *et al.* (1961), Dhawan (1969), Datta and Majumdar (1970), Tilak and Hussain (1977), Johal and Tandon (1979, 1980), Rishi and Dattagupta (1979), Johal and Sharma (1986), Johal *et al.* (1993, 2002), Johal (1998), Moza and Misra (2003), Johal and Rawal (2004), Nautiyal (2005), Negi and Malik (2006) and Johal and Jha (2007) have been reviewed. For the determination of validity of generic and specific names of the reported fish species, Taxonomic Authority List (Luca, 1988), Talwar and Jhingran (1991) and Jayaram (2010) have been followed. Synonyms have been mentioned wherever necessary but not counted in the total number of fish species as separate entity. Conservation status of the reported fish species according to IUCN criteria (1994) have been ascertained following Molur and Walker (1998) and Lakra *et al.* (2010). For the taxonomic arrangement of the reported fish species, the criteria given by Greenwood *et al.* (1966, 1967), Talwar and Jhingran (1991) and Jayaram (2010) have been adopted.

To evaluate the Jaccard's Similarity Index, the following equation has been applied:

Jaccard Similarity Index (JSI) (Jaccard, 1912):

Simple matching coefficient,

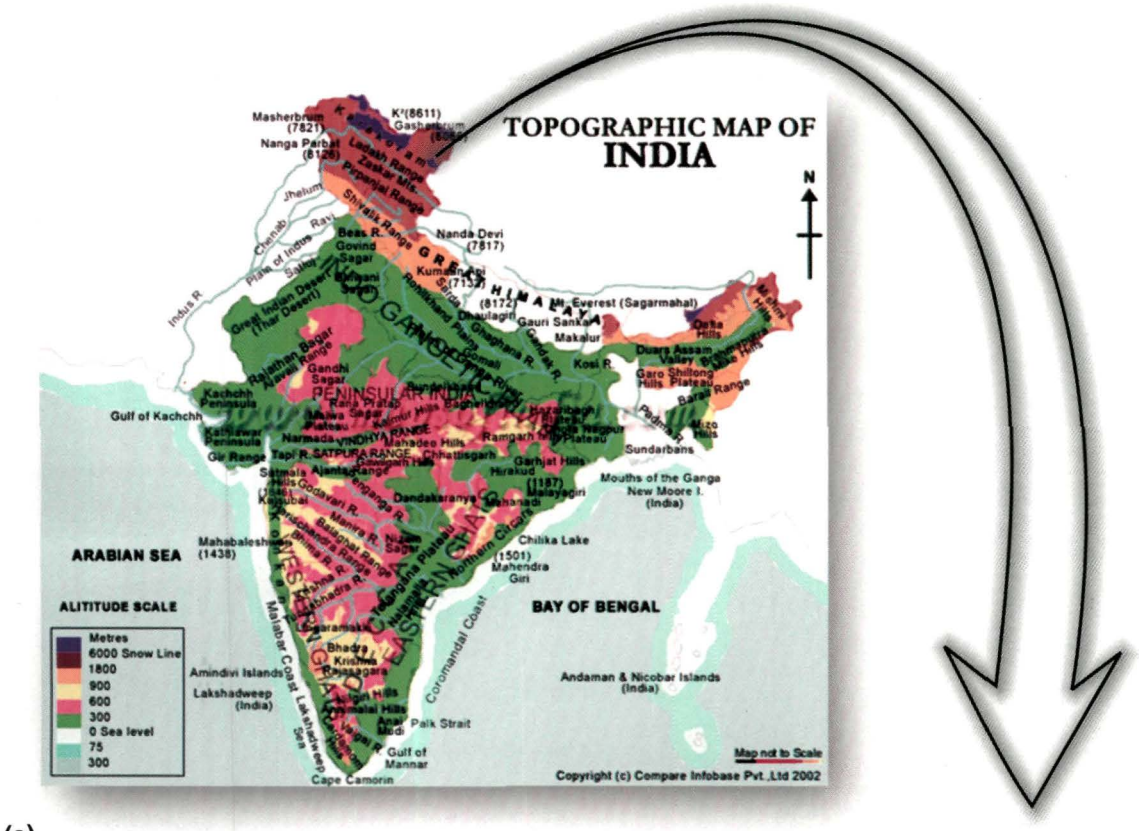
$$Csm = \frac{a + b}{a + b + c + d}$$

Where,

a = the number of species common to both states,
b = the number of species in state B, but not A,
c = the number of species in state A, but not B,
d = the number of species absent in both states.

TOPOGRAPHY

The present study covers the five states, *viz.*, Haryana, Himachal Pradesh, Punjab, Rajasthan and Uttarakhand (also known as Uttaranchal) of north-Indian zone (Fig. 1) having the geographical limits of 26° 35' 21.65" to 31° 6'12" N (Latitude) and 73° 50' 20.47" to 78° 3' 36" E (Longitude). In this region, there prevails wide range of ecological conditions which includes the Thar Desert (hottest part of India) and high mountains having dense forests, snow covered peaks in Himachal Pradesh and Uttarakhand and the air temperature varies between -50 to 50 °C. The altitude varies from 200 to 6000 msl.



(a)

(b)

Fig. 1: Detailed map of India showing distribution network of various river systems: (a) full view; (b) area under report.

The areas under report are included in the Gangetic Plains and Aravalli's Range. Large number of rivers, *viz.*, Bandi, Banganga, Baner, Beas, Chambal, Ganga, Ghagger, Luni, Mahi, Mashi, Morel, Ravi, Sutlej and Yamuna belonging to Ganges and Indus River System and Aravalli's Range supports a wide range of fish fauna. The states of Himachal Pradesh, Rajasthan and Uttarakhand have 28 small, medium and large reservoirs and numerous natural lakes fed by either some river or streams (Sugunan, 1995), which support both indigenous and introduced fish fauna. There is excellent network of irrigation canals, *viz.*, Indira Gandhi Canal, Sirhind Canal, Sirhind Feeder and Gang Canal in the states of Punjab, Haryana along with north-west parts of Rajasthan. Due to the seepage of water, large number of small and large ditches has been formed along the banks of these canals which have high productivity, therefore supporting a wide range of fish diversity, especially the minnows, small sized perches and cat fishes.

OBSERVATIONS AND DISCUSSION

The freshwaters fish fauna of five states of north-western part of India, *viz.*, Haryana, Himachal Pradesh, Punjab, Rajasthan and Uttarakhand (Fig. 1) is very rich, constituting 18.8% of the freshwater fish fauna of Indian sub-continent (Jayaram, 2010) comprising 196 fish species belonging to 10 orders, 28 families and 82 genera. The conservation status (Molur and Walker, 1998; Lakra *et al.*, 2010) of each reported fish is also mentioned (Table-1). The arrangement and classification of the fish species under report is according to the works of Greenwood et al., (1966, 1967), Johal and Tandon (1979, 1980), Talwar and Jhingran (1991) and Jayaram (2010). It is clearly evident from the table that the maximum number of fish species inhabits the waters of Punjab (117) followed by Rajasthan (107), Haryana (95), Himachal Pradesh (91) and Uttarakhand (76).

The maximum number of genera belongs to the order Cypriniformes (35), followed by the order Siluriformes (21) and Perciformes (10). The orders Mugiliformes and Synbranchiformes are represented by three genera each, whereas the order Beloniformes, Cyprinidontiformes and Osteoglossiformes includes two genera each and order Clupeiformes and Salmoniformes is represented by only one genus. Maximum number of fish species belongs to the order Cypriniformes (112) followed by the order Siluriformes (53), Perciformes (15) and Synbranchiformes (3). The order Beloniformes, Cyprinidontiformes, Osteoglossiformes and Salmoniformes includes two species each while order Clupeiformes has only one species.

Surprisingly, the areas under report have recorded fifteen exotic fish species, *viz.*, *Gambusia affinis* (Baird & Girad), *Aplocheilus lineatus* (Valenciennes), *Oreochromis massambicus* (Peters), *Osphronemus oramy* Lacepede, *Clarius gariepiunnus* (Burchell), *Carassius auratus auratus* (Linnaeus), *Carassius carassius* (Linnaeus), *Ctenopharyngodon idellus* (Valenciennes), *Cyprinus carpio* var. *communis* Linnaeus, *Cyprinus carpio* var. *specularis* Lacepede, *Cyprinus carpio* var. *nudus* Bloch, *Hypophthalmichthys molitrix* (Valenciennes), *Hypophthalmichthys nobilis* (Richardson), *Salmo trutta fario* Linnaeus and *Salmo gairdnerii gairdnerii* Richardson are also the part of the fish fauna of these states.

All these fishes have been introduced in the past for several reasons such as control of mosquito larvae, various macrophytes, phytoplankton, fish-culture, games and ornamental purposes. The introduction of these fishes has been controversial (Welcomme, 1988) as most of the exotic introductions have failed to show the desired effects and in most of the cases has been responsible for the decline in the native fish species composition and fishery stocks.

In the past there are seven fish species, *viz.*, *Puntius dukai* (Hamilton) from Uttarakhand, *Cirrhinis latia* (Hamilton) from Punjab, *Schizothoraichthys (Schizothorax) kumaonensis* (Menon) from Uttarakhand, *Schistura (Nemacheilus) stoliczkae* (Steindachner) from Himachal Pradesh, *Mystus (Callichrous) microphthalmus* (Day) from Punjab, *Silonia gangetica* (Cuvier & Valenciennes) from Rajasthan and *Acentrogobius viridipunctatus* (Valenciennes) reported from Punjab and Rajasthan that are included in the ichthyofaunal component from the areas under report, but presently, according to Jayaram (2010) these were not the valid fish species.

There were eleven fish species reported earlier from the states of Haryana, Himachal Pradesh, Punjab, Rajasthan and Uttarakhand which have now been synonymised, *e.g.*, *Labeo sindensis* (Day) syn. *Labeo dero* ((Hamilton), *Puntius tetrarupagus* (McClelland) syn. *Puntius chola* (Hamilton), *Garra mullya* (Sykes) syn. *Garra lamta* (Hamilton), *Perilampus atpar* (Hamilton) syn. *Chela cachius* (Hamilton), *Nemacheilus kangree* Menon syn. *Schistura (Nemacheilus) montanus* (McClelland), *Glyptothorax alaknandi* Tilak syn. *Glyptothorax brevipinnis* Hora, *Euglyptosternum lineatum* Day syn. *Glyptothorax cavia* (Hamilton), *Rita pavimentatus* (Valenciennes) syn. *Rita gogra* (Sykes), *Channa gachua* (Hamilton) syn. *Channa orientalis* (Bloch

Table-1: List of occurrence of fishes reported from the five states under observation.

Name of Fish Species	Name of the States of Northern India					
	Punjab	Haryana	Himachal Pradesh	Rajasthan	Uttarakhand	IUCN Status
Order - CLUPEIFORMES						
Family - Clupeidae						
1. *Gudusia chapra* (Hamilton-Buchanan)	P	P	A	P	A	LRlc
Order - OSTEOGLOSSIFORMES						
Family - Notopteridae						
2. *Chitala* (*Notopterus*) *chitala* (Ham.-Buch.)	P	P	P	P	A	En
3. *Notopterus notopterus* (Pallas)	P	P	A	P	A	LRnt
Order - BELONIFORMES						
Family - Belonidae						
4. *Strongylura strongylura* van Hasselt						
Syn. *Strongylura luira* van Hasselt	P	A	A	A	A	DD
5. *Xenentodon cancila* (Hamilton-Buchanan)	P	P	P	P	P	LRnt
Order - CYPRINODONTIFORMES						
Family - Poecilidae						
6. *Gambusia affinis* (Baird & Girard)	P	P	A	P	P	DD
Family - Aplocheilidiae						
7. *Aplocheilus* (*Haplocheilus*) *lineatus* (C. & V.)	A	A	A	P	A	DD
Order - SYNBRANCHIFORMES						
Family - Mastacembelidae						
8. *Mastacembelus armatus* (Lecepede)	P	P	P	P	P	DD
9. *Macrognathus aculeatus* (Bloch & Schneider)						
Syn. *Macrognathus aral* (Bloch & Schneider)	P	P	A	P	A	LRnt
10. *Macrognathus pancalus* (Hamilton-Buchanan)	P	P	A	P	A	LRnt
Family - Synbranchidae						
11. *Monopterus cuchia* (Hamilton-Buchanan)	P	A	A	A	A	LRnt

	Punjab	Haryana	Himachal Pradesh	Rajasthan	Uttarakhand	IUCN Status
Order - PERCIFORMES						
Family - Anabantidae						
12. *Anabas testudineus* (Bloch)	A	P	A	A	A	Vu
Family - Gobiidae						
13. *Acentrogobius viridipuntatus* (Valenciennes)	P	A	A	P	A	DD
14. *Glossogobius giuris giuris* (Ham.-Buch.)	P	P	A	P	A	LRnt
Family - Badidae						
15. *Badis badis* (Hamilton-Buchanan)	P	A	A	A	A	DD
Family - Ambassidae						
16. *Chanda* (*Ambassis*) *nama* (Hamilton-Buchanan)	P	P	A	P	A	DD
17. *Parambassis* (*Chanda*) *baculis* (Ham.-Buch.)	A	P	P	P	A	DD
18. *Parambassis ranga* (Hamilton-Buchanan)	P	P	A	P	A	DD
Family - Channidae						
19. *Channa marulius* (Hamilton-Buchanan)	P	P	P	P	A	LRnt
20. *Channa orientalis* (Bloch & Schneider)						
Syn. *Channa gachua* (Hamilton-Buchanan)	P	P	P	P	A	Vu
21. *Channa punctatus* (Bloch)	P	P	P	P	A	LRnt
22. *Channa striatus* (Bloch)	P	P	P	P	A	LRlc
Family - Nandinae						
23. *Nandus nandus* (Hamilton-Buchanan)	P	A	P	P	A	LRnt
Family - Cichlidae						
24. *Oreochromis mossambica* (Peters)	P	P	A	A	A	DD
Family - Osphronemidae						
25. *Osphronemus goramy* (Lecepede)	A	A	A	P	A	DD
Family - Belontidae						
26. *Colisa fasciatus* (Bloch & Schneider)	A	P	A	A	A	DD

	Punjab	Haryana	Himachal Pradesh	Rajasthan	Uttarakhand	IUCN Status
Order - MUGILIFORMES						
Family - Mugilidae						
27. *Liza parsia* (Hamilton-Buchanan)	A	A	A	P	A	DD
28. *Mugil cephalus* Linnaeus	A	A	A	P	A	DD
29. *Rhinomugil corsula* (Hamilton-Buchanan)	A	A	A	P	A	Vu
Order - SILURIFORMES						
Family - Amblycipitidae						
30. *Amblyceps mangois* (Hamilton-Buchanan)	P	P	P	P	A	En
Family - Bagridae						
31. *Mystus bleekeri* (Day)	P	P	P	P	A	Vu
32. *Mystus cavasius* (Hamilton-Buchanan)	P	P	A	P	A	LRnt
33. *Mystus keletius* (Cuvier & Valenciennes)	P	A	A	A	A	DD
34. *Mystus (Callichrous) microphthalamus* (Day)	P	A	A	A	A	En
35. *Mystus montanus* (Jerdon)	P	A	A	A	A	Vu
36. *Mystus oculatus* (Cuvier & Valenciennes)	A	A	A	P	A	DD
37. *Mystus tengara* (Hamilton-Buchanan)	P	P	A	A	A	DD
38. *Mystus vittatus* (Bloch)	P	P	P	P	P	Vu
39. *Sperata (Aorichthys) aor* (Hamilton-Buchanan)	P	P	P	P	A	DD
40. *Sperata (Aorichthys) seenghala* (Sykes)	P	P	P	P	A	DD
41. *Rita gogra* (Sykes)						
Syn. *Rita pavimentatus* (Valenciennes)	A	A	A	P	A	En
42. *Rita rita* (Hamilton-Buchanan)	P	P	A	P	P	LRnt
Family - Clariidae						
43. *Clarius batrachus* (Linnaeus)	P	P	A	P	A	LRnt
44. *Clarius gariepinus* (Burchell)	A	P	A	A	A	DD
45. *Glyptosternon reticulatum* McClelland	A	A	P	A	A	En
46. *Heteropneustes fossilis* (Bloch)	P	P	A	P	A	Vu
47. *Heteropneustes microps* (Gunther)	A	P	A	A	A	DD

	Punjab	Haryana	Himachal Pradesh	Rajasthan	Uttarakhand	IUCN Status
Family - Schilbidae						
48. *Ailia coila* (Hamilton-Buchanan)	A	A	A	P	A	Vu
49. *Clupeisoma gaura* (Hamilton-Buchanan)	P	P	P	P	P	Vu
50. *Eutropiichthys vacha* (Hamilton-Buchanan)	P	P	A	P	A	En
51. *Eutropiichthys* (*Pseudeutropius*) *murius* (Hamilton-Buchanan)	P	A	A	A	A	LRnt
52. *Neotropius* (*Pseudeutropius*) *atherinoides* (Bloch)	P	A	A	A	A	En
53. *Silonia silondia* (Hamilton-Buchanan)	A	P	A	P	A	Vu
54. *Silonia gangetica* (Cuvier & Valenciennes)	A	A	A	P	A	DD
Family - Siluridae						
55. *Ompok* (*Callichrous*) *bimaculatus* (Bloch)	P	P	A	P	A	En
56. *Ompok malabaricus* (Valenciennes)	P	A	A	A	A	En
57. *Ompok* (*Callichrous*) *pabda* (Ham.-Buch.)	P	P	A	A	A	Vu
58. *Wallago attu* (Bloch & Schneider)	P	P	P	P	A	LRnt
Family - Sisoridae						
59. *Bagarius bagarius* (Hamilton-Buchanan)	P	P	P	P	P	Vu
60. *Bagarius yarrelli* Sykes	P	A	A	A	A	En
61. *Gagata cenia* (Hamilton-Buchanan)	P	P	A	P	A	DD
62. *Glyptothorax alaknandi* Tilak						
Syn. *Glyptothorax brevipinnis* Hora	A	A	P	A	P	CR
63. *Glyptothorax cavia* (Hamilton-Buchanan)						
Syn. *Euglyptosternum lineatum* Day	P	A	A	A	P	En
64. *Glyptothorax conirostris* (Steindachner)	A	A	A	A	P	DD
65. *Glyptothorax dakpathari* Tilak & Hussain	A	A	A	A	P	DD
66. *Glyptothorax garhwali* Tilak	A	A	P	A	P	CR
67. *Glyptothorax gracilis* (Gunther)	A	A	P	A	A	DD
68. *Glyptothorax horai* Shaw & Shebbeare	P	A	P	A	A	DD

	Punjab	Haryana	Himachal Pradesh	Rajasthan	Uttarakhand	IUCN Status
69. *Glyptothorax indicus* Talwar	A	P	A	A	A	Vu
70. *Glyptothorax kashmirensis* Hora	A	A	P	A	A	En
71. *Glyptothorax lonah* (Sykes)	A	A	P	A	A	LRnt
72. *Glyptothorax madraspatanam* (Day)	A	A	A	A	P	Vu
73. *Glyptothorax pectinopterus* (McClelland)	A	A	P	A	P	LRnt
74. *Glyptothorax punjabensis* Mirza & Kashmir	P	A	A	A	A	DD
75. *Glyptothorax stoliczkae* (Steindachner)	A	A	P	A	A	CR
76. *Glyptothorax telchitta* (Hamilton-Buchanan)	A	P	P	P	P	LRnt
77. *Glyptothorax trilineatus* Blyth	A	A	A	A	P	DD
78. *Nangra nangra* (Hamilton-Buchanan)	A	A	A	P	P	En
79. *Nangra punctata* Day	P	A	P	A	A	DD
80. *Parachiloglanis* (*Euchiloglanis*) *hodgatri* Hora	A	A	A	A	P	Vu
81. *Pseudecheneis sulcata* (McClelland)	A	A	P	A	A	Vu
82. *Sisor rhabdophorus* Hamilton-Buchanan	P	P	A	A	A	En
Order - CYPRINIFORMES						
Family - Balitoridae						
83. *Balitora burcie* Gray	A	A	A	A	P	LRnt
84. *Bhavania australis* Jerdon	A	A	A	A	P	En
85. *Nemacheilus botia* (Ham.-Buch.)	P	P	P	P	P	LRnt
86. *Schistura* (*Nemacheilus*) *beavani* (Gunther)	A	A	A	A	P	DD
87. *Schistura* (*Nemacheilus*) *carletoni* (Fowler)	A	A	P	A	A	En
88. *Schistura* (*Nemacheilus*) *corica* (Ham.-Buch.)	A	P	P	P	P	LRnt
89. *Schistura* (*Nemacheilus*) *denisoni* (Day)	A	A	P	P	A	DD
90. *Schistura* (*Nemacheilus*) *horai* (Menon)	A	A	P	A	A	DD
91. *Schistura* (*Nemacheilus*) *montanus*(McClelland)						
Syn. *Nemacheilus kangree* (Menon)	A	A	P	p	A	En
92. *Schistura* (*Nemacheilus*) *multifasciatus* (Day)	A	A	A	A	P	Vu

	Punjab	Haryana	Himachal Pradesh	Rajasthan	Uttarakhand	IUCN Status
93. *Schistura* (*Nemacheilus*) *neilgiriensis* (Menon)	A	A	P	A	A	En
94. *Schistura* (*Nemacheilus*) *punjabensis* (Hora)	P	P	P	A	A	DD
95. *Schistura* (*Nemacheilus*) *rupecola* (McClelland)	P	A	P	A	P	LRnt
96. *Schistura* (*Nemacheilus*) *savona* (Ham.-Buch.)	A	A	A	A	P	DD
97. *Schistura* (*Nemacheilus*) *scaturigina* (McClelland)	A	A	A	A	P	Vu
98. *Schistura* (*Nemacheilus*) *semiarmatus* (Day)	A	A	P	A	A	Vu
99. *Schistura* (*Nemacheilus*) *stoliczkae* (Steindachner)	A	A	P	A	A	DD
Family - Cobitidae						
100. *Botia almorhae* Gray						
Syn. *Botia dayi* Hora	A	A	A	A	P	En
101. *Botia birdi* Chaudhuri	P	P	P	P	A	LRnt
102. *Botia dayi* (Hora)	A	A	P	A	P	DD
103. *Botia dario* (Hamilton-Buchanan)	P	P	A	A	P	DD
104. *Botia lohachata* Chaudhuri	P	P	P	P	A	En
105. *Lepidocephalichthys* (*Lepidocephalus*) *guntea* (Hamilton-Buchanan)	P	P	P	P	P	DD
Family - Cyprindae						
106. *Amblypharyngodon mola* (Ham.-Buch.)	P	P	A	P	A	LRlc
107. *Amblypharyngodon microlepis* (Bleeker)	P	A	A	P	A	DD
108. *Aspidoparia morar* (Hamilton-Buchanan)	P	P	A	P	A	LRnt
109. *Barilius barila* (Hamilton-Buchanan)	P	P	P	A	P	Vu
110. *Barilius barna* (Hamilton-Buchanan)	A	A	P	P	P	LRnt
111. *Barilius bendelisis* (Hamilton-Buchanan)	P	P	P	P	P	LRnt
112. *Barilius modestus* Day	A	A	P	A	A	DD
113. *Barilius radiolatus* (Hamilton-Buchanan)	P	A	A	A	A	DD
114. *Barilius shacra* (Hamilton-Buchanan)	A	P	P	A	P	LRnt
115. *Barilius vagra* (Hamilton-Buchanan)	P	P	P	P	P	Vu

	Punjab	Haryana	Himachal Pradesh	Rajasthan	Uttarakhand	IUCN Status
116. *Brachydanio (Danio) rerio* (Ham.-Buch.)	P	P	P	P	P	LRnt
117. *Catla catla* (Hamilton-Buchanan)	P	P	P	P	P	Vu
118. *Carassius auratus auratus* (Linnaeus)	P	A	P	A	P	DD
119. *Carassius carassius* (Linnaeus)	P	A	A	A	P	DD
120. *Chela cachius* (Hamilton-Buchanan)						
Syn. *Parilampus atpar* (Ham.)	P	P	A	P	A	DD
121. *Chela laubuca* (Hamilton-Buchanan)	P	A	A	A	A	LRlc
122. *Chagunius chagunio* (Hamilton-Buchanan)	A	P	P	A	P	DD
123. *Cirrhinus mrigala* (Hamilton-Buchanan)	P	P	P	P	P	LRnt
124. *Cirrhinus reba* (Hamilton-Buchanan)	P	P	P	P	A	Vu
125. *Cirrhinus cirrhosus* (Bloch)	P	A	A	A	P	Vu
126. *Cirrhinus latia* (Hamilton-Buchanan)	P	A	A	A	A	DD
127. *Crossocheilus latius latius* (Ham.-Buch.)	A	A	P	A	P	DD
128. *Crossocheilus latius diplocheilus* (Heckel)	P	P	P	P	P	DD
129. *Ctenopharyngodon idellus* (Valenciennes)	P	P	P	P	P	DD
130. *Cyprinus carpio communis* Linnaeus	P	P	P	P	P	DD
131. *Cyprinus carpio specularis* Lecepede	P	P	P	P	A	DD
132. *Cyprinus carpio nudus* Bloch	A	A	P	A	A	DD
133. *Devario (Danio) devario* (Hamiton-Buchanan)	P	P	P	P	P	LRnt
134. *Diptychus maculatus* Steindachner	A	A	P	A	A	DD
135. *Esomus danricus* (Hamilton-Buchanan)	P	P	P	P	P	LRnt
136. *Garra gotyla gotyla* (Gray)	P	P	P	P	P	Vu
137. *Garra lamta* (Hamilton-Buchanan)						
Syn. *Garra mullya* (Sykes)	P	P	P	P	P	DD
138. *Hypophthalmichthys molitrix* (Valenciennes)	P	P	P	P	P	DD
139. *Hypophthalmichthys nobilis* (Richardson)	A	P	A	A	A	DD
140. *Labeo angra* (Hamilton-Buchanan)	P	P	A	P	A	LRnt
141. *Labeo bata* (Hamilton-Buchanan)	P	P	P	P	A	LRnt

	Punjab	Haryana	Himachal Pradesh	Rajasthan	Uttarakhand	IUCN Status
142. *Labeo boga* (Hamilton-Buchanan)	P	P	P	P	A	LRnt
143. *Labeo boggut* (Sykes)	A	A	A	P	A	DD
144. *Labeo caeruleus* Day	P	P	A	A	A	DD
145. *Labeo calbasu* (Hamilton-Buchanan)	P	P	P	P	A	LRnt
146. *Labeo dero* (Hamilton-Buchanan)						
Syn. *Labeo sindensis* (Day)	P	P	P	P	A	Vu
147. *Labeo dussunieri* (Valenciennes)	P	A	A	P	A	En
148. *Labeo dyocheilus* (McClelland)	P	P	P	A	A	Vu
149. *Labeo fimbriatus* (Bloch)	A	A	A	P	A	LRnt
150. *Labeo gonius* (Hamilton-Buchanan)	P	P	A	P	A	LRnt
151. *Labeo nigripinnis* Day	A	A	A	P	A	DD
152. *Labeo pangusia* (Hamiton-Buchanan)	P	P	P	P	P	Vu
153. *Labeo potail* (Sykes)	A	A	A	P	A	DD
154. *Labeo rohita* (Hamilton-Buchanan)	P	P	P	P	P	LRnt
155. *Naziritor* (*Tor*) *chelynoides* (McClelland)	P	P	P	A	P	DD
156. *Osteobrama cotio cotio* (Hamilton-Buchanan)	P	P	P	P	A	LRnt
157. *Puntius amphibius* (Valenciennes)	A	A	A	P	A	DD
158. *Puntius chola* (Hamilton-Buchanan)						
Syn. *Puntius tetrarupagus* (McClelland)	P	P	P	P	P	Vu
159. *Puntius conchonius* (Hamilton-Buchanan)	P	A	P	P	A	Vu
160. *Puntius dorsalis* (Jerdon)	A	A	A	P	A	En
161. *Puntius dukai* (Hamilton-Buchanan)	A	A	A	A	P	DD
162. *Puntius filamentosus* (Valenciennes)	P	A	A	A	A	DD
163. *Puntius gelius* (Hamilton-Buchanan)	A	A	A	A	P	DD
164. *Puntius guganio* (Hamilton-Buchanan)	P	A	A	A	A	LRnt
165. *Puntius parrah* (Day)	P	A	A	P	A	En
166. *Puntius phutunio* (Hamilton-Buchanan)	P	A	A	A	P	LRlc
167. *Puntius sarana sarana* (Hamilton-Buchanan)	P	P	P	P	P	Vu

	Punjab	Haryana	Himachal Pradesh	Rajasthan	Uttarakhand	IUCN Status
168. *Punitus sarana subnaustus* (C. & V.)	P	A	A	A	A	DD
169. *Puntius sophore* (Hamilton-Buchanan)						
Syn. *Puntius* (*Barbus*) *stigma* (Day)	P	P	P	P	P	LRnt
170. *Puntius terio* (Hamilton-Buchanan)	A	P	A	P	A	LRnt
171. *Puntius ticto* (Hamilton-Buchanan)						
Syn. *Barbus stoliczkanus* Annandale						
Puntius (*Barbus*) *punctatus* Day	P	P	P	P	A	Vu
172. *Puntius waageni* (Day)	A	A	P	A	A	DD
173. *Raiamus bola* (Hamilton-Buchanan)	P	P	P	P	P	Vu
174. *Rasbora* (*Parluciosoma*) *daniconius* (Hamilton-Buchanan)	P	P	P	P	P	LRnt
175. *Salmophasia* (*Salmostoma*) *bacaila* (Hamilton)	P	P	P	P	A	LRlc
176. *Salmophasia* (*Chela*) *clupeoides* (Bloch)	A	A	A	P	A	DD
177. *Salmophasia* (*Salmostoma*) *horai* (Silas)	P	A	A	A	A	DD
178. *Salmophasia* (*Salmostoma*) *phulo* (Hamilton)	P	A	A	P	A	DD
179. *Salmophasia* (*S.*) *phulo punjabensis* (Day)	P	P	A	P	A	DD
180. *Salmophasia* (*Salmostoma*) *phulo orissaensis* Banarescu	A	A	A	P	A	En
181. *Securicula gora* (Hamilton-Buchanan)	P	P	A	P	A	DD
182. *Schizothoraichthys* (*Schizothorax*) *curvifrons* Heckel	A	A	A	A	P	Vu
183. *Schizothoraichthys* (*Schizothorax*) *esocinus* Heckel	P	A	A	A	P	LRnt
184. *Schizothoraichthys* (*Schizothorax*) *kumaonensis* (Menon)	A	A	A	A	P	LRnt
185. *Schizothoraichthys* (*Schizothorax*) *micropogon* Heckel	A	A	A	A	P	DD
186. *Schizothoraichthys* (*Schizothorax*) *nasus* Heckel	A	A	A	A	P	DD
187. *Schizothoraichthys* (*Schizothorax*) *niger* Heckel	A	A	A	A	P	Vu

	Punjab	Haryana	Himachal Pradesh	Rajasthan	Uttarakhand	IUCN Status
188. *Schizothorax richardsonii* (Gray)						
Syn. *Schizothorax plagiostomus* Heckel	P	A	P	A	P	Vu
189. *Schizothorax sinuatus* (Heckel)	A	A	A	A	P	LRnt
190. *Tor khurdee* (Sykes)	A	A	A	P	A	Vu
191. *Tor mosal* (Hamilton-Buchanan)	A	A	P	A	A	En
192. *Tor putitora* (Hamilton-Buchanan)	P	P	P	P	P	En
193. *Tor tor* (Hamilton-Buchanan)	P	P	P	P	P	En
Family - Psilorhynchidae						
194. *Psilorhynchus balitora* (Hamilton-Buchanan)	A	A	A	P	A	DD
Order - SALMONIFORMES						
Family - Salmonidae						
195. *Salmo gairdnerii gairdnerii* Richardson	A	A	P	A	P	DD
196. *Salmo trutta fario* Linnaeus	A	A	P	A	P	DD

Abbreviations: **A**= Absent; **CR**= Critically endangered; **DD**= Data deficient; **En**= Endangered; **Ham.-Buch.**= Hamilton-Buchanan; **IUCN**= International Union for Conservation of Natural Resources; **LRlc**= Threatened, Lower Risk least concern; **LRnt**= Threatened, Lower Risk near threatened; **P**= Present;
Syn.= Synonym; **VU**= Vulnerable.

& Schneider), *Strongylura strongylura* van Hasselt syn. *Strongylura lura* van Hasselt and *Macrognathus aral* (Bloch & Schneider) syn. *Macrognathus aculeaus* (Bloch)

In the earlier reports the number of fish species from the five north Indian states namely, Punjab, Haryana, Himachal Pradesh, Rajasthan and Uttarakhand seems to be quite high because there are thirty-eight fish species which have been reported from these areas under report, but they are endemic to some other area also, hence, their occurrence seems to be doubtful (Table-2). The inclusion of these doubtful fish species in the fish faunae of respective states is either on the basis of superficial morphological resemblance or wrong identification. Therefore, their presence needs further confirmation through subsequent extensive ichthyological survey respectively.

Table-2: List of fishes reported from the area under report but endemic to other areas.

S. No.	Name of fish species	Occurrence	Actual zoogeographical distribution
1.	*Strongylura strongylura* van Hasselt	Punjab	Indo-west pacific, India.
2.	*Aplocheilus* (*Haplocheilus*) *lineatus* (Cuvier & Valenciennes)	Rajashtan	Western and South-eastern regions, Raigarh district, Wayanad, etc.
3.	*Liza parsia* (Hamilton)	Rajasthan	West & east coasts entering tidal rivers and estuaries. Commonly found in West Bengal, Andaman & Bangladesh.
4.	*Mugil cephalus* (Linnaeus)	Rajasthan	Common in east and west coasts of India, ascending tidal rivers and estuaries.
5.	*Rhinomugil corsula* (Hamilton)	Rajasthan	India (Mahanadi, Chilka lake, Cauvery river systems, Pune district, Tapi river), Bangladesh, Myanmar & Nepal.
6.	*Mystus keletius* (Cuvier & Valenciennes)	Punjab	India (Coimbatore, Tirunelveli in South India, Tamil Nadu, Mysore), Sri Lanka.
7.	*Mystus montanus* (Jerdon)	Punjab	Confined to Western Ghats, Wayanad range of hills in Kerala, Kernataka, Maharashtra, Madhya Pradesh & Assam.
8.	*Rita gogra* (Sykes)	Rajasthan	In India- rivers of Deccan up to Krishna river system.
9.	*Glyptostemon reticulatum* McClelland	Himachal Pradesh	India (Kashmir, Leh, Ladakh), Pakistan & Afghanistan.
10.	*Ompok malabaricus* (Valenciennes)	Punjab	Distribution in Western Ghats from Kerala to Goa and Maharashtra.
11.	*Glyptothorax gracilis* (Gunther)	Himachal Pradesh	India (Sikkim) & Nepal.
12.	*Glyptothorax lonah* (Sykes)	Himachal Pradesh	Middle India and Western Ghats, Kerala, Madhya Pradesh, Maharashtra, Tamil Nadu.
13.	*Glytothorax trilineatus* Blyth	Uttarakhand	India (Assam, Manipur), Myanmar & Thailand.
14.	*Parachiloglanis* (*Euchiloglanis*) *hodgatri* Hora	Uttarakhand	Assam (Teesta Valley), Dargeeling, West Bengal, Nepal, etc.
15.	*Pseudechneis sulcata* (McClelland)	Himachal Pradesh	India- Brahmaputra drainage.
16.	*Balitora brucie* Grey	Uttarakhand	India (Assam, Manipur, Darjeeling), Nepal, Myanmar, etc.

17.	*Bhavania australis* Jerdon	Uttarakhand	South India, hillstreams of Mysore, Nilgiri, Annamalai hills.
18.	*Schistura* (*Nemacheilus*) *denisoni* (Day)	Himachal Pradesh, Rajasthan	Kerala, Karnataka, Eastern Ghats, South-west coast, Maharashtra.
19.	*Schistura* (*Nemacheilus*) *neilgiriensis* (Menon)	Himachal Pradesh	Nilgiri district (Pykara), Tamil Nadu, Karnataka.
20.	*Schistura* (*Nemacheilus*) *Savona* (Hamilton)	Uttarakhand	East-Himalayan range, Darjeeling, Manipur & Nepal.
21.	*Schistura* (*Nemacheilus*) *scaturigina* (McClelland)	Uttarakhand	Eastern part of India only.
22.	*Amblypharyngodon microlepis* (Bleeker)	Punjab, Rajasthan	Eastern and Southern India & Bangladesh.
23.	*Salmophasia* (*Chela*) *clupeoides* (Bloch)	Rajasthan	Sothern India (Cauvery, Krishna, Godawari), Myanmar, etc.
24.	*Crossocheilus latius latius* (Hamilton)	Himachal Pradesh, Uttarakhand	India (Maharashtra, Arunanchal Pradesh), Bangladesh, Nepal, etc.
25.	*Diptychus maculates* Steindachner	Himachal Pradesh	Indus river system, North Pakistan, Nepal & Tibet.
26.	*Labeo nigripinnis* Day	Rajasthan	Pakistan only (Sindh hills and its basin rivers).
27.	*Labeo potail* (Sykes)	Punjab, Rajasthan	Penisular India. Record from Punjab (Johal & Tandon, 1979-80) doubtful.
28.	*Puntius dorsalis* (Jerdon)	Rajasthan	Cauvery river system, Krishna river system, Andhra Pradesh, Kerela, Karnataka & South India only.
29.	*Puntius filamentosus* (Valenciennes)	Punjab	Southern India only.
30.	*Puntius sarana subnaustus* (Cuvier & Valenciennes)	Punjab	India (South of Krishna River).
31.	*Salmophasia* (*Salmostoma*) *horai* (Silas)	Punjab	Cauvery river system & Karnataka only.
32.	*Salmphasia* (*Salmostoma*) *phulo orissaensis* Banarescu	Rajashthan	Mahanadi & lower reaches in Orissa.
33.	*Schizothoraichthys* (*Schizothorax*) *curvifrons* Heckel	Uttarakhand	India (Kashmir only), Pakistan.
34.	*Schizothoraichthys* (*Salmostoma*) *micropogon* Heckel	Uttarakhand	Kashmir only.
35.	*Schizothoraichthys* (*Salmostoma*) *niger* Heckel	Uttarakhand	Kashmir valley only.
36.	*Tor khurdee* (Sykes)	Rajasthan	Peninsular India especially Karnataka, Kerela, Maharashtra, Sri Lanka, etc.
37.	*Tor mosal* (Hamilton-Buchanan)	Himachal Pradesh	Myanmar only.

The conservation status of the reported fish species from the five states under report is given in (Table-1). From the State of Himachal Pradesh there are three fishes, *viz.*, *Glyptothorax brevipinnis* Hora, *Glyptothorax garhwali* Tilak and *Glyptothorax stoliczkae* (Steindachner) that was considered as critically endangered (CR) fish species. Most of the fish species included in the conservation category are largely of endangered (En) (27) or vulnerable (VU) (39), followed by the category of lower risk near threatened (LRnt) (40). Minimum number of fish species (06) belongs to the category lower risk least concerned (LRlc). The information on the conservation status of 81 fish species constituting 41.33% of the fishes under report of the five states is not accessible. Maximum number of endangered (En) fish species occurs in Punjab (15) followed by Rajasthan (14), Himachal Pradesh (11), Haryana (8) and Uttarakhand (7) (Table-3). The number of fish species included in the category of vulnerable (VU) varies between 19-23. The number of fish species included in the category of lower risk near threatened (LRnt) varies between 21 to 34, maximum from the state of Punjab and Rajasthan (34), followed by Haryana (32), Himachal Pradesh (26) and Uttarakhand (21), whereas this range varies from 1-6 for those fishes included in the category of lower risk least concerned (LRlc) (Table-3). The data on 39 fishes from Punjab, 34 from Rajasthan, 29 each from Haryana and Himachal Pradesh and 26 from Uttarakhand cannot be documented (DD) due to absence of valid reports or documents emphasizing the conservation status so far.

From the above information relating to the conservation status, it is apparent that 65 fish species (33%) which are included in the conservation categories, *i.e.*, critically endangered (CR), endangered (En) and vulnerable (VU) need immediate conservation measures, otherwise, they would disappear from the natural waters of these states in the near future. Another 45 fish species (23%) are included in the category of lower risk near threatened (LRnt) and five fish species (2.5%) is in LRlc category need special attention of the fish biologists, conservationists and aquatic naturalists so that they may not shift themselves to the conservation categories of critically endangered, endangered and vulnerable. Further, efforts should be made to collect the biological data on 77 fish species (39%) included in the conservation category of data deficient (DD) so that their exact status and decline in population (if any) can be averted within time.

Table-3. Conservation status of the fish fauna of the north-Indian states.

Name of the Indian States	Critically endangered (CR)	Endangered (En)	Vulnerable (VU)	Lower risk near threatened (LRnt)	Lower risk least concern (LRlc)	Data deficient (DD)
Punjab	0	15	23	34	6	39
Haryana	0	8	22	32	4	29
Himachal Pradesh	3	11	21	26	2	29
Rajasthan	0	14	21	34	4	34
Uttarakhand	2	7	19	21	1	26

An attempt has been made to evaluate the similarity of fish faunae between these states under report employing the Jaccard Similarity Index (JSI) or Simple matching coefficient. It is considered that when the value of JSI is '1', both the localities have the similar fish faunae whereas there is no similarity when the value is '0'. As the value of JSI varies between 0.388-0.546 (Table-4), it is important to discuss this aspect of fish species distribution It has been observed that there are 29 fish species which are common to all the states under report (Table-5). On the basis of the values of JSI, three distinct groups of fish faunae have emerged. First group includes the states of Haryana, Himachal Pradesh, Punjab and Rajasthan having the value of JSI between 0.546-0.540. Second group of Haryana, Himachal Pradesh and Punjab having the value of JSI between 0.480-0.474. Greater dissimilarity occurred when the fish fauna of Uttarakhand is compared with the other states under report and the value of JSI has the lowest range, *i.e.*, 0.388-0.383. All these observation leads to the two unique findings, *i.e.*, firstly, the basic similarity between the five states of northern India is attributed to the presence of 29 fish

species that are common to all the states. Further, the high value of JSI is attributed to the high number of fish species in that state (Table-4).

Table-4. Showing 'Jaccard Similarity Index' (JSI) on the occurrence of fish species of the five north-Indian states.

STATES	Punjab	Haryana	Himachal Pradesh	Rajasthan	Uttarakhand
Punjab	-	0.480	0.474	0.546	0.388
Haryana			0.474	0.540	0.388
Himachal Pradesh				0.545	0.383
Rajasthan					0.388
Uttarakhand					-

The above mentioned fact that the fish fauna of Uttarakhand have least connection with that of other four states is also supported by the observations of table-5. Here, it can be easily assessed from the available data that there is close proximity between the fish faunal distribution among the two neighboring states in comparison. The table also suggests that the two states shares their borders whose geographical, cultural and socio-economic aspects are more close to each other, shows more similarity (30%-42%) in terms of common fish species available in the two states in comparison.

Table-5. Number and percentage of common fish species present in both the two states in comparison.

STATES (Fish Species = 196)	Punjab	Haryana	Himachal Pradesh	Rajasthan
Haryana	82 (42%)			
Himachal Pradesh	61 (31%)	59 (30%)		
Rajasthan	80 (41%)	77 (39%)	58 (30%)	
Uttarakhand	43 (22%)	38 (19%)	46 (23%)	36 (18%)

However, this similarity percentage index reduces considerably when the State of Uttarakhand is compared with that of Himachal Pradesh, Punjab, Haryana and Rajasthan showing 23%, 22%, 19% and 18% respectively. This observation also supports the fact that the Rajasthan is not sharing direct boundaries with Uttarakhand, hence, having least similarity in terms of fish faunal distribution when compared to other states.

Virtually, the states of Punjab, Haryana and Himachal Pradesh share their borders (Fig.1) and almost share their source of natural waters. The rivers which pass through the states of Punjab and Haryana have their origin in the Himachal Pradesh and there is intermixing of fish faunae too. The dissimilarities are due to the entirely different ecological conditions of Punjab and Haryana State close to the desert conditions in the areas touching the borders of Rajasthan State. Further, various water management practices such as the construction

of irrigation canals, *e.g.*, Indira Gandhi Canal, Sirhind Feeder and Gang Canal and the seasonal floods of south-west monsoon do carry the hill-stream fish fauna of Himachal Pradesh to the states of Punjab and Haryana to a greater extent and Rajasthan to somewhat lesser extent. Considering all these facts, it can be concluded that the four states namely Haryana, Himachal Pradesh, Punjab and Rajasthan share 52% of the fish fauna. The Uttarakhand exclusively a Himalayan hill state has the lowest number of fish species and share less than 39% of the fish fauna with the neighboring states. This state shares its small borders with only Haryana and Himachal Pradesh but not with Punjab and Rajasthan. Logically, the Jaccard Similarity Index (JSI) of Uttarakhand with other states is on the higher side and it is attributed to the low number of fish species (76) inhabiting the waters of this state and the occurrence of 29 common fish species which occur in all the states (Table-6).

Table-6. Number of fish species reported from each state (present, absent and common).

Total no. of fish species = 196	Punjab	Haryana	Himachal Pradesh	Rajasthan	Uttarakhand
Present	117	95	92	107	76
Absent	79	101	104	89	120
Common	29	29	29	29	29

The table-6 gives the indication that the State of Punjab supports maximum number of fish species because this state has three very distinct ecological regions *viz.*, sub mountainous, flat plains and part of the Thar Desert touching the state of Rajasthan. Rajasthan state also supports 107 fish species belonging to Himalayan and Aravalli's Range. The minimum number of fish species is recorded from the State of Uttarakhand because it includes exclusively mountainous and high altitude zones having very low fish diversity because of average low temperature, low productivity and fast current of water. Johal *et al.*, (2001, 2002) opined that with the increase in altitude, the fish diversity decreases also supports the present observations. The states of Himachal Pradesh and Haryana have same level of fish diversity, but Himachal Pradesh supports more number of coldwater and hillstream fishes as compared to Haryana State.

Thus, it can be concluded from the above discussion that the five states under report supports 18.8% freshwater fish fauna of Indian region because of varied ecological conditions. The similarity of fish fauna among five states is exhibited by the occurrence of 29 common fish species, which indicate that there is some contiguity of waters. Due to various water management practices, loss of fish habitat, pollution, majority of the fish species are threatened, hence, need immediate species specific conservation measures. The occurrence of 37 fish species not endemic to these states and 15 exotic fish species is a solid proof that the waters of these states have received fish fauna from various other sources, deliberately or unintentionally.

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TAXONOMY AND DISTRIBUTION OF *ATRICHUM UNDULATUM* (HEDW.) P. BEAUV. FROM KUMAON HILLS OF INDIA

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ABSTRACT

The present study is on the taxonomy, distribution and ecology of the genus *Atrichum undulatum* (Hedw.) P. Beauv. from North-Western part of Kumaon hills of India. Genus is largely holartic in distribution and in abundance under moist and semi-moist condition at different sites (Nainital, Mukteshwar, Ranikhet, Chaubatia, Almora, Artola, Jageshwar and Pithoragarh) of Kumaon hills of Uttarakhand. An exposed rock has poor distribution of *Atrichum undulatum* (Hedw.) P. Beauv. in comparison to the moist shady places. Moss *Atrichum undulatum* (Hedw.) P. Beauv. plants are soft, large and form cushions of yellowish-green to green patches.

Keywords: moss, *Atrichum undulatum* (Hedw.) P. Beauv., Distribution, Taxonomy

INTRODUCTION

Mosses are distributed abundantly in India and play a key role in the formation of natural biotic community that Prof. Goebel called it a "gold mine in Western Himalayas". Western Himalayas, is rich in moss both in frequency and abundance (Chopra, 1975). Kumaon hills of Uttarakhand, cover an area of 21,033 sq km. and lie at latitudes 28° 44' and 30° 49' and longitude 78° 45' and 81° 5' E (Map 1). Mosses are important constituents of hilly ecosystem inspite of their small size and relatively low biomass. The percentage occurrence of mosses in India is quite high than any other group of plant, about 27.5% of world mosses are present in India (Banerjee, 1978). Due to high rainfall and humidity, Kumaon hills are densely forested with majestic towering trees festooned with numerous perennial creepers (lianas), trees and forest-floor are densely covered with moss vegetation, these mosses are the important part of forest ecosystem (Rose, 1992). The topography of the hills is irregular due to valleys and plateaus of various dimensions (Singh *et al*, 2004). Kumaon hills exhibit a luxuriant growth of *Atrichum undulatum* (Hedw.) P. Beauv. in almost all the bryo-geographical units including the Western Himalayas and Eastern Himalayas of India (Kashyap, 1932; Parihar, 1962).

Genus *Atrichum* belongs to the order Polytrichales having only a single family Polytrichaceae, comprising of 23 genera and several hundred species (Buck and Goffinet, 2000), while in India Polytrichaceae family represents 21 genera (Chopra, 1981; Chopra, 1998).

Seven species of genus *Atrichum* i.e. *A. longifolium* (Cardot & Dixon), *A. flavisetum* (Mitt.), *A. aculeatum* (Cardot & P. de la Varde), *A. subserratum* (Hook) Mitt., *A. obtusulum* (C. Muell.), *A. pallidum* (Renauld & Cardot) and *A. undulatum* (Hedw.) P. Beauv has been described from India by Chopra (1975). The genus is widespread in Shimla (H.P.), Darjeeling, Kashmir (Chopra, 1975) Burma, Indonesia, China, Japan, N. Africa, N. America (Gangulee, 1969) and also across the Europe due to its large size (Bijelovic *et al*, 2004; Lin *et al*, 2005).

There seems very little information on the abundance, distribution and taxonomical study of moss *Atrichum undulatum* (Hedw.) P. Beauv. from Kumaon hills except that the genus was reported by Tewari from Nainital (Tewari *et al*, 2002). Contributions on the taxonomy of the moss *Atrichum undulatum* (Hedw.) P. Beauv. are also fragmentary. Therefore, present work is an attempt to provide a descriptive illustrated account of moss *Atrichum undulatum* (Hedw.) P Beauv from Kumaon hills. The Catherine's moss *Atrichum undulatum* (Hedw.) P. Beauv. is among the largest European terrestrial moss species (Sabovljevic *et al*, 2006).

MATERIALS AND METHODS

Seasonal trips were made for the study and collection of moss *Atrichum undulatum* (Hedw.) P. Beauv. during the year 2008-2009 from Kumaon hills. Detail of the collection sites is given in the Table-I. Moss *Atrichum undulatum* (Hedw.) P. Beauv. was collected from different sites of Kumaon hills at each season during the year and brought in tagged polystyrene bags, then they were air dried and kept in labeled packets after identification and were deposited in Bryological Laboratory of Botany Department of Bareilly College, Bareilly. Herbarium

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Line drawing illustrations were made with the help of Camera Lucida. Species was identified, on the basis of its characters as *Atrichum undulatum* (Hedw.) P. Beauv. given by Gangulee (1969), Chopra (1975) and Smith (2004).

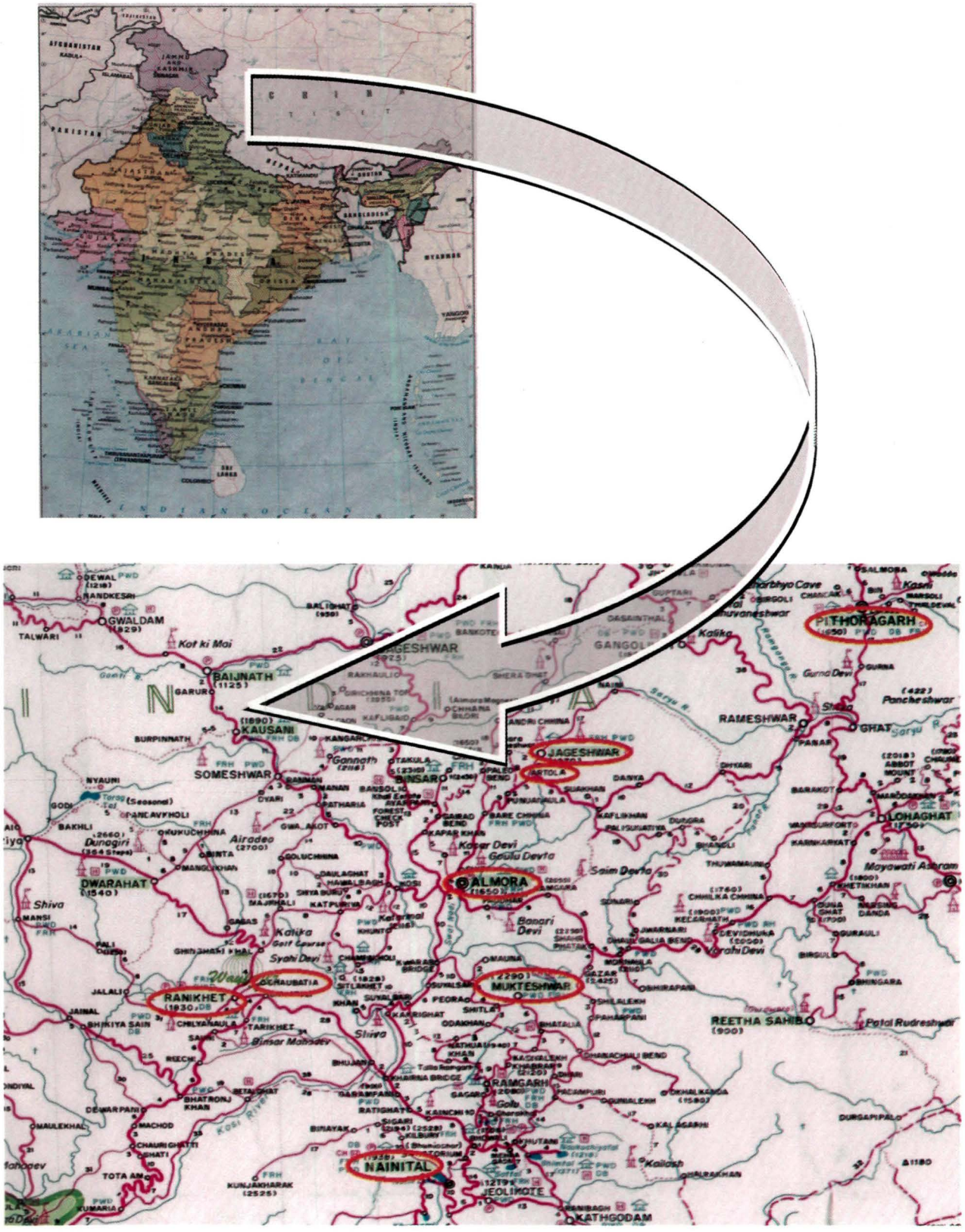
ECOLOGY AND DISTRIBUTION

Moss *Atrichum undulatum* (Hedw.) P. Beauv. exhibited luxuriant growth in Kumaon hills (Map 2). Their meta data and G.P.S. data were taken from different sites of Kumaon hills during different seasons as (relative humidity, temperature, soil *p*H with a digital infrared thermometer, thermo hygrometer, *p*H meter) given in the Table-I. It grows either solely, or in association with other mosses i.e. *Polytrichum commune, Rhodobryum roseum, Mnium cuspidatum* etc. on moist, slightly acidic soil or on rocks in shady forest floors, which are not or partially exposed to sunlight. It forms cushion of yellowish-green to green patches and scattered on loose moist soils of roadsides, waterways and are very frequent in all kind of forest covers. It exhibits luxuriant growth in rains as well as in winter but in summer plants turn to reddish-brown due to low moisture.

Moss *Atrichum undulatum* (Hedw.) P. Beauv. was found to be widely distributed and grows luxuriantly in the region of Kumaon hills i.e. in Almora (longitude 79° 30' E; latitude 29° 36' N) at the height of 1829m, Jageshwar (Alt.1870 m); Kosi (Alt. 2116m); Dinapani (Alt. 1890m); Artola (Alt. 1868m); Ranikhet (Alt.. 1790m); Chaubatia (Alt. 1820m); Nainital (Alt. 1938m); Mukteswar (Alt. 2290m) and Pithoragarh (Alt. 1635m) of Kumaon region.

Table I: Meta data & G.P.S. data of different sites surveyed at Kumaon hills during different seasons

Sites	Seasons	Latitude	Longitude	Soil *p*H	Temperature (°C)	Relative Humidity (%)
Nainital	Rainy	29° 27.209'	079° 39.210'	6.5	15	52
	Winter	29° 27.231'	079° 39.261'	6.0	12	46
	Summer	29° 27.220'	079° 39.232'	5.8	23.5	73
Mukteshwar	Rainy	29° 28.324'	079° 37.138'	5.6	12	50
	Winter	29° 28.267'	079° 37.152'	5.4	10	44
	Summer	29° 28.258'	079° 37.142'	5.3	24	75
Ranikhet	Rainy	29° 37.143'	079° 27.729'	5.9	18.8	45
	Winter	29° 37.163'	079° 27.764'	5.7	15.7	42
	Summer	29° 37.146'	079° 27.697'	5.4	25	78
Artola	Rainy	29° 37.407'	079° 49.906'	6.2	19.8	56
	Winter	29° 37.457'	079° 49.942'	5.9	13.5	53
	Summer	29° 37.427'	079° 49.920'	5.8	22	74
Jageshwar	Rainy	29° 37.595'	079° 50.230'	5.8	17	51
	Winter	29° 37.691'	079° 50.310'	5.6	14	49
	Summer	29° 37.634'	079° 50.235'	5.4	21.19	63
Pithoragarh	Rainy	29° 34.610'	80° 12.630'	6.3	22	41
	Winter	29° 34.639'	80° 12.701'	6.0	19	62
	Summer	29° 34.521'	80° 12.656'	5.8	29.19	49



1. Map showing area of Uttraranchal
2. Map showing survey sites of Kumaon

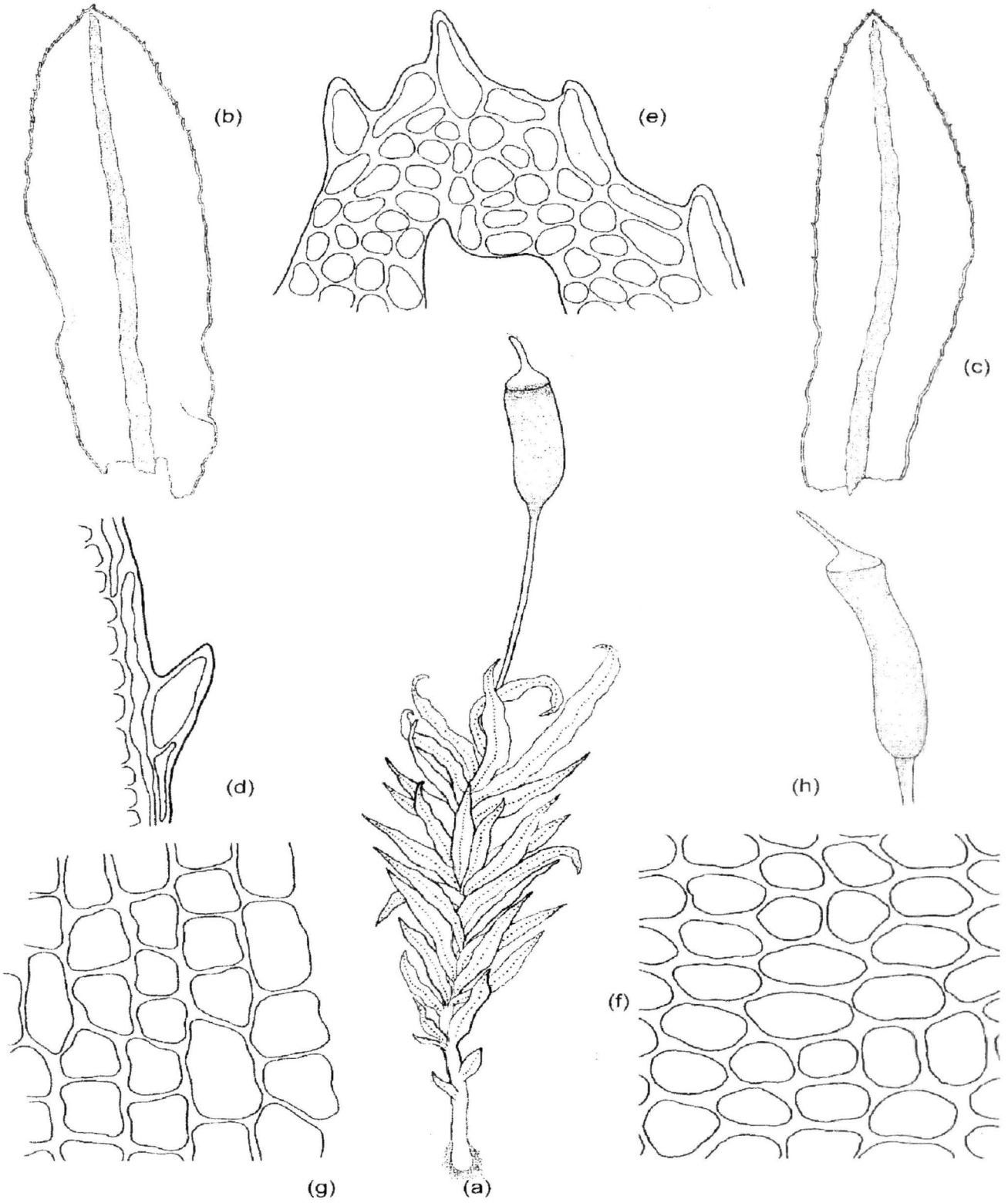


Plate-1: Figure 1 : *Atrichum undulatum* (Hedw.) P. Beauv.
(a) Whole plant 20x (b) Leaf 50x (c) Leaf 50x (d) Marginal cells 400x
(e) Leaf apex 400x (f) Mid cells 400x (g) Basal cells 400x (h) capsule 50x

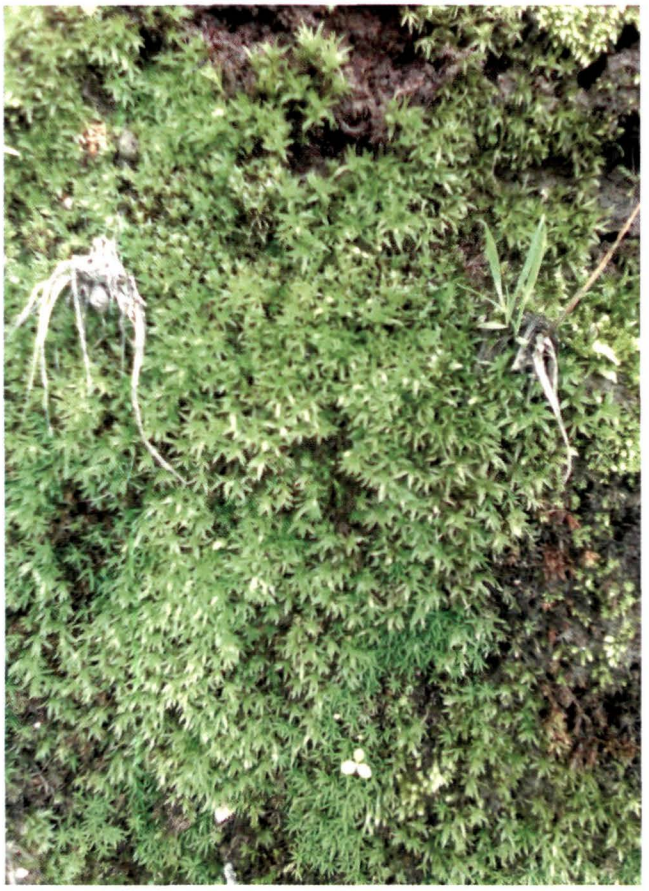


Fig. 2: Habitat of *Atrichum undulatum* (Hedw.) P. Beauv. in Jageshwar

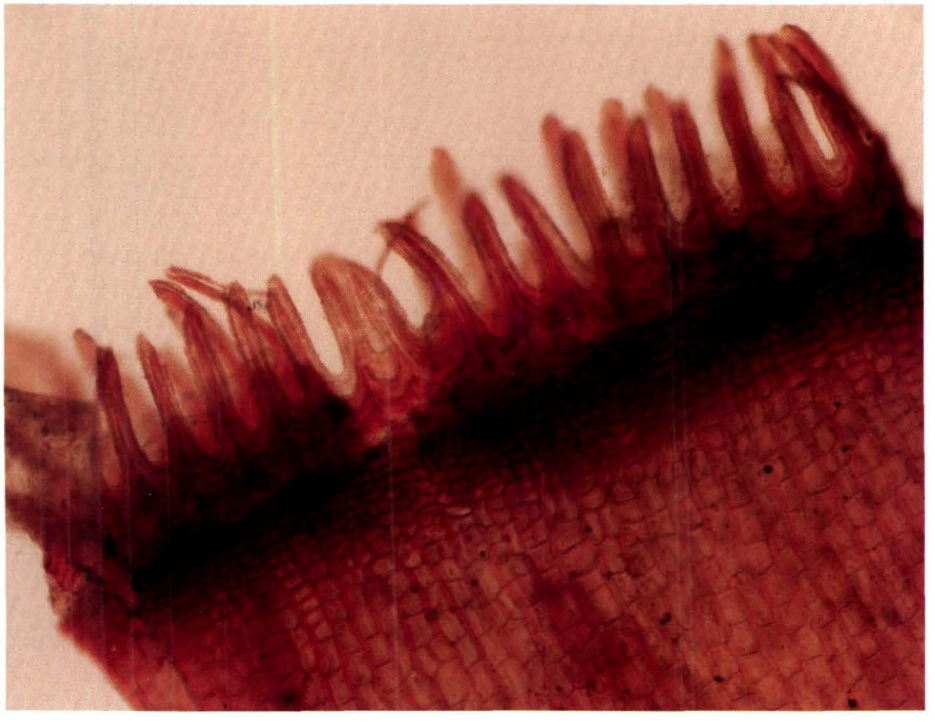


Fig. 3: *Atrichum undulatum (*Hedw) P. Beauv. peristome teeth

TAXONOMIC DESCRIPTION

Atrichum undulatum (Hedw.) P. Beauv. is an acrocarpous moss forms loose cushions of yellowish-green to green patches (Fig. 2) and is very frequent in all kind of forest area of Kumaon region.

Studied plant were robust, parocious, polyoicous or dioicous. The aerial shoots were measured up to 5.8 cm (Plate1-Fig.1a) and stem is unbranched but sometimes branched. Leaves measured 8mm long and 2.3mm in width (Plate1-Fig.1b, c) which are distantly placed but crowded towards the upper portion of the plant. These are linear lanceolate strongly crisped when dry, spreading during moist, margin of the leaves bistratose bordered with 2-3 rows of narrow cartilaginous cells and is spinosely dentate (Plate1-Fig.1d) toothed with single or double teeth in the upper acuminate 2/3 region of the leaf (Plate1-Fig.1e). Nerve with 3-6 lamellae each 2-3 cell large. Leaves are thin, soft and are rich in chlorophyll at apex. Mid rib is prominent at base. Mid cells are rounded or six sided, smooth incrassate 14-19mm wide (Plate1-Fig.1f). Basal cell are 28mm widely rectangular thin walled and smaller towards the margin (Plate1-Fig.1g). Alar cells are absent. Capsule with long setae, are in most cases sub-erect elongate-cylindrical 6mm long (Plate1-Fig.1h) and 1.0-1.2 mm in diameter, seta 1.8 cm. long, with reddish-brown. Calyptra glabrous narrow, smooth, roughened & hood-shaped with minute teeth and hairs are not present on it. The operculum is conical, long beaked, columella expanded at top into membranous epiphram joined to tips of the 32 peristome teeth (Fig. 3).

Genus *Atrichum undulatum* (Hedw.) P. Beauv. is well established and was known to grow in most of the geographical regions of India. The genus is being described in detail from kumaon region of India first time along with its taxonomical description.

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REVIEW OF COOPERATION SCHEMES FOR MANET

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ABSTRACT

Mobile Ad hoc Wireless Networks (MANET) is a self organized, infrastructure less, and multi hop network. For various network operations like packet forwarding, nodes rely on cooperation of its neighbor nodes. This *node cooperation* issue becomes very important for the successful operation of MANET. It assumes that all nodes of network are working cooperatively, but this assumption ignores the user specific requirements and attitude. For example some users view their energy resources as being limited by battery life and they misbehave to save the power of the machine by keeping their network interface down. But, such user misbehavior effect's the performance of network. This paper is focused on the various factors that effect cooperation among the various mobile nodes. Moreover a comprehensive review of several cooperation enforcement schemes has been carried out. It is found that various authors have reported that network performance increased with cooperation enforcement schemes, but only for small networks. Many issues like drop of packet due to link failure/ congestion, cause of selfishness etc. still needs attention.

Keywords: cooperation schemes, adhoc wireless networks, node behavior.

INTRODUCTION

MANET is a new generation of network offering unrestricted mobility without any underlying infrastructure. Due to frequent mobility, topology of the network changes frequently and link disconnections can occur. In MANET nodes has limited resources like battery and bandwidth. It does not have any trusted central authority. Above features of the MANET distinguish it from other communication networks. These are self-organized networks and intermediate nodes should participate to carry out the end-to-end communication. To achieve this, each node relies on its neighbor to forward the packet to the destination and accordingly issue of node cooperation becomes very important as it is a basic requirement for the operation of MANET. However, a user may misbehave due to several advantages resulting from non-cooperation, the most obvious being power saving. Cooperation is harder to enforce in MANET than in infrastructure based network due to many reasons (Al-karaki and Kamal, 2008). First, nodes can arbitrarily join and leave the network. Second, detection of misbehaving nodes is in distributed manner due to lack of central authority. Finally, user specific requirements and attitude is ignored. Nodes are classified into four categories:

a) Cooperative nodes are active in route discovery and packet forwarding with positive attitude.

b) Malicious nodes are active in both route discovery and launching attacks. These nodes intentionally try to damage the network.

c) Selfish nodes are active in route discovery, but not in packet forwarding. These nodes try to gain help from the network without willing to pay back the help received.

d) Hacker node (Mandalas, Flitzanis, Marias and Georgiadis, 2005) might try to intercept the information exchanged between the nodes. Such violation is materialized through e.g. impersonation. Both selfish and malicious nodes are considered as misbehaving nodes.

This paper presents cooperation enforcement schemes in MANET. There are basically two methods to enforce a desirable strategy a) by punishing the misbehaving nodes b) encouraging those who adopt it. It is better to consider punishment based schemes for two reasons (Al-karaki and Kamal, 2008). First, rewarding schemes are not easily extensible to scenarios in which malicious nodes are active. Second, Malicious nodes may not be interested in rewards. Instead, when they are susceptible to punishment, they tend to behave well.

The rest of the paper is organized as follows. Second section presents cooperation challenges. In section third considerations for selfish node solution are described.

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Section four categorizes cooperation enforcement schemes. Finally last section concludes the review.

CHALLENGES OF COOPERATION IN MANET

a) *Lack of association:* Cooperation gets effected as the different nodes at any instant of time can either leave the network or they can join the network

b) *Congestion in network:* Because of the congestion in the networks, sometimes a cooperative node will have to drop the packets. Though it's a cooperative node but can be treated as a selfish node by the nearby nodes.

c) *Link failure*: Because of the mobility of the ad hoc wireless network, some times there are link failure between the nodes and hence effect the cooperation.

d) *Limited battery power:* If the node will take active participation in routing and packet transmission of other nodes its battery power depletes fast and a stage will come when the participating node tend to behave as a selfish node to send its own data. So limited battery power is said to be the major factor for the nodes to become selfish.

e) *Privacy and security:* As the ad-hoc wireless network is attack prone, the nodes did not take active participation in network because of a threat to lose their confidential data.

f) Node Behavior: Malicious node and hacker nodes can disturb the cooperation.

CONSIDERATIONS FOR SELFISH NODE SOLUTION:

A solution to the selfish nodes problem should take the following into consideration (Wang and Singhal, 2005):

1) A selfish node should be identified and be punished. Just finding another route that bypasses a suspicious selfish node is not enough to force the cooperation since it encourages the selfish node not to forward packets.

2) False conviction should be avoided, i.e., a cooperating node should not be convicted as a selfish node and be punished. Avoiding false punishment is more important than identifying all selfish nodes.

3) The solution should be distributed due to the absence of a centralized server in ad hoc networks. Due to collision and interference, a node can not get an accurate observation of others and thus, we cannot rely on any node's single opinion or decision to convict a node.

4) A heavy-weight solution is infeasible. If the cost for identifying selfish nodes in terms of battery and bandwidth is very high, it may be enough to just identify suspicious nodes.

5) Other issues such as the location privilege problem should be considered. Nodes in the different locations in the network should have the same chance to serve others and be served by others.

COOPERATION ENFORCEMENT SCHEMES:

In general, mechanisms that try to mitigate and stimulate the misbehaved or non cooperative node can be classified into following four classes:

a) Reputation or motivation based schemes.

b) Virtual currency/Credit/incentive based schemes.

c) Game theory based schemes.

d) Other schemes.

A. Reputation based schemes

These models use the nodes' reputation to forward packets through the most reliable node. The reputation of node increases when it carries out rightly the task of packet forwarding that is dispatched by its neighbors, without altering their fields. The models of this categories support effective mechanism to measure the reputation of other nodes of the network. They also incorporate techniques that isolate the misbehaving nodes; i.e. those that show low reputation value (Mandalas, Flitzanis, Marias and Georgiadis, 2005). Following are some key points for reputation based schemes (Al-karaki and Kamal, 2008).

i) Trust and Reputation: Reputation rating represents how well a node behaves, and is used to decide whether the node is cooperative or misbehaving. On the other hand, trust rating represents how honest a node is, used to decide whether the node is trustworthy or not, thus the indirect reputation message from the node is accepted or not.

ii) First and Second Hand Reputation. First Hand Information (Direct Reputation) is obtained by direct observation. A node monitors the behavior of other nodes usually in one-hop to see if it works well. On the other hand, Second Hand Information (Indirect Reputation) obtains reputation information about a node from other nodes in the network. The acceptance or rejection of this information is based on the trust level of the sender node.

iii) Global and Local Reputation: Global reputation refers to the case where every node knows the reputation of every other node in the network. This is achieved by exchanging indirect reputation messages among the network. In local reputation, however, information is based only on direct observations of one-hop neighbors. Any second-hand reputation exchanges are disallowed.

In class of reputation based mechanism (Marti, Giuli, Lai and Baker, 2000) was one of first research about cooperation problem in ad hoc wireless network. They use a *watchdog* that identifies misbehaving nodes and a *pathrater* that helps routing protocols avoid these nodes. There is no penalty for non cooperative nodes in this mechanism which impose extra loads on to cooperative nodes.

CONFIDANT (Cooperation of nodes- Fairness in dynamic ad hoc network)**:** Buchegger and Le (2002) aims to isolate non-cooperative nodes through nodes' behavior. It uses first hand and second hand reputation of nodes. They add observation, detection, and reaction mechanisms to a routing protocol to exclude uncooperative nodes from the network. In this, each node consists of four components. *Monitor*, it records misbehavior of nodes by either listening to the transmission of the next node or by observing route protocol behavior and informs it *to reputation system*, which maintain a list of ratings reflecting nodes behavior. If the ratings become intolerable, the information is given to a *pathrater* which can deletes all routes containing the misbehaving node from the path cache. The *trust manager* sends an alarm message to alert others of malicious nodes, and maintains received alarms from the friend nodes and alarms produced by the node itself.

CORE (Collaborative Reputation): Michiardi and Molva (2002) proposed CORE which stimulates node collaboration through monitoring of cooperativeness of nodes. It uses first hand and second hand experiences, combined by a specialized function. In this scheme each node uses two components. *Watchdog* monitors the behavior of neighboring node with respect to a requested function, and collects observations about the execution of that function. These observations are recorded into *Reputation Table* a data structure stored in each node. Based on collected observations, each node computes reputation value for each of its neighbor. When a neighbor's reputation falls below a predefined threshold, service provision to the misbehaving node is suspended. Each row of the table consists of four entries: the unique identifier of the node, collection of recent subjective observations made on the node's behavior, a list of the recent indirect reputation values provided by other nodes and the value of reputation evaluated for a predefined function. The reputation table updates its entries based on the result generated by the watchdog.

SORI (secure and objective reputation based incentive scheme for ad hoc networks): He, Wu and Khosla (2004) combines features of first hand and those that use reputation spreading. The nodes exchange reputation information only with their neighbors. This way a non cooperative node will be punished by all of its neighbors (who share the reputation information about its misbehavior), instead of just the ones who are directly affected by this node.

OCEAN (Observation based Cooperation Enforcement in Ad hoc Networks) (Bansal and Baker, 2003): It is a layer that resides between the network and the MAC layer. It helps the nodes to make intelligent routing and forwarding decisions. It is designed on the top of DSR, but its principles can be applied to other routing protocols. It is hybrid scheme that uses both a reputation based component to detect and punish selfish behavior and micro payment component to encourage cooperation. The credit is earned for each immediate neighbor and it cannot be used to send packets in different routes. It avoids second hand (indirect reputation information) and uses only first hand (direct reputation) observations of the nodes behavior.

HEAD (Hybrid mechanism to enforce node cooperation in MANET): Jianli, Jian and Yang (2007) proposed HEAD, an improvement to OCEAN. It employs only first hand information and works on the top of DSR. *Watchdog* locating at each node keep a faulty list and selfish list. All nodes detected as misleading / malicious put into faulty list and selfish nodes into selfish list. Before deciding whether to forward the packet for a neighbor, the node checks faulty list and selfish list if neighbor lies in faulty / selfish list, the node denies the request. HEAD also keeps a reputation table in each node with various entries. Each node broadcast warning messages to its neighbor with its own faulty/selfish list.

CineMA (Cooperation Enhancement in MANETs): Frank, Martini and Plaggemeier (2004) proposed a detection based approach to increase the level of cooperation in mobile ad hoc networks. In contrast to all other detection based mechanisms, CineMA is group based. It uses first-hand information only. Thus, false accusation by

other nodes is not possible. It is the approach that calculates the level of cooperation of nodes by taking into consideration, how selfish is a node, how many packets have been dropped. This is done by calculating the ratio of received and forwarded packets. The more packets a node drops, the harder the punishment. It consists of three modules. The *watchdog module* monitors the network, prepares the collected data and passes them to the reputation system. The *reputation system* calculates the level of cooperation for each node in the network and informs the interface queue about the selfishness of nodes. The *interface queue* punishes malicious nodes by reducing their throughput. In addition, CineMA informs the routing layer about malicious nodes. Therefore, routes containing malicious nodes are avoided.

Wang and Singhal (2005): present a light weight solution for selfish node problem considering battery status in MANET. It a fully distributed solution which force's node cooperation. The solution is based on neighbor monitoring and this monitoring is on demand. In this scheme a sender node, after sending a packet stream of data, can monitor behavior of downstream node A in promiscuous mode. On detecting consecutive packets' drop, it send a broadcast message to ask for the help of A's neighbor. These neighbor nodes monitor A's behavior in promiscuous mode. On the basis of their own observation, they make judgment about node A. A is convicted as a selfish node, if a majority of neighbors accuse A. Selfish node will be punished by other nodes by dropping packets intended to and originated from such a selfish node. Routes will be re-established to bypass such a selfish node. A selfish node is re-admitted to the network, after some predetermined amount of time. Thus, a selfish node knows that the selfishness will do harm to itself and it will be forced to be more cooperative. With this scheme, nodes in the center of the network, usually consuming more battery, can have a rest and get no punishment. It broadcasts a *Battery* message to announce its low battery status, since every node can estimate its neighbor's battery consumption, it can verify the credibility of *Battery* message broadcast by the neighbor. Once a selfish node has been identified, it is punished.

Hao, Zheng and Xia (2006) proposed a cooperation enhancement mechanism based on the trusty central server and the neighbor monitoring. It may solve the spoofing attacks caused by selfish nodes changing their identities. It establishes trusty central server to solve the identity crisis problem. When the nodes frequently change identities, they cannot get any benefit. The incentive mechanism to encourage the selfish nodes obtains other nodes' trust again through the cooperation. This paper mainly consists of the four components: *Reputation Initial Value Setting*, it is used for determining the reputation initial value of the nodes, which join in the network recently. *Neighbor Cooperation Monitoring*, it describes how to monitor the behavior of each node in the network and how to detect the attacks from the malicious nodes. *Reputation Evaluation*, the reputation value, computes with the evaluation system, and finally calculates the synthetic reputation. *Reaction Mechanism*, when the node reputation value is lower than the threshold value, it may determine the node is a selfish node, then isolates the selfish node outside the network, and inform other neighbor nodes or trusty central server.

Zahra, Anisi and Torgheh (2008) proposed a scheme which uses only the first hand or direct observations for upgrading the reputations. Only first hop neighbors can checks the behavior of node and upgrade reputation of that node. The other neighbors do not have this permission. In proposed system, all nodes have same reputation at the beginning. The reputation will increase with good behavior and reduced with bad behavior. This approach encouraged cooperative nodes by using a priority processing system by which cooperative nodes can receive their services earlier than the nodes which were selfish and have no cooperation in network. The architecture of proposed approach consists of three main parts: *Checking System*: monitor one hop neighbor nodes. It registers the number of incoming and outgoing packets of each node. *Reputation system*: Calculate the rate of cooperation as a reputation value. Adds a new field to header of DSR and put á in it, where á cooperation coefficient is given as below:

α = No. of sent packets / No. of received packets
where $0 < \alpha < 1$

The values which are near to zero show that the cooperation of node is low and it is a selfish node but the values which are near to one show that the cooperation is high and as a result the reputation of node is high. *Priority processing system*: prioritizes the packet received from node based on their reputation.

Drawbacks of the reputation based systems:

Malicious nodes are avoided by *pathrater/watchdog* in route discovery process. Therefore malicious nodes are

not punished. Thus, the malicious node reaches its goal to not forward packets of other nodes. CONFIDANT and CORE punish a malicious node by excluding it from the routing phase. This works fine if all nodes use the same mechanism. But future of mobile ad hoc networks will be formed by many totally different devices, thus this assumption may be unrealistic. A malicious node will find a route and therefore can send packets to other nodes if parts of the network do not use such a mechanism or if they use different approaches. Another challenge is the consequence of false accusation or false detection of malicious behavior. It is assumed that all nodes use a mechanism to enforce cooperation. Whenever a node is punished and excluded from the route discovery phase, no traffic can be sent to that node. The question is: How long will a node be punished? It is essential that an accused node has the chance to be re-integrated into the ad hoc network. In case of false accusation, the node is still able to send data but it will suffer from a lower bandwidth. Centralized mechanisms are applicable in such networks. However, a cooperation approach should be usable both in stub and pure ad hoc networks (Frank, Martini and Plaggemeier, 2004). Table-1 represents comparison between selfishness prevention schemes with reputation based mechanism.

B. Credit/virtual currency based schemes

In credit based schemes, each node receives a payment for its cooperation in forwarding network messages and also pays other nodes which participate in forwarding its messages. Virtual currency is used to charge /reward the packet forwarding service.

In credit based class Buttyan and Hubaux (2000, 2003) present a scheme to ensure cooperation among nodes in wireless ad hoc networks. They introduce a virtual currency called *Nuglet,* which is used to charge for the transmission of packets and to reward the forwarding process (Li, 2007). A credit counter can implement the nuglet. There are two models in Nuglets (i) Packet Purse Model: in which the credit payment is deducted from the source. (ii) Packet Trade Model: in which the credit payment is deducted from the destination. The major problem in Nuglets is that it needs a tamper-proof mechanism to manage the increments and decrements of the credit counter for each node.

Janzadeh, Fayazbakhsh, Dehghan and Fallah (2009) propose *Express* a credit- based cooperation mechanism which does not rely on any tamper proof mechanism. It utilizes hash chain for its secure and computationally efficient nature. Reliable Clearance center (RCC) is a central entity which is responsible for balancing network nodes' credits. RCC opens a credit account and issued a digitally signed certificate for each node who intends to join the network. RCC is able to track the cooperativeness of the mobile nodes using the reports received via network. It might avoid renewing the certificates of the malicious or non cooperative nodes.

SPRITE (A Simple, Cheat Proof, Credit-based System): (Zhong, Chen and Yang, 2003), uses a Credit Clearance Service (CCS) that manages the rewards and credit payments for each node. SPRITE does not rely on any tamper-proof mechanism. CCS is a central entity which is responsible for balancing network nodes' credits. Source node pays to cooperative intermediate nodes. SPRITE uses digital signature for any single transaction. The authors analyzed the effectiveness of the mechanism using game theory.

*Ad hoc-VCG scheme (*Anderegg and Eidenvenz, 2003*):* is a model which deals with cooperation problem and introduces a second-best sealed type of auction. It ensures that a packet gets routed along the most energy-efficient path. A pricing question arises concerning the amount of the payment a node should ask to forward packets. Intermediate nodes declare their respective prices honestly. Honest behavior is assured by VCG mechanism. The ad hoc-VCG is robust when only one cheating node exists. It might fail in the presence of collusions of nodes who try to maximize their payments (Janzadeh, Fayazbakhsh, Dehghan and Fallah, 2009). The authors introduced a game theoretic setting for the routing layer of mobile ad hoc networks in which the communication nodes are assumed to be selfish and in which the communication nodes need to declare their cost-of energy in order to compute a cost-efficient communication path. An additional issue is the excessive overhead. It requires complete knowledge of the network topology during the route discovery phase. Finally, it does not focus on the actual payment delivery, but only on the estimation of the payments.

*In priority forwarding (*Raghavan and Snoeren, 2003) introduces incentives for nodes to cooperate in forwarding network packets. In this scheme, two types of traffic provided by the network: best-effort and priority. Network provides priority forwarding by paying credit to intermediate nodes if source node pays for forwarding

its packet, otherwise, best-effort service is provided. This work assumes that every node will provide best effort service.

iPass: An Incentive Compatible Auction Scheme (Chen and Nahrstedt, 2004). It is an auction-based incentive scheme that enables cooperative packet forward behavior in MANET. Market price of forwarding packet service is paid by each flow to the intermediate routers. The resource allocation mechanism in ipass is based on the generalized Vickrey auction with reserve pricing. iPass is a joint solution of incentive engineering and flow control in a non-cooperative MANET. The authors prove that user's truthful bidding of utility remains a dominant strategy, users and routers have incentive to participate in the scheme, and packet forwarding always leads to higher social welfare for the whole network. Table 2 summarized the features of credit based schemes.

C. Game theory based

Game theory is a branch of applied mathematics that is used in the social sciences, most notably in economics to derive the optimal strategy for every rational competitive player. The objective of game theory is to find out the *Nash equilibrium* point where a player cannot increase his/ her payoff by changing strategies while other players' strategies remain fixed. Now a day it is used in variety of areas in communication networks including packet forwarding etc.. In other words, if some nodes do not have any interest in increasing their profits but just concentrating on power saving, the game theory may not be able to model a selfish user network. Assuming rational node, all the following methods, model packet forwarding as a strategic game so that the forwarding rate may be Nash equilibrium for every node. Each algorithm in this section is based on different complicated equations, but there is a common notion behind them: Under the assumption that all nodes can change their strategies freely, if selfish behavior of a node is detected, all other nodes will change their strategies to punish it. Thus, the game will gradually be stable at one point where all nodes are satisfied (Yoo and Agrawal, 2006).

*CAP-SV (*Cai and Pooch, 2004*)*: CAP-SV (Contribution rewArd routing Protocol with Shapley Value), is presenting a novel way to encourage cooperative works-rewarding service providers according to their contributions. It achieves the objective of truthfulness. Truthfulness means that revealing true information is the best interest of the nodes in a MANET. Nodes in a MANET reduce aggregate transmission power on each hop along a route by form coalitions and use it as a gauge of the payoff. The more power saved by the redirection, the more payoff a node earns. The payment of each node in a coalition is determined by using Shapley Value, a well-known concept in game theory for allocating payoff for each member in a cooperative coalition. A player may try to cheat in a game to maximize its utility. However in a truthful mechanism, cheating cannot bring more profits to a player than telling the truth. Consequently, a node would lose the intension to lie and only play correctly. The form of wealth could be some virtual currency. Each node knows the wealth of itself and its neighbors. If it is the richest or among top richest nodes within neighborhood, it may take a break and go to sleep while others continue to work to keep up with it. The richest ones can relax until they become less rich compared to their neighbors. CAP-SV is a reactive routing protocol in which only when a source node has packets to send, is a route discovery process initiated.

GTFT (Urpi, Bonuccelli and Giordano, 2003), (Michiardi and Molva, 2003): Generous Tit-For-Tat (GTFT) and multiple-GTFT (m-GTFT) are the first relay acceptance algorithms to use game theory in MANETs. GTFT is for the case where all requests are relayed by just one node until they reach the destination, and m-GTFT is for when multiple relays exist between the source and the destination (Li, 2007). These algorithms are for a node to balance the energy consumed for other nodes with the energy used by others for it; and to find an optimal trade-off between blocking probability and power consumption. The authors study the properties of a well known strategy and demonstrate that under the energy constraints imposed to the nodes, G-TFT promotes cooperation if every node of the network conforms to it. The model provides an accurate description of the energetic constraint of a node, which is the main reason for a selfish behavior, but provides only high-level guidelines towards the design of a cooperation enforcement mechanism based on the G-TFT strategy.

Name	Reputation Information	Type	Manage	Routing	Feature	Limitations
Watchdog (Marti, Giuli, Lai and Baker, 2004)	Local	R	Distributed	DSR	Watchdog that identifies misbehaving nodes and a pathrater that helps routing protocols avoid these nodes.	False accusations are easily possible, Dependence on promiscuous listening, malicious nodes are not punished
Context-aware (Paul and Westhoff, 2002)	Global and Local	R	Distributed	DSR	Misbehavior detection in the route discovery process as well	Offline agreement on a secret number (Yoo and Agrawal, 2006)
CONFIDANT (Buchegger and Le, 2002)	Global and Local	R	Distributed	DSR	Detecting and isolating uncooperative nodes, thus making it unattractive to deny cooperation.	Dependence on promiscuous listening
CORE (Michiardi and Molva, 2002)	Global and Local	R	Distributed	DSR	Node collaboration through monitoring of cooperativeness of nodes and a reputation mechanism	High computation and communication overhead, as each successful request results in the adjustment of the reputation table and in propagating the success.
Local reputation (Yau and Mitchell, 2003)	Local	R	Distributed	Protocol Independent	Utilization of only self-experience to evaluate reputation	Ignorance of non-neighboring Nodes (Yoo and Agrawal, 2006).
Friends and foes (Miranda and Rodrigues, 2003)	Global and Local	R	Distributed	DSR	Individual relation between two nodes in reputation management	Large memory overhead (Yoo and Agrawal, 2006)
TWOACK (Balakrishnan, Deng and Varshney, 2005)	-	R	Distributed	Protocol Independent	Acknowledgment for transmission between nodes two hops away	Large message and memory overhead (Yoo and Agrawal, 2006)
RIW (Adams, Hadjichristofi and Davis, 2005)	-	R	Centralized	Protocol Independent	Three-window weighted average for reputation for smooth change of node status	Arbitrary weight without a theoretical base (Yoo and Agrawal, 2006)
SORI (He, Wu and Khosla, 2004)	Local	R	Distributed	Protocol Independent	Nodes exchange reputation information only with their neighbors. This way a non-cooperative node will be punished by all of its neighbors.	No countermeasures to prevent collusion.

OCEAN (Bansal and Baker, 2003)	Local	R,C	Distributed	DSR	It does not suffer from false rating. Saves local memory. It rejects all traffic from malicious neighbor, but gives second chance to allow these station to become useful again	OCEAN considered unfair for nodes belonging to the perimeter of the network. It is vulnerable to attack by tampering avoid-list.
HEAD (Jianli, Jian and Yang , 2007)	Local	R,C	Distributed	DSR	In order to mitigate the effect of fault accusation HEAD employs the second chance mechanism. It allows node previously considered misbehaving to become useful again.	–
CineMA (Frank, Martini and Plaggemeier, 2004)	Local	R,C	Distributed	DSR	It is a group based approach which detects malicious nodes and punishes them by reducing their throughput.	Malicious nodes can not be isolated completely by a group of nodes.
Hao, Zheng and Xia (2006)	Global and Local	R.C	Centralized and Distributed	DSR	Cooperation enhancement mechanism based on trusty central server and neighbor monitoring.	–
Wang and Singhal (2005)	Global and Local	R	Distributed	AODV	light weight solution for selfish node problem considering battery status	false conviction is not totally avoided
Zahra, Anisi and Torgheh (2008) CEPF	Local	R	Distributed	Compare with DSR	A reputation based approach which uses only first hand or direct observations. Nodes with higher priority receives their services earlier	–

(R: Reputation based, C: Credit based)

Table 1: Reputation based or motivation based schemes for selfishness prevention.

Name	Type	Manage	Routing	Features	Limitations
Buttyan and Hubaux (2002, 2003) Nuglets	C	Centralized	–	Uses virtual currency to charge for transmission of packets and as to reward forwarding node	Relies on tamper-proof hardware.
Express (Janzadeh, Fayazbakhsh, Dehghan and Fallah , 2009)	C	Centralized	DSR	Credit-based cooperation mechanism that utilizes hash chains on messages to defend against cheating nodes.	–

SPRITE (Zhong, Chen and Yang, 2003)	C,G	Centralized	DSR	Collusion prevention, uses digital signature for any transaction.	Scalability issue in message overhead
Ad hoc-VCG (Anderegg and Eidenvenz, 2003) (Yoo and Agrawal, 2006)	C,G	Centralized	DSR	Two phases of cost calculation and payment for relays.	Excessive overhead. Do not focus on actual payment delivery, but on estimation.
Priority forwarding (Raghavan and Snoeren, 2003) (Yoo and Agrawal, 2006)	C	Centralized	DSR	Two-layered service: free best-effort and priced priority forwarding	Dependence on an MN as a credit server.
Multihop cellular (Salem, 2003) (Yoo and Agrawal, 2006)	C	Centralized	Protocol Independent	Combined architecture with cellular network	Indirect communication between nodes.
Willingness to pay (Crowcroft, Gibbens, Kelly and Ostring, 2003)	C	Distributed	Protocol Independent	Adaptive price depending on status of resources	Naive trust in each node on the cost. (Yoo and Agrawal, 2006)
Truthful multicast (Wang and Li, 2004)	C,G	Distributed	Protocol Independent	Encouragement for truthful reporting in multicast routing tree	Only bi-connected networks. (Yoo and Agrawal, 2006)
PIFA (Yoo, Ahn and Agrawal, 2005) ipass	C,R	Centralized	Protocol Independent	Full compatibility to any types of routing	Dependence on node as credit server protocol. (Yoo and Agrawal, 2006)
(Chen and Nahrstedt, 2004)	C,G	Centralized	DSR	Auction-based incentive to enable cooperative packet forward behavior.	the work mainly studies flow control problem in MANET

(R: Reputation based, C: Credit based, G: Game theory)

Table 2: Credit based or Incentive based schemes for selfishness prevention

Name	Type	Manage	Routing	Features	Limitations
GTFT (Urpi, Bonuccelli and Giordano, 2003), (Michiardi and Molva, 2003)	G	Distributed	Protocol Independent	Generous nodes for others' selfishness to some degree	Need for much system information
Catch (Milgram, 1967)	G	Distributed	Protocol Independent	Sender ID of packets hidden	No proof of evolutionary stability (Yoo and Agrawal, 2006)
SLAC (Hales, 2004)	G	Distributed	Protocol Independent	Prisoners dilemma in P2P network	Need to compare node performance fairly. (Yoo and Agrawal, 2006)
Incentive scheduling (Wei and Gitlin, 2005) than non-relay node	G	Centralized	Protocol Independent	More time slots and power for relay node	Relay node actually not relaying packets. (Yoo and Agrawal, 2006)

CAP-SV (Cai and Pooch, 2004)	G,C	Distributed	CAP-SV AODV	Payment of each node in coalition is determined using Shapley Value (concept of game theory for allocating payoff for each member in a cooperative coalition)	–
Sundararajan and Shanmugam (2010)	G,C	Distributed	AODV	Nodes Participate in communication according to their residual energy .	–

(G: Game theory, C: Credit theory)

Table 3: Game theory based schemes for selfishness prevention

Mario, Ganeriwal, Aad and Hubaux (2005): In this a game-theoretic approach is used to investigate the problem of the selfish behavior of nodes in CSMA/CA networks, specifically geared towards the most widely accepted protocol in this class of protocols. By applying the model of dynamic games borrowed from game theory, the authors derive the conditions for the stable and optimal functioning of a population of cheaters. It is a distributed protocol that successfully guides multiple selfish nodes to Pareto-optimal Nash equilibrium.

Sundararajan and Shanmugam (2010), developed a game theoretic based cooperation model that observes the behavior of an intermediary selfish node, while forwarding packets for others on a route between a source and a destination. Residual Energy of the nodes is the main issue. Nodes are participating in the communication according to the value of their residual energy and getting incentive as tokens. Each node possesses a positive number of tokens initially, earns tokens when it forward packets for other, and spends tokens when it sends or receives its own packets.

Table 3 summarized the features of credit based schemes.

D. Other schemes

Baaziz and Pierre (2008) studied the impact of mobility on level of cooperation between nodes. The idea is to change the route periodically between the source to destination. To achieve different routes between source to destination node requires a frequent mobility of different nodes. Intermediate nodes will not remain same. The purpose of mobility is to deliver packets by different routes, avoid selfish intermediate nodes, load distribution and to delay the selfish behavior of nodes. Mobility in static network is achieved by virtual mobility (re-routing traffic) from time-to-time.

Sundararajan and Shanmugam (2010) introduce Selfish Avoidance Routing Protocol for MANET (SARP), it ensures a node with low residual energy and higher traffic density is not selected for routing. This approach prevents a node to become selfish in near future and enhances cooperation among the nodes of a MANET. The effect of power consumption and the current traffic density at a node is also captured with the help of drain rate. SARP is an on demand routing protocol, where routing decisions are made at each hop. The routes taken by a data packet is based on the cost of routing that packet. The proposed cost metric ensure that a node with low residual battery energy and higher traffic density is not selected for routing.

Sundararajan and Shanmugam (2009) proposed the selfish aware AODV+2ACK model to detect routing misbehavior and to mitigate their adverse effect. The main idea of the protocol is to send two-hop acknowledgement packets in the opposite direction of the routing path. To reduce additional overhead, a fraction of received data packets are acknowledged.

CONCLUSION

Cooperation of nodes in MANET becomes very vital for proper operation of the network. Various cooperation schemes based on reputation/credit/game theory have been summarized & categorized. It has been observed by various researchers that network performance increases with cooperation enforcement schemes, but only for small networks. There are many issues in cooperation schemes that still need attention, like how will cooperative node be treated after rejoining, by other nodes in the network? What is the exact cause of selfishness? Causes can be (i) node can not forward packets as it become isolated due to link failure (ii) a node can drop packets due to congestion in the network.

In such cases the reputation of nodes should no depreciate.

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SOYBEAN AS AN ALTERNATIVE OF FISHMEAL IN CLARIAS BATRACHUS DIETS FOR SUSTAINABLE AQUACULTURE

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ABSTRACT

Research work has been focused to replace expensive fish feed i.e. fishmeal (FM) with the other cheaply available plant protein source i.e. soybean supplemented with mineral premix. Both feeds were fed to the experimental fish Clarias batrachus for 45 days. This resulted in better growth performance paralleled with better digestibility of nutrients in the fish. This also resulted in less excretion of ammonia and phosphorous in the treated water, thus also alleviate pollution problems from the aquaculture. Therefore, use of soybean is recommended as an alternative to FM in fish feeds. This may help to reduce the demand for FM, without affecting growth.

Keywords: Aquaculture, digestibility, fish feed, fishmeal, fish growth, Soybean.

INTRODUCTION

The expansion of global aquaculture production is increasing the demand for aquaculture feeds. Fishmeal (FM) is the main and most critical ingredient in aquafeed production. The increasing cost of FM has encouraged feed manufacturers search for cheaper alternative protein sources such as plant proteins. (Kalla *et al.,* 2004). Though the palatability of many plant materials has merits, anti-nutritional factors (ANF's) are the most serious concern in replacing FM completely in feed formulations. The ANF's have an adverse impact on the digestion of feed and its efficiency. There are many kinds of ANF's. The most important ANF's present in plant materials are trypsin inhibitors, glucosinolates and phytate (Felix and Selvaraj, 2004).

Phytic acid is one of the most powerful ANF's in plant ingredients (Hardy, 2002). The anti-nutritional activity of phytic acid can be eliminated by the relevant enzymes i.e. phytase. The phytic acid or phytate found in cereals, legume grains and oil seed is bound with P, Ca and Mg, trace elements like Fe and Zn, protein and amino-acids. Most fishes do not possess their own enzymes to break down phytate and thus, the nutrients are released as such (undigested). That is why higher proportions of valuable nutrients from vegetable sources are not utilized by fishes and are wasted as excreta. Therefore, it is most important to remove these ANF's from plant sources before its incorporation into fish feed. Heat inactivation and water soaking are the two common detoxification methods used to overcome most of the ANF's (Garg et al., 2002).

The main plant protein sources used in catfish feeds are oilseed meals, such as soybean meal, cottonseed meal, and peanut meal. Some other oilseed meals could be used but are not generally available on a timely basis and at an economical cost per unit of protein. Due to worldwide dominance of soybean and its appreciation as quality protein, several workers have attempted to replace FM with soybean in diets formulated for several fish species (Jindal *et al.,* 2007a, b; Robinson and Menghe, 2007; Jindal, 2008 and Jindal et al., 2008 a,b,c). However, the raw beans contain many ANF's especially lectin, phytohaemoglutinin, anti-vitamins and protease (trypsin) inhibitors, which interfere with appetite, absorption and metabolism. Therefore, these ANF's have to be destroyed or inactivated by suitable treatment (121OC at 15 lb for half an hour) before incorporating the same in the fish diet (Garg *et al.,* 2002).

In addition, as compared to FM, the plant proteins are commonly deficient in lysine, methionine, phytin, tannin etc. Therefore, it is necessary to supplement it with certain amino-acids through synthetic/pure amino-acids (mineral premix and amino-acids,MPA). Each Kg of which contains Copper - 312mg; Cobalt - 45mg; Magnesium - 2.114g; Iron -979mg; Zinc - 2.13g; Iodine 156mg; DL-Methionine - 1.92g; L-lysine mono hydrochloride - 4.4g; Calcium 30% and Phosphorous - 8.25% (Jindal and Garg, 2005 and Jindal, 2008 and 2009). They provide more minerals than what is needed for growth to make up for any losses that may occur during feed manufacture or storage.

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Therefore, in the present study, efforts were made to substitute FM with soybean in the diet of Clarias batrachus and the effects were compared w.r.t. growth performance and nutrient digestibility of fish along with the excretion levels of ammonia and phosphorus in the treated water.

MATERIALS AND METHODS

Preparation of supplementary feed (plant and animal based)

Groundnut oil cake, rice bran, wheat flour, fishmeal and soybean were procured from local market of Hisar. ANF's from soybean were eliminated by heat processing (Garg *et al.*, 2002). All the ingredients were grinded, powdered and subjected to proximate analysis following AOAC (2000) (Table 1).

Two types of supplementary feeds (Fishmeal and soybean based) were prepared by mixing the basic ingredients (groundnut oil cake and oiled rice bran) and wheat flour as a binder according to Table 2. In addition, mineral premix and amino-acids @ 1% were also added to fortify the diets with minerals and amino-acids. Chromic oxide @ 1% was added to study external digestibility. The finished product is routinely tested for protein, fat and fibre, and it is periodically tested for selected micronutrients to ensure nutritional value. The feed is also checked for physical characteristics, including floatability. The proximate composition of these two supplementary feeds is given in Table 2.

Experimental design

Specimens of C. batrachus fingerlings were procured from Sultan Singh Fish Farm, Nilokheri, Haryana, India. For a week prior to their experimental use, the fish were standardized in the Fisheries Laboratory, Department of Zoology and Aquaculture, CCS Haryana Agricultural University, Hisar, Haryana maintained at a temperature of 25± 1°C. The aquaria water was renewed daily with dechlorinated water. During acclimatization period, the fingerlings were fed on supplementary feed (FM based and soybean based) alternatively.

All treatments were conducted in glass aquaria (60×30×30cm); containing 30L of chlorine free water in a replicate of three, kept in a temperature controlled laboratory. All groups of fingerlings (each group containing 20 fingerlings) were fed daily (only once) between 4:00 to 5:00 pm, @ 5% BWd-1 for the whole experimental duration of 45 days. After 4hrs of feeding, the uneaten feed was siphoned out, otherwise they will deteriorate the water of aquaria. Growth of fingerlings was monitored after every 15 days interval in terms of weight and length and feeding rate adjusted accordingly.

Table 1: Proximate analysis (% dry weight basis) of the ingredients prior to the preparation of supplementary diets (fishmeal based and soybean based)

Ingredients	Proximate composition (%)					
	Crude Protein	Crude Fat	Crude Fiber	Total Ash	Nitrogen Free Extract	Gross Energy (KJg-1)
Groundnut Oil Cake (GNOC)	35.266 ± 0.005	6.250 ± 0.003	6.000 ± 0.005	7.000 ± 0.000	45.496 ± 0.006	18.607 ± 0.003
Oiled Rice Bran (RB)	14.100 ± 0.005	10.066 ± 0.005	11.003 ± 0.005	20.556 ± 0.003	44.440 ± 0.005	14.906 ± 0.007
Wheat Flour (WF)	14.290 ± 0.029	2.666 ± 0.088	1.533 ± 0.033	1.366 ± 0.088	80.143 ± 0.407	18.177 ± 0.037
Fish Meal (FM)	42.926 ± 0.002	10.996 ± 0.003	3.493 ± 0.003	29.653 ± 0.003	12.930 ± 0.001	16.713 ± 0.000
Processed Soybean* (PS)	43.733 ± 0.008	25.603 ± 0.000	4.496 ± 0.003	3.796 ± 0.000	22.370 ± 0.001	24.298 ± 0.001

* Raw soybeans were hydrothermically processed in an autoclave at 1210C at 15 lbs for 30 min. to remove anti-nutritional factors (ANFs) (Garg et al; 2002).

\# All values are mean ± S.E. of means of 3 observations

Table 2: Ingredients and proximate composition of two supplementary feeds used for feeding the fingerlings of *C. batrachus*

Ingredients	Supplementary Feeds	
	FM based	Soybean based
Groundnut oil cake (GNOC) A1	60	60
Oiled rice bran A2 (RB)	5	5
Wheat flour B (WF)	5	5
Fishmeal C (FM)	28	-
Processed soybean D (PS)	-	28
Mineral premix and amino acidsE (MPA)	1	1
Chromic Oxide F (Cr2O3)	1	1
Proximate composition (%)		
Crude protein	40.25	40.25
Crude fat	7	9.5
Crude fiber	3.5	7.25
Total Ash	7.3	6.5
Nitrogen free- extract (NFE)	42.95	36.5
Gross energy KJ/g	19.65	19.53

A1 and A2 - used as basic feed ingredients

B used as a binder to make diets water stable

C used as supplementary feed of animal origin

D used as supplementary feed of plant origin

E used to supplement the diets with minerals and amino acids.

Each Kg contains Copper - 312mg; Cobalt - 45mg; Magnesium - 2.114g; Iron -979mg; Zinc - 2.13g; Iodine 156mg; DL-Methionine - 1.92g; L-lysine mono hydrochloride - 4.4g; Calcium 30% and Phosphorous - 8.25%

F used as external digestibility marker

Individual weight of the fish fry was recorded at the beginning and at the end of the experimental period of 45 days. The faecal matter voided by the fish was also collected from each aquaria separately and dried at 60°C for subsequent determination of digestibility.

Analytical techniques

Live weight gain (g), length gain (cm), growth percent gain, specific growth rate (SGR) in terms of length and weight were calculated using standard methods given by Steffens (1989). Apparent nutrient digestibility (APD) of diets were also calculated following Cho et al., 1982.

Physico-chemical parameters like dissolved oxygen (mg/l), temperature (°C), pH, conductivity (μ mhos cm-1), total alkalinity (mg/l), total hardness (mg/l), free CO2 (mg/l) and excretory levels of ortho- phosphate (o-PO4) and ammonical nitrogen (NH4-N) mg 100g-1 BWd -1 of aquaria water of different treatments were recorded following APHA (1998) after every 15 days interval. Parameters like dissolved oxygen, temperature and pH were recorded daily to ensure the better survival of fish.

Statistical Analysis

Data was analysed following ANOVA, at 5 percent probability level. Group means were compared by student 't' test.

RESULTS AND DISCUSSIONS

Water quality parameter

The data showing water quality parameters of different treatments are presented in Table 3. Highest dissolved oxygen (DO) concentration was observed in the groups

Table 3: Analysis of physico-chemical parameters in different treatments after every 15 days interval for an experimental period of 45 days

S.N.	Parameters	Range	
		Supplementary feeds	
		FM based	Soybean based
1	Temperature (0C)	26.0-30.5	26.5-30.0
2	Dissolved oxygen (mg/l)	4.1-4.7	4.5-5.6
3	pH	7.9-8.1	7.8-8.0
4	Alkalinity (mg/l)	252-290	249-283
5	Total hardness (mg/l)	217-241	210-232
6	Free Co2 (mg/l)	15.4-17.2	15.2-16.8
7	Electrical conductivity (μ mhos/cm)	0.52-0.55	0.50-0.51
8	Ammonical Nitrogen (mg g-1 BW of fish)	0.179-0.250	0.162-0.236
9	Orthophosphate (mg g-1 BW of fish)	0.215-0.272	0.201-0.226

Table 4: Comparative growth performance of catfish Clarias batrachus fingerlings fed on Experimental diets.

S. No.	Type of feed	Stocking density	Survival % age	Weight gain (g)	Length gain (cm)	Growth % gain in body weight	Growth/ day in % body weight	Specific growth rate (SGR) (g / day)	Specific growth rate (SGR)(cm/ day)	Apparent Protein digestibility (APD)
1	Soybean based	20	95	2.84 ±0.003	2.09 ±0.004	99.98 ±0.005	1.116 ±0.001	1.37 ±0.003	1.15 ±0.005	87.27 ±0.005
2	FM based	20	91	2.40 ±0.005	1.95 ±0.005	97.54 ±0.003	1.012 ±0.000	1.25 ±0.002	1.04 ±0.003	85.42 ±0.005

Duration of experimental period = 45 days.

All values are mean ± S.E. of means of 3 observations

Significance level is $P < 0.05$

of fingerlings fed on processed soybean based diet in comparison to group receiving FM based diet. This clearly showed better utilization of processed soybean by the growing fingerlings resulting in better growth of the fish. These results are in agreement with those of Kalla et al. (2004) and Jindal (2009). pH remained alkaline throughout the experimental period. All other parameters remained in the optimal range.

The ammonical nitrogen (NH4-N) and ortho-phosphate (o-PO4) concentration in water was found to be lower in processed soybean based troughs in comparison to FM based troughs as shown in Table 3. This clearly showed that among dry feeds, plant (soybean) based feed excrete less ammonia and phosphorus in comparison to animal (FM) based feed and thus alleviate pollution problems from the intensive aqua cultural

systems. This finding also supports high retention of nutrients in the fish fed on plant based protein. Since a lot of energy is wasted in excreting ammonia and phosphorus, when fish were fed with FM based feeds, therefore, less growth was recorded in these fish groups. These results are in agreement with those of Kalla et al. (2004); Jindal and Garg (2005); Jindal et al. (2007a, b) and Jindal (2008 and 2009).

This also indicated that unless proper cleaning and water exchange were done daily, the concentration of ammonia and phosphorus would have been much more, which would be detrimental to fingerling growth and may cause mass mortality of fingerlings.

Survival and growth

Fingerling mortality was very less and independent of experimental treatment. The growth responses of the experimental fish on supplementary feeds (FM based and soybean based) are shown in Table 4.

In the present investigation, significantly ($P<0.05$) higher growth of C. batrachus fingerlings was observed in processed soybean based fish group in comparison to FM based fish group. These results indicated that processed soybean based diet resulted in better growth of fish with better digestibility of plant origin protein sources. These results are in broad agreement with those of Kalla et al., 2004; Jindal and Garg, 2005; Jindal et al., 2007 a, b and Jindal et al., 2008 a, b, c.

CONCLUSIONS

The results showed better growth performance paralleled with better digestibility of nutrients in the fish group fed on soybean (plant protein) based diet in comparison to fish group fed on FM based diet. Further, the use of plant proteins make the aquaculture feed more environmental friendly as the metabolism of such feeds results in the lower excretion of nitrogen, phosphorous and other organic wastes in the environment. Therefore, use of soybean is recommended as an alternative to FM in fish feeds. This may help to reduce the demand for FM, without affecting growth, from the aquaculture sector in coming years.

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REVISITING THE TEXTURE SPECIFIC QUARK MASS MATRICES

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ABSTRACT

Large number of texture specific Hermitian quark mass matrices has been investigated to ascertain their compatibility with the existing quark mixing data. For both Fritzsch-like and non Fritzsch-like mass matrices, we find several interesting conclusions regarding the structural features of these as well as the associated phases.

Keywords: Texture specific quark mass matrices, hierarchy of the elements of the quark mass matrices

INTRODUCTION

Texture specific mass matrices seem to be very helpful in understanding the pattern of quark mixings and CP violation (Fritzsch, 2000; P.S. Gill, 1995). The present quark mixing data completely rule out Fritzsch-like texture 6 zero mass matrices (Fritzsch, 2000; P.Ramond,1993), whereas Fritzsch-like texture 5 zero mass matrices may have limited viability (Rasin,1998; H. Fritzsch, 2000; Ramond, 1993; P.S. Gill, 1995). Further, Fritzsch-like texture 4 zero quark mass matrices have been shown to be quite successful in interpreting the strong hierarchy of quark masses and the smallness of flavor mixing angles(Fritzsch, 2000; Gill, 1995). Likewise, in the leptonic sector also texture zero mass matrices (Gill,1998; Z.Z. Xing, 2002) have been shown to be useful in explaining the pattern of neutrino masses and mixings.

It may be noted that the quark lepton unification hypothesis (Simornov) immediately brings forth the issue of finding the simplest texture structures at the leading order, compatible with the quark and lepton mixing phenomena. In this context, in view of absence of any theoretical justification for Fritzsch-like mass matrices, it becomes essential from the phenomenological point of view to consider non-Fritzsch like mass matrices as well. In the case of neutrinos, such an exercise has been done (Xing,2004; Xing, 2002) however, a similar exercise has not been carried out for quarks, therefore it is desirable to carry out detailed investigations for non Fritzsch-like quark mass matrices also.

The purpose of the present work is to identify all possibilities of texture 6 zero, texture 5 zero as well as texture 4 zero Fritzsch-like and non Fritzsch-like Hermitian quark mass matrices which, in principle, are possible. It would also be interesting to ascertain their compatibility with the existing quark mixing data and explore the parameter space of the elements of the viable mass matrices.

To this end, we first consider typical Fritzsch-like texture specific Hermitian quark mass matrices as

$$M_U = \begin{pmatrix} 0 & A_u & 0 \\ A_u^* & D_u & B_u \\ 0 & B_u^* & C_u \end{pmatrix}, \qquad M_D = \begin{pmatrix} 0 & A_D & 0 \\ A_D^* & D_D & B_D \\ 0 & B_D^* & C_D \end{pmatrix},$$

where MU and MD correspond to up and down mass matrices respectively. These above mentioned matrices are Fritzsch-like texture 4 zero mass matrices and from these the texture 5 and 6 zero mass matrices can be easily obtained. The non Fritzsch-like mass matrices can be obtained from these matrices by shifting the position of Ci (i= U,D) on the diagonal as well as by shifting the position of zeros among the non diagonal elements.

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POSSIBILITIES OF TEXTURE 6 ZERO QUARK MASS MATRICES

To begin with, we discuss all possible combinations of texture 6 zero Hermitian mass matrices. Before counting all possibilities, in view of non zero masses of quarks, these matrices have to satisfy the conditions Trace $M_{U,D}$ 0 and Det $M_{U,D}$ 0 .One can easily check that in case of texture 3 zero mass matrices we arrive at 20 different possible texture patterns, out of which 8 are easily ruled out by imposing these conditions. The remaining 12 possible textures break into two classes, as shown in table (1), depending upon the equations these matrices satisfy. For example, six matrices of class I, mentioned in table (1), satisfy the following equations

$$C = m_1 - m_2 + m_3, \quad A^2 + B^2 = m_1 m_2 + m_2 m_3, \quad A^2 C = m_1 m_2 m_3. \qquad (2)$$

Similarly, in case of class II all six matrices satisfy the following equations

$$C+D = m_1 - m_2 + m_3, \quad A^2 - CD = m_1 m_2 + m_2 m_3 - m_1 m_3, \quad A^2 C = m_1 m_2 m_3. \qquad (3)$$

The subscripts U and D have not been used as these are valid for both kinds of mass matrices.

Matrices M_U and M_D each can correspond to any of the 12 possibilities, therefore yielding 144 possible combinations which in principle can yield 144 quark mixing matrices. These 144 combinations can be put into 4 different categories, e.g., if M_U is any of the 6 matrices from class I, then M_D can be either from class I or class II yielding 2 categories of 36 matrices each. Similarly, we obtain 2 more categories of 36 matrices each when M_U is from class II and M_D is either from class I or class II. The 36 combinations in each category further can be shown to be reduced to groups of six combinations of mass matrices, each yielding same CKM matrix

An analytical analysis of categories as mentioned above yields only 4 groups of and M_{D}, corresponding to 6 combinations each of which yield 4 CKM matrices showing hierarchical nature. Thus, the problem of exploring the compatibility of 144 phenomenological allowed texture 6 zero combinations with the recent low energy data is reduced only to an examination of 4 groups each having 6 combinations of mass matrices corresponding to the same CKM matrix.

POSSIBILITIES OF TEXTURE 5 ZERO QUARK MASS MATRICES

Considering now the case of texture 5 zero mass matrices which consist either of M_U being 2 zero and M_D being 3 zero or vice versa. Texture 3 zero possibilities have already been enumerated, therefore we consider only the possible patterns of texture 2 zero mass matrices. After taking into consideration the Trace and Determinant conditions mentioned earlier, one can check that there are 18 possible texture 2 zero patterns. These textures further break into three classes, detailed in table (2), depending upon the diagonalization equations satisfied by these matrices, however, it can be shown that the classes IV and V essentially reduce to texture 3 zero patterns. We are therefore left with only class III of texture 2 zero, that needs to be explored for texture5 zero combinations. All matrices of this class satisfy the following equations

$$C+D = m_1 - m_2 + m_3, \; A^2 + B^2 - CD = m_1 m_2 + m_2 m_3 - m_1 m_3, \; A^2 C = m_1 m_2 m_3 \qquad (4)$$

Considering class III of texture 2 zero mass matrices along with different patterns of class I and class II of texture 3 zero mass matrices we find a total of 144 possibilities of texture 5 zero mass matrices, in sharp contrast to the case if we had considered the class IV and class V also yielding 432 possibilities. Keeping in mind the hierarchy of CKM matrix, we observe that out of 144 cases, we are again left with only 4 such groups of texture 5 zero mass matrices leading to mixing matrix having hierarchical structure as that of CKM matrix.

INPUTS USED IN THE PRESENT ANALYSIS

For checking the compatibility of the above mentioned texture 6 zero and 5 zero mass matrices with the recent quark mixing data, we first consider the various inputs used in the analysis. The quark masses and mass ratios, are considered at M_Z scale (GeV) (Leutwyler, 1996) ,are:

$$m_u = 0.002\text{-}0.003, \quad m_c = 0.6\text{-}0.7, \quad m_t = 169.5\text{-}175.5, \qquad (5)$$

$$m_d = 0.0037\text{-}0.0052, \quad m_s = 0.072\text{-}0.097, \quad m_b = 2.8\text{-}3.0, \qquad (6)$$

$$m_u/m_d = 0.51\text{-}0.60, \quad m_s/m_d = 18.1\text{-}19.7. \qquad (7)$$

For the purpose of our calculations, we are giving full variation to phases Φ_1 and Φ_2 and in the case of texture 5 zero matrices the variation of the parameter D is carried from 0 to D < C, as is usually done in such analyses. Some of the CKM parameters so reproduced are to be compared with the data taken from PDG (2008) (Yao,2008), e.g.,

$$|V_{us}| = 0.2236\text{-}0.2274, \quad |V_{cb}| = .0401\text{-}0.0423, \tag{8}$$

$$sin2 = 0.656\text{-}0.706, J = (2.85\text{-}3.24)x10^{-5} \delta = (45^0 - 107^0), \tag{9}$$

where β represents the angle of the unitarity triangle and J represents the Jarlskog's rephasing invariant parameter with being the CP violating phase of the CKM matrix.

DISCUSSION OF TEXTURE 6 ZERO QUARK MASS MATRICES

Coming to the case of texture 6 zero mass matrices, we have carried out the analysis for the four groups of texture 6 zero mass matrices each corresponding to six combinations of M_U and M_D. To check the viability of these combinations, we first reproduce $|V_{us}|$ element and then other elements of CKM matrix. We find that all possible combinations of texture 6 zero are ruled out as these are not able to reproduce the CKM element $|V_{cb}|$

DISCUSSION OF TEXTURE 5 ZERO QUARK MASS MATRICES

In the case of texture 5 zero mass matrices, again we first consider $|V_{us}|$ as a constraint and then try to reproduce $|V_{cb}|$ and other elements of CKM matrix. In table (3) we have presented the output of our analysis pertaining to texture 5 zero possibilities. A general look at the table immediately reveals that only in one case we are able to reproduce $|V_{cb}|$ as well as other CKM elements in the desired range given by PDG 2008. Interestingly, this possibility corresponds to usual Fritzsch-like texture 5 zero mass matrix where M_U is of texture 2 zero and M_D is of texture 3 zero.

DISCUSSION OF TEXTURE 4 ZERO QUARK MASS MATRICES

Coming to the case of texture 4 zero mass matrices, the texture two zero possibilities have already been detailed in table (2), resulting into 324 texture 4 zero possibilities. However, due to large number of viable possibilities, in the present work we have gone into the details of only the Fritzsch-like possibility of texture 4 zero Hermitian quark mass matrices. In particular, extension of the parameter space of the elements of these matrices has been carried out to include the case of 'weak hierarchy 'amongst them along with the usually considered 'strong hierarchy' case.

The various relations between the elements of the mass matrices, given in equation (1), A_i, B_i, C_i, D_i ($i= U,D$) essentially correspond to the structural features of the mass matrices including their hierarchies. As is usual the element $|A_i|$ takes a value much smaller than the other three elements of the mass matrix which can assume different relations amongst each other, defining different hierarchies. For example, in case Di < $|B_i|$ < C_i it would lead to a strongly hierarchical mass matrix whereas a weaker hierarchy of the mass matrix implies D_i $|B_i|$ C_i It may also be added that for the purpose of numerical work, one can conveniently take the ratio Di / C_i ~ 0.01 characterizing strong hierarchy whereas D_i / C_i implying weak hierarchy.

The analysis has been carried out by incorporating the quark masses and their ratios mentioned in equations (5), (6) and (7) as well as by imposing the constraints given in equations (8) and (9). Further, we have given full variation to the phases Φ_1 and Φ_2, the parameters D_U and D_D have been given wide variation in conformity with the hierarchy of the elements of the mass matrices e.g., $D_i < C_i$ for $i= U,D$. The extended range of these parameters allows one to carry out the calculations for the case of weak hierarchy of the elements of the mass matrices as well.

To begin with, in figure 1(a) we have plotted C_U / m_t versus C_D / m_b. A look at the figure reveals that both C_U / m_t as well as C_D / m_b take values from ~ 0.55-0.95, which interestingly indicates the ratios being almost proportional. Also, the figure gives interesting clues regarding the role of strong and weak hierarchy. In particular, one finds that in case one restricts to the assumption of strong hierarchy then these ratios take large

values around 0.95. However, for the case of weak hierarchy, the ratios C_U / m_t and C_D / m_b take much larger number of values, in fact almost the entire range mentioned above, which are compatible with the data.

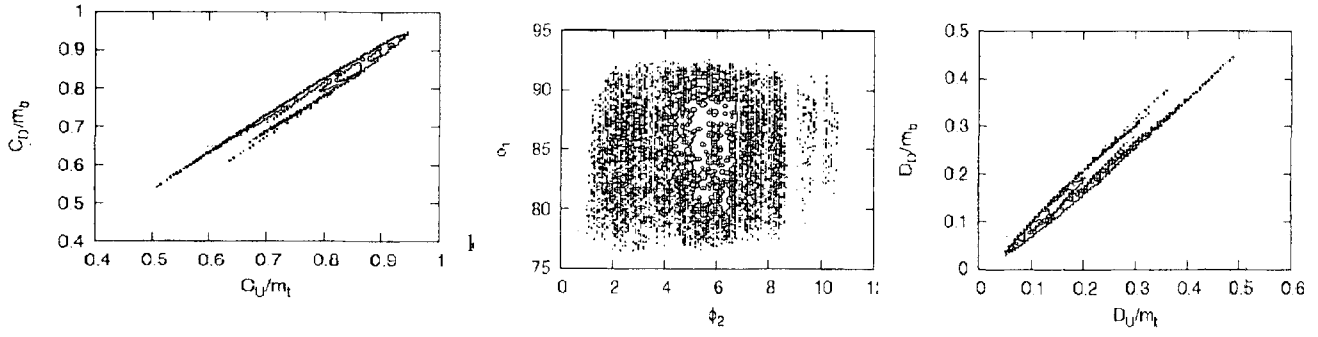

Fig. 1. Plots showing the allowed ranges of (a) C_U / m_t versus C_D / m_b , (b) Φ_1 versus Φ_2 and (c) D_U / m_t versus D_D / m_b .

In figure 1(b), we present the plot of Φ_1 versus Φ_2 Interestingly, the present refined inputs limit the ranges of the two phases to $\Phi_1 \sim 76^0$ -92^0 and $\Phi_2 \sim 1^0$ -11^0 .Keeping in mind that full variation has been given to the free parameters D_U *and* D_D, corresponding to both strong as well as weak hierarchy cases, it may be noted that the allowed ranges of the two phases come out to be rather narrow. In particular, for the strong hierarchy case one gets $\Phi_2 \sim 10^0$ whereas for the case of weak hierarchy Φ_2 takes almost its entire range mentioned above. Also, our analysis indicates that although Φ1 Φ_2, still both the phases are required for fitting the mixing data.

As a next step, we would like to emphasize the role of the hierarchy defining parameters D_U and D_D To this end, in figure 1(c) we have plotted D_U / m_t versus D_D / m_b ,representing an extended range of the parameters D_U and D_D A closer look at the figure reveals both D_U / m_t as well as D_D / m_b take values ~0.05- 0.5. The lower limit of the range i.e. when the ratios D_U / m_t and D_D / m_b are around 0.05 corresponds to strong hierarchy amongst the elements of the mass matrices, whereas when the elements have weak hierarchy then these ratios take a much larger range of values. From this one may conclude that in the case of strongly hierarchical elements of the texture 4 zero mass matrices, we have limited compatibility of these matrices with the quark mixing data, whereas the weakly hierarchical ones indicate the compatibility for much broader range of the elements.

SUMMARY AND CONCLUSIONS

To summarize, large number of texture specific Hermitian quark mass matrices have been investigated to ascertain their compatibility with the existing quark mixing data. Interestingly, all the 144 Fritzsch-like as well as non Fritzsch-like possibilities for texture 6 zero mass matrices are completely ruled out whereas in case of texture 5 zero mass matrices, out of the 432 possibilities there is one unique Fritzsch-like combination which shows limited viability, the other possibilities are again ruled out. In the case of texture 4 zero mass matrices, due to large number of viable possibilities, in the present work we have gone into the details of only the Fritzsch-like possibility of texture 4 zero Hermitian quark mass matrices. These mass matrices have been examined by considering not only the usual strong hierarchy amongst the elements of these matrices, but also for the weak hierarchy case. We find that in the case of strong hierarchy, one gets limited compatibility of these matrices with the quark mixing data, whereas the weakly hierarchical ones indicate the compatibility for much broader range of the elements. Also, one finds that both the phases are required to fit the data, in particular these come out to be $\Phi_1 \sim 76^0$-92^0 and $\Phi_2 \sim 1^0$ - 11^0 .

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Table 1. Twelve possibilities of texture 3 zero mass matrices categorized into two classes I and II, with each class having six matrices labeled as {a,b,c,d,e,f.}

	Class I	*Class II*
a.	$\begin{pmatrix} 0 & Ae^{i\alpha} & 0 \\ Ae^{-i\alpha} & 0 & Be^{i\beta} \\ 0 & Be^{-i\beta} & C \end{pmatrix}$	$\begin{pmatrix} 0 & Ae^{i\alpha} & 0 \\ Ae^{-i\alpha} & D & 0 \\ 0 & 0 & C \end{pmatrix}$
b.	$\begin{pmatrix} 0 & 0 & Ae^{i\alpha} \\ 0 & C & Be^{i\beta} \\ Ae^{-i\alpha} & Be^{-i\beta} & 0 \end{pmatrix}$	$\begin{pmatrix} 0 & 0 & Ae^{i\alpha} \\ 0 & C & 0 \\ Ae^{-i\alpha} & 0 & D \end{pmatrix}$
c.	$\begin{pmatrix} 0 & Ae^{i\alpha} & Be^{i\beta} \\ Ae^{-i\alpha} & 0 & 0 \\ Be^{-i\beta} & 0 & C \end{pmatrix}$	$\begin{pmatrix} D & Ae^{i\alpha} & 0 \\ Ae^{-i\alpha} & 0 & 0 \\ 0 & 0 & C \end{pmatrix}$
d.	$\begin{pmatrix} C & Be^{i\beta} & 0 \\ Be^{-i\beta} & 0 & Ae^{i\alpha} \\ 0 & Ae^{-i\alpha} & 0 \end{pmatrix}$	$\begin{pmatrix} D & 0 & 0 \\ 0 & C & Ae^{i\alpha} \\ 0 & Ae^{-i\alpha} & 0 \end{pmatrix}$
e.	$\begin{pmatrix} 0 & Be^{i\beta} & Ae^{i\alpha} \\ Be^{-i\beta} & C & 0 \\ Ae^{-i\alpha} & 0 & 0 \end{pmatrix}$	$\begin{pmatrix} D & 0 & Ae^{i\alpha} \\ 0 & C & 0 \\ Ae^{-i\alpha} & 0 & 0 \end{pmatrix}$
f.	$\begin{pmatrix} C & 0 & Be^{i\beta} \\ 0 & 0 & Ae^{i\alpha} \\ Ae^{-i\alpha} & Be^{-i\beta} & 0 \end{pmatrix}$	$\begin{pmatrix} C & 0 & 0 \\ 0 & 0 & Ae^{i\alpha} \\ 0 & Ae^{-i\alpha} & D \end{pmatrix}$

Table 2: Texture 2 zero possibilities categorized into three classes III, IV and V, with each class having six matrices labeled as a, b, c, d, e, f.

	Class III	*Class IV*	*Class V*
a	$\begin{pmatrix} 0 & Ae^{i\alpha} & 0 \\ Ae^{-i\alpha} & D & Be^{i\beta} \\ 0 & Be^{-i\beta} & D \end{pmatrix}$	$\begin{pmatrix} D & Ae^{i\alpha} & 0 \\ Ae^{-i\alpha} & 0 & Be^{i\beta} \\ 0 & Be^{-i\beta} & C \end{pmatrix}$	$\begin{pmatrix} 0 & Ae^{i\alpha} & Fe^{i\gamma} \\ Ae^{-i\alpha} & 0 & Be^{i\beta} \\ Fe^{-i\gamma} & Be^{-i\beta} & C \end{pmatrix}$
b	$\begin{pmatrix} 0 & 0 & Ae^{i\alpha} \\ 0 & C & Be^{i\beta} \\ Ae^{-i\alpha} & Be^{-i\beta} & D \end{pmatrix}$	$\begin{pmatrix} D & 0 & Ae^{i\alpha} \\ 0 & C & Be^{i\beta} \\ Ae^{-i\alpha} & Be^{-i\beta} & 0 \end{pmatrix}$	$\begin{pmatrix} 0 & Fe^{i\gamma} & Ae^{i\alpha} \\ Fe^{-i\gamma} & C & Be^{i\beta} \\ Ae^{-i\alpha} & Be^{-i\beta} & 0 \end{pmatrix}$
c	$\begin{pmatrix} D & Ae^{i\alpha} & Be^{i\beta} \\ Ae^{-i\alpha} & 0 & 0 \\ Be^{-i\beta} & 0 & C \end{pmatrix}$	$\begin{pmatrix} 0 & Ae^{i\alpha} & Be^{i\beta} \\ Ae^{-i\alpha} & D & 0 \\ Be^{-i\beta} & 0 & C \end{pmatrix}$	$\begin{pmatrix} 0 & Ae^{i\alpha} & Be^{i\beta} \\ Ae^{-i\alpha} & 0 & Fe^{i\gamma} \\ Be^{-i\beta} & Fe^{-i\gamma} & C \end{pmatrix}$
d	$\begin{pmatrix} C & Be^{i\beta} & 0 \\ Be^{-i\beta} & D & Ae^{i\alpha} \\ 0 & Ae^{-i\alpha} & 0 \end{pmatrix}$	$\begin{pmatrix} C & Be^{i\beta} & 0 \\ Be^{-i\beta} & 0 & Ae^{i\alpha} \\ 0 & Ae^{-i\alpha} & D \end{pmatrix}$	$\begin{pmatrix} C & Be^{i\beta} & Fe^{i\gamma} \\ Be^{-i\beta} & 0 & Ae^{i\alpha} \\ Fe^{-i\gamma} & Ae^{-i\alpha} & 0 \end{pmatrix}$
e	$\begin{pmatrix} D & Be^{i\beta} & A^{i\alpha} \\ Be^{-i\alpha} & C & 0 \\ Ae^{-i\alpha} & 0 & 0 \end{pmatrix}$	$\begin{pmatrix} 0 & Be^{i\beta} & Ae^{i\alpha} \\ Be^{-i\beta} & C & 0 \\ Ae^{-i\alpha} & 0 & D \end{pmatrix}$	$\begin{pmatrix} 0 & Be^{i\beta} & Ae^{i\alpha} \\ Be^{-i\beta} & C & Fe^{i\gamma} \\ Ae^{-i\alpha} & Fe^{-i\gamma} & 0 \end{pmatrix}$
f	$\begin{pmatrix} C & 0 & Be^{i\beta} \\ 0 & 0 & Ae^{i\alpha} \\ Be^{-i\beta} & Ae^{-i\alpha} & D \end{pmatrix}$	$\begin{pmatrix} C & 0 & Be^{i\beta} \\ 0 & D & Ae^{i\alpha} \\ Be^{-i\beta} & Ae^{-i\alpha} & 0 \end{pmatrix}$	$\begin{pmatrix} C & Fe^{i\gamma} & Be^{i\beta} \\ Fe^{-i\gamma} & 0 & Ae^{i\alpha} \\ Be^{-i\beta} & Ae^{-i\alpha} & 0 \end{pmatrix}$

Table 3: Texture 5 zero combinations and their corresponding predicted values pertaining to $|V_{cb}|, |V_{ub}|, sin2, \delta, J$.

M_U	M_D	$\|V_{cb}\|$	$\|V_{ub}\|$	$sin2$	δ^0	$JX10^{-5}$
I_i	III_i	0.09- 0.28	0.005- 0.02	0.44- 0.60	60- 100	10- 110
II_i	III_i	0.14- 0.26	0.008- 0.02	0.46- 0.59	65- 93	0.27- 0.97
III_i	I_i	0.0401- 0.0423	0.0032- 0.0041	0.656- 0.701	55- 100	2.4- 3.8
III_i	II_i	0.06- 0.29	0.003- 0.02	0.51- 0.54	45- 88	4.8- 110

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ULTRASTRUCTURE OF THE CYCLOID FISH SCALE OF *PUNTIUS SARANA SARANA* (HAMILTON-BUCHANAN, 1822)

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ABSTRACT

The use of fish scale, one of the hard parts is in vogue for age and growth studies in commercial fish species in the western countries. On the basis of information on age growth in conjunction with length-weight data, major fishery management decisions such as optimum exploitation of the stocks of the commercial fish species, ascertaining of fishable legal size and conservation strategies are taken.

Puntius sarana sarana (Hamilton-Buchanan), commonly known as 'Puthi' is one of the minor carps and constitutes the miscellaneous fishery in most of the natural water bodies of North India. Though it is not a culturable fish, but the information on the correct age determination will be useful to promote its populations in its natural habitat. Considering this fact, an attempt has been made to describe the ultrastructure of the scale of this fish species so that the age of this fish species is correctly evaluated. The emphasis is on the structure of circuli, annuli and the chromatophores using SEM technique. The outcome of the present investigations will be helpful in the exact determination of age and growth studies and may be in the separation of populations on the basis of the structure of circuli and lepidonts.

Keywords: Scale, *Puntius sarana sarana* (Hamilton-Buchanan), fish scale, SEM, ultrastructure.

INTRODUCTION

Fish scale which is one of the hard parts of teleostean fishes, has been widely used for classification (Goodrich, 1907,1908; Cockrell, 1910,1915; Matsui, 1949; Smith *et al.*, 1972; Roberts, 1993), phylogeny (Lagler, 1947; Kobayashi, 1952, 1953; Roberts, 1993; Schultze, 1996), identification up to family and species levels (Lagler, 1947; Tandon and Sharma, 1977; Tandon and Chaudhary, 1983-84; Johal and Kaur, 2004; Johal and Dhiman, 2007), stock determination (Carlander, 1943; Landu, 1979; Shackelton and Johnson, 1988) and age determination and growth studies (Johal *et al.*, 1984; Hollander, 1986; Johal and Tandon, 1992; Tandon and Johal, 1996; Johal *et al.*, 2001, 2006). Recently fish scale has been employed as pollution indicator with great precision and accuracy (Johal and Dua, 1994; Johal and Grewal, 2006; Johal and Ravneet, 2004, 2007; Darafsh *et al.*, 2008).

In India, the lepidological studies on freshwater fishes are scarce. Tandon and Johal (1996) compiled the data on the Indian fish species and opined that age composition data based on scale method are very useful for the management of the stocks of the commercial fishes in nature and conservation of endangered fish species. Further old fishery regulations such as legal fishable size and fishing effort can be reviewed on the basis of scale studies. To arrive at conclusions, it is appropriate to study the detailed study of the scale employing the most advance technique(s) so that age can be determined accurately. Keeping this fact in mind, the scale of one of the minor carps, *Puntius sarana sarana* (Hamilton-Buchanan) has been studied under optical microscope and Scanning Electron Microscope (SEM).

Puntius sarana sarana (Hamilton) commonly known as 'Puthi' is of common occurrence in the natural water bodies of northern India up to Krishna river system, Bangladesh, Myanmar, Nepal and Pakistan (Jayaram, 2010). It constitutes the miscellaneous fishery and is considered as excellent food fish. In natural water bodies it can attain the maximum size of 31.00 cm total fish length (Talwar and Jhingran, 1991); however, Tandon and Johal (1983) reported that rarely some specimens can attain the size of 38.00 cm total fish length.

Considering the economic importance of this minor carp as commercial fish species some attempts have been made to study its various biological aspects. (Sinha, 1972). Murty (1976) and Tandon and Johal (1983) employing scale method for age and growth studies from Lake Kolleru, Andhra Pradesh, Sukhna Lake, Chandigarh and end part of the river Ghagger located in western part of Rajasthan

The perusal of literature has revealed that till now the ultrastructural details of the cycloid scale of *Puntius*

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sarana sarana (Hamilton-Buchanan) have not described so far, hence the present studies have been undertaken. The outcome of the present studies shall help in understanding the basic features of the scale, hitherto unknown and to pin point the exact features of the true annulus so that the age of this can be determined accurately. Further, the present studies will eliminate the errors which normally occur due to under or over estimations of age and back-calculated lengths.

MATERIALS AND METHODS

Scale samples of *Puntius sarana sarana* (Hamilton-Buchanan) were collected from the commercial catches of Harike wetland, Punjab, (74° 54'E; 31° 10' 15" N) during April 1988 – May 1990. Each scale was removed in the field with the help of ordinary tweezers from the left side of the fish, below the dorsal fin, above the lateral line preferably from the second or third row. Each scale was stored in the envelope and the other relevant data such as date of collection, weight (gms.), total and standard lengths (mm), exact location and the sex of the fish. The scales were brought to the laboratory for further studies. The extraneous matter was removed by rubbing the scales between finger tips and giving 4-5 washings in tap water and dried in the desiccator for three to four days. Invariably during this process the margins of the scale curl and to avoid this curling, the scales were tied between the microslides during the process of drying.

The dried scale or piece of the dried scale was fixed on the metallic stub with the help of double stick tape in such a way that the shinning side or ventral surface of the scale faces the surface of the metallic stub. As the scale is non-conducive material, they were coated with layer of gold having the thickness of 100 A° in a gold coating unit. This process overcome the problems of over charging and beam damage. The gold coated scales were viewed under vacuum in Jeol JSM-6100 Scanning Electron Microscope at an accelerating voltage of 20 kv. For the preparation of photographic image, the image formed by back-scattered electrons was used.

Scales were also viewed under optical microscope preferably the binocular at the magnification of 10X, and Carl Zeiss Scale Reader Model DL 5.3 at the magnifications of 10X and 17X.

OBSERVATIONS AND DISCUSSION

The cycloid scale of *Puntius sarana sarana* (Hamilton-Buchanan) is flat and roughly rectangular having posterior side pointed. The surface of the scale attached to the body skin is shiny is termed as ventral surface, whereas the opposite is dorsal and rough. The ultrastructure of the scale is described from the dorsal surface. The scale has four distinct regions namely, anterior, posterior, left and right laterals. The scales are arranged on the body of the fish in an overlapping manner. The anterior part of the scale is covered or overlapped by the posterior part of the preceding scale. The posterior part is exposed and is covered by skin epithelium. In literature, the anterior and posterior parts are also referred as cephalic and caudal parts respectively. As already mentioned for describing the detailed structure of the scale of *Puntius sarana sarana* (Hamilton-Buchanan) the dorsal surface has been the subject of study.

The scale has distinct focus which is present in the posterior half of the scale and it is the point from where the scale makes its beginning (Fig. 1a, b, c). Around the focus, there are present growth lines called circuli (sclerites) (Fig. 1d). The shape of the circuli corresponds to the shape of the scale. The circuli are more distinct in the anterior part than the posterior part. In the beginning, the circuli are disorganized and do not follow circular arrangement. Little away from the focus, regular and distinct formation of the circuli has been observed. The beginning of the organized circuli indicates the end of the larval period or it indicates the hatching time when the fish has come out of the vitelline membrane of the egg. From the focus, three types of radii originate viz., primary, secondary and tertiary (Figs. 1a-d). The radii cut the circuli at right angles. The radii are more prominent in the anterior and lateral regions of the scale. On the dorsal surface of the scale, there are present annuli which indicate the age of the fish.

In figures. 2a-f, the ultrastructure of the dorsal side of the scale *P. sarana sarana* (Hamilton-Buchanan) using SEM is presented. In figures 2a-d the details of the circuli are given. The inter-circulus distance varies from 20-60 µm. It is more in the lateral regions and less in the anterior region (Figs. 1d, 2a-b). In the annular zone (Fig. 2b), two or three circuli break down and are disorganized. When this mechanism is exhibited on the all sides of the scales, it is termed as 'true annulus' or 'annual mark'. It is formed during the months of July-August and coincides with the spawning or breeding act of the fish, therefore, it is also termed as 'spawning or breeding mark'. As this fish in the Northern part of India breeds once in a year during south-west monsoon and the annual

mark is formed once in a year, therefore, the number of 'annual marks' or spawning marks' or breeding marks' indicate the age of fish in years.

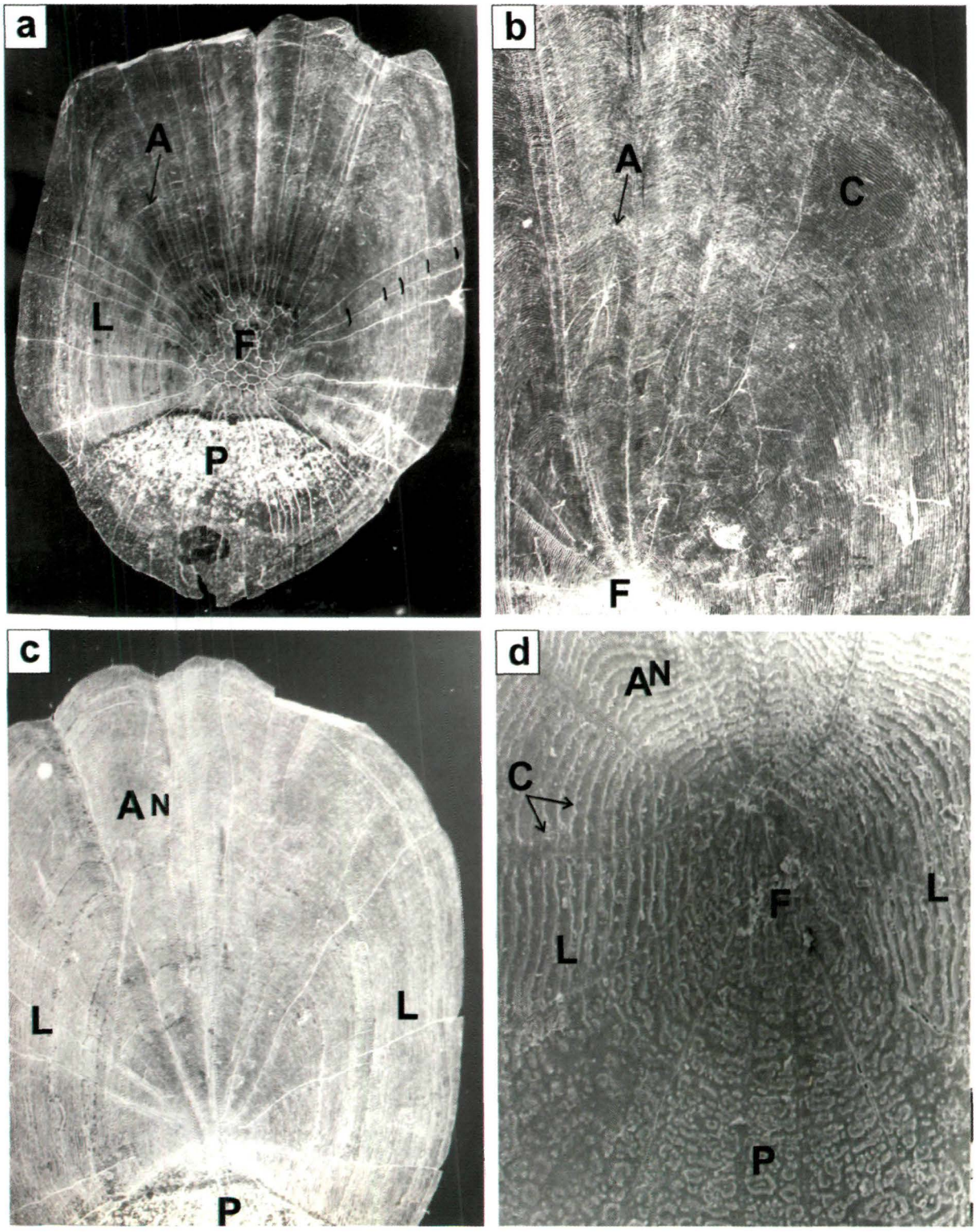
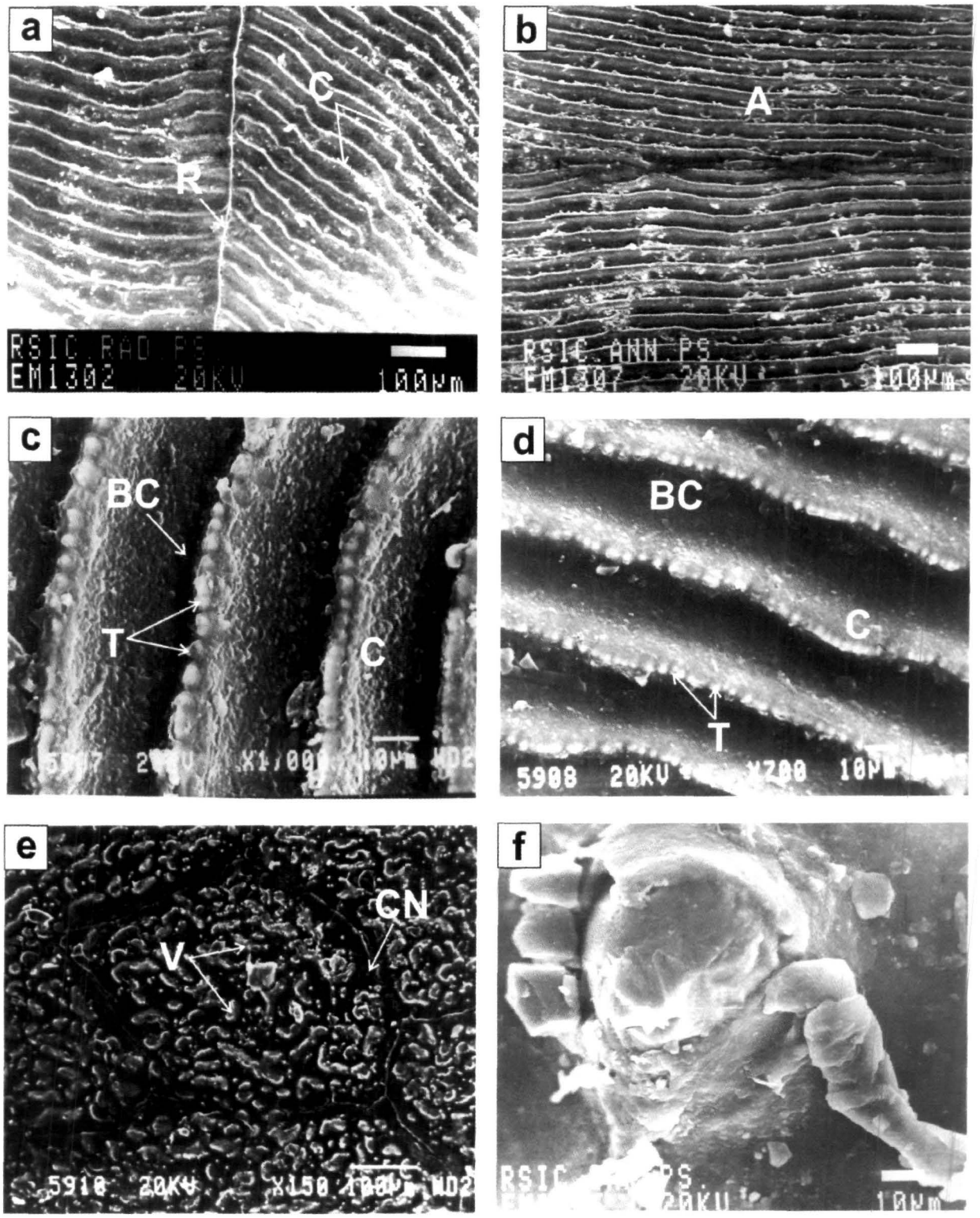


Fig. 1a-d: Scale of *Puntius sarana sarana* (Hamilton-Buchanan) under optical microscope. a) Entire scale, dorsal part, age 5 yrs. focus part absent due to regeneration, 10X; b) Right antero-lateral part showing focus, radii and annulus, 16X; c) Posterior part and antero-lateral part, 20X; d) Focus region, 30X.

Abbreviations : A-Annulus; AN; Anterior side C - Circuli; F-Focus;
L-Lateral side; P-Posterior side



Figs. 2a-f: SEM micrographs of dorsal side of scale of *Puntius sarana sarana*. a) radii of circuli; b) circuli showing annulus; c&d) details of cirucli armed with poorly developed tubercles; e) details of microvilli; f) details of the skin epithelium covering the posterior part of exposed part of the sale.

Abbreviations : A - Annulus; BC - Base of circuli; C - Circuli, CN - Canaliculi;
V - Microvilli; R - Radii; T - Tubercles

The circuli present on the dorsal surface of the scale have broad bases and pointed surfaces (Figs. 2c, d). The general surface of each circuli is rough and has the deposition of organic granules. The upper surface of each circulus has globule like structures. The dorsal surface of the posterior region of the scale is covered by the epithelial layer (Figs. 1a, 1d, 2e, 2f). The region of the scale is thicker than the anterior and lateral regions because the calcium salts are being deposited continuously without any hindrance and resistance. As a result of the deposition of the calcium salts, this region is harder and the circuli are less distinct and lack radii. The SEM investigations have revealed that the epithelium is thrown into microvilli (Fig. 2e) of various shapes and sizes. The microvilli are arranged in groups which exhibit hexagonal shape. In this region, there is present canal system (Fig. 2e) for the movement of water, nutrients and the waste materials. Each microvillus consists of several cells having club shaped appearance (Fig. 2f).

The structure of cycloid scale has been described by various workers (Delamater and Courtenay, 1973; Lanzing and Higganbotham, 1974; Girand *et al.*, 1978: Lippitsch, 1992; Sire, 1985; Johal and Agarwal 1997; Sire *et al.*, 1997; Johal and Kaur, 2004; Jawad, 2005; Johal, 2005; Johal and Chadha, 2005; Johal and Dhiman, 2007; Johal and Ravneet, 2007). The morphology of the scale has been employed for the phylogenetic relation, classification and species identification (Goodrich, 1907, 1908: Kobayashi, 1952, 1953; Norman, 1975; Roberts, 1993; Sharma, *et al.*, 2005; Johal and Dhiman, 2007). The lateral line scales on the basis of lateral line canal has been employed for the identification of some marine and freshwater fishes by Tandon and Chaudhary (1983-84) and Johal and Kaur (2004) respectively. The ultrastructure of the fish scale of various fish species has been described by Johal and Dua (1994), Johal and Sawhney (1997), Johal and Grewal (2006). Johal and Thomas (2000) described the sexual dimorphism on the basis of scale structure in *Barilius bendelisis* one of the hillstream fish inhabiting the cool water of western Himalayas.

The general structure of the scale of *P. sarana sarana* (Hamilton-Buchanan) is similar to that of carps with minor differences, however, great variations have been observed at ultrastructural levels e.g., location of focus, circuli architecture, presence or absence of lepidonts or tubercles, shape, size of tubercles, microvilli structure on the posterior or exposed part of the scale and shape, size, rows and number of ctenii incase of ctenoid scale.

It is concluded that the fish scale can be an excellent tool in the identification in fishes up to species level in addition to conventional tools especially in those fish genera where the number of species is enormous and the morphological and meristic characters are either confusing or overlapping. Considering all these points in mind, the ultrastructure of the cycloid scale of *Puntius sarana sarana* (Hamilton-Buchanan) has been described employing SEM technique. The genus *Puntius* Hamilton is widely distributed in India, Pakistan, Nepal, Bangladesh, Sri Lanka, Burma, Thailand and its members occur in abundance in Southern continental China and adjunct islands and Africa. In present day India, the occurrence of 75 fish species of the genus *Puntius* Hamilton has been reported (Jayaram, 2010) and the maximum number species are endemic to Western Ghats. Good numbers of fish species of this genus are excellent aquarium fishes because of their small size and varied color patterns; hence, they have great economic importance too. It is opined that the present observation will initiate the use of new tool in fish identification and shall form the base line data for the identification of fish species belonging to the genus *Puntius* Hamilton which poses enormous problems.

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FEED MANAGEMENT FOR THE GROWTH OF FISH *CHANNA PUNCTATUS* (BLOCH.)

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ABSTRACT

To manage feed for optimum growth in *Channa punctatus* four diets (D - D) with varying protein levels (33.0%, 39.5%, 43.0% and 46.5 %) using processed soybean (PS) as the major protein source were formulated. Studies have revealed an increase in live weight gain (g), per cent gain in body weight, specific growth rate, apparent protein digestibility and energy retention in the fingerlings fed on a managed feed containing 39.5% crude protein in comparison with the fish fed on other feeds containing low or high protein levels. Carcass composition also revealed high accumulation of protein, fat & energy in fish fed on a managed feed containing 39.5% protein, while those of moisture and ash contents remained low. Excretory levels of ammonical nitrogen and ortho-phosphate were found significantly ($P<0.05$) low in the groups of fish fed on feed containing 39.5% protein. Hence, the feed containing processed soybean @ 200g/kg of diet with 39.5 % crude protein level resulted in optimum growth of *C. punctatus* along with minimal pollution of the culture system.

Keywords- Ammonical nitrogen, carcass, excretion, protein digestibility, ortho-phosphate

INTRODUCTION

Fish feeds constitute the major fraction of the operational cost in both intensive and semi intensive culture system globally. In the past, the traditional feed was commonly given to fish with the ingredients like rice bran, groundnut oil cake, mixed/soaked in water in equal proportion. This feed does not contain enough nutrients required for the good health of the fish. Further, these nutrients are not only low in energy but also yield a lot of wastes in the treated water. Therefore, to reduce pollution and increase fish growth a nutritionally balanced diet is necessary for enhancing not only the growth but also nutritionally rich fish flesh, such fishes also fetch better price in the market.

In comparison to agriculture, aquaculture has been found to be a better productive venture. In recent years, there has been much consideration for the supplementary feeds which are a combination of different protein rich sources of plants and animal origin. Fish production with the use of supplementary feed is invariably higher than even the livestock rearing. Now days, these constitutes more than 50% of the operating cost in aquaculture. The major feed constitutes are the total proteins. Unlike mammals, the proteins in the fishes act both as structural components and as an energy source.

Protein requirement of fishes is uniformly high irrespective of their food habits (Vielman *et al.*, 2000, Jindal and Garg, 2005 and Jindal, 2011a,b). Growth in cultivable fishes is primarily influenced by quality (amino acid composition) and quantity of proteins in the formulated feeds which is being managed through proper feeding of fishes. The ultimate aim of artificial feeding in aquaculture is to manage feeding for maximum protein deposition with minimum inputs of feeds and at minimum cost. Any reduction in feed cost through dietary management is, therefore, crucial for the development and well being of the industry.

Conventional fish meal (FM) continues to be a primary protein source in formulated feeds. But its rising cost, uncertain availability and unreliable quality have led to the scientific search for alternate sources. The utility of plant protein sources (such as soybean, canola, mustard *etc.*) to replace FM is being managed meticulously (Kalla and Garg, 2004, Jindal and Garg, 2005, Robinson and Menghe, 2007, Jindal *et al.*, 2007a, b, 2009).

Channa punctatus is regarded as an excellent table fish having good growth. It is also very hardy fish which can survive in extended periods of hot and cold temperature and in marginal water conditions. Moreover, this fish is easily available in Haryana and surroundings.

The present investigation was, therefore, planned to work out the protein requirements for optimum growth of fingerlings of *C. punctatus* by using processed soybean (PS) as the major plant protein source in the formulated diets. Effect of PS diets on post prandial excretion of

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ammonical nitrogen (NH_4-N) and ortho- phosphate (o-PO4) in the treated water were also examined.

MATERIAL AND METHODS

Experimental diets

Groundnut oilcake, oiled rice bran and soybean seeds were arranged from the local market of Hisar, Haryana, India. Soybean seeds were cleaned, autoclaved (at 121.6° C at 15 lb pressure for 30 min.) to remove anti-nutritional factors (ANF's) such as trypsin inhibitors, haemoglutinins, lectins and phytic acid (Garg *et al.*, 2002). The proximate composition of the ingredients used in the formulation of diets is shown in Table 1.

All these ingredients were mixed to formulate four iso-caloric diets (D_1 - D_4) by supplementing the formulated diets with a mineral premix and amino acids (MPA). The ingredient composition and proximate composition of these four diets are shown in Table 2.

Experimental design

Fingerlings of *C. punctatus* (mean body weight 12.93 ± 0.70) were obtained from fish dealers of Hisar during April-May and acclimated in the laboratory of Department of Zoology and Aquaculture, Chaudhary Charan Singh Haryana Agricultural University, Hisar, for a minimum period of 7 days and were fed *ad libitum* on a feed containing processed soybean (31% protein level) as the protein source between $08^{00} - 09^{00}$h.

Study was conducted in transparent glass aquaria (60 × 30 × 30 cm), containing 30L of chlorine free water, in replicate of three containing 20 fingerlings each. All the replicates were kept in the laboratory where the temperature was maintained at 20±1° C and a lighting schedule of 12:12h light. All groups of fish were fed daily only once between $08^{00} - 09^{00}$ h, at the fixed feeding rate of 2% BWd^{-1} during the experimental period of 45 days.

Fish were bulk weighed every 10th day and feeding rates were managed accordingly. In order to maintain water quality, about half of the water in the aquaria was replenished daily with the water which had been previously equilibrated to the desired temperature (20-25°C).

Fish fingerlings were fed with respective diets and thereafter, the uneaten feed was siphoned out and stored separately for calculating the feed conversion ratio (FCR). Faecal matter was collected by pippeting following the method of Spyridakis *et al.* (1989) every morning. The pooled faecal samples were dried in an oven maintained at 60°C for subsequent analysis. Individual weight of the fish fingerlings was recorded at the beginning and at the end of the experiment.

Analytical Techniques

The feed ingredients, experimental diets, faecal samples and fish carcass were analyzed following the procedure of AOAC (2000). Chromide oxide levels in the diets as well as in the faecal samples were estimated spectrophotometrically following the method of Furukawa and Tsukhara (1966).

Growth parameters (such as live weight gain (g), growth per cent gain, growth per day per cent body weight and specific growth rate) were calculated using standard methods (Steffens, 1989). Apparent nutrient digestibilities (APD) of the diets were calculated according to Cho *et al.* (1982) as follows:

$$APD = 100 - \frac{100 \times \%\ Cr_2O_3 \text{ in diet} \times \%\ \text{nutrient in faeces}}{\%\ Cr_2O_3 \text{ in faeces} \times \%\ \text{nutrient in diet}}$$

Table 1 : Proximate composition of feed ingredients (% dry Matter)

S. No.	Name of the ingredient	Crude Protein	Crude Fat	Crude Fiber	Total Ash	Nitrogen Free Extract	Gross Energy (KJ/g)
1	**Hydrothermically treated soybean**	49.743 ± 0.000	15.967 ± 0.033	3.100 ± 0.058	3.800 ± 0.057	27.393 ± 0.120	22.763 ± 0.002
2	**Rice bran (oiled)**	12.873 ± 0.485	1.760 ± 0.155	19.957 ± 0.266	20.676 ± 0.333	44.733 ± 0.732	11.410 ± 0.025
3	**Groundnut oil cake**	43.490 ± 0.736	8.933 ± 0.067	7.833 ± 0.033	7.000 ± 0.057	32.740 ± 0.623	19.430 ± 0.046

All Values are mean ± S.E. of mean

Table 2: Formulation of diets using different feed ingredients

Ingredients	Diet number			
	D_1	D_2	D_3	D_4
Groundnut Oil Cake, a1	60.0	60.0	60.0	53.0
Rice bran, a2	23.0	13.0	3.0	-
Processed soybean, a3	10.0	20.0	30.0	40.0
Chromic oxide (Cr_2O_3)*	1.0	1.0	1.0	1.0
Binder (Carboxyl methyl cellulose)**	5.0	5.0	5.0	5.0
Mineral mix and amino-acids***	1.0	1.0	1.0	1.0
Proximate composition (%)				
Crude Protein	33.0	39.5	43.0	46.5
Crude Fat	9.7	8.6	8.4	6.4
Crude Fiber	9.4	9.4	8.6	6.5
Total Ash	9.1	8.4	6.5	6.8
Nitrogen free extract (NFE)	38.9	34.0	33.5	33.9
Gross Energy (KJg^{-1})	18.3	18.6	19.2	19.3

a1 & a2 – used as basic feed ingredients

a3- used as main protein source

* Cr_2O_3- used for estimating apparent digestibility

**Binder- used to make the diets water stable.

*** mineral premix and amino acid (MPA) supplemented @ $10gkg^{-1}$ diet

(each Kg contains Cu – 312 mg; Co – 45 mg; Mg – 2.114 g; Fe – 979 mg;

Zn – 2.13 g; I – 156 mg; DL-Methionine – 1.92g;

L-lysine mono hydrochloride – 4.4 g; Ca – 30%; P – 8.25%)

Water Quality Parameters

Water samples for the determination of water quality parameters were obtained in replicates of three from each treatment at 10 days interval. Water temperature (°C) was recorded daily using digital thermometer. pH, conductivity and dissolved oxygen were monitored using multiline F-set-3 (E. Merck Ltd., Germany). Other parameters like ammonical nitrogen and ortho-phosphate excretion by fish in treated water were determined (APHA, 1998) after every 10 days interval.

At the end of feeding trials, water samples from each aquarium were collected at 2h interval for the estimation of excretory levels of ammonical nitrogen (NH_4-N) and ortho-phosphate (o-PO_4) following APHA (1998) and calculated as follows:

$$\text{Ammonical nitrogen excretion} = \frac{NH_4\text{-N } (mgl^{-1}) \text{ in water}}{(mgg^{-1}BWd^{-1}) \quad \text{fish biomass (g) per L of water}}$$

$$\text{Ortho-phosphate production} = \frac{o\text{-}PO_4 \ (mgl^{-1}) \text{ in water}}{(mgg^{-1}BWd^{-1}) \quad \text{fish biomass (g) per L of water}}$$

Statistical analysis

Data was analysed following ANOVA, Duncan Multiple Range Test at 5 per cent probability level. Group means were compared by student 't' test.

RESULTS AND DISCUSSIONS

Physico-chemical parameters of water

The pH of the aquaria water fluctuated between 7.1 to 7.5. Dissolved oxygen (DO) contents ranged between 5-7 mg/l (Table 3). High DO content in diet D_2 (39.5% protein level) showed better utilization of diet by the fish; hence better growth was managed (Jindal *et al.*, 2007b).

A significant ($P<0.05$) decrease in NH_4-N and o-PO_4 was observed in the receiving waters with increase in the protein levels of the diets (Table 3) but in groups of fish fed on diets above 39.5% protein, again increase in NH_4-

Table 3: Changes in water quality parameters in different treatments

Parameters	Diet No. D_1	D_2	D_3	D_4
Dissolved oxygen (DO) mg/l	5.55 – 5.60	5.70- 5.85	5.45 – 5.57	5.36 – 5.55
pH	7.50 – 7.60	7.40 – 7.50	7.15 – 7.20	7.20 – 7.30
Water temperature (°C)	25.0 - 26.0	25.0 – 26.0	25.0 – 25.0	25.0 – 26.0
Ammonical nitrogen (NH_4-N) excretion (mg/100gBW of fish)	1.378 – 1.467	1.205 – 1.256	1.576 – 1.688	1.796 – 1.832
Ortho-phosphate (o-PO_4) excretion (mg/100gBW of fish)	1.435 – 1.478	1.363 – 1.389	1.496 – 1.538	1.503 – 1.540

All values are mean ± S.E. of mean of 3 observations.
The table shows range of water quality parameters (min. – max.)

Table 4: Effect of formulated diets on the growth performance and nutrient utilization in fish *C. punctatus*

Diet No.	Weight gain (g)	Growth % gain in Body Weight	Growth/ day (%) Body Weight	Specific Growth Rate (SGR)	Feed Conversion Ratio (FCR)	Apparent Protein Digestibility (APD)	Survival (%)
D_1	5.593^A ± 0.179	82.889^A ± 1.310	0.784^A ± 0.020	1.793^A ± 0.021	2.445^A ± 0.079	86.077^A ± 0.404	94.4
D_2	7.973^B ± 0.038	99.637^B ± 0.489	0.939^B ± 0.007	1.954^B ± 0.007	2.009^B ± 0.011	92.040^B ± 0.221	96.0
D_3	6.226^C ± 0.003	87.979^C ± 0.055	0.860^C ± 0.000	1.870^C ± 0.000	2.231^C ± 0.001	90.077^C ± 0.419	95.2
D_4	6.136^D ± 0.043	85.561^C ± 0.388	0.854^C ± 0.006	1.864^D ± 0.006	2.235^C ± 0.015	88.970^D ± 0.342	94.9

All values are mean ± S.E. of mean of 3 observations.
Data was analyzed by Duncan Multiple Range Test.
Mean with same letter in the same columns are not significantly ($p>0.05$) different

N and o-PO_4 excretion was observed. This is because when protein levels in the diets exceeds the limits of digestibility (above 200g/kg of diet) get deaminized and are excreted as ammonia in the aquaria water (Jindal, 2011a, b). Therefore, these results clearly showed that plant based (soybean) diets at 39.5% protein level less deteriorate the polluted water by excreting less ammonia and phosphorus.

In the present experiments, the peaks in the excretion of NH_4-N were observed at 8h of post-feeding (Fig. 1). But the maximum excretion of o-PO4 was observed at 6h of post-feeding (Fig. 2). These results were in agreement with those of Kalla and Garg (2004), Jindal and Garg (2005), Robinson and Menghe (2007), Jindal *et al.* (2007 a,b, 2009).

Growth and digestibility parameters

Feeding trials have revealed low mortality in all dietary treatments. Survival rate of fingerlings were also found to be significantly ($P<0.05$) highest (96.0%) in the group of fingerlings fed on D_2 containing 39.5% protein level

Table 5: Proximate carcass composition (% fresh weight) of fish *C. punctatus* fed on four experimental diets from D_1 to D_4

Diet No.	Moisture	Crude Protein	Crude Fat	Total Ash	Nitrogen Free Extract	Gross Energy (KJg^{-1})
D_1	75.070^{A} ± 0.034	15.711^{A} ± 0.022	4.496^{A} ± 0.014	2.984^{A} ± 0.005	1.738^{AC} ± 0.001	5.790^{A} ± 0.011
D_2	74.807^{B} ± 0.006	16.333^{B} ± 0.073	4.826^{B} ± 0.006	2.979^{A} ± 0.017	1.055^{B} ± 0.068	5.935^{B} ± 0.004
D_3	74.890^{B} ± 0.020	15.097^{A} ± 0.141	4.570^{C} ± 0.010	3.190^{B} ± 0.006	1.023^{B} ± 0.130	5.736^{C} ± 0.015
D_4	75.750^{C} ± 0.005	14.701^{C} ± 0.082	4.475^{D} ± 0.010	3.157^{B} ± 0.010	1.916^{C} ± 0.096	5.573^{D} ± 0.006

All values are mean ± S.E. of mean of 3 observations.
Data was analyzed by Duncan Multiple Range Test.
Mean with same letter in the same columns are not significantly ($p>0.05$) different

diet. Significantly ($P<0.05$) highest values in weight gain (g), growth per cent gain in body weight and specific growth rate (SGR%/day) were observed in group of fish fed on D_2 containing 39.5% protein (Fig. 3). However a significant ($P<0.05$) decrease in weight gain took place in the groups of fish fed on diets D_3 and D_4 containing 43.0% and 46.5% protein, respectively (Table 4).

Literature reported growth depressing effect of high dietary protein levels fed diets (above 40% crude protein level) for several fish species like, snakehead (Samantaray and Mohanty, 1997), walking catfish, *Clarias batrachus* fingerlings (Jindal, 2011b) and Indian catfish *H. fossilis* fingerlings (Jindal ,2007) and on other carnivorous fish species (Hepher, 1988).

Decrease in growth performance of fish at high protein levels may be attributed either to insufficient non-protein energy (NRC-NAS ,1983) or to the inhibitory effects of high dietary protein on the production/release of digestive enzyme (such as protease etc.). Inhibitory effects of high dietary protein levels on intestinal enzyme activity have already been shown by Kalla and Garg, 2004, Jindal and Jain, 2008 and Jindal and Yadava, 2009.

Feed conversion ratio (FCR) remain significantly ($P<0.05$) low in fish fed on diet D_2 containing 39.5% crude protein in comparison with the fish fed on low (33.0%) or high protein (43.0 and 46.5%) diets. Likewise, apparent protein digestibility (APD) was significantly ($p<0.05$) high in fish fed on D_2. (Table 4)

The growth and digestibility parameters were found to be negatively correlated with NH_4-N and o-PO4 excretion. This is one of the reason for the better growth of fish fed on diet D_2 (at 39.5% protein level).These results are in agreement with those of Kalla and Garg, 2004, Robinson and Menghe, 2007, Jindal *et al.*, 2007 a, b, 2009 and Jindal, 2011a,b.

Effect of formulated diets on carcass composition

The changes in the carcass composition indicated that the protein accumulation in the carcass increased up to the protein level of 39.5% (Diet D_2), but as the protein levels in the diets increased it started decreasing. These results indicated that optimum protein level in the diet resulted in higher growth, better digestibility with greater efficiency of protein deposition in the carcass (Table 5). On the other hand, moisture and ash contents were reported low in the groups of fish fed on diet having 39.5% protein level (D_2) as observed by Shearer, 1994, Kalla and Garg, 2004, Jindal and Garg, 2005 and Jindal, 2007, 2011b.

CONCLUSIONS

Fish have the ability to handle protein in excess of that needed for growth and maintenance by deaminizing amino acid bronchially and excreting ammonia. Protein levels above optimum requirements may results in decreased growth rates because of a reduction in dietary energy available for growth due to energy required to deaminate and excrete excess absorbed amino acids. Present studies on *Channa punctatus*, thus, revealed that when protein levels in the diet exceeds the limits of digestibility (above 200g/kg of diet) get deaminized and

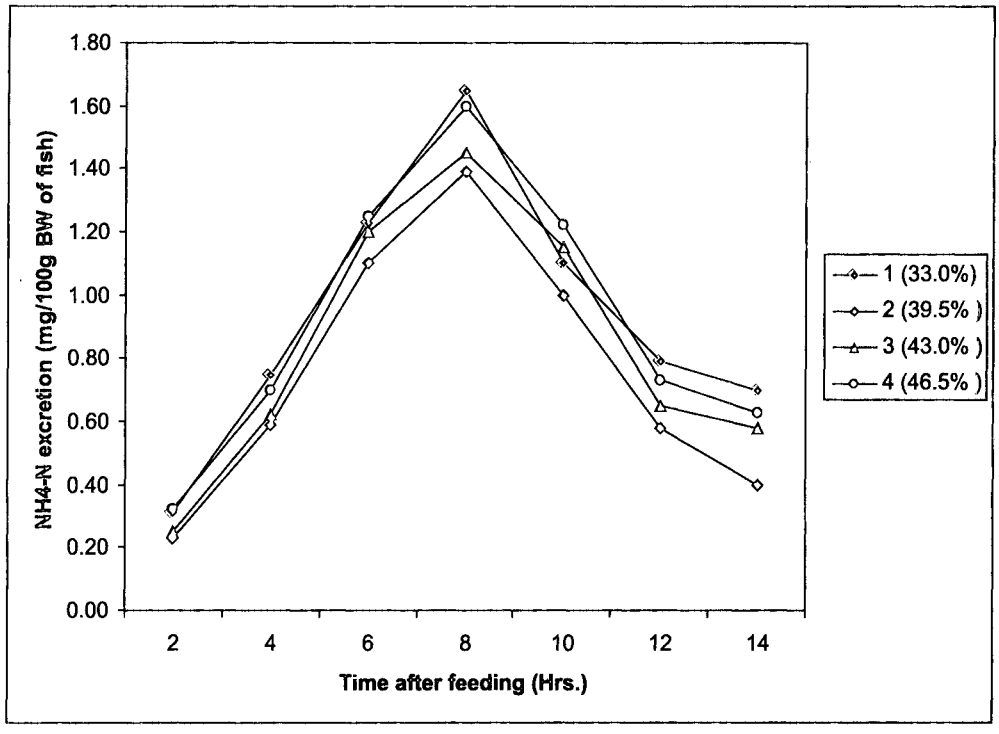

Fig. 1: Diurnal excretory pattern of ammonical nitrogen (NH_4-N) in treated waters in fish *C. punctatus* fed on formulated diets D_1 to D_4

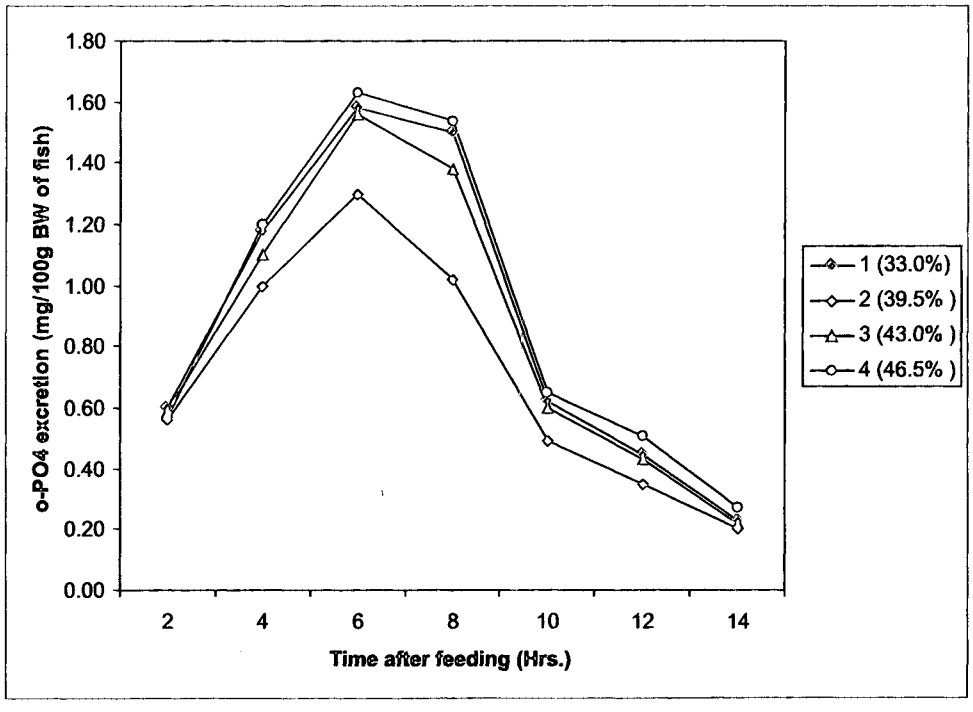

Fig. 2: Diurnal excretory pattern of ortho-phosphate (o-PO_4) in treated waters in fish *C. punctatus* fed on formulated diets D_1 to D_4

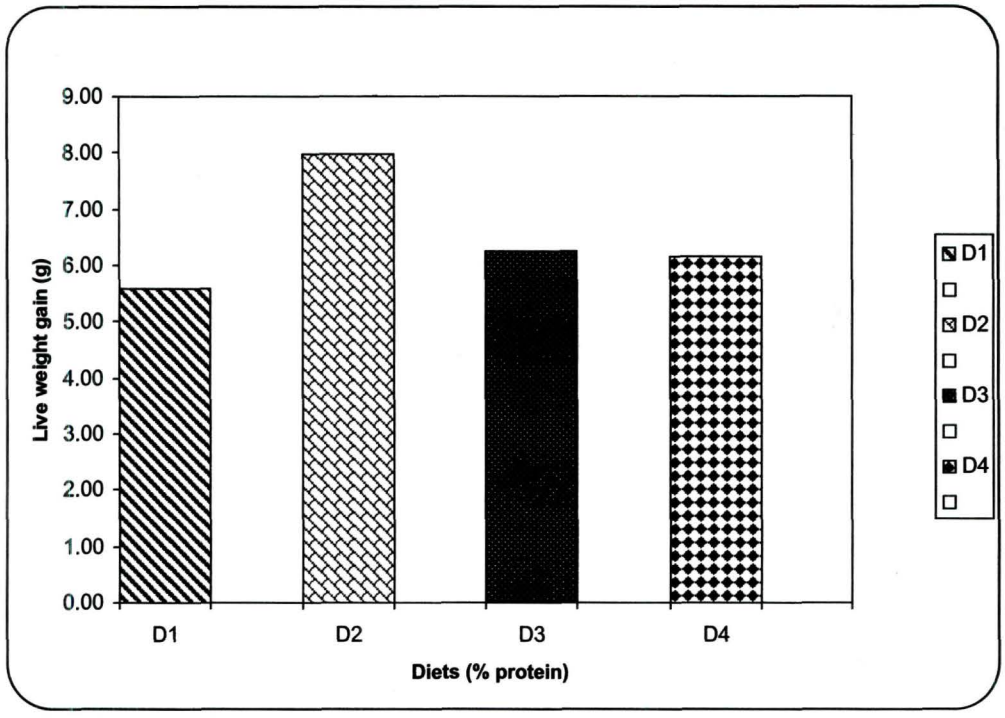

Fig. 3: Weight gain (g) in *C. punctatus* fed on different formulated diets (D_1 to D_4)

are excreted as ammonia in the culture water, which can lead stress to the fish and thus results in reduced growth.

The results of this study clearly demonstrated that PS supplemented with MPA can be recommended as a dietary protein source in the diet of *C. punctatus*, up to the protein levels of 39.5%. Such a management not only save total feed cost, but would certainly reduce excretion of ammonia and phosphorous. Hence, this technology will help to alleviate pollution problems in the intensive aqua-culture systems.

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EFFECT OF SEASONAL VARIATION ON SEMEN CHARACTERISTICS AMONG DIFFERENT BREEDS OF BULL UNDER THE AGRO-CLIMATIC CONDITION OF WEST BENGAL, INDIA

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ABSTRACT

Seasonal effect on bull semen is important to ensure the quality of cryo-preserved semen straws used in artificial insemination programme. A total of 5844 semen samples from 19 bulls, maintained under farm condition were collected during September, 2005 to March, 2008 at the Frozen Semen Bull Station, Salboni, Midnapore (West), West Bengal, India. The best season for frozen semen straw production was observed during Summer (April – May) and Monsoon (June-July) as most of the semen characteristics viz- Initial Motility, (81.20±0.13 %), Post thaw motility (71.71±0.13 %), Live sperm (81.91±0.10%), Acrosome integrity(84.15±0.13 %) and HOS response(64.16±0.13 %) were significantly ($P<0.01$) best in the Summer and Sperm concentration (892.04±8.23 $x10^6$) was significantly ($P<0.05$) best in the monsoon. Whereas the volume of semen per ejaculate (5.40±0.04 ml) were significantly ($P<0.01$) high during dry season (Oct- Nov).

Keywords *:* Cryo-preserved semen, Acrosome integrity, HOS response, Artificial insemination (AI).

INTRODUCTION

Accurate semen analysis is a critical component of any assisted reproductive technology. The semen characteristics are the major factors that influence conception rate in cattle and therefore, a determinant of efficiency of reproduction. Although Artificial insemination (AI) using frozen semen emerged 50 years back, fertility rate with A I technology is less and unpredictable. The knowledge of trend of seasonal influence on semen characteristic would help to know requirement of bulls to meet the demand for frozen semen and to provide any suitable additional managerial requirements time to time. It is essential to know the seasonal influence on seminal attributes in different breeds of bull under the agro-climatic condition of West Bengal, India.

Studies on seasonal variations in semen quality of dairy bulls have been started since many decades but there are varied results. Weather by *et al.* (1940) reported monthly averages of semen volume, concentration, and longevity was generally higher in June and July than in other months, but the differences among the months were very slight. Whereas, an observation, made on bull in Kenya, indicates the volume and motility of sperm were both low from May to August (Anderson, 1941). Volume of semen, percentage of live sperm, and percentage of resistant sperm increased during May to September but concentration and percentage of abnormal sperm did not change (Lasley, 1943). Seasonal variations have been shown to have effects on semen production and its quality (Vincent, 1972; Meyerhoeffer *et al.*, 1976; Parkinson, 1987). Godfrey *et al.* (1990) also stated that season affected sperm concentration in all breeds and significantly ($P<0.05$) decreases during the winter in Brahman Bull and during the summer in Nebraska Hereford (NH) and Montana Hereford (MH) bulls. The significant seasonal variation occurred in the incidences of sperm head abnormalities and total sperm abnormalities (Söderquist *et al.*, 1996) while Sarder *et al.* (2000) observed in a study that the volume of semen does not differ with season but other value were higher in summer compared to the winter and autumn season. Brito *et al.* (2002) opined neither ambient temperature and humidity nor month (season) significantly affected sperm production and semen quality in Brazil while Bhat *et al.* (2004) reported after a thorough study on seasonal effect on seminal attributes that the seminal volume, mass activity and number of semen straws produced were significantly high ($P<0.05$) during monsoon. Mostari *et al.* (2005) opined that seasons had significant ($P<0.05$) effect on semen volume, initial and post- thawing sperm motility and pH of semen but not on sperm motility of diluted semen and fertility. Simmental bulls had significantly higher percentages of major sperm defects during the summer than the winter (Nichi *et al.*, 2006). Sarder (2007) observed that, summer season, ambient temperature groups of >29°C

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and Relative humidity groups of >85% are suitable for excellent semen characteristics.

The present study was conducted to understand the effect of seasonal variation on semen characteristics like volume of semen ejaculates, concentration of spermatozoa, initial motility of spermatozoa, post thaw motility of spermatozoa, live and dead sperm count, acrosome integrity and hypo osmotic swelling of spermaozoa among the different breeds of bull at various temperature, humidity and rainfall in different seasons of the year under the agro-climatic condition of West Bengal, India.

MATERIAL AND METHODS

Time and place of study :-The study was undertaken at 'Frozen Semen Bull Station' of Composite State Animal Husbandry Farm, Salboni, Dist .-West Midnapore, West Bengal, India under the management of Paschim Bango Go Sampad Bikash Sanstha (ISO 9001:2000, HACCP) Govt. of West Bengal. The whole study covers the period from September, 2005 to March, 2008.

Four Jersey, four cross bred Jersey (50%), two cross bred Holstein (50%), two Red Sindhi, three Sahiwal and four Gir bulls were used in this study. These animals were regular herd sires in Frozen Semen Bull Station (FSBS), Salboni and ranged in age from three to seven years.

Animals and data used :-The Bulls were housed in individual 10 X 12 feet stalls and had access at will to 12 X 40 feet extended pen. The Bulls are placed under compulsory exercise with the Bull exerciser situated with in the Farm. The animals had no access to pasture and were maintained on Bull Concentrate feed ('Epic' brand of W.B Dairy and Poultry development Corporation, Salboni plant) at the rate of 3 kg to 5 kg per day per animal depending upon the body weight. Chaffed green fodder and paddy straw at 3:1 ration are included in the ration at the rate of 20 kg to 30 kg daily per animal according to the body weight. 30 gms of mineral mixture were also added with the daily ration for each animal. All the bulls were kept under identical condition of management, feeding (seasonal fodder) and watering.

The ambient temperature, relative humidity were recorded with in the farm campus at Agronomy section. The whole year was divided into 6 (six) season,

a) Winter (Dec-Jan),b) Spring (Feb-March), c) Summer (April- May), d) Monsoon (June-July), e) Autumn(Aug-Sept) and f) Dry(Oct-Nov). Climatological information of the farm location during the experimental seasons of the years is summarized in table 1.

Semen collection, evaluation and preservation : Semen was collected by artificial vagina (A V) method from every bull twice in a week. Artificial Vagina was prepared appropriately having proper temperature (40-44^{0}C), pressure and softness. A total number of 5844 semen samples from 19 bulls were collected during the period of study. Of 5844 ejaculates 1814 from 100% Jersey bull, 1208 from 50% Jersey x 50% Indigenous, 750 from 50 % Holstein-Friesian x 50% Indigenous, 489 from Red-sindhi, 638 from Sahiwal and 945 from Gir bulls were obtained. Immediately after collection the semen samples were placed in a beaker containing lukewarm water (37^0 C) and were examined for different semen characteristics (table.2).

Volume of the ejaculates were measured directly from graduated collecting tube and recorded in ml. **The concentration of spermatozoa** (million/ml) in fresh semen was determined by using a photometer instantly. The Photometer (Imv- France) was also calibrated with the help of Neubour Haemocytometer at regular interval. The semen sample below the concentration of 500 x 10^6 spermatozoa per ml of neat semen were not considered for further dilution and Frozen Semen Straw production.

The initial motility of sperm was evaluated in small drop of diluted semen placed on clean, pre-warmed slide covered with a cover slide and examined at magnification of 400 X under phase contrast microscope. Sperm motility was scored on the basis of the percentage of spermatozoa with normal forward progressive movement while these showing circling movement or oscillating at one place were regarded as immotile. The initial motility of spermatozoa having more than 70% of progressive motile sperm was accepted for dilution and further freezing process.

Post Thaw motility : The straw containing frozen Semen was thawed in a water bath at 37°C - 38°C for 10 – 20 seconds. The sperm motility was always evaluated at 37°C using Phase-Contrast Microscope at 400 X magnification. Post thaw motility with more than 50 % progressive motile spermatozoa is considered otherwise the batches are discarded.

Live and Dead Sperm Count : Using Eosin 5% and Nigrosin 10% the semen samples on the glass slides were stained to calculate the percentage of unstained spermatozoa (Live sperm). A maximum of 333 sperms are generally calculated to find out the live sperm by

Table 1. Meteorological data recorded during the study period from Sept, 2005 to March, 2008.at Salboni CSAHF, West Midnapore,(W.B)INDIA (Lattitude-22"37'49.11" N; Longitude-87"17'44.31" E.)

Season	No. of observation	Ambient Temperature^{0}C * Maximum	Minimum	Relative Humidity %* Morning	Noon	Rainfall (mm)
Winter (Dec-Jan)	172	24.62±2.98 (18.2-28.4)	14.28±2.38 (10.2-18.4)	69.55±2.87 (62-80)	56.47±7.67 (41-82)	3-6
Spring (Feb-March)	165	30.28±4.78 (20.1-38.6)	21.26±3.78 (15.0-29.2)	66.96±5.92 (52-80)	54.45±12.8 (30-83)	2-36
Summer	169	37.48±2.59	28.56±1.72	68.25±3.8	58.02±7.0	4.4-30.4
(April-May)		(31.4-42.4)	(24.6-35.3)	(61-82)	(40-70)	
Monsoon (June-July)	175	33.87±2.61 (28.2-40.2)	27.89±2.89 (21.6-34.4)	73.6±3.65 (64-82)	71.1±6.88 (58-85)	25-300
Autumn (Aug-Sep)	171	34.82±2.98 (24.8-39.0)	27.67±2.32 (21.0-34.8)	76.04±2.92 (70-83)	74.31±5.33 (60-84)	9-245
Dry (Oct.-Nov)	158	29.94±3.74 (22.0-39.8)	21.94±4.06 (16-29.8)	72.15±4.67 (42-81)	63.16±8.44 (53-82)	5-60
Overall	1010	0.0±0.0 (18.2-42.4)	0.0±0.0 (10.2-35.3)	0.0±0.0 (42-83)	0.0±8.02 (30-85)	

*Data is presented as Mean±SD (Range)

Table-2. Number of Semen collection per season

Sl No.	Breed	No. of Bull 1	Collections Winter (Dec-Jan)	Spring (Feb-March)	Summer (April-May)	Monsoon (June-July)	Autumn (Aug-Sep)	Dry (Oct-Nov)	Total
1	JERSEY	4	315	334	230	237	360	338	1814
2	C B J	4	223	245	156	174	208	202	1208
3	CBHF	2	133	135	89	110	144	139	750
4	R Sindhi	2	85	92	84	79	67	82	489
5	SAHIWAL	3	125	126	86	86	105	110	638
6	G I R	4	152	165	160	153	159	156	945
	Total	19	1033	1097	805	839	1043	1027	5844

using following formula.

Percentage of Live sperm = No. of unstained sperm X 3/10.

The semen samples containing at least 80% of live sperm are considered for freezing.

Acrosome integrity Test : A thin smear of frozen semen on glass slide was air dried and immersed in 5% formaldehyde for 30 minutes at 37°C. After washing the slide in distilled water and dried in air, it is immersed in Giemsa stain for 3 hours at 37°C. [Giemsa stain (3ml) mixed with 2 ml of Sorenson phosphate buffer at pH 7 with 45 ml of distilled water] and then the slide was washed in water and dried in air for examination. It was examined under DIC microscope with oil immersion objectives (Watson, 1975). A total of 100 spermatozoa per sample were counted to classify the acrosome integrity at 'intact', 'altered' or completely loss. The sample containing more than 30 – 40 % of acrosomal alteration are not accepted.

Hypo-Osmotic Swelling Test (HOST) : Hypo-osmotic swelling (HOS) medium is prepared with equal volume of sodium citrate solution (0.367 gm of sodium citrate in 50 ml of triple glass distilled water) and fructose solution (0.675 gm of Fructose in 50 ml of triple glass distilled water). 0.1 ml of frozen thawed semen was mixed with one ml of HOS medium and incubated the mixture at 37°C for 30 minutes. One drop of incubated semen-HOS media mixture was placed on glass slide under cover slide and examined with the help of DIC microscope for the detection of tail curling of spermatozoa (Jayendram *et al.*, 1984). Good semen sample containing about 60 – 70 % of spermatozoa with tail curled in frozen thawed semen was accepted .

$$\text{Percentage of Spermatozoa with tail curling} = \frac{\text{no. of tail curled sperm X100}}{\text{total no sperm counted}}$$

Statistical analysis: Collected data were analyzed using "MSTAT" computer program to compute analysis of variance (ANOVA) according to Steel and Torrie (1984).

RESULTS

The observation on the effect of seasonal variations of semen characteristics among 19 (Nineteen) bulls of different breeds between the period from September 2005 to March 2008 are presented in this study. To predict the status of semen quality, both pre and post freezing evaluation were carried out with non-conventional methods like acrosome integrity test, hypo-osmotic swelling test and the observations are presented in this study. Seasonal variations in the semen quality of bulls used for the study are presented in table 3. No significant differences (P> 0.05) of semen characteristic between the bulls were observed due to seasonal variations.

Volume of semen. The overall highest volume of semen was found (Table 3) during dry season (5.40±0.04 ml) followed in order by spring (5.22±0.03 ml), summer (5.20±0.04 ml), monsoon (5.09±0.03 ml), autumn (5.07±0.04 ml) and winter (4.90±0.03 ml). The values of dry season were significantly (P<0.01) affected by season (Table. 4).

Sperm concentration. The best sperm concentration was obtained significantly (P<0.05) in monsoon (892.04±8.23 million/ml) and significantly (P<0.01) lowest in autumn (812.30±6.23 million/ml) (Table 4). The values during other seasons were 882.51±6.58 million/ml in dry, 869.26±7.17 million/ml in winter, 848.69±6.20 million/ml in spring and 837.08±7.55 million /ml in summer.

Initial sperm motility. The variation was observed in initial sperm motility significant (P<0.01) among the seasons (Table 3a). The peak value of motility was observed in summer (81.20±0.13 %), next in order were in the autumn (80.33±0.12 %), monsoon (80.33±0.13 %), spring (80.06±0.11 %), dry (79.37±0.12 %) and winter (78.43±0.12 %).

Post thaw motility. The results of post-thaw motility in different seasons are displayed. Significant (P<0.01) variation in post thaw motility was observed between summer and other seasons (Table 3a). The best motility was observed in summer (71.71±0.13 %) followed in order by autumn (70.98±0.13 %), monsoon (70.97±0.12%), spring (70.64±0.12%), dry (70.21±0.12%) and winter (69.03±0.11 %).

Live and Dead sperm count. The statistical analysis (Table 3a) of this parameter shows (P<0.01) the maximum live spermatozoa (81.91±0.10 %) during summer followed by 81.17±0.11 % in monsoon, 81.11±0.09 % in autumn, 80.87±0.09 % in spring, 80.06±0.10 % in dry and 79.06±0.09 % in winter

Acrosome integrity test. Observations on acrosome integrity test indicates the significantly (P<0.01) highest percentage of intact acrosome in summer (84.15±0.13 %) against the overall seasonal values (Table 3a). The other seasonal values of intact acrosome were observed as 83.71±0.12 % in autumn, 83.62±0.09 % in spring, 83.58±0.13 % in monsoon, 83.47±0.12 % in winter and

Table -3. Mean (±SE) of Volume (ml), Initial motility(%), Sperm concentration (x 10^6),Post thaw motility(%), Live sperm(%), Intact Acrosome(%) and HOS positive(%)semen samples from 19 bulls at different season of the year

Semen Characteristics	Season						
	Winter (n=1033)	Spring (n=1097)	Summer (n=805)	Monsoon (n=839)	Autumn (n=1043)	Dry (n=1027)	Over all (n=5844)
Volume (ml)	4.90±0.03	5.22±0.03	5.20±0.04	5.09±0.03	5.07±0.04	5.40±0.04*	5.15±0.01
Initial Motility(%)	78.43±0.12	80.06±0.11	81.20±0.13*	80.33±0.13	80.33±0.12	79.37±0.12	79.95±0.05
Sperm concentration ($x10^6$)	869.26±7.17	848.69±6.20	837.08±7.55	892.04±8.23**	812.30±6.23	882.51±6.58	856.84±2.83
Post thaw motility(%)	69.03±0.11	70.64±0.12	71.71±0.13*	70.97±0.12	70.98±0.13	70.21±0.12	70.59±0.05
Live sperm (%)	79.06±0.09	80.87±0.09	81.91±0.10*	81.17±0.11	81.11±0.09	80. 06±0.1	80..70±0.04
Intact Acrosome(%)	83.47±0.12	83.62±0.09	84.15±0.13*	83. 58±0.13	8 3. 71±0.12	82.79±0.13	83.56±0.05
H O S positive (%)	63.29±0.10	63.62±0.12	64.16±0.13*	63.54±0.12	63.71±0.13	62.69±0.12	63.50±0.05

*P<0.01, **P<0.05, n= no. of observations

82.79±0.13 % in dry season.

Hypo-osmotic swelling test. The highest significant (P<0.01) percentage of hypo-osmotic swelling positive spermatozoa were found (Table 3a) during summer (64.16±0.03 %) followed in order by autumn (63.71±0.13 %), spring (63.62±0.12 %), monsoon (63.54±0.12%), winter (63.29±0.10 %) and dry season (62.69±0.12%).

DISCUSSION

Season have significant effect (P<0.01 & P<0.05) on most of the semen characteristics. **The volume of semen ejaculates** was significantly (P<0.01) highest (5.40±0.04 ml) during dry season (October– November) followed by second highest (5.22±0.03 ml) during spring (February-March) compared to lowest (4.90±0.03 ml) during winter season(December-January). The highest significant (P<0.05) sperm concentration was observed in monsoon (892.04±8.23 million/ml) and lowest significant (P<0.01) concentration were recorded during autumn season. The present results conform to those of Mostari *et al.* (2005), Vincent (1972), Meyerhoeffer *et al.* (1976), Parkinson (1987) and Sarder (2007) except the observation on semen volume. Mostari *et al.* (2005) recorded the highest volume of semen ejaculates during summer season, while Brito *et al.* (2002) opined neither ambient temperature nor humidity nor month (season) significantly affected sperm production and semen quality which differs also from the present observation. But the observation recorded by Ruttle *et al.* (1975) as the larger semen volume during spring and lowest in winter season is very much close to the present study.

The number of semen straws produced were significantly high (P<0.05) during monsoon (Bhat *et al.*, 2004), and corroborates with the present observation on sperm concentration, as more straws are produced from the semen with more sperm concentration.

The initial motility of spermatozoa was found significantly (P<0.01) highest (81.20±0.13 %) in summer which again supports the findings of highest post thaw motility (71.71±0.13 %) in the same season and the observation was in agreement with Mostari *et al.* (2005), as they found the highest motility in summer. Lowest initial motility (78.43±0.12 %). was recorded in winter season in this study which also confirm the findings of Swanson and Herman (1944) who concluded that initial motility and useful viability was lower in winter than in spring and summer.

The percentage of live, intact acrosome and HOS positive spermatozoa were also found significantly (P<0.01) highest during summer and further confirms that the summer is the best season for the production

Table- 4. ANOVA showing the effect of season on Volume , Initial motility, Sperm concentration ,Post thaw motility, Live sperm, Acrosome Integrity and Hypo-Osmotic swelling (HOS) of semen

Source of variances	d.f	Volume of semen		Initial Motility		Sperm Concentration.		Post thaw motility		Live sperm		Acrosome Integrity		HOS	
		MSS	'F'	MSS	'F'	MSS	'F'	MSS	'F'	MSS	'F'	MSS	'F'	MSS	'F'
Season	5	0.543	15.94	17.142	18.54	17072.523	7.005	15.638	17.289	18.811	18.26	3.729	8.330	4.547	8.696
Bulls	18	0.018	0.54	0.163	0.177	65381.359	26.827	0.168	0.187	0.167	0.162	0.687	1.536	0.717	1.373
Season x Winter	1	0.589	17.32	21.903	23.69	1465.808	0.601	23.062	25.494	25.355	24.613	0.061	0.137	0.400	0.767
Season x Spring	1	0.052	1.52	0.108	0.117	630.280	0.258	0.026	0.028	0.288	0.279	0.043	0.096	0.146	0.280
Season x Summer	1	0.029	0.847	14.918	16.13*	17382494	7132.5	11.890	13.15*	13.861	13.455*	3.415	7.628*	4.089	7.822*
Season x Monsoon	1	0.034	0.993	1.356	1.46	11772..64	4.830**	1.348	1.490	2.122	2.060	0.007	0.016	0.009	0.019
Season x Autumn	1	0.049	1.458	1.349	1.46	18843.092	7.731*	1.424	1.575	1.605	1.558	0.218	0.488	0.411	0.788
Season x Dry	1	0.235	6.89*	3.219	3.48	6261.298	2.569	1.347	1.488	3.795	3.684	5.577	12.46*	6.308	12.06*
Monsoon x Dry	1	0.922	27.09	8.755	9.46*	862.798	0.354	5.388	5.958**	11.594	11.255*	5.992	13.386*	6.812	13.030*
Error	108	0.034	-	0.925	-	2437.0839	-	0.904	-	1.030	-	0.447	-	0.522	-

*P<0.01, **P<0.05

of good quality semen. This is in the same meanings with the observations of Sarder (2007). But it differs from the findings of Söderquist *et al.* (1996) as they observed the incidence of sperm head abnormalities and total sperm abnormalities were significantly higher during the warmer seasons (spring, summer) compared with the colder ones (autumn, winter). These discrepancies may be due to the fact that the months comprising the seasons in this study, were not exactly similar in the season of their study and also the difference of ambient temperature and humidity between seasons of this study and their study.

There is a tremendous scope of improving the present situation through crossbreeding or grading up of the non-descript as well as indigenous low producing cattle population , preferably with optimum and steady conception rate through artificial insemination with quality frozen semen. The outcome of the work have a commendable role for a successful artificial insemination programme to assess the requirements of desired bull at different seasons of a year. It will also help to predict the managerial requirements at different season to fulfill the demands of field activity on artificial insemination on different breeds and quality of frozen semen straw. Maximum number of bulls can be utilized at the best season for production of optimum quality as well as quantity of frozen semen. The results of the present investigation would be a milestone for the advancement of artificial insemination technology.

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NOTES ON FRESH WATER CHLOROCOCCALES OF UDAIPUR DISTRICT, RAJASTHAN, INDIA

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ABSTRACT

The major water bodies were surveyed for enlisting various Chlorococcalean members as major part of planktonic Algae of Udaipur District, Rajasthan. Forty two taxa were recorded during the study period March 2004 to April 2007. The present work is the first record of Chlorococcales from the study area.

Keywords. Algae, Lakes, planktons, blooms.

INTRODUCTION

The present work is an outcome of exploring algal diversity of South Rajasthan. The work on algal diversity of south Rajasthan is very scanty 'therefore' the present work deals with the systematic and ecological study of Chlorococcales, a major part of planktonic algae. The work on Chlorococcales has been carried out from different regions of India by many workers like Bruehl and Biswas (1926), Gonzalves and Joshi (1946), Biswas (1949), Mitra (1951), Gupta (1956), Bharati (1964), Philipose (1967), Hortobadyi (1969), Patel (1970), Patel and Isabella (1977), Shashi kant and Anand (1978), Pandey et al (1983)

Udaipur is situated towards the south of Rajasthan State on longitude 73°42' East and latitude 24°35' North. It is popularly known as the city of Lakes and is flanked by a number of artificial lakes like Jaisamand, Fateh Sagar, Lake Pichhola, Lake Badi etc. For the present study above water bodies were selected. The period of surveyed ranged from March 2004 to April 2007.

MATERIAL AND METHODS

The samples from various lakes and neighboring places were collected by using planktonic nets of various pore sizes. Microalgal samples were centrifuged and cultured, if not identified. The identification was done by standard methods and keys (Philipose 1967; Fritsch 1935, Prescott, 1962; Bold and Wynne, 1985; Palmer, 1980; Dillard 1999.)

SYSTEMATIC ENUMERATION

Golenkinia radiata Chodat (P I. Fig. 23) ; Philipose, 1967, 102, Fig. 27,

Cells solitary, spherical, with the entire cell wall covered by a number of long bristles, chloroplast cup shaped and with a pyrenoids. Cells 3.7-9.37 µmmm wide and bristles of unequal length ranging from 29 to 37mm.

Habitat- Planktonic in small tank near Jaisamand, May, April 2004.

Chlorella vulgaris Beijerinck (Pl. II Figs.16, 17, 18); Philipose, 1967,173, Figs. 82a,b, c, d.

Cells ovoid with usually a single chloroplast is in form of a parietal plate. Cells diameter ranging from 1.37 to 2.7mm

Habitat – With algal bloom after rainy season, Doodh Talai, Udaipur during August- September 2004 & 2005.

Ankistrodesmus spiralis (Turner) Lemmermann (PII. Fig.11); Philipose, 1967, 210, Fig. 119 a,b,c.

Acicular cells in group of 8-16, sometime two, cells spirally twisted round one another in the median region, but free at the ends. Cells up to 4.5 mm broad and up to 35mm.

Habitat - planktonic in Fateh Sagar, Swaroop Sagar, Pichhola Lake, Doodh Talai, and it is a part of algal bloom, July and August 2004 and 2005.

Ankistrodesmus spiralis (Turner) Lemmermann var. *fasciculatus* G.M. Smith (Pl.II Fig.10);Philipose, 1967, 211, Fig. 119 d.

Cells curved or sigmoid, twisted around one another and united in colonies of 50- 200 cells, cells with the median portion of the cells in contact and apices free. Cells diameter up to 4.7mm, up to 60 mm long. Colonies

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diameter ranging from 89-190mm.
Habitat- Jaisamand,Udaipur July-August 2004.

Ankistrodesmus falcatus (Corda) Ralfes var. *duplex* (Küetz.) G.S.West (**P I, Fig 28**) Ahmed *et al,* 1983, p. 400, Pl. III, f. p

Cells aggregated, planktonic, longitudinally arranged and joined by side walls. Cells fusiform, straight, 2.5-5.4mm in diameter and 35.0-43.5mm long

Habitat - Drainage of Udai Sagar, Udaipur Oct, 2006

Selenastrum gracile Reinsch (Pl.I, Fig. 25) Philipose, 1967, P. 219, Fig.128.

Cells loosely aggregated, lunate, with acute apices. Cells 3.5-6.0mm broad at middle and 11.5-21.0mm long

Habitat- Planktonic in tank, Science College campus, MLSU, Udaipur Dec. 2004

Chlorococcum infusionum (Schrank) Meneghini (Pl.II. Figs. 12, 13); Philipose, 1967- 73, Fig. 1.

Cells usually spherical, rarely ovoid or elongated and of variable dimensions, solitary or in flat irregular colonies, chloroplast like a hollow sphere with a notch on one side and with a single pyrenoid. Cells 10-109mm, rarely up to 135 mm in diameter.

Habitat- In crude culture of moist soil of swaroop sagar,Udaipur June 2004.

Coelastrum microporum Naeg.(Pl.I, Fig.24); Philipose1967, P. 228, f. 135

Colonies usually 16 celled or more, 35.0-62.0 mm in diameter. Cells ovoid sometimes spherical with sheath, 7.0-12.5mm wide.

Habitat- Planktonic in Udai sagar,Udaipur Oct. 2006.

Actinastrum hantzschii Lagerheim (Pl. II, Fig. 21); Philipose, 1967, P. 217, f. 125 a-c.

Alga colonial or sometimes solitary cells, number of cells not fixed in colony, cells fusiform, slightly tumid at middle and tapering towards both ends. Cells 4.7-6.7mm wide at middle and 23.5-26.9mm long. The specimen differs from type in having irregulare arrangement of cells instead radial arrangement.

Habitat- Planktonic in flowing water, Uday Sagar,Udaipur, Oct. 2006.

***Characium acuminatum* A. Braun ex Küetzing (Pl. I Fig. 7); Philipose,1967, p. 83.**

Planktonic cells oblong with the apex in the form of a short acuminate beak, stalk short and with a basal knob-like thickening. Cells 5.26 mm broad and 19-72 mm long.

Habitat: Doodh Talai, Fateh Sagar, Udaipur attached on *Spirogyra* sp.& *Cladophora* sp. Collected during all seasons of 2004-05.

Shroederia planctonica Skuia. (Pl. II Fig.7); ; Philipose, 1967, P.90, f. 18.

Cells tumid at middle and acute at apices. Cells 7.8-15.0 mm broad and up to 37.0 mm long.

Habitat- Planktonic in pond, way to Kumbhalgargh Sanctuary, Rajasamand 2005.

Tetraedron minimum (A.Braun) Hansgirg (Pl. II Fig.19); Philipose, 1967,138, Fig.53a,b,c.

Cells small and quadrangular with the sides concave and angles rounded cell wall smooth. Cells 4.7- 9.6mm wide. Habitat – Jaisamand lake near Udaipur, July, August 2004.

Tetraedron regulare Küetz. (Pl. II, Figs. 14, 15); Philipose, 1967, P. 145, f. 60, a-d, f.

Cells 12.5-22.0mm wide and spines 6.0-6.5mm long; Habitat- Pichhola Lake, Udaipur Jan. 2006.

Pediastrum integrum Naegeli (Pl.I, Fig. 26, 27). (Philipose, 1-67, P. 112)

Colonies 8-16 celled, not perforated, marginal cells with two short stumpy processes. Inner cells usually pentagonal 11.5-34.0 mm wide. Previously not recorded from Indian region.

Habitat- Planktonic in small pond, way to kumbhalgargh Sanctuary, 2005.

Pediastrum simplex Meyen (Pl. II Fig. 1) . Philipose 114, f. 36 a-c, 1967.

Colonies circular to oval, generally 16-32 cells. Inner side of marginal cells nearly straight, outer side produced into a gradually tapering process, side concave, inner cells polygonal, cells in contact with adjacent ones and usually without intercellular spaces. When present, intercellular spaces very small and few in numbers, cells wall smooth or punctate to granulate. Cells up to 12 mm broad and length vary from 14 mm to 28 mm.

Habitat - The alga was collected during September from all Lakes of Udaipur 2005.

Pediastrum simplex var. *duodenarium* (Bailey) Rabenh (Pl.II Fig. 9); Philipose, 1967, 115, f. 36 d-h.

Specimen with large intercellular spaces, cells arranged in a ring at the periphery, inner face of marginal cells concave, outer face prolonged into a single delicately tapering process, sides of marginal cells also concave, interior cells similar to the marginal cells but with shorter processes, cell wall finely punctate or smooth. Colonies usually found in 16 cells. Cell width ranging from 9.34 to 27.5 mm length up to 47.7mm.
Habitat- planktonic in Swaroop Sagar,Udaipur September 2005.

Pediastrum duplex Meyen (Pl.II Fig. 1); Philipose, 1967, 121, f. 43 a-b,.

Planktonic, colonies usually of 16-32, sometimes of 4, 8, 64, or 128 cells with small lens-shaped perforations between cells, inner cells quadrate to angular and not in contact at the central portion of the side walls, inner side of marginal cells concave, outer side produced into two short truncate processes. Cells 8-21 m in diameter,16- celled colonies up to 90 mm in diameter.
Habitat: found in all stagnant water bodies Udaipur July-August2004-05

Pediastrum duplex var.*subgranulatum* Rociborski (Pl. II Fig. 3); Philipose, 1967, 125, f. 43 c, j.

Colonies usually with 16-32 cells. Cell wall granulated. Cells width 12-18 mm length up to 40mm; Habitat-planktonic in Lake Bari and Jaisamand, Udaipur Oct 2005.
Pediastrum duplex var. *clathratum* (A. Braun) Lagerheim (Pl.II Fig.4); Philipose, 1967, 123, f. 43 e, f.

Cells with deeply emginates sides and larger intercellular spaces than the type species. Other chacteristicts same as *P. duplex*

Habitat- planktonic form found in all study areas of Udaipur district, 2005- 06.

Pediastrum boryanum (Turnip) Meneghini (PII Fig.6); Philipose, 1967, 118 f. 40 a.

Cells width up to 27.5mm and length up to 10.5 mm.

Habitat - Doodh Talai, Pichhola Lake, Udaipur 2005-06.

Pediastrum tetras var. *excisum* (Rabenh.) Hansgirg (Pl. I Fig.22); Philipose, 1967, 129, f. 45 f.

Cells length ranging from 7 to 15 mm. Eight celled colonies 20-30 mm and 16 colonies up to 50 mm in diameter; Habitat- small pond in Fateh Sagar, Udaipur January 2006.

Pediastrum tetras var. *tetraodon* (Corda) Hansgirg (Pl.II Fig.5) ; Philipose 129, f. 45 d, e, g. 1967.

Cell diameter up to 20mm; Habitat - small ditches near Ashoke Nagar, Udaipur July, 2006.
Pediastrum ovatum (Ehr.) A. Braun (Pl.II Fig.8); Philipose, 1967, 115, f. 37 a-g.

Cell wall smooth of identified specimen. Four-celled colony up to 70.5mm, and 16 celled colony up to 97.5mm in diameter. Cells 9.4- 15.7 mm broad and 16-25 mm long.
Habitat - Swaroop Sagar April 2004.

Hydrodictyon reticulatum (Linn.) Lagerheim (Pl.II Fig.20); Philipose, 1967, p. 134, f. 48.

Cells up to 250 mm broad and up to 1.5 cm long. Nets up to 20 cm long.

Habitat – Near Swaroop sagar, Fateh sagar, surrounding area of Jaisamand march 2005-06.

Hydrodictyon indicum Iyenger; Iyenger, 1925, P.316, Pl. 1-4; Philipose, 1067, P. 134, f. 49.

Cells are uninucleate in early stage (13.6mm wide and 32.7mm long) and become coenocytic on maturity, 672.0-682.0mm wide and 1 cm or longer. Cell wall bilayered but short knob like projections not observed; Habitat- Swaroop sagar, Udaipur Nov. 2004.

Scenedesmus acuminatus (Lagerheim) Chodat (Pl. I, F.4); Philipose, 1967, P. 251, f.161.

Cells 3.5 – 4.0 mm wide and 20.0-24.0 m long.

Habitat- Plannktonic in Fateh Sagar,Udaipur July 2005.

Scenedesmus bijugatus (Turnip) Küetzing (Pl. I Fig.5); Philipose , 1967, p. 252, f. 164, c, e, f .

Cells up to 7.89 mm broad, 21.04 mm long; Habitat. Doodh Talai, Udaipur. July 2005

Scenedesmus bijugatus var. *bicellularis* (Chodat) Philipose (Pl.I Fig.7); Philipose, 1967, p. 253, f. 164, d, n, o, f .

Single cells are more prominent in crude culture. Cells up to 4.47 mm broad and 10.3m long; Habitat- found in crude culture of Doodh Talai, July 2005.

Scenedesmus bijugatus var. *graevenitzii* Bernard (Pl.I Fig.8); Philipose, 1967, p. 253, fig. 164, a, b.

Cells 4.7- 8.1mm broad and up to 19.5mm long

Habitat – as plankton in all study area, 2005-06.

Scenedesmus bijugatus var. *alternans* (Reinsch) Hansgirg.(Pl. I Fig. 9); Philipose, 1967, p. 255, fig. 164, g.

Length of cells up to 14.5mm and width up to 5.0mm Habitat- as plankton in small pond near Jaisamand February 2005.

Scenedesmus quadricauda (Turnip) Brebisson (Pl.I Fig. 10); Philipose, 1967, p. 283, Fig. 187 a.

Cells 3-7 µm broad, 9-18.5 µm long. Spines 6.5 – 15 µm long.

Habitat- Fateh sagar, Pichhola Lake, Doodh Talai, Swaroopsagar, Jaisamand

Scenedesmus quadricauda var. *longispina* (Chodat) G. M. Smith (Pl.I fig. 13); Philipose, 1967, p. 285, Fig. 187 b,c.

Cells 4.0µm broad, up to 11.5 µm long. Spines generally more than 10.5 µm.

Habitat- ponds near Nimach Mata Mandir, April 2006.

Scenedesmus quadricauda var. *quadrispina* (Chodat) G. M. Smith (Pl.I fig. 14); Philipose, 1967, p. 285, Fig. 187 d, j.

Cells 3.7-6.7µm broad, up to 9.6µm long.

Habitat - small tank near Lake Jaisamand, July 2005.

Scenedesmus quadricauda var. *maximum* W. et G. S. West (Pl.I fig. 16); Philipose, 1967, p. 286, Fig. 187 g.

Cells up to12 µm broad, up to 28µm long. Spines 21-28µm long.

Habitat- small stagnant water body, near Science College. January 2006.

Scenedesmus bernardii G. M. Smith (Pl.I fig. 6); Philipose, 1967, 251, Fig. 162 a, b.

Cells 4.47 µm wide and 23.5 µm long; Habitat- mid way of Lake Bari, September 2005.

Scenedesmus obliquus (Turnip) Küetzing (Pl.I fig. 1); Philipose, 1967,248, Fig. 159 a,b,c

Cells 2.73- 4.47 µm broad and up to 21 µm long.

Habitat- Sewage Canal near Fateh Sagar, august 2004-05.

Scenedesmus dimorphus (Turnip) Küetzing (Pl.I Fig. 2,3); Philipose, 1967,249, Fig. 160 a, b, c

Cells 5.57 µm broad and 18-25 µm long.

Habitat - found in all study area during July, August 2004-05.

Scenedesmus arcuatus var. *capitatus* G. M. Smith (Pl.I fig. 15, 19); Philipose, 1967, 257, Fig. 166, d-i.

Cells 7.89 µm broad and up to 16-21µm long

Habitat- identified from crude culture of Sewage Canal, Fateh sagar February 2005.

Scenedesmus abundans (Kirchner) Cohodat (Pl.I fig. 11); Philipose, 1967, 278, Fig. 184 a-d.

Cells 2-7 µm broad, 6-15 µm long. Spines 3.5 – 8 µm long.

Habitat; Swaroop sagar, Fateh sagar 2005-06.

Scenedesmus abundans var. *brevicauda* G. M. Smith (Pl. I fig. 12); Philipose, 1967, 279, Fig. 184, e.

Cells up to 2.73 µm and up to 6.5µm long. Four celled colony up to 10.7 µm long and 6.5µm broad; Habitat - Lake Jaisamand and adjacent area, Feb 2005.

Summary – Chlorococcales were abundantly found in various lakes and other places of Udaipur district. During survey of algal community the most common Planktonic and bloom creating species are *Scenedesmus quadricauda* (Turnip) Brebisson, *Scenedesmus abundans* (Kirchner) Chidat , *Scenedesmus bijugatus* (Turnip) Küetz, *Coelastrum microporum* Naeg., *Pediastrum simplex* Meyen, *Pediastrum duplex* Meyen , *Ankistrodesmus spiralis* (Turner) Lemmemann, *Chlorella vulgaris* Beijerinck, *Hydrodictyon reticulatum* (Linn.) Lagerheim .

Pediastrum species were commonly found in oligotrophic water bodies like lake Jaisamand and Bari, *Scenedesmus* species create bloom condition in eutrophic water bodies like Swaroop Sagar , Ahar river (Both receive sewage of City) while *Chlorella vulagaris* Beijerinck abundantly found in Mesotrophic water body Doodh Talai and Pichhola lake. *Hydrodictyon* species create bloom condition and form thick blanket on water surface in slowly running water bodies or most commonly in drainage of various lakes like Udai Sagar, Jaisamand during Dec. to March. It was also noted that Chlorococcalean bloom replaced by Cyanobacterial bloom after winter season. *Characium acuminatum* A. Braun ex Küetzing found attached on hydrophytes or filamentous algae like *Cladophora* spp. and *Pithophora* spp. The presence of higher population of chlorococcales in organic rich water bodies is also a method of self-purification (e.g. degradation of surfactants), which has been observed by many workers.

PLATE-I

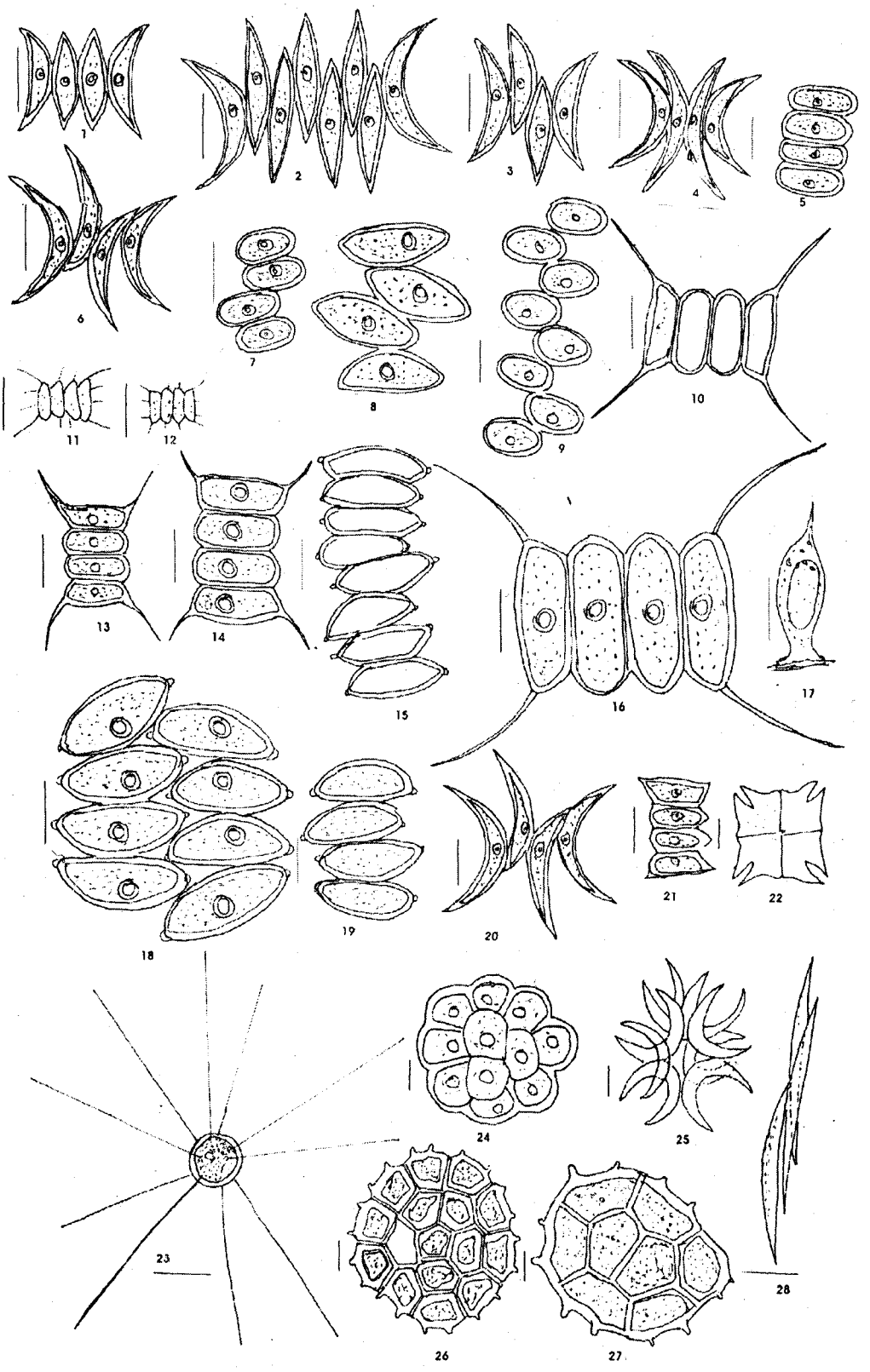
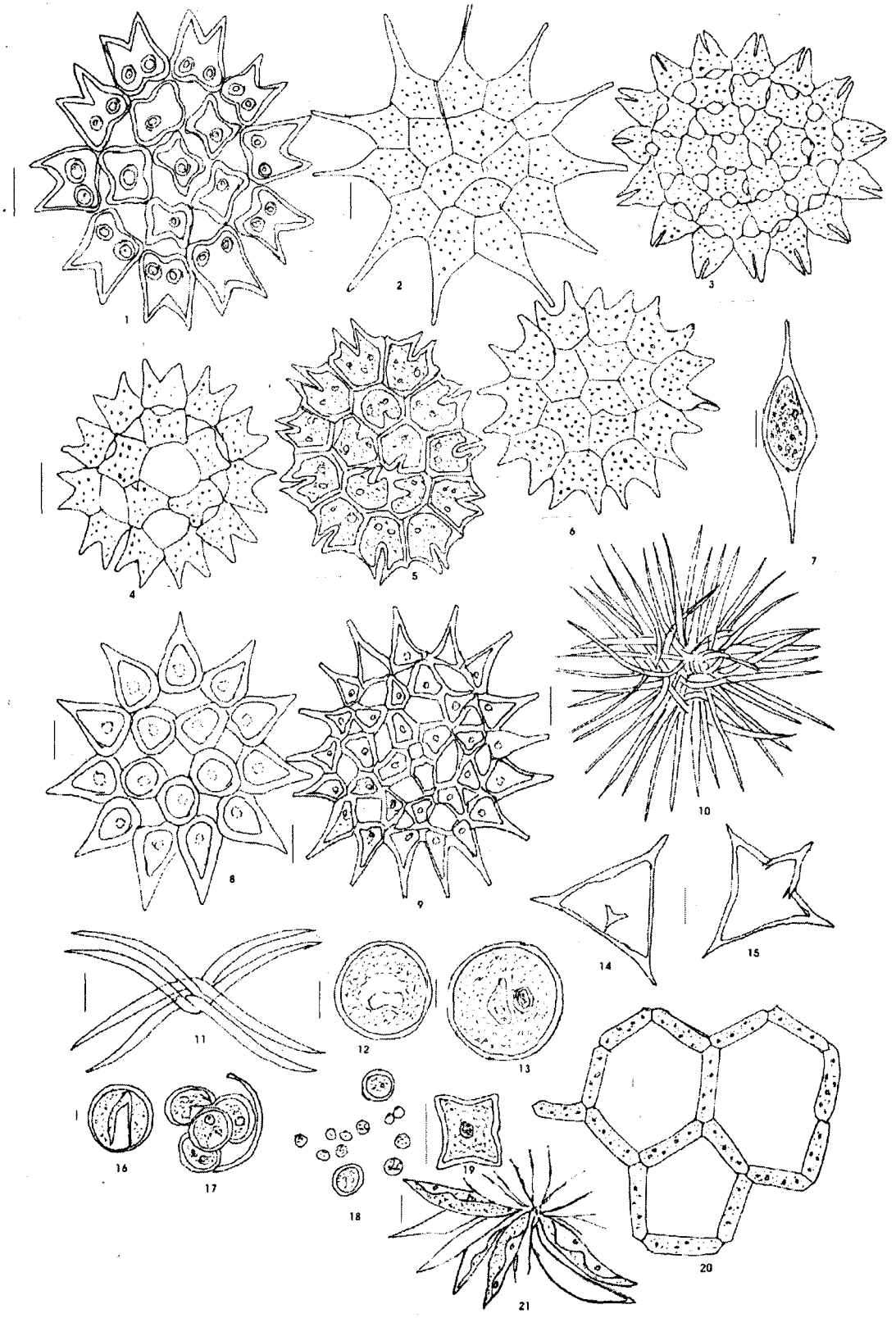


PLATE-II



LEGENDS OF PLATES

Plate-I

1. *Scenedesmus obliquus* (Turnip) Küetzing
2. *Scenedesmus dimorphus* (Turnip) Küetzing
3. *Scenedesmus dimorphus* (Turnip) Küetzing
4. *Scenedesmus acuminatus*(Lagerheim) Chodat
5. *Scenedesmus bijugatus* (Turnip) Küetzing
6. *Scenedesmus bernardii* G.M. Smith
7. *Scenedesmus bijugatus* var. *bicellularis* Chodat
8. *Scenedesmus bijugatus* var. *graevenitzii* Bernard
9. *Scenedesmus Bijugatus* var. *alternans* (Reinsch) *Hansgirg*
10. *Scenedesmus quadricauda* (Turnip) Brebisson
11. *Scenedesmus abundans* (Kirchner) Chodat
12. *Scenedesmus abundans* var. *brevicauda* G.M. Smith
13. *Scenedesmus quadricauda* var. *longispina* (Chodat) G.M. Smith
14. *Scenedesmus quadricauda* var. *quadrispina* (Chodat) G.M. Smith
15. *Scenedesmus arcuatus* var. *capitatus* G.M. Smith
16. *Scenedesmus quadricauda* var. *maximum* W. et G.S. West
17. *Scenedesmus acuminatum* A. Braun ex Kuetzing
18. *Scenedesmus arcuatus* var. *capitatus* G.M. Smith
19. *Scenedesmus arcuatus* var. *capitatus* G.M. Smith
20. *Scenedesmus bernardii* G.M. Smith
21. *Scenedesmus obliquus* (Turnip) Kuetzing
22. *Pediastrum tetras* var. *excisum* (Rabenh) Hansgirg
23. *Golenkinia radiate* Chodat
24. *Coelastrum microporum* Naeg.
25. *Selenastrum gracile* Reinsch
26. *pediastrum integrum* Naegeli
27. *Pediastrum integrum* Naegeli
28. *Ankistrodesmus falcatus* var. *duplex* (Kuetz.)G.S. West

Scale denotes at 10 µm (each bar)

Plate-II

1. *Pediastrum duplex* Meyen
2. *Pediastrum simplex* Meyen
3. *Pediastrum duplex* var. *subgranulatum* Rociborski
4. *Pediastrum duplex* var. *clathratum* (A. Braun) Lagerheim
5. *Pediastrum tetras* var. *tetraodon* (Corda) Hansgirg
6. *Pediastrum boryanum* (Turnip) Meneghini.
7. *Shroederia planctonica* Skuja
8. *Pediastrum ovatum* (Ehr.) A. Braun
9. *Pediastrum simplex* var. *duodenarium* (Bailey) Rabenh
10. *Ankistrodesmus spiralis* (Turnip) Lemmermann
11. *Ankistrodesmus spiralis* var. *fasciculatus* G.M. Smith
12. *Chlorococcum infusionum* (Schrank) Meneghini
13. *Chlorococcum infusionum* (Schrank) Meneghini
14. *Tetraedron regulare* Küetz.
15. *Tetraedron regulare* Küetz
16. *Chlorella vulgaris* Beijerinck
17. *Chlorella vulgaris* Beijerinck
18. *Chlorella vulgaris* Beijerinck
19. *Tetraedron minimum* (A. Braun) Hansgirg
20. *Hydrodictyon reticulatum* (Linn.) Lagerheim
21 *Actinastrum hantzschii* Lagerheim

Scale denotes at 10 µm (each bar)

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PERIPHERAL GIANT CELL GRANULOMA OF THE ORAL CAVITY

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ABSTRACT

Peripheral giant cell granuloma is a non-neoplastic lesion representing a local hyperplastic reaction to injury or inflammation. It is known to be a reactive soft tissue lesion that develops only within the oral cavity, with a slight predilection for female sex. The usual localization for peripheral giant cell granuloma is the premolar region and the crest of the edentulous ridge. A possible hormonal influence for peripheral giant cell granuloma has been postulated. In the past, peripheral giant cell granuloma was called peripheral giant cell reparative granuloma. Subsequently, however, the term reparative was discarded because of lack of evidence to support the concept that it occurred in response to the healing process.

Keywords: peripheral, granuloma, epulis, giant cell.

INTRODUCTION

Epulis is a nonspecific term applied to tumors and tumor like masses of the gingiva. Histologic examination of epulides indicates that the vast majority are the focal fibrous hyperplasias, peripheral ossifying fibomas, pyogenic granulomas or peripheral giant cell granulomas. Specifically, it is a term used to describe subepithelial tumifications of the gingiva or alveolar mucosa, with or without ulceration. Therefore, lesions occurring on the gingiva but exhibiting distinct epithelial changes, such as verruca vulgaris, papilloma and primary squamous cell carcinoma are not epulides. Primarily, however epulides represent reactive hyperplasias of connective tissue cells of gingiva or superficial periodontal ligament (Madhusudan *et al.*, 2010).

Chronic trauma can induce inflammation, produce granulation tissue with endothelial cells, chronic inflammatory cells and fibroblasts proliferation and manifests as an overgrowth called reactive hyperplasia. These tumor-like lesions are not neoplastic, but they indicate a chronic process in which an exaggerated repair occurs (granulation tissue and formation of scars) following injury. Reactive hyperplastic lesions are categorized to several groups. Peripheral giant cell granuloma is one of the most frequent giant cell lesions of the jaws and originates from the connective tissue of the periosteum or the periodontal membrane. Other names of this lesion are peripheral giant cell tumor, osteoclastoma, reparative giant cell granuloma, giant cell epulis and giant cell hyperplasia of the oral mucosa. The characteristics and clinical behavior of peripheral giant cell granuloma may vary in different populations and be difficult to predict, reflecting different environmental influences, lifestyles, and racial factors, assessment of which may help in the diagnosis and management. Information regarding gender, age, signs, and symptoms may be useful and lead to an early diagnosis and proper management, preventing further damage to hard and soft tissues of involved areas (Shadman *et al.*, 2009).

DISCUSSION

Dentists frequently encounter patients complaining from an oral lesion. Giant Cell Lesions (GCL) form an important group of oral lesions. Late diagnosis and treatment of GCL will result in extension of the lesion and difficulties in treatment. Some dentists, however, lack the expertise in this regard due to lack of sufficient information and knowledge about clinical pathology. GCLs have a uniform appearance in histopathology: a gathering of multi-nuclear giant cells over a background of mononuclear cells, fibrohistiocytes, big fibroblasts, and extravasated red blood cells . However, their clinical appearance is variable. GCLs subtypes are Peripheral Giant Cell Granoluma (PGCG), Central Giant Cell Granoluma (CGCG), Cherubism, and Aneurysmal Bone Cyst (ABC) (Mohajerani et al., 2009).

There are two types of giant cell granuloma: peripheral and central (Shrimani and Arshad, 2008). Peripheral giant cell granuloma is one of the common lesions seen in the oral cavity and appears as localized tumor like enlargement of the gingival (Madhusudan *et al.*, 2010). This lesion has been reported to account for 5.1% to 43.6% of reactive gingival overgrowths (Nedir *et al.*, 1997).

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Etiology

The presence of calculus, periodontitis, periodontal surgery, ill fitting dentures, overhanging restorations, and tooth extractions have been considered to be a factor involved in the development of this pathological entity. The association between tooth loss and peripheral giant cell granuloma does not seem to be clearly established, and not all peripheral giant cell granulomas involving edentulous regions follow recent tooth extractions (Nedir *et al.*, 1997).It originates from the connective tissue of the periosteum or from the periodontal membrane (Falaschini *et al.*, 2005)

The term 'giant cell lesions', covers three nosologic entities: giant cell granuloma, brown tumor and cherubism. All are highly controversial, both etiologically (neoplastic or reactive) and regarding their denomination (giant cell granuloma, reparative giant cell granuloma, or giant cell tumor). Histologically, the three lesions are characterized by the presence of abundant giant multinucleated cells. Some studies have investigated the etiology of this disease, concluding that the three entities have some similar cytologic and histologic characteristics, and when evaluating the expression of the c-Src gene have established that this may be implicated in the development of these three pathologies include under the term 'giant cell lesions (Blanca et al., 2007).

Role of sex hormones

In order to be directly under hormonal influence, it is presumed that the particular tissue would contain receptors for these hormones. Studies have shown that many tissues/organs and certain tumors contain receptors for the ovarian hormones, and are responsive to fluctuations in hormone levels. El Atter and Hugoson (1974) showed estrogen metabolism in unhealthy gingiva was three times more than normal gingiva and the activity of responsible enzyme of these reactions increased gingival inflammation. Mohamed (1974) showed absorption of progesterone by rabbit's gingiva. In 1982, Vittek could find progesterone receptors in rabbit's gingiva and progesterone and estrogen receptors in human's gingiva, and in 1992 Forabasco showed estrogen receptors in oral mucosa. Shirani and Arshad (2008) studied the relationship between circulating levels of sex hormones and peripheral giant cell granuloma. They were unable to demonstrate direct action of sex hormones on peripheral giant cell granuloma. Their results showed no significant difference between blood levels of these hormones and normal quantities but clinical aspects confirmed the relationship between these lesions and sex hormones. It is important to note that higher frequency of these lesions on maturity, pregnancy and menstruation periods doesn't mean that patients with peripheral giant cell granuloma have high blood level of these hormones. Secondary influences of sex hormones with background of low oral hygiene or other stimulation factors are important factor in etiology of peripheral giant cell granuloma (Shrimani and Arshad, 2008).

Manifestation of hyperparathyroidism:

In rare instances, PGCG is an oral manifestation of hyperparathyroidism without obvious central bony involvement. While this is an unusual initial presentation, hyperparathyroidism should be considered when multiple lesions are found or if repeated recurrences are documented despite adequate treatment. A parathyroid tumor or chronic renal disease may result in excess production of the parathyroid hormone that stimulates the formation of a giant cell lesion. In addition, children with X-linked hypophosphatemic rickets, a condition that is associated with subclinical hyperparathyroidism, are at increased risk for developing this entity (Flaitz, 2000).

Age

The peripheral giant cell granuloma occurs throughout life, with peaks in the incidence during the mixed dentitional years and the 30-40 year old age group (Subarnbhesaj, 2006). Cases of peripheral giant cell granuloma have been documented in children, where the lesion appears to be more aggressive, with absorption of the interproximal crest area, displacement of the adjacent teeth and multiple recurrences (Falaschini *et al.*, 2005).

SEX

It is more frequent in women than in men. However Bhaskar *et al* (1971), Salum *et al* (2008), Zhang *et al.*, (2007) and also Murat *et al.*, (2004) reported a slight predilection for the male sex. But in some studies, peripheral giant cell granuloma had an equal prevalence in both genders (Shadman *et al.*, 2009).

Site

Affects largely the lower jaw (55%) than in the upper jaw (Falaschini *et al.*, 2005).The usual localization for peripheral giant cell granuloma is the gingival tissue in premolar region and the crest of the edentulous ridge. It is never found on mucosa that is not attached to bone (Falaschini *et al.*, 2005).

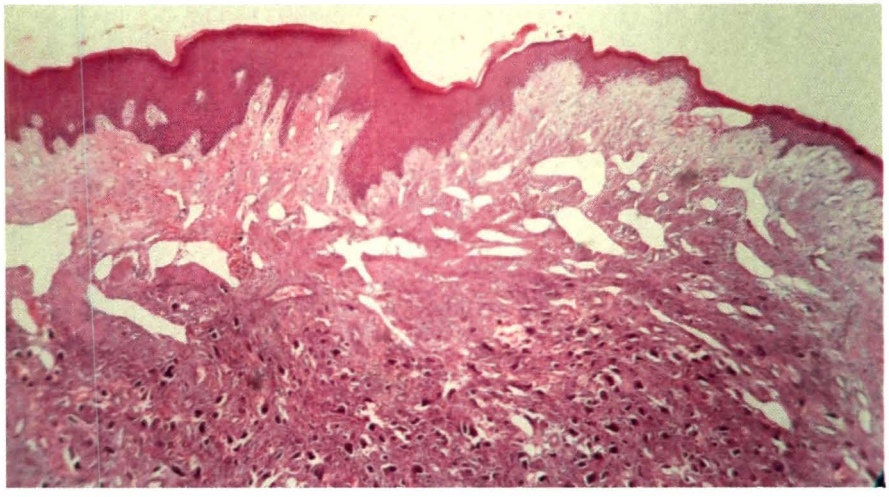

Fig.1: 10x magnification showing superficial epithelium and numerous giant cells in the connective tissue.

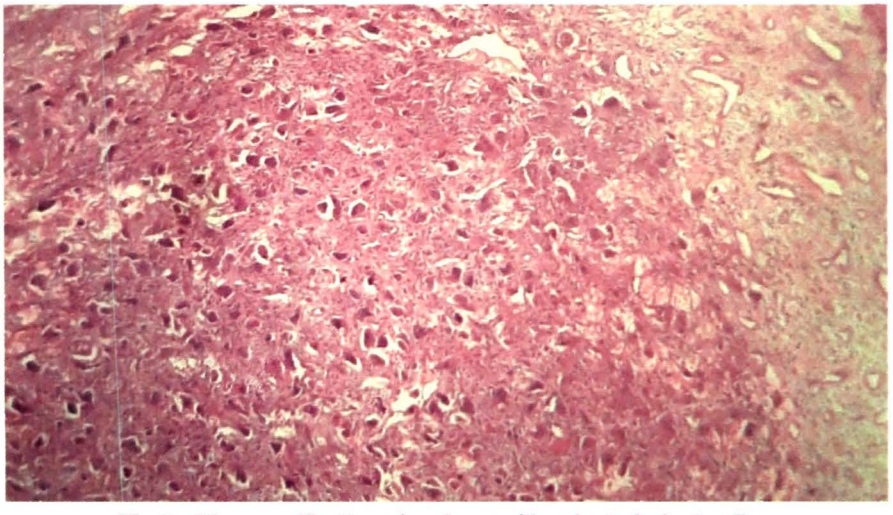

Fig.2: 10x magnification showing multinucleated giant cells.

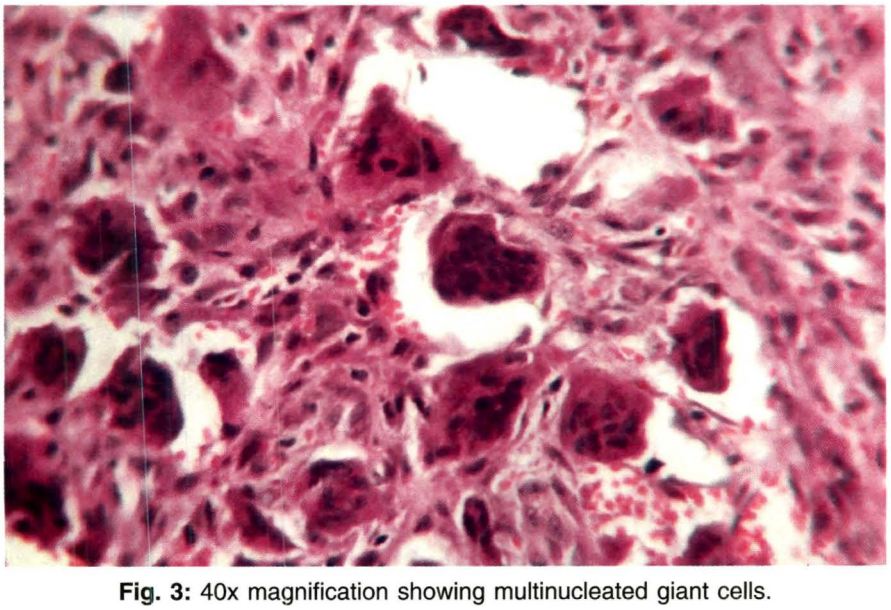

Fig. 3: 40x magnification showing multinucleated giant cells.

Clinical appearance

Clinically it is similar to the pyogenic granuloma (Neville, 2003). The lesion can be sessile or pedunculated. Clinically, it manifests as a soft to firm, bright nodule or as a sessile or pediculate mass, which is predominantly bluish red with a smooth shiny or mamillated surface (Falaschini *et al.*, 2005). The lesion ranges in size from small papules to enlarged masses, though reportedly rarely exceeding 2 cm in diameter, and are generally located in the interdental papilla, edentulous alveolar margin, or at marginal gum (Falaschini *et al.*, 2005). It is basically asymptomatic, in fact pain is not a common characteristic, unless they interfere with occlusion, in which case they may ulcerate and become infected (Shadman *et al.*, 2009). Secondary ulceration due to trauma may give the lesion a focal yellow zone as a result of the formation of a fibrin clot over the ulcer (Regezi *et al.*, 2003). The consistency of lesions depends on the age of lesions because as time passes, maturation of lesions (increasing in collagen fibers) occurs and consistency shifts from soft to firm (Shadman *et al.*, 2009).

Pediatric significance

In children, reactive oral lesions such as the PGCG can demonstrate a rapid growth rate and reach significant size within several months of initial diagnosis. These soft tissue nodules may be quite aggressive and resorb bone, interfere with eruption of teeth and produce minor to moderate tooth movement. Radiographs are important for diagnosis to confirm that this giant cell lesion arises within the oral mucosa and does not represent a central bony lesion with cortical perforation and soft tissue extension. Early detection of the PGCG results in more conservative surgery with less risk for tooth and bone loss (Flaitz, 2000).

Radiographic features

Although the peripheral giant cell granuloma develops within soft tissue, "cupping" superficial resorption of the underlying alveolar bony crest is sometimes seen. On occasion, it may be difficult to determine whether the mass arose as a peripheral lesion or a central giant cell granuloma eroding through the cortical plate into the gingival soft tissues. There may be little radiographic evidence of some lesions in teeth bearing areas because lesions may be small and primarily in the soft tissues. Larger lesions exhibit a superficial erosion of the cortical bone surface and may demonstrate some widening of the adjacent periodontal space. Close examination of the area may reveal small spicules of bone extending vertically into the base of the lesion. In edentulous areas, the cortical bone exhibits a concave area of a resorption beneath the lesion, often referred to as "saucerisation". Radiographs are important to determine if the lesion is of gingival origin or of central origin with extension to the surface (Subarnbhesaj, 2005).

Histologic features

Central giant cell granulomas are histologically similar to peripheral giant cell granuloma; however, central giant cell granulomas present frequent bone and tooth resorption (Nedir *et al.*, 1997).

The stroma of peripheral giant cell granuloma contains various cell types (Nedir *et al.*, 1997). Histologically, peripheral giant cell granuloma presents as a not-well circumscribed mass, constituted by fibrillar collagenous stroma containing two types of mononuclear cells (spindle and ovoid cells) and interspersed numerous multinucleated giant cells "osteoclasts-like" or larger than typical osteclasts, having rarely normal bone resorptive function as shown in figure 1, figure 2 and figure 3. Sometimes these cells are also localized in the internal wall of vessels (Falaschini *et al.*, 2005). The giant cells may contain only a few nuclei or up to a dozen nuclei; others demonstrate small, pyknotic nuclei (Subarnbhesaj, 2005). They are two types: the most common have lightly eosinophilic cytoplasm and large vesicular nuclei with prominent nucleoli while the other type has much more densely stained cytoplasm and pyknotic and densely haematoxyphilic nuclei. The latter are probably a degenerative form of the first type (Eveson, 2006). The mononuclear stromal cells may originate from fibroblasts and cells of histiocytic origin. The origin of giant cells has still been a source of controversy: in fact some authors suggest that they arise secondary to an alteration of the endothelial cells of the capillaries others as a consequence of a traumatic mechanism (Falaschini *et al.*, 2005). Palacios et al suggested that giant cell formation to be a fusion of hystiocytes, endothelial cells and fibroblasts. Falaschini *et al.*,(2005) found diffuse presence of CD68 (antigen most widely distributed in monocyte/macrophages lineage at various differentiation stages as well dendritic cells and osteoclasts) in a fraction of round mononuclear stromal cells and in mononuclear giant cells. There result confirmed that these latter may derive from osteoclasts (Falaschini *et al.*, 2005). There is also strong evidence that these cells are osteoclasts as they have been shown to possess receptors for calcitonin and were able to excavate bone in vitro (Subarnbhesaj, 2005). The study of Lim and Gibbins in 1995 confirmed that the multinucleated giant

cells reacted strongly for a monoclonal antibody MB1 which reacts with lymphocytes and a proportion of T cells and monocytes. The MB1 antibody has previously been shown to be expressed by osteoclasts in fetal bone (Subarnbhesaj, 2005).

There is also a growing body of opinion that giant cells may simply represent a reactionary component of the lesion and are derived via blood stream from bone marrow mononuclear cells and may be present only in response to an as yet unknown stimulus from the stroma. This concept is based on the results of some more recent studies using cell culture and transplantation in which the giant cells have been found to be short-lived and to disappear early in culture in contrast to the active proliferation of the stromal cells (Subarnbhesaj, 2005).

A study by Willing *et al.*, (2001) revealed that the stromal cells secrete a variety of cytokines and differentiation factors, including monocyte chemoattractant protein-1 (MCP1), osteoclast differentiation factor (ODF), and macrophage-colony stimulating factor (M-CSF). These molecules are monocyte chemoattractants and are essential for osteoclast differentiation, suggesting that the stromal cell stimulates blood monocyte immigration into tumour tissue and enhances their fusion into osteoclast-like, multinucleated giant cells. Furthermore, the recently identified membrane-bound protein family a disintegrin and metalloprotease (ADAM) is considered to play a role in the multinucleation of osteoclasts and macrophage-derived giant cells from mononuclear precursor cells (Subarnbhesaj, 2005).

In a study by Bo Liu *et al.*,(2003) in situ hybridization was carried out to detect the mRNA expression of the receptor activator of NF-kappaB ligand (RANKL), a newly identified that is shown to be essential in the osteoclastogenesis, its receptor RANK (receptor activator of NF-kappaB ligand) and its decoy receptor osteopritegrin (OPG). They concluded that RANKL, OPG and RANK expressed in these lesions may play important roles in the formation of multinucleated giant cells (Subarnbhesaj, 2005).

Hemorrhage, hemosiderin pigment, inflammatory cells and newly formed bone or mature calcified material throughout the cellular stroma can be seen. Lesions may be covered stratified squamous epithelium and ulcerated in some cases (Nedir *et al.*, 1997). The overlying epithelium is ulcerated in about 50% of the cases (Neville, 2010) .A zone of dense fibrous connective tissue representing a pseudocapsule usually separates the giant cell proliferation from the superficial epithelial surface (Madhusudan *et al.*, 2010). Myofibroblasts and either macrophages or their precursors, as well as Langerhans cells are also present (Nedir *et al.*, 1997).

Differential diagnosis

In adults the differential diagnosis of peripheral giant cell granuloma particularly involves giant cell tumour : nonossifying fibroma which differs from peripheral giant cell granuloma lesions in consistency and colour; pyogenic granuloma which is difficult to distinguish from peripheral giant cell granuloma lesions; central giant cell granuloma which is an expansive and destructive intraosseous lesion that can perforate the cortex, mimicking peripheral giant cell granuloma; chondroblastoma which, localized in the gum, may provoke irregular bone destruction below the exophytic lesion; odontogenic cyst; parulis, which is frequently associated with a necrotic tooth or with periodontal disorder; haemangioma cavernosum, which is distinguished from peripheral giant cell granuloma lesions by their pulsatile nature; fissured epulis (Falaschini *et al.*, 2005). Gingival lesions in children that mimic the PGCG are the pyogenic granuloma, parulis and peripheral ossifying fibroma. The pyogenic granuloma may be difficult to differentiate from the PGCG based on clinical features alone. In general the pyogenic granuloma presents as a soft, friable nodule that bleeds freely with minimal manipulation. Unlike the PGCG, displacement of teeth and resorption of alveolar bone are not observed.

Another erythematous nodule of the gingiva is the parulis, which is associated with an entrapped foreign body, a gingival pocket and/or a nonvital tooth. Pain and the expression of a purulent exudate with fluctuation in lesion size help to differentiate this inflammatory disease from the PGCG. The peripheral ossifying fibroma is a reactive gingival growth that shares similar clinical features as the PGCG. Although this reactive lesion is often ulcerated and inflamed, it lacks the purple or blue discoloration that is commonly associated with the PGCG. Identification of small flecks of calcification within the tumescence on a radiograph aids in diagnosing the peripheral ossifying fibroma, when present. The final consideration based on the red or blue discoloration of the soft tissue nodule is a hemangioma. Although many hemangiomas are congenital lesions, some vascular malformations increase in size during childhood. Brisk bleeding, increased warmth of the tissue and blanching upon palpation are characteristic of this vascular entity (Flaitz, 2000).

Treatment and recurrence

The treatment of choice is surgical excision with the suppression of the underlying etiologic factors. The periosteum must be included in the excision to prevent recurrences. Curettage in addition to the excision to remove the base of the lesion also has been suggested (Falaschini *et al.*, 2005). Recurrences of peripheral giant cell granuloma have been reported in 5% to 70.6% of cases. These wide variations are probably attributable to the surgical technique used, since recurrences re-excised to the periosteum did not recur again (Nedir *et al.*, 1997). In this case because of the large size of the surgical defect, a grafting procedure is needed to cover the exposed periosteum and roots of the incisors(Flaitz, 2000).

The treatment for these types of lesion has frequently been a subject of interest for the scientific community. Thus, some authors, based on the presumed similarities existing between giant cell granulomas, brown tumors in hyperparathyroidism, and some proliferative vascular lesions, have administered calcitonin and interferon alpha as a basis of treatment for this pathological entity. Unfortunately, on occasions the result has not been as successful as was first anticipated, producing severe secondary complications such as erythematous lupus, and pancreatitis secondary to the administration of interferon alpha due to the difficulty in managing these types of drug (Blanca et al., 2007).

CONCLUSION

An early and precise diagnosis of peripheral giant cell granuloma, based on the clinical, radiological findings and histological study, allows conservative management with a lower risk for the teeth and adjacent bone (Falaschini *et al.*, 2005).

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SHARP DECLINE IN FISH DIVERSITY OF HARYANA STATE (INDIA) - A SERIOUS CONCERN!

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ABSTRACT

The knowledge of fish species composition of an area and its change over a time period is one of the prerequisites for the evaluation of aquatic health of the water bodies. Keeping this fact in mind, an extensive ichthyofaunal survey of Haryana was undertaken during the years 2003-2006 and it has been found that at present the state supports 60 fish species belonging to 7 orders, 18 families and 36 genera. Fish fauna of Haryana is a mixture of hillstream, typical riverine and brackish water fish species. The shocking observation is that during the last four decades, this state has lost 31 fish species and 16 new fish species have been recorded for the first time. Five exotic fish species which have been introduced for some specific purposes such as fish culture, control the macrophytes, phytoplankton and mosquito larvae have slipped in to natural waters and have established themselves. This is not a healthy sign because they compete with the native fish species having the similar ecological niches. Rest of the eleven fish species are found in hillstream habitats in the sub-mountainous region having fast water current with high oxygen content. One perch, *Anabas testudineus* (Bloch) has been recorded for the first time. It is considered that this perch has been introduced as a fry along with the fry of other culturable fishes by the State Fisheries Department. The disappearance of 31 fish species is more shocking. Ninety percent of the disappeared fishes are riverine, minnows which prefer clean, shallow and highly oxygenated water. The absence of two freshwater eels indicated that banks of the rivers are ecologically disturbed. It is suggested that to conserve the fish diversity of Haryana State, river rehabilitation programme for the improvement of fish habitat be initiated at the earliest.

Keywords: Freshwater fish diversity, Haryana, decline, exotic fish species, restoration of fish habitat.

INTRODUCTION

The Indian subcontinent supports 1042 freshwater fish species belonging to 283 genera, 71 families and 16 orders (Jayaram, 2010). This document (Jayaram, 2010) is a compilation of data published by earlier workers, hence, it gives no information on the decline or enhancement of fish diversity in India or various regions over a time period. The documentation on Indian freshwater fish diversity was initiated during early nineteenth century by Hamilton-Buchnan (1822) on the most sacred river Ganges, however, some information is documented on fish diversity and bionomics of some Indian commercial fishes in Hindu sculptures (Hora , 1951). In the subsequent years, several workers undertook similar ichthyofaunal surveys (Day, 1875-1878; Misra, 1962; Dattamunshi and Srivastava, 1988) without emphasizing the change over a time period in freshwater fish diversity as a result of various water management practices, loss of fish habitat, decline in the natural spawning grounds, soil erosion and the impact of the introduction of exotic fish species.

It is considered that the appearance and disappearance of freshwater fish species or change in freshwater fish composition of an area clearly indicate the aquatic health of the waterbodies (Coad, 1980-81). Considering this fact, an extensive ichthyofaunal survey of Haryana State (Johal and Jha, 2007, 2010) has been conducted during the period 2003-2006 with the financial assistance from Haryana State Fisheries Department to evaluate the present status of fish diversity in this state and to ascertain the change in fish diversity over a period of time.. This is the beginning of such studies on regional basis and hope in future similar studies will be conducted in the other India states too. It must be mentioned here that at this moment this type of study cannot be undertaken on national basis due to numerous constrains such as man-power, financial support and moreover the lack of interest by the scientific community.

TOPOGRAPHY

The state of Haryana (27° 39' to 30° 55' N and 74° 28.8' to 77° 36.5' E; Area 44,212 km^2) is surrounded by Shivalik hills in the north and Yamuna river in the east. The Aravalli range forms southeast boundary of the state. In the north, the river Ghaggar enters the state and runs along the north-western boundary of the state (Fig.1). The

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fishery resources of Haryana include river length of 510 km (Yamuna river 305 km and Ghagger 205 km), 12,900 ha of lotic waters which include ponds, marshy lands, small reservoirs and water logged areas. In addition saline water covering about 2800 km^2 in the districts of Gurgaon, Faridabad, Hissar, Sirsa, Rohtak, Sonepat, Karnal, Kurukeshtra, Jind, Mohindergarh and Bhiwani support the hardy fish species, which can tolerate wide range of temperature salinity variations.

MATERIALS AND METHODS

Fishes were collected at frequent intervals from various aquatic sources such as rivers, streams (in the sub mountains region), lakes, canals, small and large reservoirs, pools, brackish waters and village ponds with the help of local fishermen using cast-net, gill net, drag net and hand net of various mesh sizes. Immediately after collection, fishes were preserved in 10% formaldehyde solution and brought to the laboratory. In the laboratory, preserved fishes were washed in tap water to remove the extraneous matter and again preserved in 10% formaldehyde solution or 70% ethanol (only small size fishes). Collected fishes were identified following the works of Johal and Tandon (1979, 1980), Talwar and Jhingran (1991) and Jayaram (2010).

RESULTS AND DISCUSSION

The Haryana State which includes sub-mountainous area surrounding the Shivalik hills, typical planes (Gangetic), Aravalli range and touches the Thar Desert in southeastern part of the state supports 60 freshwater fish species belonging to 7 orders, 18 families and 36 genera (Johal and Jha, 2007, 2010). The present day fish fauna of this state is a mixture of hillstream, typical riverine and brackish water fish species clearly indicating that this state has varied ecological conditions. This list also includes seven exotic fish species, which have been introduced some time back in confined waters for specific purposes such as pond fish production, eradication of macrophytes and to control the algal blooms in ponds having high nitrogen content and mosquito larvae (Welcomme, 1988)). Due to the mismanagement of fish culture practices, callous attitude of the fish farmers and state fisheries department, all the exotic fishes have entered the natural waters severely affecting the native fish species composition because of their high adaptability to the new surroundings, high growth rate and high fecundity. Their presence in natural water bodies is not viewed positively (Johal and Tandon, 1983;Welcomme, 1988).

The ichthyofauna of Haryana State has been described in the past by several workers (Tandon and Sharma, 1965; Rishi and Dattagupta, 1979; Agarwal, 1982; Rishi and Shah, 1982; Johal and Rawal, 2004). The latest compilation on the fish fauna of this state based on the data published during the years 1969-1982 (Johal and Rawal 2004) indicated that there is a possibility of the occurrence of 82 fish species. No doubt that this figure is on the lower side when the occurrence of fish species occurring in the neighboring states viz., Himachal Pradesh (Tilak and Hussain, 1977; Johal, 1998,2001), Punjab (Johal and Tandon, 1979,1980), Rajasthan (Dattagupta *et al.*, 1961; Mathur, 1952; Datta and Majumdar , 1970; Ajithkumar and Vijayan,1988; Johal *et al.*, 1993) and Uttrakhand (Johal, 2001; Negi and Malik, 2006) is considered.

The findings of the extensive ichthyofaunal survey conducted during the period 2003-2006(Johal and Jha, 2007, 2010) are shocking and revealed that during the last four decades, Haryana State has lost 31 fish species and 16 new fish species have been recorded for the first time. If the in-depth analysis of this time scale change in fish species composition is done, the overall picture seems to be very gloomy. The critical analysis of this data is as follows:-

As already mentioned that 16 new fish species have been collected during the years 2003-2006, out of which five are exotics namely, *Cyprinus carpio specularis* Lacepede, *Hypophthalmichthys nobilis* (Richardson) belonging to the order Cypriniformes: *Clarias gariepinnus* (Burchell) order Siluriformes; *Gambusia affinis* (Baird & Girard) order Cyprinidontiformes and *Oreochromis mossambicus* (Peters) order Perciformes. The occurrence of these five exotics in the natural waters is not a healthy sign for the natural fish fauna of Haryana State. The culture of *H. nobilis* (Richardson) and *C. gariepinnus* (Burchell) is banned by the Government of India, because of their adverse affects on the native fish fauna. *Cyprinus carpio specularis* Lacepede has very notorious history as it breeds four times in confined waters and two times in natural waters of North India and form the dominant population in the new surroundings (Johal and Tandon, 1983). It is omnivore and growth rate is very high. This fish is recommended for composite culture under extensive aquaculture practice and monoculture under intensive pisciculture practice (Welcomme, 1988). *G. affinis* (Baird & Girard) was introduced in Indian freshwaters for the control of mosquito larvae to minimize the occurrence of malaria. Somehow this fish made its entry into natural waters and feeds on the eggs of the minnows in natural waters,

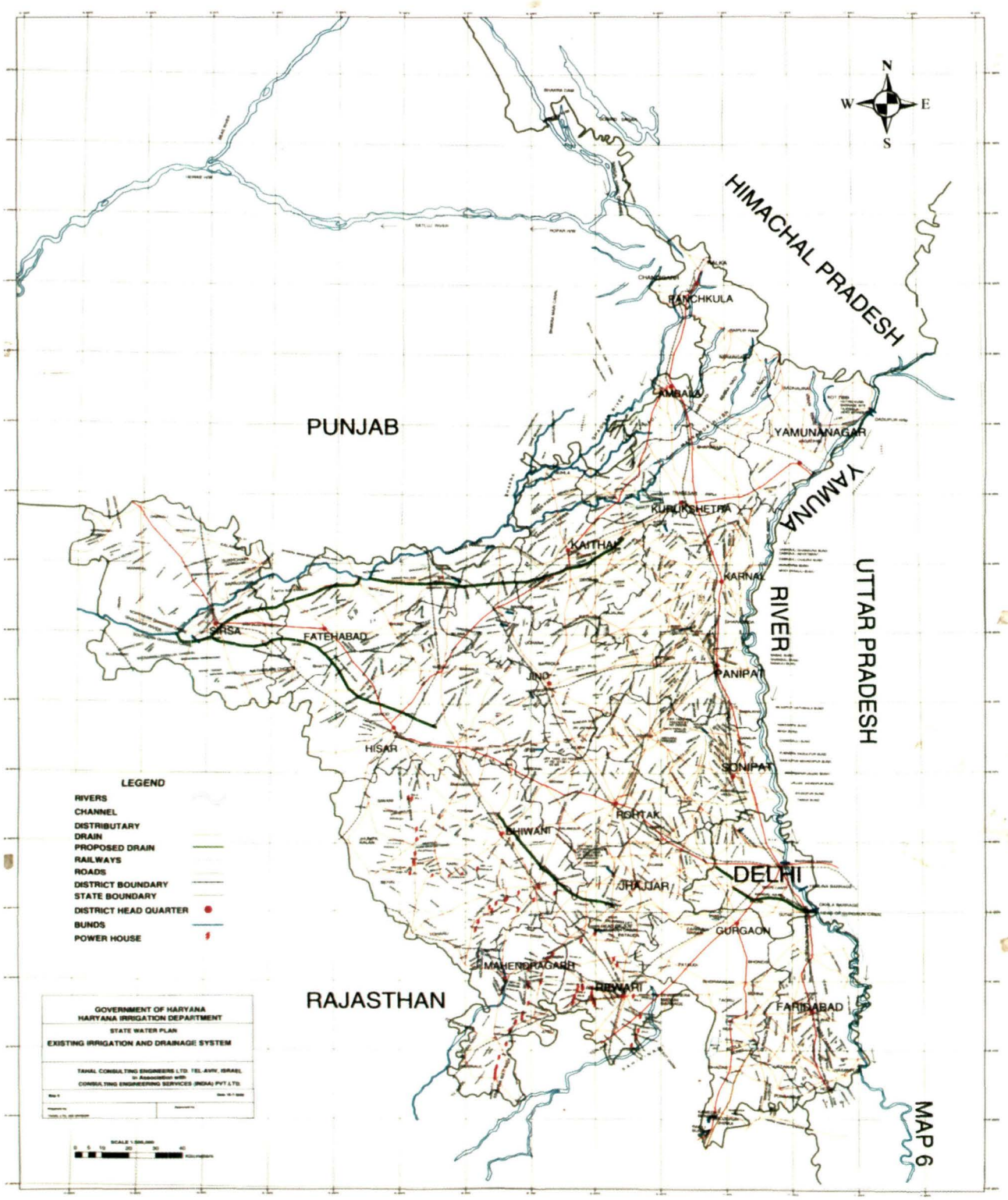

Fig. 1: Map of Haryana state

reducing the population of this very important component of the Indian freshwater fish fauna. Recent fish faunal surveys have indicated that Indian freshwaters support large number of minnow having the feeding habits similar to that of *G. affinis* (Baird & Girard) and moreover they do not feed on the eggs of minnows, hence, these minnows should be introduced in the waters having mosquito larvae to eradicate them. *Clarias gariepinnus* commonly known as Thai magur is originally inhabitant of South Africa is a voracious carnivore and has played havoc with pisciculture practices and has eradicated the native fish fauna when find its way to natural waters. *Oreochromis mosammbicus* (Peters) commonly known as Tilapia has the tendency to from dwarf populations both in confine and natural waters due to inbreeding. It competes as far as food requirements are concerned very vigorously with the entire group of minnows and minor carp species (Welcomme, 1988).

These five exotics are accepted as part of the native fish fauna of Haryana by the State Fisheries department, but they are inimical to the native fish fauna of the state and their presence is undesirable in the natural waters not only in the Haryana but in Indian freshwaters.

Out of the rest eleven newly recorded fish species, eight fish species viz., *Chagunius chagunio* (Hamilton-Buchanan), *Garra gotyla gotyla* (Gray), *Neomacheilus corica* (Hamilton-Buchanan), *Schistura punjabensis* (Hamilton-Buchanan) belonging to the order Cypriniformes; *Amblyceps mangois* (Hamilton-Buchanan), *Glyptothorax indicus*, Talwar (syn. *G.burmanicus* Prasad & Mukerjee), *Glyptothorax telchitta* (Hamilton-Buchanan) belonging to the order Siluriformes are the inhabitants of foothills of Himalayas, prefer water having high current, high level of oxygen, low temperature and are reported from the nearby hill states such as Himachal Pradesh (Tilak and Hussain, 1977; Johal, 1998) and Uttrakhand (Johal, 2001; Negi and Malik, 2006). Normally, all these fishes breed during pre-southwest monsoon period and are washed downstream during southwest monsoon floods. Some of the hillstream fishes even reach southwest part of Thar Desert through floods of the Ghagger River and the irrigation canals, but do not breed there (Johal *et al.*, 1989, 2000). The occurrence of these eleven fishes is considered to either seasonal because their availability is only for a short time period and is restricted to the sub- mountainous areas of the rivers Ghagger and Yamuna, but anyhow, they are the part of Haryana's fish fauna and being recorded for the first time.

The two cyprinids namely *Labeo angra* (Hamilton-Buchanan) and *Puntius terio* (Hamilton-Buchanan) are endemic to the natural freshwater of North India and have been reported from the neighbouring state of Punjab (Johal and Tandon, 1979, 1980). The new report of a climbing perch *Anabas testudineus* (Bloch) in the natural waters is the result of its introduction in the oxygen deficient water along with the seed of another culturable fish species. Authors are of the view that the presence of these three newly reported fishes is the consequence of half-hearted earlier ichthyofaunal surveys.

When the issue of disappeared fish species is considered critically, the picture is very gloomy and gives the clear reflection that there is a great threat to the fish habitat and the riverine conditions are depleting at a very fast rate. In table-1, the name of the fishes which have disappeared during the last four decades along with their habitat is given.

From table 1, it is apparent that the fish species which have disappeared from the natural waters of Haryana State during the last four decades belong to four orders namely, Osteoglossiformes (2), Cypriniformes (15), Siluriformes (10) and Perciformes (4). Out of these four orders the species belonging to the order Cypriniformes

Table 1. Name of the fish species disappeared during the last four decades from the natural waters of Haryana along with their habitat. (Classification after Jayaram 2010).

S. No.	Scientific name	Habitat
	Order-Osteoglossiformes	
1.	*Notopterus notopterus* (Pallas)	Riverine
2.	*Chitala chitala* (Hamilton-Buchanan) Order-Cypriniformes	Riverine
3.	*Esomus dancricus* (Hamilton-Buchanan)	Minnow
4.	*Danio devario* (Hamilton-Buchanan)	Minnow
5.	*Brachydanio rerio* (Hamilton-Buchanan)	Minnow
6.	*Rasbora daniconius* (Hamilton-Buchanan)	Minnow
7.	*Puntius conchonius* (Hamilton-Buchanan)	Minnow

8.	*Labeo boga* (Hamilton-Buchanan)	Riverine
9.	*L. caeruleus* Day	Riverine
10.	*L. gonius* (Hamilton-Buchanan)	Riverine
11.	*L. pangusia* (Hamilton-Buchanan)	Riverine
12.	*Schimatorhynchos nukta* (Sykes)	Hillstream
13.	*Securicula gora* (Hamilton-Buchanan)	Minnow
14.	*Schizothoraichthys progastus* (McClelland)	Hillstream
15.	*Nazitor chelynoides* (McClelland)	Hillstream
16.	*Botia dario* (Hamilton-Buchanan)	Loach
	Order-Siluriformes	
17.	*Rita rita* (Hamilton-Buchanan)	Riverine
18.	*Mystus vittatus* (Bloch) Riverine, side pools, clear water.	
19.	*Ompok bimaculatus* (Bloch)	Riverine
20.	*O.pabda* (Hamilton-Buchanan)	Riverine
21.	*Clupisoma garua* (Hamilton-Buchanan)	Riverine
22.	*Eutropiichthys vacha* (Hamilton-Buchanan)	Riverine
23.	*Neotropius atherinoides* (Bloch)	Riverine
24.	*Silonia silonidia* (Hamilton-Buchanan)	Riverine
25.	*Sperata aor* (Hamilton-Buchanan)	Riverine
26.	*Bagarius bagarius* (Hamilton-Buchanan)	Riverine
	Order-Perciformes	
27.	*Parambassius baculis* (Hamilton-Buchanan) Glass fish	Shallow riverine pools
28.	*Colisa lalia* (Hamilton-Buchanan) Aquarium fish	Shallow riverine side pools
29.	*Macrognathus aral* (Bloch & Schneider)	Stone cervices along river banks
30.	*Macrognathus pancalus* (Hamilton-Buchanan)	Stone cervices along river banks

are considered to be more sensitive to the even slightest change in environmental conditions, hence, in the present case, this order is represented by maximum number of fish species. Habitat-wise, fish species which are exclusively riverine are more in number i.e., 16 (S. Nos. 1-2, 8-11, 16, 18, 20-27 of table 1). These fish species start their lives in the open rivers during southwest monsoon, feed, breed and ultimately die here. They assume medium to enormous sizes and have varied feeding habits from exclusively herbivore to carnivore. There are six minnows (S. Nos. 3-7, 13 of table 1), which never exceed in size more than 15.00cm, feed on plankton, prefer shallow pools having high oxygen level and rich in planktonic populations. They breed profusely, mostly during pre-monsoon period, hence, occur abundantly (Johal, 2001). They are the favourite food of piscivore and carnivore fishes, especially the cat fishes belonging to order Siluriformes and a carp, *Tor tor* (Hamilton-Buchanan) and birds. Their disappearance can be directly correlated with the decline in the populations of piscivore birds and fish species. Most of the minnows e,g., species belonging to the genera *Puntius* Hamilton,, *Barilius* Hamilton and some glass fishes are considered to be excellent aquarium fishes because they are often brightly coloured, with colour stripes and bars. They have short life span varying between 1-2 years especially the *Puntius sarana* (Ham.)and other species of genera belonging to *Puntius* Hamilton and *Barilius* Hamilton.

This list of disappeared fishes also include three typical hillstream fishes viz., *Schimatorhynchos nukta* (Sykes), *Schizothoraichthys progastus* (McClelland) and *Nazitor chelynoides* (McClelland) and one loach, *Botia dario* (Hamilton-Buchanan) which prefer fast water current having high oxygen content and feed on the algae and lichens covering the stones lying in the hillstreams by scrapping. The disappearance of two freshwater eels viz., *Macrognathus aral* (Bloch & Schneider) and *M. pancalus* (Hamilton-Buchanan) clearly indicates that their habitat which consists of stone cervices, no more exists. Both the freshwater eels are carnivore. Absence of two perches namely *Parambassius baculis* (Hamilton-Buchanan) and *Colisa lalia* (Hamilton-Buchanan) is attributed to absence of shallow riverine side pools which is the preferred habits of these two perches.

In Haryana, mainly two rivers viz., Ghaggar and Yamuna having the linear lengths of 205 km and 305 km

respectively in this state support 70-75% of Haryana's fish diversity as per the earlier and present reports (Rishi and Dattagupta, 1979; Agarwal, 1982; Rishi and Shah, 1982; Johal and Rawal, 2004; Johal and Jha, 2007, 2011). The rivers Ghaggar and Yamuna used to support good number of hillstream fishes, some food fishes such as mahseers, minnows, loaches, freshwater eels and small size perches up to 60-70 km from the point of entry from the Siwalik range of lower Himalayas. During the last four decades, there has been lot of construction activity due to urbanization, deforestation, soil erosion and discharge of sewage in the Ghaggar river stretch in the vicinity of Pinjore, Panchkula and Ambala towns and the release of industrial effluents in the river Yamuna in the vicinity of Yamunanagar town are responsible for the drastic decline in the fish populations and other aquatic organisms in the downstream part upto 20-30 km. The water of the river Yamuna around Yamunanagar is virtually black and can be declared as 'aquatic desert'. Even the major part of the river bed remains almost dry except during south-west monsoons, hence, there is (has been) sharp decline in the fish diversity.

The river morphology of almost all the rivers has the similar basic features (Welcomme, 1983,1985; Jhingran, 1991) i.e., the main river bed and on the bank sides there are flood prone areas. The flood prone areas overflow with water during monsoons. During the pre and post monsoon periods there are large number of small ponds, ditches having different surface area in the flood prone areas. The water level of these small ponds and ditches is maintained either through the seepage or invariably they are connected to the main river bed by means of small channels. These very small water bodies are very important from fish diversity point of view because they support majority of the minnow fish species and act as feeding grounds for the larval stages of large size fishes which inhabit the main river bed. During floods, these minnows enter the main river bed and become the food of large size piscivore or carnivore fish species residing here.

The absence of twelve typical riverine fish species e.g., *Notopterus notopterus* (Pallas), *Chitala chitala* (Hamilton-Buchanan), *Tor tor* (Hamilton-Buchanan), *Rita rita* (Hamilton-Buchanan), *Ompok bimaculatus* (Bloch), *O. pabda* (Hamilton-Buchanan), *Clupisoma garua* (Hamilton-Buchanan), *Eutropiichthys vacha* (Hamilton-Buchanan), *Neoptrpius atherinoides* (Bloch), *Silonia silonida* (Hamilton-Buchanan), *Sperata aor* (Hamilton-Buchanan) and *Bagarius bagarius* (Hamilton-Buchanan) gives ample indication that in the State of Haryana, the existence of typical riverine conditions which provide breeding and feeding grounds for most of the fish species both commercial and non-commercial, is in question.

What can be done to enhance the fish diversity of Haryana State or to reverse the declining trend in this regard? It is big questions mark. It is suggested that **'Fish Habitat Restoration Programmes'** (Welcomme, 1983; Cowx and Welcomme, 1998) involving state government, religious bodies, NGO's and other stake holders must be initiated without delay. Haryana is leading in pisciculture state in India. For the genetic improvement or the stock improvement of the culturable fish species especially the Indian carps, farm produced fish species has to be cross bred with the wild populations. With the loss of river habitat, wild fish species of the Indian major carps will not be available and there is apprehension that in the near future pisciculture practices in the state of Haryana may be drastically affected. It must be mentioned here that aspect has been ignored by most of the State Fishery administrators. In case due to some financial constrains or social reasons, the entire river length cannot be covered under Fish Habitat Restoration Programme, some stretches having maximum fish diversity can be selected for this purpose.

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A REVISED MODEL FOR CONCERN BASED REQUIREMENT ENGINEERING

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ABSTRACT

Identification of requirements plays very important role for developing software which can satisfy all of the needs of a user. A software developer requires resolving a number of functional as well as non functional requirements from design to implementation phase of software development. Keeping in view the overall interest /need of the end user proper implementation of all requirements must be done. A number of models have been suggested for Requirement Engineering, but already existing approaches have the limitations in representing together the functional and non functional requirements of the software. This paper proposes a concern based model which can collectively represent the functional as well as non functional requirements and helps the developer to take a decision by observing them to together.

Keywords: Aspect Oriented, Concern, Functional Requirements, Non Functional Requirements, Traceability

INTRODUCTION

The development of the software project depends on the collection of requirements. Once the requirements have been collected they are thoroughly analyzed to find the interdependency among them (if any). A number of models have been suggested for Requirement Engineering like "Theme" [8] and "Goal Oriented" [3] etc. These approaches have the limitation of representing together the functional and non functional requirements of the software. Identification, separation, analysis and implementation of requirements are very important during the phases of software development. For satisfaction of end user (client) the implementation of his/her requirements is mandatory. Requirement Engineering is one of the branch which deals with the analysis of requirements. Because of the importance of requirements and their implementation we try to draw a model which can represent the requirements in an easily observable manner. The model is build by considering the basics of Aspect Oriented Software Programming and Aspect Oriented Requirement Engineering. Aspect Oriented Requirement Engineering provide support not only for recognizing the presences of non-functional concerns , but explicitly distinguishes cross cutting (functional and non functional) concerns and present them as an independent group which need separate/additional treatment procedure[16]. We build the model by considering the requirement as one of the concern, a concern can be defined as "The part of a software system relevant to particular concept, goal or purpose [7]" or "A concern is anything a stake holder may want to consider as a conceptual unit including features, non-functional requirements and design idioms."[14]

An effective requirement engineering approach must reconcile the need to achieve the separation of concerns with the need to satisfy broadly scoped requirements and constraints [4]. During requirement life cycle in Requirement Engineering the properties that a software must exhibit have to be elicited[16]. The elicited properties and set of operations to be performed are organized in operational context and they produce the synthesis of the requirements. These requirements need to be as far as possible correct, complete and feasible [16]. To achieve the above properties of requirements the analyst has to go for number of discussion rounds with the client. The selected sets of requirements are documented formally so that it can be assigned to the people who involved in the software development. The set of requirements collected at first phase is always prone to change during the development phases; it may be because of change in user's requirements or of some technical constraints. The adoption of changes in requirements with the primary requirements and their effect on the set of other requirements is required to be handled properly.

COMPARISON WITH THE EXISTING APPROACHES

The problem which we are trying to solve through our proposed model was also identified and some solutions were suggested by the few more authors, brief overview of solutions suggested by them is discussed in this

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section. All of the suggested solutions are in context with Requirement Engineering (RE) approaches, such as goal-oriented technique and viewpoints. In goal oriented approaches as discussed in KAOS [1] and i* [9] goal is a target which is supposed to be achieved. It treats the concern in two dimensional systems i.e. functional and non functional. KAOS uses a formal language which includes first order temporal logic with real time constraints to specify critical path of the system. Goals help in fixing up of defects (if any) in the system i* approach models the organizational requirements and consider the goals and soft goals modeling concept as its dimensions. Soft-goal is used as a representation of non functional requirements which we expect to satisfy within acceptable limits. Another requirement engineering approach is based on the viewpoints is PREVIEW [11], it also consider the separation of cross cutting property. In this approach analysis is conducted by using a set of concerns intended to correspond broadly to the overall system goal. The application of this method also identifies the concerns in terms of viewpoints as functional requirements and PREVIEW specific notion of concerns express the non-functional properties. An Aspect Oriented Requirement Engineering model presented in [6] is based on treating PREVIEW concerns as adoptions of the aspect – oriented programming [20] notion of aspects, and it carries the analysis of broadly scoped properties against a base set of viewpoints. Our proposed model uses the strengths of the model suggested in [5], mainly the informal composition rules with concerns – specific actions and operators and the effective support for establishing relations between all categories of requirements.

Above discussion of the Requirement Engineering based approaches make it clear that the existing approaches support the analysis of system requirements from the viewpoint of non-functional properties but do not provide support for cross - cutting functional properties i.e. combination of functional and non-functional properties. The approaches suggested in this paper are well capable to manage this unresolved issue.

TECHNICAL ASPECTS OF MODEL

Goal:

A goal is an objective that the system under consideration should achieve. It can be formulated at different levels of abstractions and covers concerns i.e. functional and non functional [14].

Soft Goal:

A soft goal represents non functional requirements we expect to satisfy within acceptable limits [14], or a goal that has neither a clear cut definition nor precise criteria for documentation whether it has been satisfied [16].

Composition:

The points where two or more requirements are combined together and the overall result after this very point depend on the collective execution of requirements implementations [14].

Cross Cut Points:

The point in the software system where the functional as well as non functional requirements crosses each other [16].

Identification and Representation of Functional Concerns:

Because the development of the whole software system depends mainly on the functional parts and hence their identification is mandatory. In the proposed model the functional concerns are represented by FCi.

Identification of Non Functional Concerns:

The identification of non functional concern is done as secondary task and represented by NFCi.

Analysis and Decision Support:

The overall selected/identified functional and non functional concerns are required to analyze together so that their interrelationship can be understood by analyst and the decision regarding their effect on each other and how to proceed further from this point cab be taken.

Traceability:

Preservation of traceability between the artifacts of the software lifecycle is necessary quality for understandable, main table and manageable recorded software [16]. The model provides the easiest way to keep the functional and non functional requirements traceable by the user.

Proposed Model:

The model is based on the identification and implementation of the above discussed technical aspects. Change in the rules for almost all of the business is basic need of market, keeping in view of this programmer's have to develop the software which are volatile in nature, and are very easy to adapt and evolve as per needs. To make a system adaptable and evolvable it is essential for it to determine the effect of all

requirements. Existing Requirement Approaches, focus on all the nonfunctional requirements only. Functional requirements which may affect the other functional and non functional requirements may not be dealt with properly. In respect to that the model proposed in this paper deals with all the functional as well as non functional concerns in well defined manner. Here the concern imply to any coherent collection of requirements. As shown in Fig. 1 concerns can be placed at various levels with equal importance/functionality. By treating the concerns at different levels with equal importance it is possible to identify the set of base concerns use to project the influence of another concerns. Because of flexibility of this multi dimensional model it is capable to handle both crosscutting functional and non functional

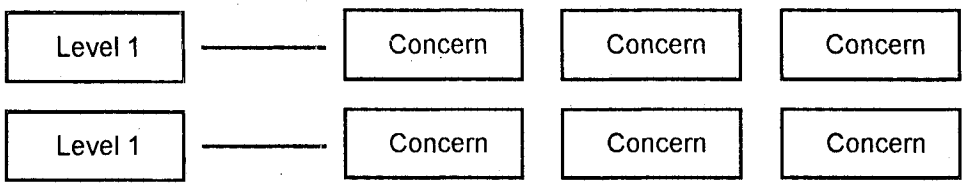


Fig. 1 : Concern Space

requirements.

Our proposed model is shown in Fig.2. At the beginning it identifies and specifies the concerns. Identification is done by synthesis existing requirement's elicit mechanism as viewpoint [2], use case [10] and goals [20]. The concerns for identification are specified by templates.

The next step is to identify Relationship among the concerns by relating them together through matrix as shown in Table 1 and Table 2. Relationship can be identified using technique such as domain analysis [13] , ethnography [19] and natural language processing [15] . From the matrix shown in Table 1 it can be seen that which concerns influence other concerns. It is also emphasized here that we are not concentrating on any exact kind of relationship between two concerns. After identifying the relationship among the concerns, next we specify projection of concerns on each other. This step corresponds to the granularity of individual requirements not about the one who encapsulate them. Hence it is possible to specify how requirements among concern in query effect the constrains and behavior of

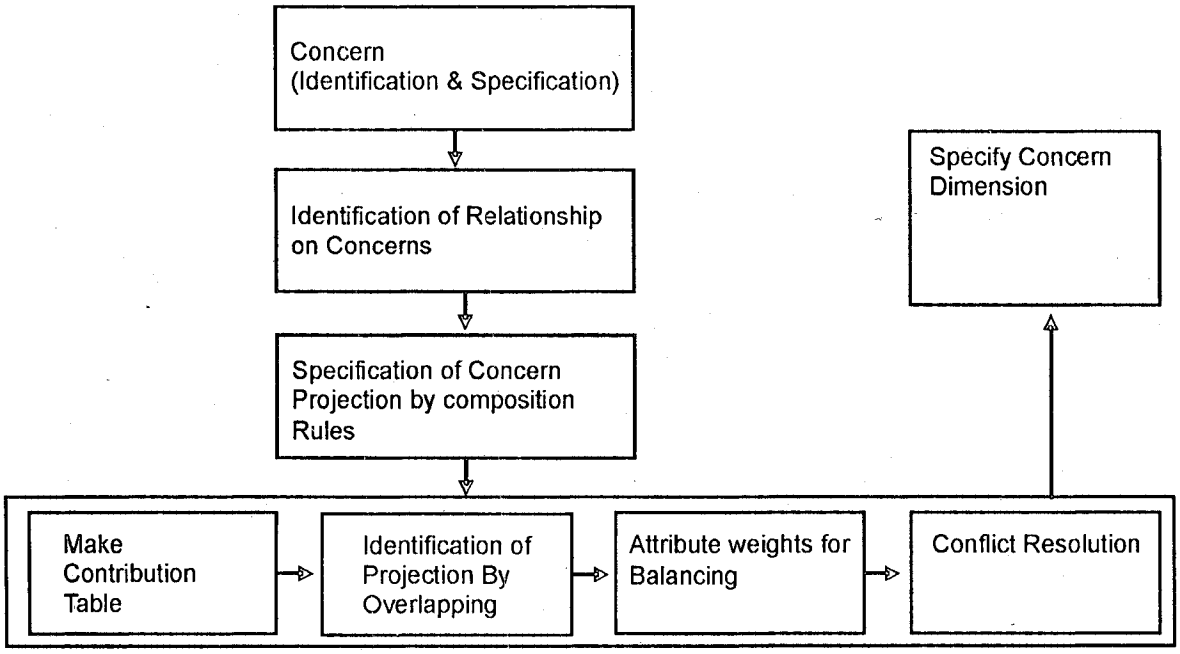


Fig. 2: Uniform Treatment of concerns

other concerns. This all help in bypassing the need for unnecessary negotiations among stakeholders for cases where there might be an apparent tradeoff between two concerns but, in-fact, different isolated requirements are being influenced by them. On the specification of various projections against composition rules, identification and resolution of conflicts among concerns can be carried out. It can be done as:

1. Developing a contribution matrix as shown in Table 2. In this table the + and – symbol among cross section of various concerns shows their contribution among each other. Diagonal line is left blank as there is no relationship among the same concerns.
2. Overlapping the rows/columns on each other along the diagonal line of the matrix. The result of folding (imagined) can be marked as in the respective cells as shown in table 3. It provides the reflected projections finally.
3. Contributing weights to all those concerns which give a –ve contribution to any other concern. Each weight is considered as a number between [0...1] and represent its priority w.r.t. concern on which it is projected.
4. Resolving conflicts with stakeholders using above discussed methods.

   Resolution among conflicts may demand the rework of the requirement specification. In the cases like this there may be the need of revision o projections. This all goes on repeating until all the conflicts are resolved to full. Last action is the identification of dimensions among concerns. As discussed in [2] the dimensions of concerns may affect the latter on steps of development and generally identified as:

a) **Mapping:** It is identified that a concern might map onto a simple method, decision architecture, object/component, design/ implementation aspects etc.

b) **Influence:** A concern might affect the other steps of SDLC e.g. availability of resources may affect the system architecture; mobility may affect the specification architecture and design.

From the set of available requirements the goal of software system are identified and are kept in a separate list of functional requirements uniquely by assigning each requirement an id as FC_1 , FC_2,.....FC_i and so on. In the similar fashion the non functional requirements are also generated by considering each element uniquely and assigning each requirement an id as NFC_1, NFC_2 ...NFCi and so on. Both of the above selected requirements are represented in the form of a Plane (table) one for functional Table-1 and one for non functional Table-2 as shown below:

	FC_1	FC_2	FC_3
FC_1		+	
FC_2			+
FC_3			

Table 1: (Functional Requirements)

	NFC_1	NFC_2	NFC_3
NFC_1			
NFC_2			–
NFC_3	+		

Table 2: (Non-Functional Requirements)

By using these tables the interdependency or independency among the concerns can be represented easily. To reflect the dependency among the concerns some mark can be placed in the boxes where concerns cross each other.

To know about the dependency/relationship a functional concern with a non functional concern we have to place the two table representation on each other and mark the boxes at the crossing for the functional and non functional concerns.

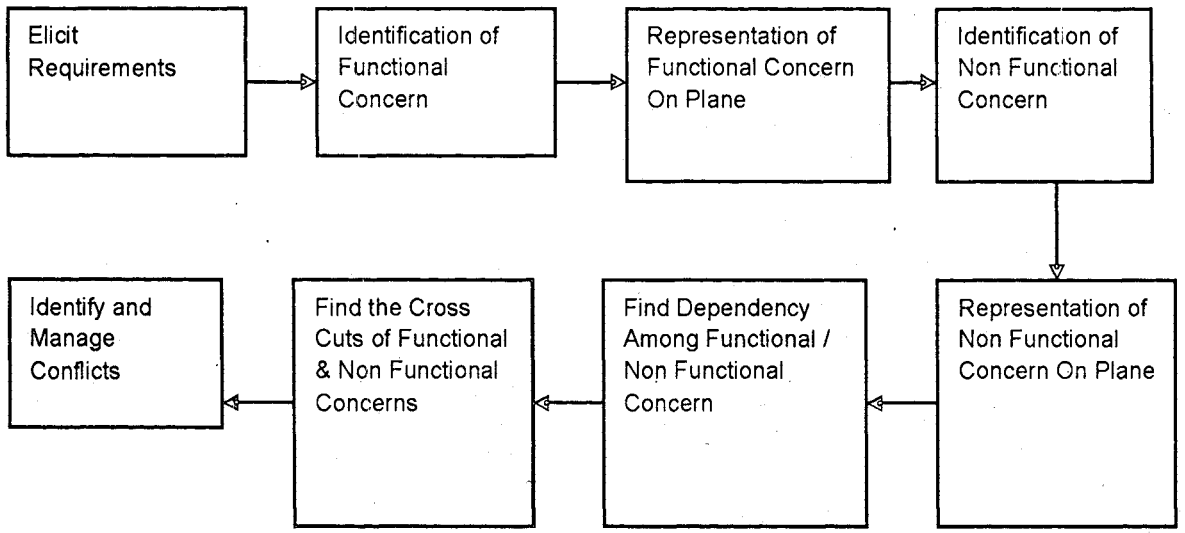
	FC_1	FC_2	FC_3
FC_1		*	
FC_2			*
FC_3			

Table 3: (Concerns for Functional Requirements)

	NFC_1	NFC_2	NFC_3
NFC_1			
NFC_2			*
NFC_3	*		

Table 4: (Concerns for Functional Requirements)

The collected representation of the functional and non functional concerns in the single figure also makes it possible to find the point of compositions. From this representation it is also possible to take a decision about conflicts if (any) occur during the analysis. All of the remarks about the functional and non functional concerns can be collected in the form of tables. The steps to be followed for the proposed model are



Model for Concern Based Requirement Engineering

RELATED WORK

Hyperspaces [17] and Cosmos [18] also support this type multi dimensional separation of concerns. In [17] the decomposition is done by using hyperslices where concerns are organized as per multiple dimensions and further each dimension is partitioned by concerns generally of same type. At the requirement level our model can be seen as specific instantiation of hyperspaces models. The entity concern can be perceived as hyperslices and composition rules can be seen a specific instances of hypermodules. On the other hand the discussion in [18] is a concern space modeling schema. Concern can be treated as any matter of interest in the system. The term concern space is considered as an organized representation of concerns and their relationships. An aspect oriented requirement engineering method with target at component based software development is discussed by Grundy [12]. This approach helps by providing a categorization of diverse aspects of systems where each component provides to end users. In Theme/Doc [1], provides support for Aspect Oriented Analysis. As per this the analysis is carried out firstly by identifying a set of actions from the requirements list where the crosscutting behavior is identified. The theme is prepared by collection of structure and behaviors that collectively represent one feature. This all is helpful for identification of requirements in documents and our approach complements this work by considering not only the identification of work but also their specification and compositions.

CONCLUSION

In this paper, we have proposed a model based on identification of functional and non-functional requirements, representing them separately in terms of matrix so that just by their overlapping we can identify their interdependency and can resolve the related issues

easily. This implementation is in direct context with existing Requirement Engineering approaches. Uniform transformation of concerns in their model make it possible for us to define the projections of each concern on any set of other concerns it relates to. By overlapping the resulting contribution matrix we can obtain a set of reflected projections which are then used for analyzing the contribution of multiple concerns towards one particular concern. Rest of the models provides no support for the same.

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REPRODUCTIVE BIOLOGY OF DISTYLOUS *SPERMADICTYON SUAVEOLENS* ROXB. (RUBIACEAE)

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ABSTRACT

Spermadictyon suaveolens is a woody and perennial shrub exhibiting distyly. Distyly is a population attribute where half of the plants had long styles and short stamens and are known as pin while the other half had long stamens and short styles and are known as thrum. The stigma showed dimorphism of its papillae size with longer pin papillae and shorter thrum papillae. Pollen dimorphism was observed in size, and sculpturing with pin pollen smaller in size than thrum and showing small sized sculpturing. The iso-plenthy (1:1 ratio) of pin and thrum plants in natural populations indicated out-breeding nature of the species. The species showed self-incompatibility. The simultaneous anthesis, synchronised anther dehiscence and stigma receptivity and overlapping period of the activity of pollinators of pin and thrum plants lead to efficient pollination.

Keywords: Distyly, Pin, Thrum, Receprocal herkogamy, Isoplenthy.

INTRODUCTION

Heterostyly is a population attribute displaying two to three levels of the style length coupled with the alternate stamen lengths. The former condition is termed as **distyly** and the latter as **tristyly**. In populations of distylous species about half of the plants bear flowers with long style and short stamens while the other half possess flowers with short style and long stamens. Following Lewis (1943), who used Darwin's (1877) terminology for **distylous** *Primula* to *Linum,* the long-styled flowers are known as **pin** and the short-styled ones as **thrum**.

In the present case *Spermadictyon suaveolens* of family Rubiaceae showing distyly has been investigated. Rubiaceae contain only distylic heteromorphism (Bir Bahadur, 1968). It has more di-stylous genera than any other family of the flowering plants and even all the families combined together have less heterostylous genera than Rubiaceae (Vuilleumier, 1967; Bir Bahadur, 1968; Ganders, 1979).

MATERIAL AND METHODS

Spermadictyon suaveolens is found throughout India ascending from 1000 m up to an elevation of 1900 m. It is an erect spreading shrub 1-3 m in height. It was found on hill slopes on road sides and along streams upto an elevation of 1250 m in Solan district (H.P.). The leaves are oppositely stalked, roughly pubescent, lanceolate, 6 cm to 20-cm in length and foetid when crushed.

The morphological dimensions of the distylous floral reproductive parts (stamens and styles) of *S. suaveolens* were large enough and were measured on a scale in the field. The **size of pollen grains** was measured using acetolysed pollen grains following Schoch Bodmer's (1940) technique. The **wall pattern** of the pollen grains were studied and photographed under scanning electron microscope at Panjab University Chandigarh. **Pollen fertility** was estimated as percentage of morphologically normal pollen grains which got stained in lectophenol-analine blue stain (Ornduff, 1979). **Stigmatic papillae length** was measured following staining techniques adopted by Datta and Naug (1967). The population attributes were assessed and analysed from 5 populations in Solan district of Himachal Pradesh both by random sampling and point sampling through nearest neighbour analysis. Morph data of each population was subjected to ÷2 test (Fisher and Yates, 1963) to verify the statistical equality of pin and thrum plants. The distribution pattern of the pin and thrum morphs in a population was ascertained through analysis by the nearest neighbour method (Pielou, 1966). Phenology and pollination biology was studied in the field at one hour interval for three days. Pollinators were observed during the peak flowering season. Insects involved were collected and got identified from entomologist of Zoology Deptt. Panjab University, Chandigarh. Controlled pollinations (self, intra and inter-morph) were resorted to in the field by using butter paper bags for bagging and capsule/seed formation determined to arrive at incompatibility system. The cytological studies on the pollen tube behaviour of pin and thrum

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pollen on the gynoceium of both the morphs were made following Martin's (1959) technique.

RESULTS

a) Morphological Features of Distyly:

The distylous flowers of *S. suaveolens* are blue lilac, 2 cm long and arranged in small bracteate head like clusters at the end of short forking branches forming terminal pubescent panicles. Although the flowers of both the morphs were blue lilac, pin flowers were lighter in colour than the thrum flowers. This feature helped in the identification of the two morphs from a distance in the field. In exhibiting distyly the stigma height of long-styled pin flower (1.8 cm) corresponded with the exerted stamens (1.8 cm) of thrum and so did the short styled thrum stigma height (1.2 cm) with the inserted stamens (1.2 cm) of pin (Table 1; Figs. 1 and 2). A glimpse at serial number 5 of Table 1 reveals that on an average the ratio of thrum stigma height to pin stigma height was nearly 1: 1.5. The ratios of stigma height to stamen length were distributed in two discrete classes. In the thrum morph this ratio was less than one that is, the stigma was situated below the anthers whereas in the pin morph it was greater than one as the stigma was situated above the anthers (Fig. 3). The five clefted stigma of both the morphs was of dry type. No difference in the length of stigmatic clefts was visible between the two morphs. Pin stigma had longer papillae as compared to thrum stigma. The average length of pin stigmatic papillae was 72.28 µm and that of thrum was 41.04 µm. (Table 1).

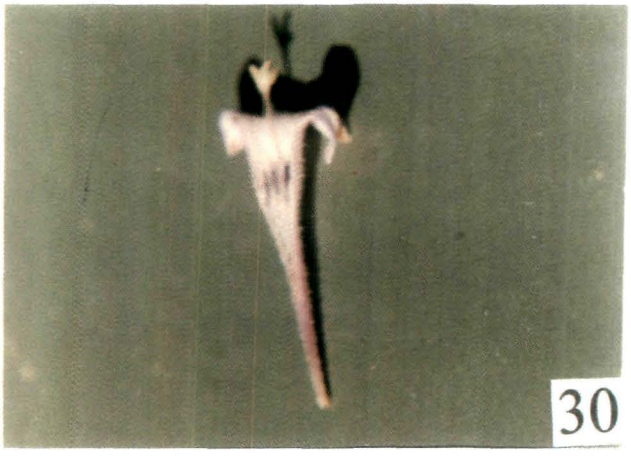

Fig. 1: Pin Flower

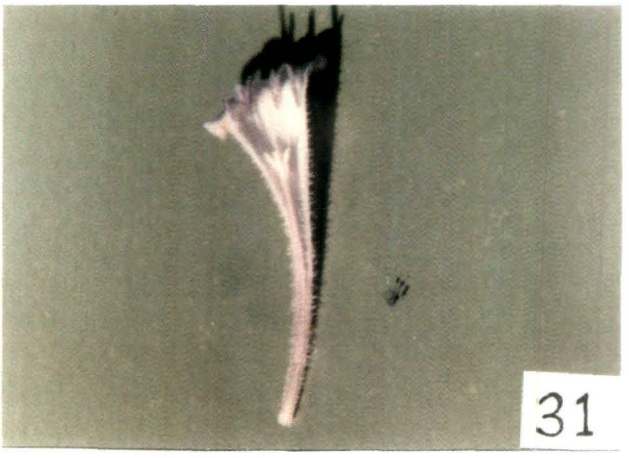

Fig. 2: Thrum flower

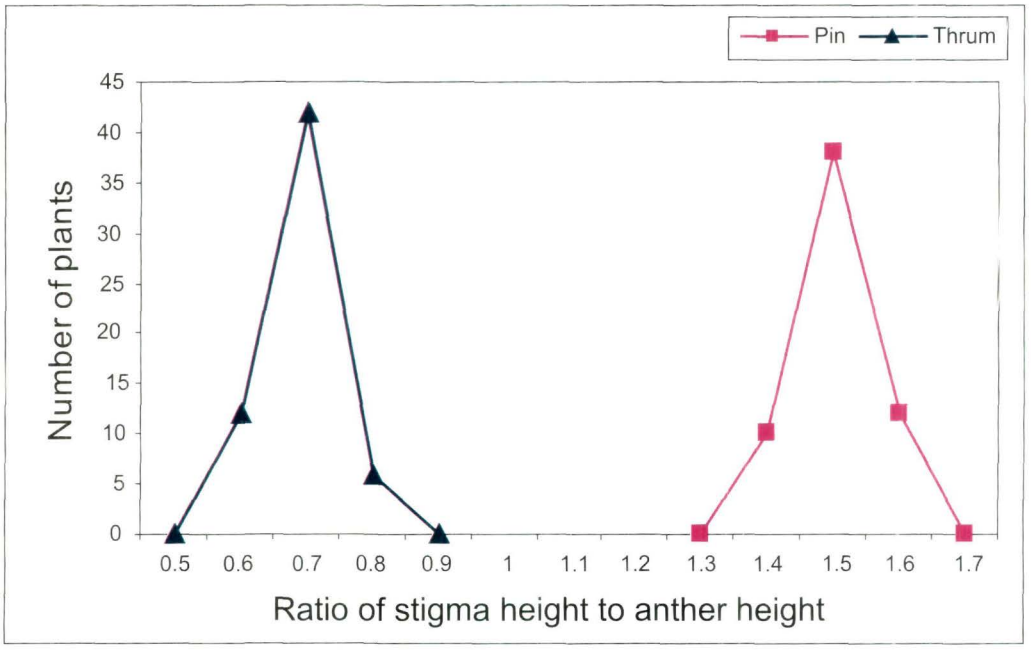

Fig. 3: Frequency distribution of the ratio of stigma height to anther height in Pin and Thrum morphs in *S. sauveolens.*

The pollen grains of both the morphs were reticulate, 3 – colpate and oblate spheroidal. The thrum pollen grain (40.72 µm) was larger (Fig. 5) than pin pollen grains (37.72 µm) (Fig. 6). The pin and thrum pollen revealed an overlap in their size between 36.20 µm and 44.30 µm. Only 12 % of pin pollen and 4% of thrum pollen did not overlap in size (Fig. 6). The percentage of pollen fertility was comparable in pin (92%) and thrum (90%) morphs. There were 4500 pollen grains in anthers of the thrum flower compared to 5200 in pin (Table 1) showing a ratio of 1:1.15.

Table 1: Morphological features of distyly in *S. suaveolens.*

S. No	Characters	Morphological Dimensions	
		Pin	Thrum
1	Mean Stamen length in cm ± SD (Range; sample number).	1.2 ± 0.10 (1.0-1.4; 30)	1.8 ± 0.14 (1.5-2.1; 30)
2	Mean Pollen size (µm) ± SD (Range; sample number)	37.72 ± 3.07 (32.40 - 44.30; 100).	40.72 ± 3.02 (36.20 - 48.30; 100).
3	Average number of Pollen grains per flower (Range; sample number)	5200 (4500 -6000; 100)	4500 (4000 - 5000; 100)
4	Pollen fertility	92%	90%
5	Mean stigma height (cm) ± SD (Range; Sample number)	1.8± 0.12 (1.5-2.0; 30)	1.2 ± 0.1 (1.0-1.4; 30)
6	Mean size of stigmatic papillae (µm) ± SD(Range; sample number)	72.28 ± 9.44 (56.50 - 92.60; 100)	41.04 ± 3.33 (36.20 - 52.50; 100)
7	Stigma type	Dry	Dry
8	Corolla colour	Light blue lilac	Dark blue lilac

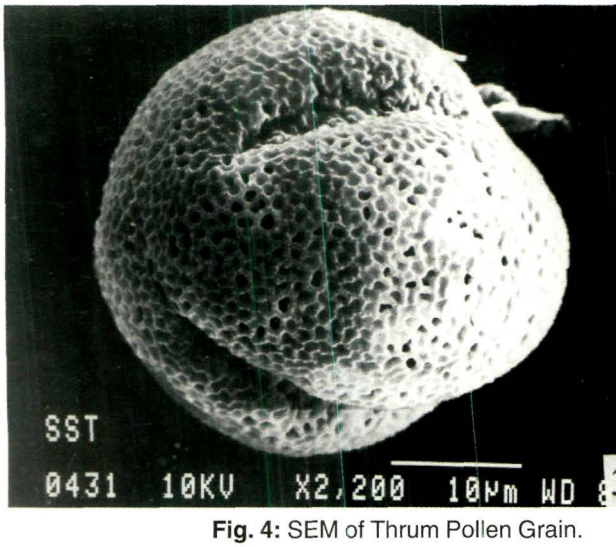

Fig. 4: SEM of Thrum Pollen Grain.

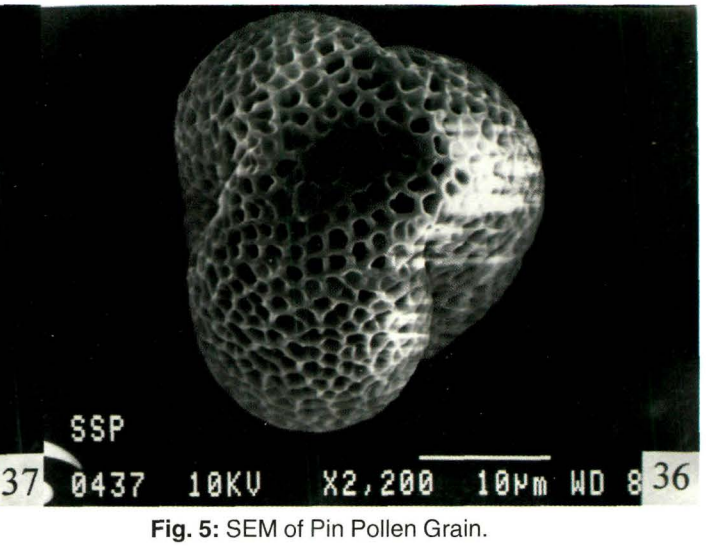

Fig. 5: SEM of Pin Pollen Grain.

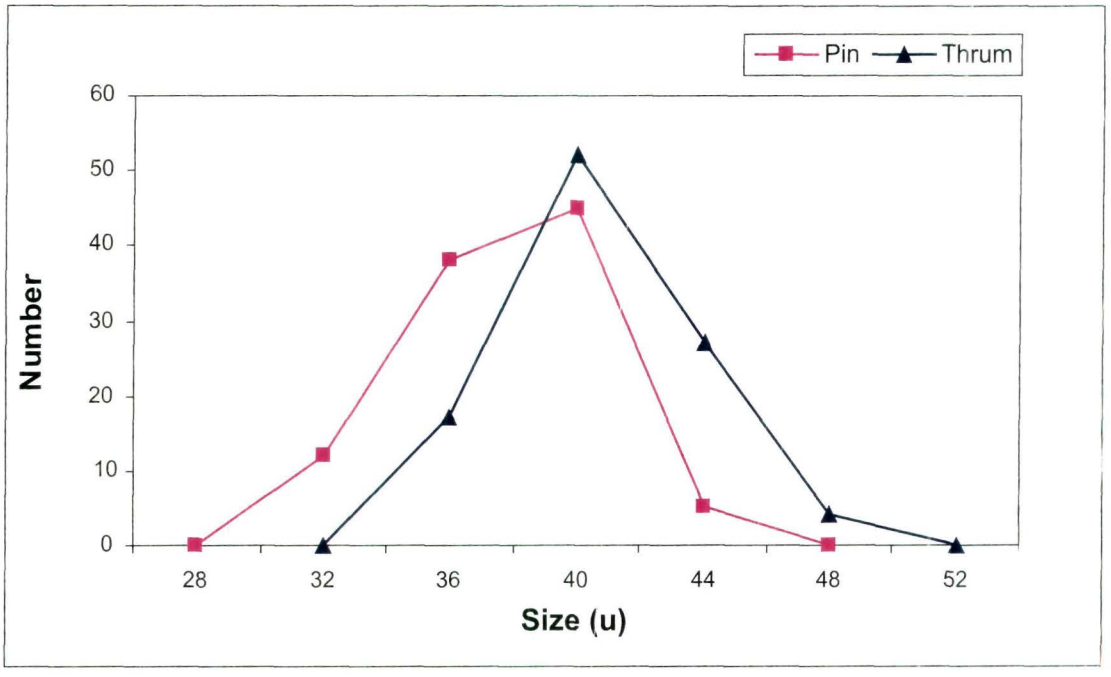

Fig 6.: Frequency distribution of pin and thrum pollen sizes in *S. suaveolens*

The intra-morph deviation in style length and level of anther insertions in *S. suaveolens* was very small, and therefore, no overlapping in the height of stigma and anthers between the two morphs was observed (Fig. 7) suggesting the absence of homostyly. Homostyles were never observed in the field during the current investigations.

b. Population Composition:

The populations of *S. suaveolens* were analysed for their structure from different localities in Solan district (H.P.). Random sampling in these localities and nearest neighbour analysis was carried out in one of these populations to study the population composition and dispersion of pin and thrum plants respectively.

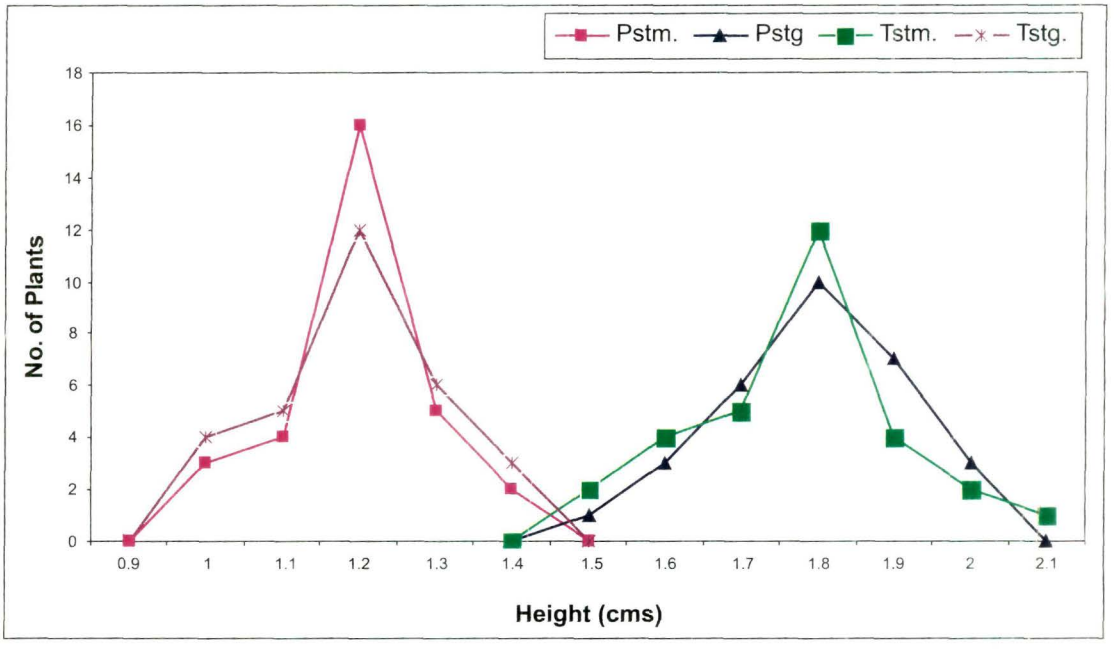

Fig. 7: Frequency distribution of stigma and anther heights in Pin and Thrum morphs in *S. sauveolens*. (30 flowers sampled for each floral morph; Tstm – Thrum stamen, Tstg – Thrum stigma, Pstm – Pin stamen, Pstg – Pin stigma).

i) **Random sampling** was made in five natural populations of *S. suaveolens*. A total of 1291 plants were counted in these populations with 646 pin plants and 645 thrum plants. A glance at Table 2 reveals that thrums exceeded pins in number in four populations (÷2 - 0.018 to 0.468) and only in one population, number 2 (÷2 - 2.358), pins outnumbered thrums. The value of ÷2 for all the populations was less than 3.84 hence its statistical analysis revealed that pin and thrum plants were present in 1:1 ratio (isoplenthy) in all the populations. These data are suggestive of the out-crossed nature of the species.

Table 2: Composition of natural populations of *S. suaveolens*.

S. No	Species/Locality	Altitude	Number of Pin plants	Number of Thrum plants	T/P Ratio	x^2 values
1	Parwanoo I	650 m	82	91	1.11	0.468
2	Parwanoo II	650 m	145	120	0.83	2.358
3	Parwanoo III	650 m	308	316	1.03	0.102
4	Koti	1000 m	26	27	1.04	0.018
5	Jabli	1200 m	85	91	1.07	0.204
	Total		**646**	**645**		

ii) The **nearest neighbour analysis** was carried out in Parwanoo I population of Solan district of H.P. In this population 49 pin plants had thrum as the nearest neighbour compared to 54 thrum plants having pin as the nearest neighbour. Similarly, 37 thrum plants had thrum as the nearest neighbour compared to 33 pin plants having pin as the nearest neighbour (Table 3).

Table 3: Nearest-neighbour relationship in Parwanoo population of *S. suaveolens*.

POPULATION	No. of Pins	No. of Thrums	Percent Pins	Ratio	Nearest - neighbour relationship				H (Index of Diversity)
					P-P	T-T	P-T	T-P	
Parwanoo I	**82**	**91**	**47%**	**1:1**	**33**	**37**	**49**	**54**	**0.69**

The distribution of pins to pins and thrums to thrums was found to be less than expected on random basis while that of pins to thrums and thrums to pins was found to be more than expected on random basis. These data on the nearest - neighbour analysis of the populations of this species done through Shanon-Wiener Index (Pielou, 1966) suggested that pin and thrum plants were randomly distributed which aided insect pollinators in causing legitimate pollinations.

c. Floral Biology:

Flowering phenology, pollination biology and seed production and dispersal has been studied in Jabli population of Solan district in H.P.

(i) Flowering phenology: - The flowering season in *S. suaveolens* extended over four months from September to December. An individual plant continued to bear flowers nearly throughout the flowering period. The flowering was at its peak in the last week of October. The flowering pattern of pins and thrums was similar.

With the onset of flowering the leaves started withering from the base of the plant upwards. New leaf primodia made their appearance a few days after complete

defoliation of the plant. In an inflorescence 3-5 flowers opened per day. It took five days for a bud to develop into a flower. The flowers opened synchronously in pin and thrum morphs. Individual flowers opened only on sunny days between 8.00 hrs and 11.30 hrs. Anther dehiscence occurred from 9.00 hrs to 12.30 hrs, when white powdery mass of pollen was observed all around the anthers. The insect activity started around 10.00 hrs and continued up to 13.00 hrs. The un-pollinated flowers withered the next day while in the pollinated flowers the ovary swelled noticeably after about a week of pollination to eventually turn into a fruit. The examination of marked flowers revealed the mean period of anthesis to be 5.3 days for pin and 5.5 days for thrum, 4 and 6 days being minimum and maximum respectively for both the morphs.

(ii) Pollination Biology: - The flowers of *S. suaveolens* are blue lilac in colour, have fragrance due to the presence of nectar and these features make them attractive for the insect visits. The insects that were seen to visit the flowers of *S.suaveolens* included bumble bees *(Bombus sp.)*; Indian honey bees (*Apis cerana*); rock bees (*Apis dorsata, Andrena sp.*); butterflies and thrips (Table 4). No insect activity was observed on cloudy or rainy day. *S.suaveolens* was seemingly an important source of food for these insects as none of the other co-inhabiting taxa was seen to be flowering synchronously in any of the studied populations of this species.

The most conspicuous insect that visited the flowers of this species frequently during the field observations was **bumble bee**. The visits of these insects to the various flowers of both the morphs were random. The bumble bee during visit to a flower spent three to five seconds and then moved to another flower either of the same plant or that of the other plant may it be a pin or thrum. When the bumble bee visited a flower it sent its proboscis into the narrow corolla tube for the collection of nectar. On its visit to pin flower, some of the thrum pollen already deposited at the proximal part of the proboscis which the insect had gathered during its previous visit to thrum flower got deposited on the exerted pin stigma. When the proboscis was further squeezed past the corolla tube for the collection of nectar, the dehisced pin pollen from the inserted pin stamens got struck to the distal end of the proboscis. Similarly, when the insect visited the thrum flower the dehisced thrum pollen at the mouth of the corolla got attached at the base of the proboscis and the pin pollen sticking at the distal part of the proboscis got simultaneously deposited on the inserted thrum stigma. If the movements of the bumble bee were erratic during this process, the dehisced pollen from the exerted thrum stamens fell on the thrum stigma. Similarly if the tip of the proboscis carrying pin pollen touched the pin stigma it might dust the pin pollen on it. This process of pollination was repeated several times when the bumble bee during the course of its movement visited flowers of the two morphs. The see-saw placement of reproductive floral parts of *S.suaveolens* aided bumble bees to mainly account for legitimate pollinations. However, in the process as explained above some proportion of illegitimate pollinations could also occur.

Indian honey bee and **Rock bee** were the other two insects that accounted for sufficient legitimate pollinations. The frequency of visits of these insects was low as compared to bumble bees and butterflies. The Indian honey bee has a small proboscis, which in combination with its head was used in the pollination process. When the bee inserted its head into the corolla tube of the pin flower to collect nectar the pin pollen got attached to its proboscis and on the visit to thrum flower the thrum pollen got deposited on its head. In affecting the pollination process the proboscis and head of honey

Table 4: Pollinators of *S. suaveolens.*

S. No.	Insect species	Frequency	Insect's body part used.
1.	Bumble bee (*Bombus* sp.)	Abundant	Proboscis
2.	Indian Honey bee (*Apis cerana indica)*	Frequent	Proboscis and head.
3.	Rock bee (*Apis dorsata* and *Andrena* sp.)	Common	Head and thorax
4.	Butterflies	Abundant	Proboscis
5.	Thrips	Abundant	Whole body.

bee behaved in the same way as the distal and proximal regions of the proboscis respectively of the bumble bee in causing legitimate pollinations. Illegitimate pollinations could also occur similarly as explained for the bumble bee. The rock bees affected pollination in the same way as the Indian honey bee but instead used its head and thorax.

The **butterflies** of white and brown colour also visited the flowers to feed on nectar through their long and coiled proboscis. Transfer of pollen from pin and thrum and vice versa took place by the use of proboscis in the way as described above for other insect species. The large number of butterflies helped in conserving energy in this process and was effective in ensuring legitimate pollinations. However as in case of other insects because of the vigorous activities of these insects and due to disturbances illegitimate pollinations might also occur.

Thrips are very small insects whose movements were primarily restricted to a flower. These insects come out only when the flowers were disturbed. A maximum of five thrips (range from 1 to 5) were observed in a single flower. These insects were always covered with pollen and were found in flowers of both the morphs. Since these insects were mostly confined inside the flower and could fly only very small distances, they mostly accounted for self-pollination. Intra-morph and inter-morph pollinations might occur when the branches or plants of the same morph or of the other morph adjoined each other.

iii) Fruit and seed production and dispersal: The fruits started maturing by December and nearly all of them matured in January when they dried up and turned brown. The dried mature fruits remained for long on the plants. The seeds were dispersed by wind during the month of February and the remaining were blown over small distances before rains or even during the rainy season. When the plants grew near the streams the dispersal of seeds was found to also occur by water and the plants were seen growing on the banks of the streams.

Table 5: Capsule and seed formation in nature in *S.suaveolens.*

S. No	Morph	Total number of flowers tagged	Number of capsules formed	Percentage of flowers producing capsules	Mean number of seeds per capsule
1	Pin	40	11	27.5	4.2
2	Thrum	40	11	27.5	4.0

The fruits of *S. suaveolens* are arranged in umbelliform heads and are five lobed. The fruit production in nature was calculated to be 27.5% both in pin and thrum morphs. The average number of seeds per capsule was 4.2 in pin and 4.0 in thrum (Table 5).

d) Compatibility Relationship:

The phenomenon of incompatibility in *S. suaveolens* was tested in Jabli population of Solan district in H.P. This was made possible by making self, intra and inter-morph pollinations and then studying the behaviour of pollen tube on stigma and in the style, and studying the seed set pattern of selfed, intra-morph and inter-morph cross pollinated flowers.

(i) Pollen - Pistil Interactions: - Twenty treatments each involving selfing intra-morph crosses and inter-morph crosses were made to study the behaviour of pollen tubes on the stigma and in the style. In selfing and intra-morph crosses, although the pollen were seen to germinate on the stigma, the emerging pollen tubes remained restricted on the stigmatic surface where they bent and burst. In inter-morph crosses pollen tubes pierced through the stigma, transversed towards the end of the style, and were seen entering the ovary.

(ii) Fruit/seed production following artificial pollinations: - Selfed and intra-morph (Pin X Pin and Thrum X Thrum) pollinated flowers (20 each) showed complete failure of seed set indicating presence of strong incompatibility. Inter-morph (Pin X Thrum and Thrum X Pin) pollinations were 100% successful in both the morphs. Out of a total of five ovules in ovary the average seed set per capsule in pin X thrum crosses was 4.0 while it was 4.2 in thrum X pin crosses (Table 6).

Table 6: Capsule and seed formation following experimental pollinations in *S. suaveolens*.

S. No	Type of pollination	Number of flowers pollinated	Number of capsules formed	Percentage of flowers producing capsules	Mean number of seeds per capsule
1.	P self	20	0	0	0
2.	T self	20	0	0	0
3.	P x P	20	0	0	0
4.	T x T	20	0	0	0
5.	P x T	20	20	100	4.0
6.	T x P	20	20	100	4.2

It is thus evident as studied through both cytological and seed set pattern that distyly associated with strong incompatibility system with sexual reproduction occurred only between pin and thrum reproductive parts.

DISCUSSION

Spermadictyon sauveolens shows distyly in having flowers with stigma and anther heights in pin and thrum morphs showing reciprocal correspondence (reciprocal herkogamy), inter-morph pollen and stigmatic papillae dimorphism in pin and thrum morphs and presence of self-incompatibility. Similar findings have been reported for other distylous species studied from diverse families (Darwin, 1877; Vuillieumier, 1967; Ganders, 1979; Richards, 1986; Barrett and Richards, 1990; Barrett, 1992; Dulberger, 1992; Lloyd and Webb, 1992; Barrett and Cruzen, 1994). *S. sauveolens* has regular and actinomorphic flowers with floral tubes. Findings suggest presence of heterostyly in flowers with such characteristics rather than those with strongly zygomorphic flowers (Ganders, 1979; Barrett, 1992; Dulberger, 1992; Lloyd and Webb, 1992). Its flowers possess darker coloured thrum corollas compared to pin corollas (Tables 1). The question arises if dimorphism in colour of corolla has something to do with the need for additional attraction for insects in the pollination process in the thrum morph due to its flowers having deep-rooted stigma that too located in the tube shaped flower. The pollinators of these species did not show particular preference for the flower of any morph making it difficult to conclude as to what role corolla colour has in the pollination process.

The pin morph of *Spermadictyon suaveolens* has longer stigmatic papillae than thrum morph in the currently studied species as in most other distylous taxa (Vuilleumier, 1967; Dulberger, 1974; Ganders, 1979). The thrum pollen grains were larger than pin pollen grains a feature characteristic of distylous species (Darwin, 1877; Vuilliumier, 1967; Ganders, 1979; Dulberger, 1992). It did not show marked pollen size dimorphism, pin to thrum pollen size ratio is 1:1.08 (Table 1). In Rubiaceae as in the presently studied species the pin-thrum pollen size ratio in the earlier investigated species has been found to be low for example 1:1.14 in *Oldenlandia umbellata* (Bir Bahadur,1964), 1:1.06 in *O.scopulorum* (Bir Bahadur (1966) and 1:1.20 in *Hedyotis caerulea* (Ornduff, 1977).

The equality in representation of the pin and thrum plants (1:1 ratio) in the sampled natural populations of the investigated distylous species (Table 2) is a common feature of the population structure of to-date studied self-incompatible distylous species (Ornduff, 1970, 1971; Ornduff and Perry, 1964; Barrett, 1992). Deviation from such a ratio in the distylous species of the family is known in *Hedyotis nagricans* (Levin, 1974) and *Hedyotis caerulea* (Ornduff, 1980). This isoplenthy is the consequence of the presence of self and intra-morph incompatibility which has been experimentally demonstrated in the presently studied taxa. Such a ratio is expected if one of the two morphs is heterozygous dominant and the other one homozygous recessive. The genetically studied self-incompatible distylous species are known to display one morph (usually pin) to be homozygous recessive (ss) and the other morph (often thrum) to be heterozygous dominant (Ss) (See Ganders, 1979; Barrett, 1992). Haldane (1938) conjectured that

in case illegitimate pollinations in pins and thrums were successful to produce off springs, this would have resulted in excess of pins if pins are homozygous recessive which is usually the case. Li (1955) and Barrett (1992) have interpreted that completely self-incompatible distylous species show negative assortative mating, and 1:1 ratio of the compatible phenotypes is arrived at in one generation only.

The comparison of nearest neighbours for pin and thrum morphs of the populations studied (Table 3) reveals random segregation of morphs. Levin (1974) while studying spatial distribution of morphs in *Hedyotis nagricans* was able to show significant random morph segregation in the populations. Such segregation of pin and thrum morphs in the distylic species have been observed in other species also (Ornduff and Weller, 1975; Ganders, 1976). This observed random segregation of morphs in populations of studied species play an effective role in their pollination process, as they are dependent on insects, which efficiently bring about legitimate pollinations between the randomly distributed pins and thrums.

The concurrent opening of flowers of pin and thrum morphs in the observed species, synchrony in their anther dehiscence during duration of anthesis, followed by the transfer of adequate legitimate pollen by insect pollinators to the receptive stigmas of both the morphs is an adaptation to ensure compatible pollination so essentially required due to the presence of self- and intra-morph incompatibility. The characteristics of colour, production of nectar and fragrance of flowers constitute visual, food and olfactory cues respectively which serve to attract the appropriate insects to accomplish the pollination process. The maximum activity of insects on any day during the period of anther dehiscence and stigma receptivity provides favourable situation for the success of pollination.

The reciprocal herkogamy in the distylous flowers aid in carrying of pin and thrum pollen at suitable body parts of the insect. These are consequently transferred by the insects (other than thrips) to the alternate stigmas located at corresponding height to accomplish legitimate pollination. Similar methodology of legitimate transfer of pollen in the breeding system of various distylous species has been reported by Darwin (1877) and Ganders (1979). However in this process, due to some disturbance or different behaviour of the insects on their visit to flowers illegitimate pollen grains also get deposited on the stigmas of both the morphs.

The combination of the activity of all the insects listed for each of the investigated species is essential in the pollination process as the missing of one or the other insect may result in shift from complimentary transfer of pollen to unidirectional one leading to the shift of the reproductive biology from distylous to dioecious or some other system. The unidirectional transfer of pin pollen to thrum stigma has resulted in shift to dioecious reproductive system in many distylous species especially of Rubiaceae (Beach and Bawa, 1980).

The present observations of complete inhibition of pollen tubes and complete lack of fruit formation following the self- and intra-morph pollinations (Table 6) in the pin as well as thrum morphs demonstrate that the species is self-incompatible. This finding is in agreement with the concept developed by Darwin (1877) that distyly is associated with self-incompatibility, subsequently confirmed in species of diverse families (See Vuilleumier, 1967; Ganders, 1979; Richards, 1986; Barrett, 1992; Barrett and Curzan, 1994).

The distylic syndrome having reciprocal herkogamy with dimorphic stigmatic papillae and pollen grains with self and intra-morph incompatibility is working successfully in *Spermadictyon suaveolens* by acquisition of adaptations during the course of its evolution which can be seen by its widespread distribution.

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CONTRIBUTIONS OF AND η' PROTON SPIN PROBLEM IN CHIRAL CONSTITUENT QUARK MODEL

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ABSTRACT

The non-relativistic quark model (NRQM) predictions of some spin and flavor parameters are in sharp conflict with the observations made from deep inelastic scattering experiments. For example, these experiments indicated that only 30% of the spin is carried by the valence quarks. Besides this, along with the observation $\bar{u} - \bar{d} \neq 0$ there are other spin and flavor dependent quantities which could not be explained by NRQM. These contradictions are referred to as "Proton spin problem". These issues get resolved, to some extent, in Chiral constituent quark model (¿CQM) which incorporates the basic features of NRQM and chiral symmetry. The implications of the latest data pertaining to $\bar{u} - \bar{d}$ asymmetry and the spin polarization functions on the contributions of singlet Goldstone Boson η' within ¿CQM with configuration mixing for explaining the "proton spin problem" have been investigated. It is found that the present data favors smaller values of the coupling of singlet Goldstone Boson η' as compared to the corresponding contributions from π, K and η Goldstone bosons. It seems that a small non-zero value of the coupling of η' $(\zeta \neq 0)$ is preferred over $\zeta = 0$ phenomenologically.

Keywords: Chiral, Constituent, Quark, Model (¿CQM) Proton, Spin

1. Introduction:

Understanding of QCD, the most valuable theory of strong interactions, in the low energy limits has been a challenging task; however, considerable progress has been achieved in this regime through the calculations of QCD using lattice techniques. The lattice gauge theory calculations have given strong evidence for the *confinement* as well as existence of chiral symmetry breaking in the non-perturvative regime of QCD. This has given strong impetus to the formulation of the models such as Chiral quark model, instanton model etc., for studying the low energy features of hadrons. One of the most enigmatic feature of the low energy hadron physics has been the remarkable success of the **non relativistic quark model** (**NRQM**), first introduced by Rujula, Georgi and Glashow [1] in explaining a large amount of hadron data.The NRQM, can now be considered as an intermediate phenomenological model that fits the experimental data and incorporates several of the key features of QCD. Based on every simple assumptions, such as, point like quarks carrying spin ½, valence quark structure of hadrons, single quark transitions etc., the NRQM along with the color spin-spin forces has given a remarkable fit to the hadron spectroscopy data including some of the very subtle features of the data such as neutron charge radius, N – Δ mass difference, photohelicity amplitudes, baryon magnetic moments, etc.. The success of NRQM led Weinberg, Manohar and Georgi [2] to formulate **chiral constituent quark model** (¿CQM), which has strong linkage with QCD Lagrangian.

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Europian Muon collaboration (EMC) in deep inelastic scattering (DIS) experiment [3], interestingly found that valence quarks of proton carry only about 30% of proton spin which is quite in contradiction with the predictions of NRQM. Viewed from the point of overwhelming success of NRQM in explaining hadronic data led many to christian the above problem as "*proton spin crisis*". The EMC observations were confirmed by several other experimental groups. Apart from the problem faced by NRQM with regard to spin structure of nucleon, the NRQM also faced problem regarding its flavor structure, for example, the strange quark content measured in the pion nucleon sigma ($\sigma_{\pi N}$) term as well as the $\bar{u}-\bar{d}$ asymmetry measured in the DIS data. The contradictions observed in reference to spin and flavor structure of proton are referred to as "**PROTON SPIN PROBLEM**". The problem becomes more intriguing when one realizes that NRQM is able to give fairly good description of magnetic moments of octet baryons using the assumption that magnetic moments of valence quarks are proportional to the spin carried by them. Thus, finding appropriate spin and flavor structure of nucleon becomes important issue to be resolved.

2. NRQM and Proton spin problem: NRQM is based on the assumption that hadrons are made up of point like valence quarks carrying spin ½, with baryons and mesons consisting of three quarks and quark-antiquark combinations, respectively. Quarks do not appear as free particles but are confined within color neutral i.e., color singlet states. These quarks are supposed to be interacting through confining potential, several of these have been used, the most popular being Coulombic+linear and the harmonic oscillator potential, the latter facilitating exact solutions. The color wavefunctions of mesons are: $|M\rangle = \frac{1}{\sqrt{6}}|qqq\rangle$, and that of baryons are: $|M\rangle = \frac{1}{\sqrt{6}}|qqq\rangle$.Taking into account all the degrees of freedom the baryon wavefunction can be factorized as: $\psi_{3q} = \psi_{colour} \times \psi_{flavor} \times \chi_{spin}$.

Based on these simple assumptions, NRQM has been applied successfully to huge amount of low energy hadronic data. In particular, Isgur *et. al.* [4], have shown that NRQM incorporating color spin-spin interaction is remarkably successful in this context. Some of the well known successes of NRQM are as follows:

- It is not only successful in explaining the flavor J^{PC} (spin parity-charge conjugation), quantum number of hundreds of hadrons but is also able to give a fair description of their masses, including mass splitting within the multiplet.
- Based on single quark transition rule, it is able to provide fairly satisfactory description of large number of strong decay of hadrons, electromagnetic decays, as well as their weak decays.
- It is able to provide a highly satisfying description of subtle features such as: Δ–N splitting, non-zero neutron charge radius, photohelicity amplitudes, magnetic moments of octet and decuplet of baryons, etc..

For the present purpose, the effect of color spin-spin forces in the proton wavefunction, referred to as configuration mixing, can be expressed as [5]

$$|B\rangle_{config} = \cos\phi\,|56,0^+\rangle_{N=0} + \sin\phi\,|70,0^+\rangle_{N=2}, \tag{2.1}$$

Where,

$$|56,0^+\rangle_{N=0} = \tfrac{1}{\sqrt{2}}(\varphi',\chi' + \varphi'',\chi'')\psi^s, \tag{2.2}$$

$$|70,0^+\rangle_{N=2} = \tfrac{1}{2}[(\varphi'\chi' + \varphi''\chi'')\psi'(0^+) + (\varphi'\chi' - \varphi''\chi'')\psi''(0^+)], \tag{2.3}$$

The spin and unitary spin wavefunctions χ, φ' and φ'' for proton are

(2.4) $\chi' = \frac{1}{\sqrt{2}}(\uparrow\downarrow\uparrow - \downarrow\uparrow\uparrow)$, $\chi'' = \frac{1}{\sqrt{6}}(2\uparrow\uparrow\downarrow - \uparrow\downarrow\uparrow - \downarrow\uparrow\uparrow)$

(2.5) $\varphi'_B = \frac{1}{\sqrt{2}}(udu - duu)$, $\varphi''_B = \frac{1}{\sqrt{6}}(2uud - udu - duu)$.

In order to understand the proton spin problem, we have worked out in detail the spin and flavor structure of proton within NRQM with and without configuration mixing. The quark spin polarization is defined as [6]:

(2.6) $\Delta q = q^+ - q^-$,

where $q^{+(-)}$ is the number of q quarks with spin up (down). The sum of Δq's gives the total spin carried by the quarks, for example, $\Delta\Sigma=\Delta u+\Delta d+\Delta s$, where $\Delta\Sigma$ is twice the spin of the proton. The spin structure for baryon is defined as

(2.7) $\hat{B} \equiv \langle B|\square|B\rangle$,

where N is the number operator corresponding to different quark flavor with spin up and spin down and is expressed as

(2.8) $\square = n_{u^+}u^+ + n_{u^-}u^- + n_{d^+}d^+ + n_{d^-}d^- + n_{s^+}s^+ + n_{s^-}s^-$,

with the coefficients of the $q^{\pm}$ giving the number of $q^{\pm}$ quarks. Using the above number operator, we can calculate for proton, the number of u quarks with spin up u^+, for example, $u^+ = \langle 56,0^+|n_{u^+}|56,0^+\rangle$. As the operator does not affect the spatial part of the wavefunction, therefore, using Eq. (2.2), we can write

(2.8) $u^+ = \frac{3}{2}\langle \chi'\varphi' + \chi''\varphi''|n_{u^+}(3)|\chi'\varphi' + \chi''\varphi''\rangle$.

Using the explicit form of χ and φ wavefunctions, one finds $u^+ = \frac{5}{3}$ for the case of proton, similarly, we can find u^-, d^+ and d^-, for example

(2.10) $u^- = \frac{1}{3}, d^+ = \frac{1}{3}, d^- = \frac{2}{3}$.

Thus, using Eq. (2.6), the contribution by each of the quark flavors to the proton spin polarization can be written as:

(2.11) $\Delta u = \frac{4}{3}, \Delta d = \frac{-1}{3}, \Delta s = 0$.

Using Eq. (2.7), the spin structure for Eq. (2.1) is given as

(2.12) $\hat{p}_{config} = \cos^2\phi(\frac{5}{3}u^+ + \frac{1}{3}u^- + \frac{1}{3}d^+ + \frac{2}{3}d^-) +$
$\sin^2\phi(\frac{4}{3}u^+ + \frac{2}{3}u^- + \frac{2}{3}d^+ + \frac{1}{3}d^-)$

The spin polarization functions are therefore given as

(2.13) $\cos^2\phi[\frac{4}{3}] + \sin^2\phi[\frac{2}{3}]$, $\Delta d_{val} = \cos^2\phi[\frac{-1}{3}] + \sin^2\phi[\frac{1}{3}]$, $\Delta s_{val} = 0$.

The typical set of the phenomenological result for the quark spin polarizations, as found by EMC and other experiments, are as follows:

(2.14) Δu=0.85, Δd=-0.41, Δs=-0.07,

to be compared with the predictions of NRQM, for example

(2.15) Δu=1.33, Δd=-0.33, Δs=0,

where, the total polarization $\Delta\Sigma=\Delta u+\Delta d+\Delta s$ is normalizes to 1 in the case of NRQM. The total spin polarization, from Eq. (2.14), corresponds to $\Delta\Sigma=0.38$, showing a good deal of disagreement with NRQM expectations.

Apart from the above mentioned inadequacy of NRQM, the problem of NRQM becomes more acute when one confronts it with some of the well known sum rules involving spin polarization functions having weak Q^2 dependence and are derived rigorously from QCD using operator product expansion, renormalization group invariance and isospin conservation. For example, the Bjorken sum rule (BSR), relates the flavor non-singlet component Δ_3, isovector axial current obtained using SU(3) flavor symmetry from the neutron β-decay G_A/G_V and the spin polarization functions of proton, is given as

(2.16) $$BSR: \Delta_3 = G_A/G_V = \Delta u - \Delta d.$$

Experimentally, the value of G_A/G_V is 1.267, to be compared with the NRQM value 1.66, again indicating the inadequacy of NRQM. Similarly, the Ellis-Jaffe sum rule (EJSR), relating the flavor non-singlet component Δ_8 and the spin polarization functions, has the form

(2.17) $$EJSR: \Delta_8 = \Delta u + \Delta d - 2\Delta s$$

If one assumes $\Delta s=0$, we get $\Delta\sum = \Delta_8$ which is observed to be strongly violated by data. This implies that there is a significant contribution to the proton spin by the polarized strange quark components in the sea. The measured value of the pion-nucleon sigma term $\sigma_{\pi N}$ suggests a surprisingly large fraction of strange quark. When SU(3) breaking effects are taken into account, the fraction f_s, defined as the strange quark and antiquark number divided by the sum of the quark numbers, is of the order of 0.10, indicating the failure of NRQM which predicts it to be zero.

Apart from the spin polarization functions, the valence quark structure of NRQM is inadequate to describe the flavor structure of the proton. For example, the Gottfried sum rule (GSR), in terms of antiquark densities as $\bar{u} - \bar{d} = -0.118 \pm 0.026$, indicates that the nucleon sea is quite asymmetric with respect to $\bar{u}$ and $\bar{d}$ quark contents. This is in contrast to the symmetric expectation from NRQM. These inadequacies of NRQM are to be referred as "PROTON SPIN PROBLEM". The situation is still further intriguing for NRQM's description of magnetic moments of octet baryons using the assumption that magnetic moments of valence quarks are proportional to the spin carried by them. Thus, finding the appropriate spin and flavor structure of the nucleon becomes an urgent issue to be resolved.

3. χCQM and emission of GB:

The chiral constituent quark model (χCQM), as formulated by Manohar and Georgi [2], has recently got good deal of attention [6, 7, 8, 9] as it is successful in not only explaining the "proton spin crisis" [3, 10, 11, 12] through the emission of a Goldstone boson (GB) but is also able to account for the $\bar{u} - \bar{d}$ asymmetry [13, 14, 15], existence of significant strange quark content $\bar{s}$ in the nucleon, various quark flavor contributions to the proton spin [7], baryon magnetic moments [6, 7] and hyperon β-decay parameters etc..

Recently, it has been shown that configuration mixing generated by spin-spin forces [1, 4, 16], known to be compatible with the χCQM [17, 18, 19], improves the predictions regarding the quark distribution functions and the spin polarization functions

[20]. Further, χCQM with configuration mixing (henceforth to be referred as χCQM_{config}) when coupled with the quark sea polarization and orbital angular momentum (Cheng-Li mechanism [21]) as well as "confinement effects" is able to give an exellent fit for the violation of Coleman Glashow sum rule [22].

The scope of χCQM, as developed by Weinberg, Manohar and Georgi [2], was extended by Cheng and Li [6] to resolve the "proton spin problem" [23]. They realized that the key to understand the problem lies in generating an appropriate quark sea in the proton through the chiral symmetry breaking mechanism. They considered the following chiral fluctuations for the quark sea generation through the emission of Goldstone boson (GB) by a given valence quark $q^{\pm}$

(3.1) $$q_{\pm} \to GB^0 + q'_{\mp} \to (q\bar{q}') + q'_{\mp},$$

where the superscript $\pm$ indicates the helicity of the quark. The $q\bar{q}'$ and $q'_{\mp}$ represent the "sea" associated with the quark $q_{\pm}$. The production of the $q\bar{q}'$ pair is shown in Figure 3.1 In the SU(3) symmetric model, they demonstrated that this picture can account for the observed spin and flavor structure in the nucleon in terms of two parameters: the probability of the above fluctuation of the pion GB to be controlled by the parameter a and the ratio of singlet and octet coupling ζ. The agreement of Cheng and Li improves further when symmetry breaking effects, attributed to the difference between strange and non-strange quarks, are taken into considerations.

It is not difficult to understand the success of χCQM in resolving the "proton spin problem' in terms of its basic tenets. The depolarization of the valence quarks can be understood from Eq. (3.1). Since both the q and $\bar{q}'$ quarks of the "quark sea" in the equation are unpolarized, the polarization of the entire quark sea must be given by the $q'_{\mp}$, which is opposite to the initial quark helicity state. This naturally leads to a negatively polarized sea and is in qualitative agreement with the phenomenological observation that the entire spin is not carried by the valence quarks and is less than one. The negatively polarized sea q makes

(3.2) $$\Delta\Sigma = \Delta\Sigma_{val} + \Delta\Sigma_{sea}$$

less than one as observed in the DIS experiments.

To understand the proton flavor structure in χCQM, it can be seen that the valence u quark more likely produces $\bar{d}$ whereas the valence d quark tends to produce $\bar{u}$ through the intermediate state of GBs, for example, $u \to \pi^+ d \to u\bar{d}d$ and $d \to \pi^- u \to d\bar{u}u$. This naturally leads to a proton "quark sea" having more $\bar{d}$ than $\bar{u}$ because there are two valence u quarks and only one valence d quark. This accounts for the $\bar{u} - \bar{d}$ asymmetry as measured by the deviation from the GSR and by the cross section difference of the Drell-Yan process on proton and neutron targets. As both u and d quarks can produce $\bar{s}$, we have significant strange quark content f_s, as indicated by the value of the pion nucleon sigma term.

In recent work, Cheng and Li [21] have discussed in detail the reason why NRQM is able to give a reasonably good description of baryon magnetic moments without satisfying the data pertaining to the spin polarization functions. In this context, they have shown that this longstanding puzzle can be resolved if, apart from the contribution of the sea polarization, the intermediate GBs also have angular momentum and therefore contribute to the magnetic moments as well. Interestingly, they found that there is a significant cancellation between the sea polarization and the orbital angular momentum of the GBs, giving the magnetic moments in terms of the valence constituent

quarks alone. To make the χCQM, which is SU(3) symmetric, more realistic, one can add a singlet of η' to the octet of the GB. The Lagrangian is now U(1) symmetric, however, such a symmetry is not observed in nature. To include the broken U(1) symmetry, the η' boson should come with a coupling constant g_8 for the other octet GBs. This is realized by adding the SU(3) scalar interaction $L = g_1 \bar{q} \frac{\eta'}{\sqrt{3}} q$ to the Lagrangian, where g_1 is the coupling constant for the η' bosons. Thus, the effective Lagrangian describing interaction between quarks and nonet of GBs (consisting of octet and singlet) can be expressed as

$$L = g_8 \bar{q} \Phi q + g_1 \bar{q} \frac{\eta'}{\sqrt{3}} q = g_8 \bar{q} (\Phi + \zeta \frac{\eta'}{\sqrt{3}} I) q, \tag{3.3}$$

where $\zeta = g_1 / g_8$, g_1 and g_8 are the coupling constants for the singlet and octet GBs, respectively, I is a 3×3 identity matrix and

$$q = \begin{pmatrix} u \\ d \\ s \end{pmatrix}. \tag{3.4}$$

The GB field which includes the octet and the singlet GBs is written as

$$\Phi = \begin{pmatrix} \pi^0 + \beta \frac{\eta}{\sqrt{6}} + \zeta \frac{\eta'}{\sqrt{3}} & \pi^+ & \alpha k^+ \\ \pi^- & -\frac{\pi^0}{\sqrt{2}} + \beta \frac{\eta}{\sqrt{6}} + \zeta \frac{\eta'}{\sqrt{3}} & \alpha k^0 \\ \alpha k^- & \alpha \bar{k}^0 & -\beta \frac{2\eta}{\sqrt{6}} + \zeta \frac{\eta'}{\sqrt{3}} \end{pmatrix} \tag{3.5}$$

SU(3) symmetry breaking is introduced by considering $M_s > M_{u,d}$ as well as by considering the masses of GBs to be nondegenerate $M_{k,\eta} > M_\pi$ [8, 9, 21], whereas the axial U(1) breaking is introduced by $M_{\eta'} > M_{k,\eta}$ [6, 8, 9, 21]. The parameter $a(=|g_8|^2)$ denotes the transition probability of chiral fluctuation of the splittings $u(d) \to d(u) + \pi^{+(-)}$, whereas $\alpha^2 a, \beta^2 a$ and $\zeta^2 a$, respectively denote the probabilities of transitions of $u(d) \to s + K^{-(0)}, u(d,s) \to u(d,s) + \eta$, and $u(d,s) \to u(d,s) + \eta'$.

Recently, it has been pointed out that the new measurement of both the $\bar{u}/\bar{d}$ asymmetry as well as $\bar{u} - \bar{d}$ asymmetry by the NuSea Collaboration [14] may not require substantial contribution of η' [9]. As the contribution of η' not only has important implications for the χCQM but also has deeper significance for axial U(1) anomaly as well as nonperturbative aspects of QCD including the effects of gluon anomaly on the spin polarizations [24], it therefore becomes interesting to understand the extent to which its contribution is needed in the χCQM to fit the data pertaining to the "proton spin problem".

The purpose of the present communication is to phenomenologically estimate the contribution of η' GB by carrying out a fine grained analysis of "proton spin problem" within χCQM_{config} which also includes the implications of the latest E866 data. Further, it would be interesting to fine tune the contribution of η', expressed through the parameter

ζ, by studying its implications on spin polarization functions and quark distribution functions.

The details of χCQM_{config} have already been discussed in Ref. [20], however to facilitate the discussion as well as for the sake of readability of the manuscript, some essential details of χCQM with configuration mixing have been presented in the sequel. As has already been discussed that spin-spin forces generate configuration mixing [1, 4, 16] which effectively leads to modification of the spin polarization functions [20]. The most general configuration mixing in the case of octet barayons [4, 16, 25] can be expressed as

$$(3.6)\quad |B\rangle = (|56,0^+\rangle_{N=0}\cos\theta + |56,0^+\rangle_{N=2}\sin\theta)\cos\phi + (|170,0^+\rangle_{N=2}\cos\theta' + |70,2^+\rangle_{N=2}\sin\theta')\sin\phi,$$

where ϕ represents the $|56\rangle - |70\rangle$ mixing, θ and θ' respectively correspond to the mixing among $|56,0^+\rangle_{N=0} - |56,0^+\rangle_{N=2}$ states and $|70,0^+\rangle_{N=2} - |70,2^+\rangle_{N=2}$ states. For the present purpose, it is adequate [16, 20, 26] to consider the mixing only between $|56,0^+\rangle_{N=0}$ and $|70,0^+\rangle_{N=2}$ states and the corresponding "mixed" octet of baryons is expressed as

$$(3.7)\quad |B\rangle \equiv \left|8,\frac{1^+}{2}\right\rangle = \cos\phi|56,0^+\rangle_{N=0}\sin\phi|70,2^+\rangle_{N=2}$$

for details of the spin, isospin and spatial parts of the wavefunction, we refer the reader to reference [5]. To study the variation of the χCQM parameters and the role of ζ in obtaining the fit, one needs to formulate the experimentally measurable quantities having implications for these parameters as well as dependent on the unpolarized quark distribution functions and the spin polarization functions. We first calculate the spin polarizations and the related quantities which are affected by the "mixed" nucleon. The spin structure of a nucleon is defined as [6, 8, 9]

$$(3.8)\quad \hat{B} \equiv \langle B|N|B\rangle$$

Here $|B\rangle$ is the nucleon wavefunction defined in eq.(3.7) and N is the number operator given by

$$(3.9)\quad N = n_{u^+}u^+ + n_{u^-}u^- + n_{d^+}d^+ + n_{d^-}d^- + n_{s^+}s^+ + n_{s^-}s^-,$$

where $n_{q\pm}$ are the number of $q_\pm$ quarks. The spin structure of the "mixed" nucleon, defined through the Eq.(3.7), is given by

$$(3.10)\quad \left\langle 8,\frac{1^+}{2}|N|8,\frac{1^+}{2}\right\rangle = \cos^2\phi\langle 56,0^+|N|56,0^+\rangle + \sin^2\phi\langle 70,0^+|N|70,0^+\rangle.$$

The contribution to the proton spin in χCQM_{config}, given by the spin polarizations defined as $\Delta q = q^+ - q^-$, can be written as

$$(3.11)\quad \Delta u = \cos^2\phi[\frac{4}{3} - \frac{a}{3}(7 + 4\alpha^2 + \frac{4}{3}\beta^2 + \frac{8}{3}\zeta^2)] + \sin^2\phi[\frac{2}{3} - \frac{a}{3}(5 + 2\alpha^2 + \frac{2}{3}\beta^2 + \frac{4}{3}\zeta^2)]$$

$$(3.12)\quad \Delta d = \cos^2\phi[-\frac{1}{3} - \frac{a}{3}(2 - \alpha^2 - \frac{1}{3}\beta^2 - \frac{2}{3}\zeta^2)] + \sin^2\phi[\frac{1}{3} - \frac{a}{3}(4 + \alpha^2 + \frac{1}{3}\beta^2 + \frac{2}{3}\zeta^2)]$$

$$(3.13)\quad \Delta s = -a\alpha^2.$$

After having formulated the spin polarizations of various quarks, we consider several measured quantities which are expressed in terms of the above mentioned spin polarization functions. The quantities usually calculated in the χCQM are the flavor non-singlet components Δ_3 and Δ_8, obtained from the neutron β-decay and the weak decays of hyperons respectively. These can be related to Bjorken sum rule [27] and Ellis-Jaffe sum rule [28] as

(3.14) BSR: $\Delta_3 = \Delta u - \Delta d$,

(3.15) EJSR: $\Delta_8 = \Delta u + \Delta d - 2\Delta s$.

Another quantity which is usually evaluated is the flavor singlet component of the total quark spin content defined as

(3.16) $2\Delta\sum = \Delta u + \Delta d + \Delta s$.

Apart from the above mentioned spin polarization we have also considered the quark distribution functions which have implications for ζ as well as for other χCQM parameters. For example, the antiquark flavor contents of the "quark sea" can be expressed as [6, 8, 9]

(3.17) $\bar{u} = \frac{1}{12}[(2\zeta + \beta + 1)^2 + 20]a$, $\bar{d} = \frac{1}{12}[(2\zeta + \beta - 1)^2 + 32]a$

$\bar{s} = \frac{1}{3}[(\zeta - \beta)^2 + 9\alpha^2]a$,

and

(3.18) $u - \bar{u} = 2$, $d - \bar{d} = 1$, $s - \bar{s} = 0$.

The Gottfried sum rule [15] is expressed as

(3.19) $I_G = \frac{1}{3} + \frac{2}{3}\int_0^1 [\bar{u}(x) - \bar{d}(x)]dx = 0.254 \pm 0.005$.

In terms of the symmetry breaking parameters a, β and ζ, this deviation is given as

(3.20) $[I_G - \frac{1}{3}] = \frac{2}{3}[\frac{a}{3}(2\zeta + \beta - 3)]$.

Similarly, $\bar{u}/\bar{d}$ [14, 29] measured through the ratio of muon pair production cross sections σ_{pp} and σ_{pn} , is expressed in the present case as follows

(3.21) $\bar{u}/\bar{d} = \frac{(2\zeta + \beta + 1)^2 + 20}{(2\zeta + \beta - 1)^2 + 32}$.

Some of the important quantities depending on the quark distribution functions which are usually discussed in the literature are as follows

(3.22) $f_q = \frac{q + \bar{q}}{[\sum_q (q + \bar{q})]}$, $f_3 = f_u - f_d$, $f_8 = f_u + f_d - 2f_s$

The χCQM_{config} involves five parameters: a, α, β, ζ and ϕ. Before carrying out the detailed analysis involving quantities which are dependent on ζ, to begin with we have fixed some of the χCQM parameters. The mixing angle ϕ is fixed from the consideration of neutron charge radius [16, 25, 30]. It has been shown [6, 9] that to fix the violation on Gottfried sum rule [15], we have to consider the relation

(3.23) $\bar{u} - \bar{d} = \frac{a}{3}(2\zeta + \beta - 3)$,

which constraints the parameters a, ζ and β when the data pertaining to $\bar{u} - \bar{d}$ asymmetry [14] is used.

4. Results and Discussions:

The parameters α and β suppress the emission of K and η as compared to that of pions as these strange quark carrying GBs are more massive than the pions. However, because of the very small mass difference between them, the suppression factors α and β are taken to be equal. In Table 1, we summarize the input parameters and their values.

In Table 2, we have presented the various spin dependent phenomenological quantities which are affected by the variation of the symmetry breaking parameters. In the Table, to highlight the particular values of a and ζ, we have presented the results for their different values. A general look at the Table shows that the results for all the quantities affected by the inclusion of ζ get improved in the right direction for lower values of ζ. In fact, for the case of $a = 0.13$ and $\zeta = -0.10$, we are able to get a perfect fit for Δ_3 and Δ_8 .

Further, the results corresponding to quark distribution functions having implications for the symmetry breaking parameters have been presented in Table 3. In general both for $\zeta = 0$ and $\zeta = -0.10$, we are able to obtain an excellent fit, however in the case of $\bar{u}-\bar{d}$, $\bar{u}/\bar{d}$ and f_3/f_8, the non-zero (small) value of ζ gives a better fit that $\zeta = 0$. A closer scrutiny of the table reveals several interesting points. Δ_3 and Δ_8 from Table 2 as well as f_3/f_8 from Table 3 perhaps suggest that a small non-zero value of ζ gives a better fit than the zero value of ζ. In the case of $\Delta\Sigma$ (Table 2), it seems that ζ=0 is a preferred value. However, as has been discussed earlier in χCQM [31] that the flavor singlet component of the spin of proton $\Delta\Sigma$ receives contributions from various sources such as gluon polarization and gluon angular momentum, therefore, we cannot conclude that $\zeta = 0$ is preferred over $\zeta \neq 0$. In this context, we would like to mention that the above contribution of η' is in agreement with the experimental value of $\Delta\Sigma$ in case we consider the contribution of the effects of gluon polarization and gluon angular momentum through gluon anomaly [31]. The results corresponding to small values of ζ including ζ=0 clearly show better overlap with the data after the latest $\bar{u}-\bar{d}$ asymmetry measurement [14]. In the χCQM, it is difficult to think of a mechanism wherein the contribution of η' or the ninth GB becomes zero. However, a small value of ζ looks to be in order from phenomenological considerations pertaining to the different GBs. For example, in case we consider the coupling of the GB corresponding to the pion, K, η an η' mesons being inversely proportional to the square of their respective masses, we find that their couplings are of the order $a\alpha^2 \approx 0.02, a\beta^2 \approx 0.02$ and $\zeta^2 \approx 0.001$, for a~0.13 which strangely agrees with our values obtained through the fit. These findings are also in agreement with the suggestions of Cheng and Li [6] who have advocated that the η' contribution corresponds to the non-planar contributions in the $1/N_c$ expansion.

To summarize, we have investigated in detail the implications of the latest data pertaining to $\bar{u}-\bar{d}$ asymmetry and the spin polarization functions on the singlet Goldstone Boson η' within χCQM with configuration mixing for explaining the "proton spin problem". We find that the lower values of ζ are preferred over the higher values. Specifically, in the case of $\Delta_3, \Delta_8, \bar{u}-\bar{d}, \bar{u}/\bar{d}$ and f_3/f_8, it seems that the small non-zero value of ζ is preferred over ζ=0.

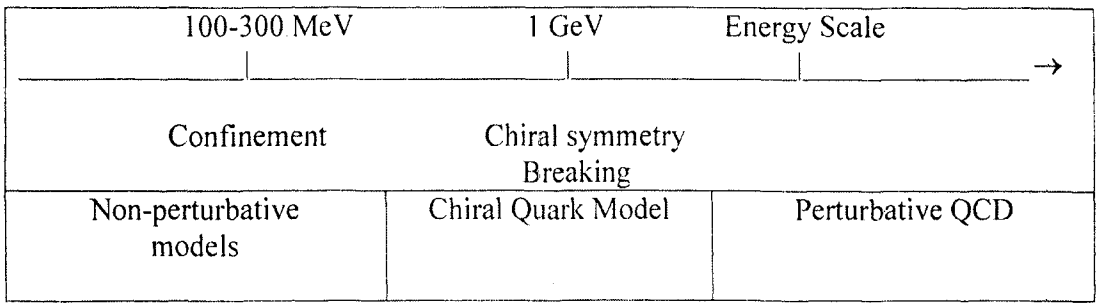

Figure 1.1: The energy scale in which χCQM works.

TABLE 1: Input parameters and their values used in the analysis.

Parameter →	ϕ	a	α	β	ζ
Value	20^0	0.1	0.4	0.7	$-0.3-\beta/2$
	20^0	0.13	0.4	0.4	$0.15-\beta/2$
	20^0	$0.345/(3-\beta)$	0.4	0.4	0

TABLE 2: The phenomenological values of the spin polarizations and dependent parameters.

Parameter	Data	χCQM_{config}		
		$a=0.1$ $\zeta=-0.65$	$a=0.14$ $\zeta=0$	$a=0.13$ $\zeta=-0.10$
Δu	0.85 ± 0.05[8]	0.95	0.91	0.91
Δd	-0.41 ± 0.05[8]	-0.31	-0.35	-0.36
Δs	-0.07 ± 0.05[8]	-0.02	-0.02	-0.02
Δ_3	1.267 ± 0.0035[29]	1.27	1.26	1.27
Δ_8	$0.58\pm.025$[29]	0.67	0.60	0.59
$\Delta\Sigma$	$0.19\pm.025$[29]	0.31	0.27	0.28

TABLE 3: The quark flavor distribution functions and dependent parameters.

Parameter	Data	χCQM		
		$a=0.1$ $\zeta=-0.65$	$a=0.14$ $\zeta=0$	$a=0.13$ $\zeta=-0.10$
$\bar{u}$	-	0.168	0.25	0.23
$\bar{d}$	-	0.288	0.366	0.35
$\bar{s}$	-	0.108	0.07	0.07
$\bar{u}-\bar{d}$	-0.118 ± 0.015[14]	-0.1.8	-0.116	-0.117
$\bar{u}/\bar{d}$	0.67 ± 0.06[14]	0.58	0.68	0.67
I_G	0.254 ± 0.005	0.253	0.255	0.255
f_u	-	0.655	0.677	0.675
f_d	-	0.442	0.470	0.466
f_s	0.10 ± 0.06[30]	0.061	0.039	0.039
f_3	-	0.213	0.207	0.209
f_8	-	0.975	1.07	1.06
f_3/f_8	0.21 ± 0.05[6]	0.22	0.19	0.20

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