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PROCEEDINGS

OF

PAKISTAN CONGRESS OF ZOOLOGY

Volume 23, 2003

TWENTY THIRD PAKISTAN CONGRESS OF ZOOLOGY

held under auspices of

THE ZOOLOGICAL SOCIETY OF PAKISTAN

at

University of Arid Agriculture, Rawalpindi

March 03 2003 to March 05, 2003

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OF

PAKISTAN CONGRESS OF ZOOLOGY

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University of Arid Agriculture, Rawalpindi, hosted the 23rd Pakistan Congress of Zoology (International).

The Zoological Society of Pakistan expresses its deep gratitude to the Vice Chancellor, University of Arid Agriculture, Rawalpindi and Chairman, faculty members and students of the Departments of Zoology and Biological Sciences for extending warm hospitality.

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TWENTY THIRD PAKISTAN CONGRESS OF ZOOLOGY (INTERNATIONAL)

UNIVERSITY OF ARID AGRICULTURE, RAWALPINDI

MARCH 3 – 5, 2003

PROGRAMME

MONDAY, MARCH 3, 2003

08:30 AM	Registration	
09:30 AM	Inauguration by	Dr. A.Q. Khan, Special Advisor to Prime Minister on Strategic Affairs.
11:30 AM	Refreshment	

JOINT SESSION I: (Plenary Lectures)

12:00 AM	Prof. Dr. Muhammad Akhtar, FRS **New Insights into the Control of Androgen Biosynthesis.**
01:00 PM	Lunch and Prayer

HALL 1

SECTION I: CELL BIOLOGY, BIOCHEMISTRY GENETICS, MOLECULAR BIOLOGY, PHYSIOLOGY, GENETICS

SESSION I

	Chairperson:	Prof. Dr. Shahzad A. Mufti
	Co-chairperson:	Dr. Azra Sultana
02:00 AM	Paper reading	
04:00 PM	Tea Time	

SESSION II

	Chairperson:	Prof. Dr. M. Anwar Malik
	Co-chairperson:	Dr. Tasawar H. Khan
04:15 PM	Paper reading	
06:00 PM	Prayer	

SESSION III

	Chairperson:	Prof. Dr. Shamsuddin Shaikh
	Co-chairperson:	Dr. Abdul Hamid
06:15 AM	Paper reading	
08:00 PM	Dinner	

HALL 2

SECTION IV: PARASITOLOGY

SESSION I

	Chairperson:	Prof. Dr. Fatima Mujeeb Bilqees
	Co-chairperson:	Dr. Z. Tasawar
02:00 PM	Paper reading	
04:00 PM	Tea Time	

SESSION II

	Chairperson:	Prof. Dr. M. Suleman
	Co-chairperson:	Dr. Aly Khan
04:15 PM	Paper reading	
06:00 PM	Prayer	

SECTION IV: FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY, MARINE BIOLOGY

SESSION I

	Chairperson:	Prof. Dr. M.A. Hafeez
	Co-chairperson:	Dr. Abdul Aleem Khan
06:15 PM	Paper reading	
08:00 PM	Dinner	

TUESDAY, MARCH 4, 2003

JOINT SESSION II: (Plenary Lectures)

09:00 AM Dr. Sagir M. Ahmed, Department of Zoology, University of Dhaka, Dhaka, 1000, Bangladesh.
Toxic Cyanobacteria Bloom and the Occurrence of Mirucystin from Freshwater Eutrophic Ponds in Bangladesh.

09:30 AM Dr. Movio Carenza, Institute, CNR, Via romea 4, 35020 Legnaro-Padova, Italy.
Recent Developments in the use of Polymeric Materials in Biotechnology and Biomedicine.

HALL 1

SECTION I: CELL BIOLOGY, BIOCHEMISTRY, GENETICS, MOLECULAR BIOLOGY, PHYSIOLOGY, GENETICS

SESSION IV

	Chairperson:	Prof. Dr. A.Q. Ansari
	Co-chairperson:	Dr. Muhammad Ali
10:00 AM	Paper reading	
11:00 PM	Tea Break	

SESSION IV (Continued)

	Chairperson:	Prof. Dr. A.Q. Ansari
	Co-chairperson:	Dr. Muhammad Ali
11:15 AM	Paper reading	
01:00 PM	Lunch and Prayer	

SESSION V

	Chairperson:	Prof. Dr. A.M. Cheema
	Co-chairperson:	Dr. Nematullah
02:00 PM	Paper reading	
04:00 PM	Tea Break	

SESSION VI

	Chairperson:	Prof. Dr. A.R. Shakoori
	Co-chairperson:	Dr. Wasim Ahmad
04:15 PM	Paper reading	
06:00 PM	Prayer	

SECTION II: PEST AND PEST CONTROL

SESSION VII

	Chairperson:	Dr. Sanaullah Khattak
	Co-chairperson:	Dr. Y. Ahmad
06:15 PM	Paper reading	
07:00 PM	Poster Session	
08:00 PM	Dinner	

HALL 2

SECTION IV: FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY, MARINE BIOLOGY

SESSION II

	Chairperson:	Dr. Muhammad Ayub
	Co-chairperson:	D. Syed Shahid Ali
10:00 AM	Paper reading	
11:00 PM	Tea Break	

SESSION III

	Chairperson:	Mr. Abdul Aziz Khan
	Co-chairperson:	Dr. Naeem Ahmad Khan
11:15 AM	Paper reading	
01:00 PM	Lunch and Prayer	

SESSION IV

	Chairperson:	Prof. Dr. S.I.H. Jafri
	Co-chairperson:	Dr. Iayat Ali Shahjehan
02:00 PM	Paper reading	
04:00 PM	Tea Break	

SESSION V

	Chairperson:	Prof. Dr. Q.B. Kazmi
	Co-chairperson:	Dr. A.A. Aradalah
04:15 PM	Paper reading	
06:00 PM	Prayer	

SESSION VI

	Chairperson:	Prof. Dr. Muhammad Arshad
	Co-chairperson:	Dr. R.R. Ghazi
06:15 PM	Paper reading	
07:00 PM	Poster Session and SZP Executive Council Meeting	
08:00 PM	Dinner	

WEDNESDAY, MARCH 5, 2003

JOINT SESSION III: (Plenary Lectures)

09:00 AM Dr. Christoph Tourenq, National Avian Research Centre, ERWDA, P.B. 45553, Abu Dhabi, UAE.
Can the Asian Houbara Population Survive the Current Hunting and Poaching Pressure?

HALL 1

SECTION III: ENTOMOLOGY

SESSION I

	Chairperson:	Prof. Dr. Imtiaz Ahmad
	Co-chairperson:	Dr. Muhammad Afzal
09:00 AM	Paper reading	
11:00 AM	Tea Time	

SESSION II

	Chairperson:	Prof. Dr. M.S. Wagan
	Co-chairperson:	Dr. S. Kamaluddin
11:00 AM	Paper reading	
01:00 PM	Lunch and Prayer.	

HALL 2

SECTION V: FISHERIES, ECOLOGY, WILDLIFE, FRESHWATER BIOLOGY, MARINE BIOLOGY

SESSION VII

	Chairperson:	Prof. Dr. Afsar Mian
	Co-chairperson:	Mr. Basharat Ahmad
09:00 AM	Paper reading	
11:00 AM	Paper reading	
01:00 PM	Lunch and Prayer.	
02:30 PM	Concluding Session	
4:00 PM	General Body Meeting	

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CITATIONS

LIFE TIME ACHIEVEMENT AWARD 2003

Prof. Dr. Muzaffer Ahmed
Professor Emeritus, Department of Zoology, University of the Punjab, Lahore

Born in Sitapur, India on 20th September 1920. Received education at the Aligarh Muslim University, India (M.Sc. 1941), Imperial Agricultural Research Institute, New Delhi, India (ASSOC. I.A.R.I. 1943) and the University of Chicago, USA (Ph.D. 1948)

Member of Sigma XI Society of America (1946), Founder Fellow of the Zoological Society of Pakistan (1968), Fellow of the Pakistan Academy of Sciences (1970), Founder member of the Pakistan Congress of Zoology (1980).

Recipient of Gold Medal awarded by the Pakistan Academy of Sciences (1979), Gold Medal jointly awarded by the United States Department of Agriculture and the Pakistan Agricultural Research Council and Izaz-i-Kamal awarded by the President of Pakistan (1993).

Joined the University of the Punjab in 1949 as Senior Lecturer and retired in 1980 as Mian Afzal Husain Professor of Zoology, was appointed Professor Emeritus in 1983.

Served as Head/Chairman of the Department of Zoology for 17 years. During this period, built an excellent research oriented Zoology Department with Ph.D. programmes in several disciplines.

Editor, Pakistan Journal of Zoology (1969-1992), Chief Editor (1993 to date), Editor, Proceedings of Pakistan Congress of Zoology (1980 to date). He had been a member of the Advisory Board of the Annals of Entomology, India and a Communication Editor of the Science and Technology of Termites, a Chinese publication.

His publications on termites extensively referred to by foreign scientists in their research papers and books.

RECIPIENT OF ZOOLOGIST OF THE YEAR AWARD 2003*

Quddusi B. Kazmi
Director, Marine Reference Collection & Resource Centre,
University of Karachi.

Dr. Quddusi B. Kazmi began her career as Research Assistant at Karachi University in 1965 after completing her Bachelor's degree with a first class. Later she pursued her masters in Marine Zoology and wrote a thesis on the taxonomy of marine shrimps of Pakistan, which was later published by FAO. For her M.Phil, she submitted a dissertation "Studies on the Stomatopods of Pakistan" which was published as a handbook. Her doctoral dissertation on the marine crabs of Pakistan was highly appreciated by Prof. L.B. Holthuis and Dr. R.W. Ingle. During this period of study she worked at the Natural History Museum, London and studied the old collection of Crustacea collected from Karachi and housed at the British Museum since 1898. Her M.Phil. and Ph.D. dissertations are recommended as Reference Books in the syllabus of Marine Zoology of Karachi University. She has guided several of M.Sc., M.Phil. and Ph.Ds. She has attended several international and national conferences. In July 2002 she represented Pakistan at Taiwan for ICOC. She has organized several conferences and seminars. She has been selected as resource person at WWF, NIO and PASTIC Workshops. Dr. Kazmi has received a gold medal from different scientific organizations, a best paper

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*Other nominees of this award were Dr. Sanaullah Khattak Khan, Prof. Dr. S.I.H. Jafri and Prof. Dr. Habib ul Hassan.

award, a star women of the year award and productivity award of Ministry of Science and Technology.

Dr. Kazmi has written 4 books, 3 monographs, 5 illustrated keys and 100 research papers published in local and international Journals and 50 popular articles. Her main interests are marine crustaceans but mollusks, echinoderms, nemerteans, fishes, tunicates, echuirids, kinorynchans and Acaris are also included in her studies and she is the editor of 3 Proceedings of International Conferences on aquatic life and biannual Journal “Pakistan Journal of Marine Sciences”.

Dr. Kazmi is a Partner to Expert Centre for Taxonomic Identification (ETI) at Amsterdam where a CD-ROM developed on Crabs contains illustrations prepared by her. She has contributed a chapter in Encyclopedia of Pakistan on fishes of Pakistan, being published by the Oxford University Press. She has been member of several editorial boards, academic bodies, chairperson of conference sessions and reviewer of papers of local and foreign journals.

RECIPIENT OF
PROF. A.R. SHAKOORI GOLD MEDAL 2003*

Dr. Bilquees Gul
Assistant Professor
Department of Botany, University of Karachi, Karachi

Dr. Bilquees Gul, Assistant Professor of Botany, University of Karachi has spent about 9 years in research and teaching. She was awarded the degree of M.Sc. (1993) by the University of Karachi and Ph.D. (1998) by the Brigham Young University, USA. She is the recipient of Brigham Young University Teaching Assistantship (1995-1998), and Brigham Young University merit Scholarship (1995-1998).

Dr. Gul was appointed as a Lecturer of Plant Ecology (1994-1995) in Jinnah University for Women, Karachi. She joined the University of Karachi in 1999 as a research coordinator in the Halophyte Biology Laboratory, Department of Botany, on a National Science Foundation, USA research project on Ecophysiology of Coastal Halophytes. She was appointed as Full time Cooperative Lecturer in Botany Department in 2000 and Assistant Professor of Botany in 2002.

Dr. Gul is the author of 46 research papers in well-known national and international journals. As a Master's student in the University of Karachi in 1993 she worked on a project on demography, osmoregulation, germination

*Other nominees of this award were Dr. Amjad Javed and Dr. Shahid Mahboob Rana.

and seed bank dynamics of *Arthrocnemum indicum*. Her research primarily focuses on ecological management of intertidal, coastal and inland saline ecosystems. Her specific research interest includes the utilization of the areas destroyed by water logging and salinity by using unconventional agricultural crops. Dr. Gul has participated in many national and international conferences and presented papers. She has organized the 2nd International Symposium on High Salinity Plants under the 8th National Conference of Plant Scientists, November, 1-5, 2002.

RECIPIENT OF
PROF. DR. MIRZA AZHAR BEG GOLD MEDAL 2003*

Dr. Muhammad Afzal
Assistant Professor
Department of Agricultural Entomology, University of Agriculture, Faisalabad

Dr. Muhammad Afzal, Assistant Professor at University of Agriculture, Faisalabad passed his Matriculation in 1980 from Government Guru Nanak High School, Nankana Sahib by securing 1st division. He then jointed Government College, Lahore and completed his F.Sc. pre-medical in 1st division and then jointed University of Agriculture, Faisalabad from where he passed B.Sc. (Hons.) Agric. And M.Sc. (Hons.) Agric. Entomology by securing 1st division in both these examination. For his Ph.D. dissertation he opted an important field of Acarology and specialized in predatory mite fauna of the family Phytoseiidae. He described 35 new species of the genus *Phytoseius* from different localities of Pakistan. These species are very important predators of phytophagous mites, small soft bodied insects and their eggs, thus providing a potential source of material for biological control strategies in future. He has 43 research publications.

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*Other nominees of this award were Mr. Amjad Pervez and Dr. Muhammad Ali.

RECIPIENTS OF GOLD MEDALS AWARDED BY ZOOLOGICAL SOCIETY OF PAKISTAN

1. **Mujib Memorial Gold Medal 2003**

   This Gold Medal is awarded, every year, to a student of Karachi University, standing first in the M.Sc. Zoology examination with specialziation in Parasitology.

   This year's award went to Miss Noreen Aleemuddin.

2. **Mohd Afzal Hussain Qadri Memorial Gold Medal 2003**

   This Gold Medal is awarded, every year, to a student of Karachi University, standing first in the M.Sc. Zoology examination. This year's award was not given.

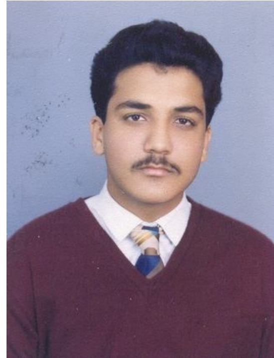
3. **Prof. Imtiaz Ahmad Gold Medal 2003**

   This Gold Medal is awarded, every year, to a student of Karachi University, standing first in the M.Sc. Zoology examination, with specialization in Entomology. This year's award went to Ms. Sobia Shabbir.

Ms. Sobia Shabbir

4. **Muzaffar Ahmad Gold Medal 2002**

   This Gold Medal is awarded, every year, to a student of Punjab University, standing first in the M.Sc. Zoology examination. This year's award went to Mr. Muhammad Faisal Rehmatullah.



Muhammad Faisal Rehmatullah

5. **Prof. Dr. S.N.H. Naqvi Gold Medal 2003**
   This Gold Medal is awarded, every year, to a student of Karachi University obtaining Ph.D. degree in Zoology with specialization in Toxicology. This year's award was not given.

6. **Ahmad Mohiuddin Memorial Gold Medal 2003**
   This Gold Medal is awarded, every year, to a student of University of Sindh, Jamshoro, standing first in the M.Sc. Zoology examination. This year's award went to Miss Asma Salahuddin Shaikh.

7. **M.A.H. Qadri Memorial Gold Medal 2003**
   This Gold Medal is awarded, every year, to a student of Karachi University obtaining Ph.D. degree in Zoology with specialization in Parasitology. This year's award went to Dr. Noor-un-Nisa.

Dr. Noor-un-Nisa

Proc. Pakistan Congr. Zool., Vol. 26, pp. 111-121, 2006.

SEXUAL DIMORPHISM IN SPHAEROMATID ISOPODS (SPHAEROMATIDAE) RECORDED FROM PAKISTAN COAST, NORTHERN ARABIAN SEA

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Abstract.- Sexual dimorphism is one of the important and striking diagnostic features in sphaeromatid isopods. In all genera primary dimorphism is evident between ovigerous and non-ovigerous females and males based on the presence or absence of secondary sex characters *i.e.* appendix masculine and penes in males, oostegites in females. Uptill now sixteen species in 11 genera belonging to the family Spaheromatidae have been recorded and described from the Pakistani waters. Among eleven genera, four genera *Clianella* Boone, 1923; *Cymodoce* Leach, 1814; *Paracilicaea* Stebbing, 1910 and *Paracerceis* Hansen, 1905 show pronounced sexual dimorphism. In the present work these differences in the male and female individuals of the same species and genera have been discussed and shown by drawings, on the basis of which in the past they were supposed to belonging to different species.

Key words: Crustacea, Decapoda, Isopoda, Flabellifera, Sphaeromatidae, Pakistan.

INTRODUCTION

The Isopoda is one of the nine order of Peracarid crustaceans, including the Flabellifera, the family Sphaeromatidae belong to the sub-order Flabellifera the second largest suborder of marine isopod crustaceans.

This family is represented in the interdial fauna of Karachi coast according to Bruce (1995) the family Sphaeromatidae comprises over 90 genera and 400 species in 3 sub-families. In the present collection it is represented by following taxa. Sphaeromatinae, Dynameninae. The main taxonomic character separating the two sub-families in the form of pleopods 4 and 5. In Sphaeromatinae, pleopods 4 and 5 wth transverse folds on endopods only; pleotelson apex usually entire without a notch or foramen. In the Dynameninae pleopods 4 and 5 with transverse folds on both rami, pleotelson usually with apical notch or foramen.

Also it is divided into 3 groups on the basis of pleopod structures. Eubranchiatinae (flat gilled). Bowman (1981) and Iverson (1982) replaced

the incorrectly formed sub-family names. They replaced the name Eubranchiatinae with Dynameninae based on the eubranchiate genus *Dynamene*. Hemibranchiatinae containing, the genus *Sphaeroma* the type genus of the family Sphaeromatidae became Sphaeromatinae. Sexual dimorphism is one of the most striking features of hemibranchiate isopod specimens collected from different localities of Karachi coast, in all genera and species including between ovigerous and non-ovigerous females and males due to the presence or absence of secondary sex characters, appendix masculine and penes in males and oostegites in females. Ovigerous females generally carry the developing eggs in ventral brood pouch formed by large plate like oostegites on certain thoracic coxae. In males with pleopod 2 appendix masculine and penes. The hemibranchiate genera which show a truly marked sexual dimorphism are *Cymodoce, Clianella* and *Paracilicaea.*

Mostly the male and female individuals of the same species differ markedly in appearance and create great problems in the identification. Many species belonging to these genera have so different male and female that in the past they were supposed to belong to different species.

Type material has been deposited in the Museum of the Department of Zoology, University of Karachi.

SYSTEMATICS

Family: *Sphaeromatidae* Latreille, 1825
Genus: ***Clianella*** Boones, 1923
(Fig. 1).

Clianella amblysina (Pillai, 1954): 11.
Dynoides amblysinus Pillai, 1954: 11; *Dynoides amblysinus* Pillai, 1965: 80, Figs. 23-39; *Clianella brucei* Harrison and Holdich, 1984: 366; Bruce, 1990: 551; Kussakin and Malyntina, 1993: 1174, Fig. 24; *Clianella amblysina* Javed and Yousuf, 1995.

Material

Adult male, 4.2 mm; 2 ovigerous females 2.8-3.0 mm; 14 October, 1994.4 adult males, 3.5-4.0 mm; 4 non-ovigerous females, 2.5-3.2 mm; March, 2002. All the material examined was collected from the coast of Bulleji, Karachi.

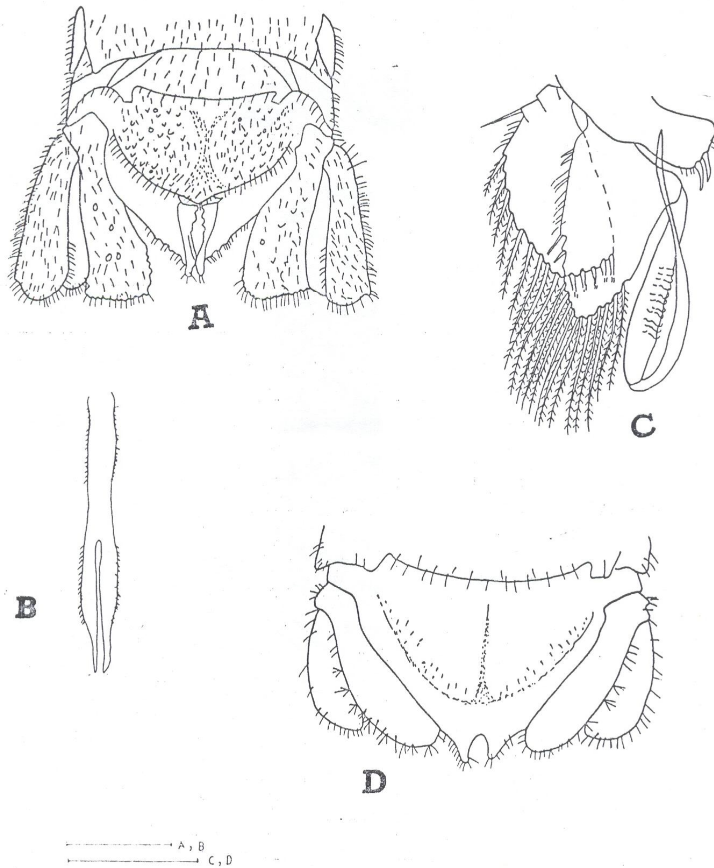

Fig. 1. *Clianella amblysina* (Pillai, 1954) Adult male, 4.2 mm; A, pleotelson, dorsal view; B, penes; C, pleopod 2; ovigerous female, 3.1 mm; D, pleotelson, dorsal view; Scale lines=1 mm.

Description

Body surface of adult male (Fig. 1A), rough, covered with short and few slightly long setae. Pleotelson apex (Fig. 1A), with an elongate notch, which is subtriangular when seen ventrally. Dorsally (Fig. 1A) each lateral side of the notch covered by small hanging distal projection of pleotelson. Penes (Fig. 2B) long fused at base tapering slightly before bifurcation, and again tapering to acute apices, Pleopod 2 (Fig. 3C) with appendix masculine long, reflected anteriorly, proximal portion broad, lateral surface bearing scales,

reflected portion narrow, tapering to acute apex. Uropod (Fig. 1A) large, thick, extending well beyond the apex of pleotelson endopod; flat, distal margin truncate with short setae, lateral margin slightly concave; exopod entire, lateral margin strongly deflected medially, bearing short setae on the outer surface on margins. Ovigerous female generally smaller than adult male. Pleotelson (Fig. 1D) with dome bearing weak longitudinal depression, tubercles and setae, apex with simple elliptical notch, having no dorsal denticles. Uropod rami thin with fine marginal setae, not extending as far as pleotelson tip, exopod shorter than endopod, with subcircular apices. Pereonites 2-4 bearing membranous oostegites at base of each pereiopod right 3-4 oostegites overlapping the left ones in the midline.

Remarks

Present material agrees closely with the description and illustrations given by Holdich and Harrison (1984) except that the dorsal surface of cephalosome and pleon is rough and pubescent. In adult male, the margins are so close that the notch appears closed posteriorly.

Distribution

Known from Lizard and Magnetic Islands of Queensland coast, Pacific Ocean (Harrison and Holdich, 1984), Hong Kong, China Sea (Bruce, 1990), Cape Comorion and Vizhingom, Indian Ocean (Pillai, 1954, 1965). Present material extends the known range northwords in the Indian Ocean to northern Arabian Sea.

Genus: ***Cymodocea*** Leach, 1814

Cymodocea spinula Yousuf and Javed, 2001
(Fig. 2)

Cymodoce spinula Yousuf and Javed, 2001.

Material

Adult male, 13.2 mm; 7 September, 1989; 2 non-ovigerous females 9.0-mm; 1 December, 1999; Bulleji, 1 adult male, 10.9 mm, 11 April, 1983; 1 non-ovigerous female, 7.0 mm; 7 September, 1989; 1 non-ovigerous female 6.9 mm; 11 December, 1993; Manora, Karachi, Pakistan.

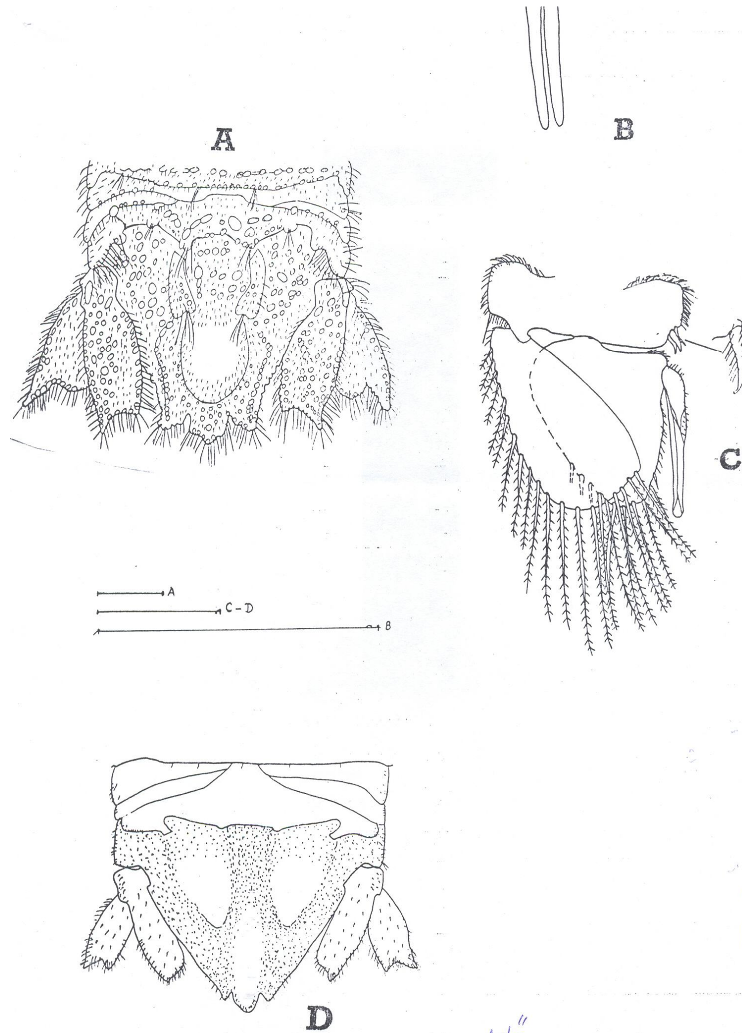

Fig. 2. *Cymodoce spinula* Yousuf and Javed. 2001. Adult male, 13.2 mm; A, pleotelson, dorsal view; B, penes; C, pleopod 2; non-ovigerous female, 9.0 mm; D, pleotelson, dorsal view; Scale lines=1 mm.

Description

Body surface of male (Fig. 2A) punctate and pubescent, pleotelson and uropods tuberculate, pleotelson (Fig. 2A) with an anterior submedian pair of longitudinal ridges, each dilating posteriorly, into a bifid structure,

posteriorly a large smooth domed boss, having a longitudinal row of tubercles on either sides. Penes (Fig. 2B) long, separate at base, tapering to narrowly rounded apex. Pleopod 2 with appendix masuline (Fig. 2C), extending slightly before the distal margin of endopod.

In female body surface lacking setae and tubercles. Pleotelson (Fig. 2D) bearing a low, longitudinal, posteriorly directed bulge on either side of midline, posterior portion without domed boss. Uropods very short, not extending to pleotelson apex, dorsal surface with weak pubescence, rami with short, fine marginal setae, exopod thin and lamellar, slightly shorter than endopod, distal margin with indentation and both lateral angles acutely produced, distomedian angle of endopod rounded and distolateral angles acutely produced.

Remarks

Bruce (1997) observed that the species of *Cymodoce* sensu stricto show two distinctive types of uropod morphology. Many of the Indo-Pacific species have a uropodal endopod that is round in cross section, with the exopod rarely extending to the tip of the endopod. *Cymodoce spinula* differs from the Australian species in having a short and straight appendix masculine, shorter and morphologically distinct uropodal rami, a pointed median tooth at the pleotelson apex.

Distribution

Cymodoce Leach, 1814 is a large genus with a worldwide distribution in all Oceans from the tropics to temperate waters (Harrison and Holdich, 1984). In the Indian Ocean the genus has been recorded from the southern and eastern coasts of Africa (Barnard, 1914, 1920, 1955; Stebbing, 1910), from the Red Sea Nobili, 1906, from Southern Indian (Pillai, 1965; Srinivisan, 1959), and Western Australian (Harrison and Holdich, 1984). This species from Pakistan, therefore, falls within the known range of the genus.

Genus: ***Paracerceis*** Hansen, 1905

Paracerceis sculpta Holmes, 1904
(Fig. 3)

Dynamene sculpta Holmes, 1904: 300-302, 1. 34.

Ciliacea sculpta Richardson, 1905: 318-319; Stebbing, 1905: 35.
Paracerceis sculpta Richardson, 1905; IX; Menzies, 1962: 340, 341, Fig. 2; Miller, 1968: 9, 14; Pires, 1981: 219, 220; Harrison and Holdich, 1982; 440-442.
Sergiella angra Pires, 1980: 212-218: 219-220.

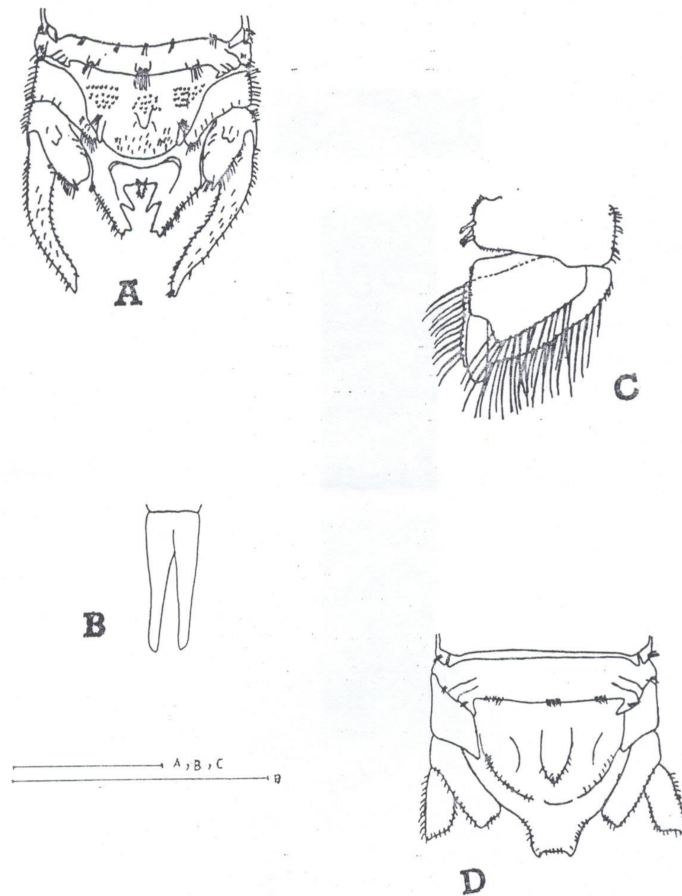

Fig. 3. *Paracilicaea kejii* (Javed, 1990) Adult male, 10 mm; A, pleotelson, dorsal view; B, penes; C, pleopod 2; ovigerous female, 7.0 mm; D, pleotelson, dorsal view; Scale lines=1 mm.

Material

Adult male, 7.00 mm, 2 females, 5.00 mm from Port Qasim, Expanded by Harrison and Holdich (1982).

Description

Body noticeably convex; coxal plates directed ventrally. Pleotelsonic notch (Fig. 3A) with a lateral pair of tubercles and single distomedian setose tubercle. Penes separate, short, (Fig. 3B) with circular tips. Appendix masculine (Fig. 3C) arising from internal margin of endopod of pleopod 2, extending beyond ramal apex to semicircular tips. Ovigerous female (Fig. 3D) with mouth parts metamorphosed. Brood pouch formed from four pairs of oostegites arising from bases of pereiopods 1 to 4. Uropodal rami (Fig. 3A) densely stose, almost uniramus, endopod highly reduced, exopod well developed, long, slender and curving inward.

Non-ovigerous female: 3.00 mm.

Pleotelson (Fig. 3D) with three fairly developed and setose longitudinal ridges, apex, extended and setose, distal margin broadly notched. Uropodal rami subequal, not extending beyond the apex of pleotelson (Fig. 3D). Sexual dimorphism pronounced specially in the morphology of uropod rami and also in the dorsal ornamentation of pleon and pleoteleson (Fig. 3D).

Remarks

Present specimens agree closely with the description and illustration given by Harrison and Holdich (1982) except that dorsal surface and lateral margins of both sexes are setose, while the specimens recorded from California have setae (Richardson, 1905) on posterior margins of pereonites 6 and 7 only.

Distribution

This species has previously been recorded from California, Brazil, Mexico, Egypt, all coast of Europe, Venice Mediterranean, Townsville and Queensland, now its range extends to Port Qasim, Karachi, Pakistan, in the Indian Ocean. The material was collected from the algae attached to a pole of the wharf, having the salinity 34%° and temperature 30°C.

Genus: ***Paraceilicaea*** Stebbing, 1910

Paracilicaea kejii Harrison and Holdich (1984)
(Fig. 4)

Paracilicaea kejii Javed (1990)

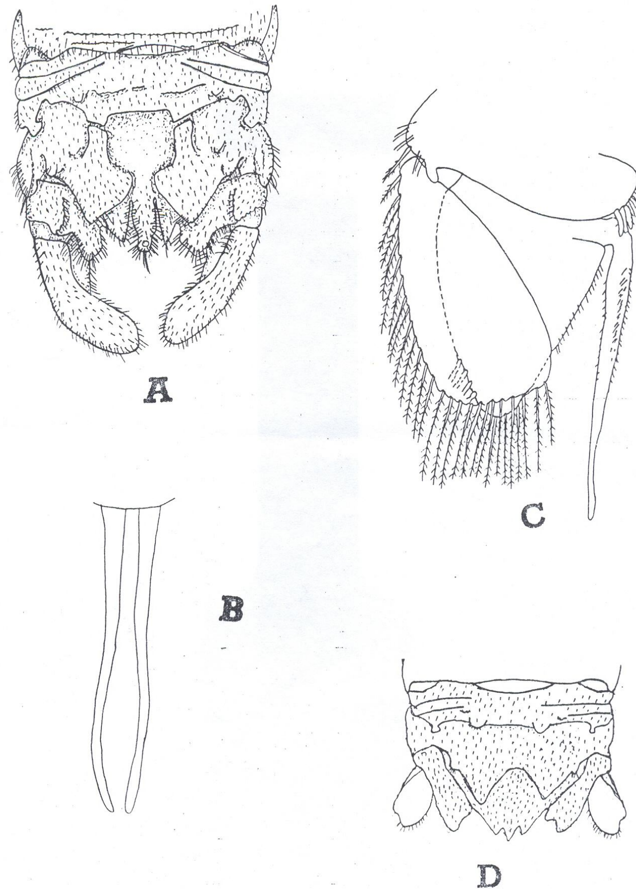

Fig. 4. *Paracerceis sculpta* (Holmes 1904) Adult male; A, pleotelson, dorsal view; B, penes; C, pleopod 2; ovigerous female; D, pleotelson, dorsal view; Scale lines=1 mm.

Material

Adult male, 10 mm; 1 ovigerous female 7.0 mm; 27 August, 1998; 2 adult males, 9.0-10 mm, 7 November, 1999; 1 non-ovigerous female, 8.5 mm; 17 January 2001; 1 ovigerous female 8.5 mm; 1 December, 2002. Bulleji, Karachi, Pakistan.

Description

The dimorphic characters of this species in adult males (Fig. 4A) have

strong ornamentation of pleotelson including the presence of highly developed bosses, tubercles, spines and setae. The uropod of male is extremely dimorphic in being almost uniramous and densely setose. The endopod is highly reduced while exopod is well developed, long, apex entire. Females differs from adult males in having less sculpted pereon and pleotelson (Fig. 4A). The uropods are biramous (Fig. 4A) with subequal and lamellar rami extending as far as pleotelson. The penes slender (Fig. 4B), separate to base. Appendix masculine sublinear, arising from interno-proximal angle of endopod of pleopod 2 (Fig. 4C) and extending beyond ramal apex. The ovigerous females, pleotelson (Fig. 4D) with a dome having median longitudinal depression, apex with an elongate notch. Dorsally each lateral side of the notch extends medially with a row of minute denticles.

Remarks

As already stated by Harrison and Holdich (1984) and Dr. Javed (1990) in *Paracilicaea* species sexual dimorphism in pronounced, especially in the form of uropods and in the dorsal ornamentation of the telson.

So it is impossible to identify the females of this species immediately because they are so different from adult males in having subequal and notched rami of uropod, pleotelson and without square-shaped cavity, distal trilobed portion convex and without thick fringe of setae.

Distribution

East Africa, Australia and Pakistan.

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REFERENCES

BARNARD, K.H., 1914. Contribution to the crustacean fauna of South Africa. 3. Additions to the marine Isopoda with notes on some previously incompletely known species. *Ann. S. Afr. Mus.*, **10**: 325, pl. 27-39.

BARNARD, K.H., 1920. Contribution to the crustacean fauna of South Africa. Further additions to the list of the marine Isopoda. *Ann. S. Afr. Mus*., **17**: 319-438.

BARNARD, K.H., 1955. Additions to the fauna list of South African Crustacea and Pycnogonida. *Ann. S. Afr. Mus*., **43**: 1: 1-107.

BOONE, P.L., 1923. New marine Tanaid and Isopod Crustacea from California. *Proc. Biol. Soc. Washington*, **36**: 147-156.

BOWMAN, T.W., 1981. *Thermosphaeroma millieri* and *T. smithi*, new sphaeromatid isopod crustaceans from hot springs in Chihuahua, Mexico, with a review of the genus. *J. crust. Biol*., **1**: 105-122.

BRUCE, N.L., 1990. *New records of Isopod crustaceans (Flabellifera) from Hong Kong*, pp. 549-554. Proc. Second Inter. Mar. Biol. Works. Hong Kong University Press.

BRUCE, N.L., 1995. Addition to the fauna – list of South African Crustacea and Pycnogonida. *Ann. S. Afr. Mus*., **43**: 1-107.

BRUCE, N.L., 1997. A new genus of marine Isopod (Crustacea: Flabellifera: Sphaeromatidae) from Australia and the Indo-Pacific Ocean. *Mem. Mus. Victoria*, **56**: 145-234.

HARRISON, K. AND HOLDICH, D.M., 1984. Hemibranchiate sphaeromatids (Crustacea: Isopoda) from Queensland, Australia, with a world-wide review of the genera discussed. *Zool. J. Linn. Soc*., **81**: 275-387.

HOLMES, S.J., 1904. Remarks on the sexes of Sphaeromids with a description of a new species of *Dynamene. Calif. Acad. Sci. Proc., Ser*., *3. Zool*., **3**: 296-306. pl. 34.

IVERSON, E.W., 1982. Revision of the isopod family Sphaeromatidae (Crustacea: Isopoda: Flabellifera). 1. Subfamily names with diagnoses and key. *J. crust. Biol*., **2**: 248-254.

JAVED, W., 1990. A new species of hemibranchiate sphaeromatid isopod of the genus *Paracilicaea* from Pakistan with the study of development stages of the male. *Hydrobiologica*, **199**: 21-28.

JAVED, W. AND YOUSUF, F., 1995. A new species and a new record of *Sphaeromopsis* Holdich and Jones, 1973 from Pakistan waters (Isopoda: Sphaeromatidae). *Pakistan J. mar. Sci*., **4**: 51-58.

KUSSAKIN, O.G. AND MALYNTNA, M.V., 1993. Sphaeromatidae (Crustacea: Isopoda: Flabellifera) from the South China Sea. *Invert. Tax*., **7**: 1167-1203.

LATREILLE, P.A., 1825. *Families Naturelles du Regne Animal, Exposes Succinetement et dans un Ordre Analytique avec l'Indication de leurs Genres*. Paris: 570.

LEACH, W.E., 1814. Crustaceology. *Brewster's Edinburgh Encyclopedia*, **7**: 383-437.

NOBILI, G., 1906. Nuovi Bopiridi. *Atti Accad. Sci. Torino*, **41**: 1098-1113.

PILLLAI, N.K., 1954. A preliminary note on the Tanaidacea and Isopoda of Travancore. *Bull. Centr. Res. Inst*., *University of Trivandrum* **3c**: 1-21.

PILLLAI, N.K., 1965. Isopods of the family Sphaeromatidae from the littoral waters of South India. *Crustaceana*, **9**: 75-89.

SRINIVASAN, T.K., 1959. On a species of wood-boring isopod *Exosphaeroma madrasensis* from Madras. *J. Timber Dryer' Preserver's Assoc. India*, **5**: 20-23.

STEBBING, T.R.R., 1910. Isopoda from the Indian Ocean and British East Africa. *Trans. Linn. Soc. London*, *(2 Zoology),* **4**: 83-122.

YASMEEN, R. AND JAVED, W, 2001. A new record of *Paracerceis sculpta* (Holmes, 1904) (Sphaeromatidae: Isopoda) from Pakistan, Northern Arabian Sea. *Pakistan J. mar. Sci*., **10**: 43-49.

YOUSUF, F. AND JAVED, W., 2001. *Cymodoce spinula*, a new species of sphaeromatid isopod from Pakistan waters. *Crustaceana,* **74**: 853-860.

Proc. Pakistan Congr. Zool., Vol. 23, pp. 13-24, 2003.

PHYSICO – CHEMICAL AND BIOLOGICAL STUDY OF RAINWATER POOLS, LONI KOT, THANA BOLA KHAN AND KOHISTAN REGION OF DISTT, DADU, SINDH, PAKISTAN

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Abstract.- Water samples of rainwater and rainwater pools at Loni Kot beside Super Highway, Distt. Dadu were collected during October 1999 to June 2000 and analyzed for water quality assessment along with flora and fauna. The rainwater indicated conductivity and total dissolved solids (TDS) of 18 – 64 µS/cm and 12 – 40 mg/L, respectively as compared with 213–404 µS/ cm and 175 – 330 mg/L of rainwater pool. The rainwater did not indicate any flora and fauna, however, after keeping water for one month, some blue green algae, *Chroococcus minor, Lyngbya limnatica, Merismopedia tenuissima* were identified. In the rainwater pools, *Chroococcus minor*; *Merismopedia tenuissima*, *Oscillatoria lemmermannii, Gloeocapsa lithophila* (Ereeg.*)* Hotterb, *Lyngbya limnetica* with 22 species of Cyanophyta, 24 species of Chlorophyta, 4 species of aquatic plants and 14 species of zooplankton were recorded.

Key words: Water chemistry, zooplankton, rainwater pools, cyanophyta, chlorophyta.

INTRODUCTION

The rainwater in the Sindh region is very low and unpredictable (3 to 6 inches) and is mostly absorbed quickly within the soil or drain down to river, canal or lakes, where the soil bed is calcareous and rocky. The water may be collected at some places in the form of pools, where water could remain upto 4 to 9 months, depending upon the soil texture, climate conditions and the rainfall.

A few water pools were observed in Loni Kot area, about 50 km from Hyderabad toward Karachi, near Super highway located with in the latitudes 25.17° – 25.22° N and longitudes 68.5° – 68.13° E. The pools are used as a source of drinking water for human, cattle, birds and agriculture purposes. In Sindh some studies are available on the natural and artificial lakes, like Hub

(Iqbal, 1986), Kinghar and Haleji (Jahangir *et al.*, 2000), Sonehro and Mehro (Leghari *et al*., 2000) and Bakar (Leghari and Khukawar, 1999). The present work examines the water quality, flora and fauna of rainwater pools and compares it with that rainwater collected.

MATERIALS AND METHODS

A few rainwater pools were observed near Loni Kot, along super highway from Hyderabad to Karachi, about 50 km from Hyderabad and 110 km from Karachi. The area is dry and arid with low rainfall. The water is collected in natural and artificial depressions within rocky bed with water depth of about 1 to 6 feet. The area of the pools was observed about 100 × 50 meters. The water was sampled two times during October 1999 to June 2000. The water samples were collected from the side of the pool. The samples were analyzed in the laboratory. The fresh rainwater was collected in the empty and open desiccators with a diameter of 1 foot during the rainfall in October 1999. The water samples were transferred to the plastic bottles of 1.5L capacity.

The temperature of water and air was measured at the site with mercury thermometer. Transparency of rainwater was measured with Sacchi disc. pH was recorded with Orian 420A pH meter. Conductivity, salinity and TDS were recorded with WTW (LF) 320-conductivity bridge. Alkalinity, chloride and hardness were determined by titrimetry using standard hydrochloric acid (0.01N), silver nitrate (0.01N) and EDTA (0.01M), respectively. Dissolved oxygen was evaluated by Winkler method. Nitrate was determined by spectrophotometry, using Hitachi 220 spectrophotometer. Nitrate was determined by using brucine sulphate derivatising reagent (APHA, 1980). Sodium, calcium, magnesium and potassium were determined by air – acetylene flame using Varian Spectr AA – 20 atomic absorption spectrometer at the conditions recommended by the manufacturer. Sodium, potassium, calcium and magnesium were determined at 589, 766.5, 422.7 and 285.2 nm, respectively, in triplicate; with delay time 3 seconds and integration time 3 seconds. The flora and fauna were collected by plankton net # 25 µm. the algal filaments and higher aquatic plants were collected by hand picking method. Identification were carried out by using keys for algae (Desikachary, 1959, Prescott, 1962), aquatic plants *Spermatophyta* (Cook, 1996), zooplankton (Ward and Whipple, 1959; Muzino, 1964).

RESULTS AND DISCUSSION

Temporary puddles of rain are formed almost everywhere, but the rainwater is absorbed quickly into the ground without formation of any particular ecosystem. However, at some places the rain is collected in depression in rocky areas. The pools may persist for 4 to 9 months and develop a particular flora and fauna. The depressions are generally having some dried detritus from previous wet phase and it is supplemented by the collection of fresh rain in the pools.

Two water pools were sampled and analyzed. Pool 1 was sampled twice and pool 2 four times during 1999 – 2001, Pool 1 was shallow with turbid water, which indicated Sacchi depth of up to 1.5 feet. Conductivity and total dissolved solids were observed within 201 – 347 μS/cm and 130 – 222 mg/L. Chloride, alkalinity and hardness were observed within 38 – 53 mg/L, 35 – 38 mg/L and 51 – 62 mg/L, respectively. The water remains in contact with sufficient amount of air and dissolved oxygen was observed up to 8.6 mg/L. The metal, sodium, potassium, calcium and magnesium decreased in the following sequence: Ca > Na > Mg > K.

The water of pool 2 was transparent with water depth of 3.5 – 8 feet. The conductivity and TDS were observed 213 – 404 μS/cm and 136 – 257 mg/L (Table I). The conductivity and TDS values were due to the dissolution of the salts from the soil. The rainwater collected in glass tank indicated conductivity and TDS within 18 – 64 μS/cm and 12 – 40 mg/L (Table III).

Plankton quickly develops from dry cysts or spores dispersed by wind. Most common are motile algae, *Euglena* sp., *Chlamydomonas* sp., blue green algal and zooplankton species growing from the bottom (Osborne and McLaclan, 1985). Thirty eight species of phytoplankton were identified with 16 species of Chlorophyta and 22 species of Cyanophyta. Among these, *Cosmarium impersulum,Cosmarium quardrum* (Figs. 1, 2)*, Spirogyra rhizoides, Spirogyra subsalsa, Spirogyra micropunctata, Mougeotia viridis, Mougeotia* sp., *Chlorella vulgaris, Chlorococcum humicola, Oocystis pusilla, Coelastrum microporum, Pediastrum tetras, Closterium leiblernii* and *Trachelomonas* sp. were dominant in both the water pools (Figs. 1-16).

TABLE I.- PHYSICO – CHEMICAL ASSESSMENT OF RAINWATER POOL AT LONI KOT (SUPER HIGHWAY).

No.	Parameters	1	2
		12-12-1999	**19-07-2001**
1	Time	16.0	14.30
2	Temperature of air (°C)	36	34
3	Temperature of water (°C)	32	28.5
4	Color	Turbid	Turbid
5	pH	8.54	7.65
6	Conductivity (μS/cm)	201	347
7	Salinity (g/L)	0.1	0.1
8	TDS (mg/L)	130	222
9	Chloride (mg/L)	38.5	53
10	Secchi visibility (ft.)	1.5	1.25
11	Total alkalinity (as mg/L $CaCO_3$)	35	38
12	Hardness (as mg/L $CaCO_3$)	51	62
13	Sulphate (mg/L)	-	17
14	Dissolved oxygen (mg/L)	8.6	-
15	Chemical oxygen demand (mg/L)	107	-
16	Nitrate (mg/L)	-	0.5
17	Sodium (mg/L)	27	31
18	Potassium (mg/L)	7	4
19	Calcium (mg/L)	36	46
20	Magnesium (mg/L)	9	12

[1]Rainwater artificial pool, 40 km away from Hyderabad towards Karachi at super highway adjacent to Fawad Petroleum from the South side. Pool is about 600 m long, 250 m wide and about 1.9 m deep.
[2]Same sampling location, but the sample collection was carried out one week after rainfall.

TABLE II.- PHYSICO – CHEMICAL ANALYSIS OF RAINWATER SAMPLES TAKEN FROM LONI KOT, THANA BOLA KHAN, KOHISTAN REGION OF DISTT. DADU, SINDH, PAKISTAN

S. No	Parameters	Date of sampling			
		10–10-1999	**14–12-1999**	**16–3-2000**	**17–6-2000**
1	Temperature of air (°C)	36	27	39	40
2	Temperature of water (°C)	29	23	36	36
3	Transparency (inch)	48	50	40	39
4	pH	8.4	8.2	8.2	8.5
5	Total alkalinity (mg/L)	182	163	226	238
6	Chlorides (mg/L)	160	195	250	200
7	Conductivity (μS/cm)	213	306	404	402
8	TDS (mg/L)	136	164	259	258
9	Depth of water (feet)	8	6	4	3.5

Fig. 1-48. Flora and fauna of rain water pools of Loni kot Area of Thana Bolakhan District Dadu Sindh Pakistan. 1, *Cosmarium imperssulum*; 2, *Cosmarium caelatum* Ralf; 3, *Oedogonium curvum* (Pringsh) var.Curvum; 4, *Bulbochaete* sp; 5, *Spirogyra singularis* Nordst; 6, *Cylindrospermum muscicola* Kutz; 7, *Spirogyra cf.micropunctata* Transseau; 8, *Microcoleus tenerrimus* Gomont; 9, *Anabaena volzii* Lemm; 10, Unknown fungal spores; 11, *Chlorococcum humicola* (Naeg) Rabenhorst; 12, *Oocystis pusilla* Hansgrig; 13, *Rivularia aquatica* (De wild) Geitler; 14, *Oscillatoria hamelii* Fremy; 15-16, *Coelastrum microporrum* Naegeli.

Figs. 1-48. Continued: 17, *Stigeoclonium polymorphum* (Franke) Heering; 18, *Spirogyra sabsalsa* Kutz; 19, *Lyngbya truncola* Ghose; 20-21, *Oscillatoria nigra* Vauch; 22, *Oscillatoria simplicissma* Gom; 23, *Gloeocapsa haematodes* Kutz; 24-25, *Lepadella amphitropis* Harring; 26, *Difflugia globulosa* Dujan; 27, *Difflugia constricta* Ehrenberg; 28, *Trachelomonas trhopoensis* Vanorya; 29, *Pediastrum tetras* (Her) Ralfs; 30, *Anabaena variabli* Kutz; 31, *Closterium leibleinii* Kutz; 32, *Chlorella vulgaris* Beyerinck.

Figs. 1-48. Continued: 33, *Bulbochaete tenuis* (Wittrock) Hirn; 34-35, *Bulbochaete tenuis* (Wittrock) Hirn; 36, *Rivularia aquatica* (De wild) Geitler; 37, *Cosmarium* sp; 38, *Bulbochaete denticulate* Wittrock; 39, *Bulbochaete* sp; 40, *Oscillatoria princeps* Vauch; 41, *Microcystis aeruginosa* Kutz; 42, *Bulbochaete tenuis* (Wittrock) Hirn; 43, *Microcoleus vaginatus* (Vauch.) Gom; 44, *Cylindrospermum muscicola* Kutz; 45, *Nitella hyalina* Ag; 46, *Chara zeylanica* Wild; 47, A. *Paracyclops fimbriatus* Poppe, B. 1 Antenna, C. 5th Leg; 48, A. *Diaptomus* sp., B. 1 Antenna, C. 5th Leg.

TABLE III.- PHYSICO–CHEMICAL ASSESSMENT OF RAINWATER FROM SINDH UNIVERSITY AND HYDERABAD CITY.

No.	Parameters	1 10-7-2001	2 12-7-2001	3 11-8-2000
1	Time	16.20	5.20	-
2	Temperature of air (°C)	30	30	29
3	Temperature of water (°C)	27	29.5	27
4	Colour	Transparent	Transparent	Transparent
5	pH	6.5	6.6	7.35
6	Conductivity (µS/cm)	18	28	64
7	Salinity (g/L)	ND	ND	ND
8	TDS (mg/L)	12	18	40
9	Chloride (mg/L)	2	3	8
10	Total alkalinity (as mg/L $CaCO_3$)	1	1	6
11	Hardness (as mg/L $CaCO_3$)	14	13	17
12	Sulfide (mg/L)	ND	ND	ND
13	Sulphate (mg/L)	-	-	6
14	Dissolved oxygen (mg/L)	9	8.9	8.1
15	Chemical oxygen demand (mg/L)	ND	ND	ND
16	Total nitrogen (mg/L)	ND	ND	ND
17	Total phosphate (mg/L)	ND	ND	ND
18	Nitrate (mg/L)	ND	ND	ND
19	Sodium (mg/L)	-	-	4.8
20	Potassium (mg/L)	-	-	ND
21	Calcium (mg/L)	-	-	8.3
22	Magnesium (mg/L)	-	-	ND

ND = Not detected - = Not determined

Sampling stations
[1,3]Institute of Chemistry, University of Sindh, Jamshoro.
[2]Sindh University Colony, University of Sindh, Jamshoro.

The zooplanktons identified in the present study comprised 15 species representing four groups: Protoza 2, Rotifera 7 and Copepoda 2. Rotifera were observed dominant with *Brachionus falcatus, Keratella valga var tropica* and *Monostyla acus* in the decreasing order. The *Difflugia globusa, Difflugia costricta* (Figs. 26-28) of Protoza was present in large quantity. During the rainy season the quantity of fauna may decrease due to dilution factor (Whitton, 1975). The quantity of fauna and flora increased from winter to summer season due to low rainfall and effective consumption of the nutrients, contributed by human and animal activity (Viner, 1973).

TABLE IV.- FLORA AND FAUNA OF RAINWATER POOLS AT LONI KOT, THANA BOLA KHAN, DISTT. DADU, SINDH, PAKISTAN.

No.	Species	Rainwater pond	Rainwater
	Cyanophyta		
1	*Anabaena variables* Kutz	++	-
2	*Anabaena volzii* Lemm	++	-
3	*Chroococcus minor* (Kutz) Nagali	++	+
4	*Cylindrospermum muscicola* Kutz	+	-
5	*Gloeocapsa haematodes* Kutz	+	+
6	*Gloeocapsa lithophilus* Hotterb	+	+
7	*Lyngbya limnetica* Lemmerman	++	+
8	*Lyngbya trucicola* Ghose	+	-
9	*Merismopedia glauca* (Ehren) Nageli	++	+
10	*Merismopedia tenuissima* Lemm	++	+
11	*Microcoleus tenerrimus* Gom	++	-
12	*Microcoleus vaginatus* (Vauch) Gomont	++	+
13	*Microcystis aeruginosa* Kutz	++	-
14	*Nostoc commune* Vauch.	++	-
15	*Oscillatoria hamelii* Fremy	+	-
16	*Oscillatoria lemmermannii* Wolosz.	++	-
17	*Oscillatoria nigra* Vauch	++	-
18	*Oscillatoria princeps* Vauch	++	-
19	*Oscillatoria simplicissima* Gom	+	-
20	*Rivularia aquatica* (De Willd) Geitler	++	-
21	*Spirulina maior* Kutzing	+	-
22	*Spirulina subsalsa* Oerstd	++	-
	Chlorophyta		
23	*Bulbochaete denticulatum* Wittrock	++	-
24	*Bulbochaete* sp. 1	+	-
25	*Bulbochaete* sp. 2	+	-
26	*Bulbochaete tenuis* Hirn	++	-
27	*Chara zeylanica* Wild	+++	-
28	*Chlorella vulgaris* Bej	++	-
29	*Chlorococcum humicola* (Neag) Rab.	+++	-
30	*Closterium leibleinii* Kutz	+	-
31	*Coelastrum microporum* Neag.	++	-
32	*Cosmarium caelatum* Ralfs	+	-
33	*Cosmarium imperssulum* Elfv	++	-
34	*Cosmarium quadratum* Ralfs	++	-
35	*Cosmarium* sp.	+	-
36	*Mougeotia* sp	++	-
37	*Mougeotia viridis* (Kutz) Wittrock	++	-
38	*Nitella hyaline* (DC) Ag	++	-

Continued

No.	Species	Rainwater pond	Rainwater
39	*Oedogonium curvum* (Pring.) var. curvum.	++	-
40	*Oocystis pusilla* Hansg	++	-
41	*Pediastrum tetras* (Ehr.) Ralf	++	-
42	*Spirogyra micropunctata* Transeau	+	-
43	*Spirogyra rhizoide* Randhwa	++	-
44	*Spirogyra singularis* Nordst	++	-
45	*Spirogyra subsalsa* Kutz	+	-
46	*Stigeoclonium polymorphum* (Frank)Heering	++	-
47	*Trachelomonas acanthostoma* (Stokes) Defl.	+	-
	Bryophyta		
48	*Riccia* sp. (present on moist soil).	++	- -
	Aquatic plants (Spermatophyta)		
49	*Cyperus articulatus* Lem.	++	- -
50	*Cyperus corymbosus* Rottb.	+	- -
51	*Marsilea minuta* Lemm.	++	- -
52	*Najas graminea* Del.	+	- -
53	*Najas minor* Allioni	++	- -
	Zooplankton		
	Phylum Rotifera		
1	*Brachionus forficula* Wierzejski	+	-
2	*B. falcatus* Zacharias	+	-
3	*Keratella valga* var. *f. tropica* Apstein	+	-
4	*Lepadella amphitrops* Heering	+	-
5	*Manostyla pygmaea* Daday	+	-
6	*Monostyla furcata* Murray	+	-
7	*M. aecus* Harring		
8.	*Difflugia globulosa* Dujan	++	-
	Phylum Arthropoda		
	Order: Copepoda		
1.	*Paracyclops fimbriatus* Poppei	+	-
2.	*Microcyclops* sp.	+	-
3.	*Diaptomus* sp.	+	-

-, absent; +, present; ++, abundant; +++ dominant

In phytoplankton, *Oscillotria princeps, Oscillatoria nigra, Oscillatoria hamelii, Oscillatoria princeps, Oscillatoria* sp. *Mersmopedia tenuissima, Lyngbya* sp. *Spirulina major, Microcystis aeruginosa, Anabaena volzi* and *Anabaena variabiIis* of Cyanophyta were present in abundance. *Cosmarium*

impersulum, Cosmarium quadrtum, Spirygra rahizoides, *Chlorococcum humicola, Chlorella vulgaris, Oocystis pussila, Coelastrum microporum, Pediastrum tetres,* among *Closterium leibleinii* were found planktonic green algae (Chlorophyta) in the pools. While *Spirogyra rhizoides, Spirogyra subsalsa*. *Mougeotia* sp., *Oedogonium curvum*, *Bulbochaete denticulatum*, *Bulbochaete tenuis*, *Stigeoclonium polymorphum* and *Rivularia aquatica* (Figs. 33-36) were epiphytic on the Rizobenthos. *Chara zeylanica*, *Nitella hyaline* (Figs. 45-46) was rhizobenthos. The aquatic plants identified as emergent plants were *Marsilea minutea* and *Cyperus* sp. were observed on the margin of the ponds along with *Riccia* sp. which associated with *Nostoc commune*, *Phormedium* sp., *Lyngbya martensiana*, *Microcoleus vaginatus*, *Lyngbya aestuarii* and *Lyngbya bergei* (Figs. 8-9, 13-14).

To conclude water collected in the pools of Lonikot is suitable as a source for drinking and agricultural purposes, may be because of low amount of soluble salts in the soil bed within the region. The water indicated normal algal flora and fauna of fresh water.

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REFERENCES

APHA., 1980. *Standard methods for the examination of water and wastewater*, 15th Ed., Washington D.C.

COOK, C.D., 1996. *Aquatic and wet land plants of India* (Reprinted in 1978), William Dawson and Sons, London, pp. 778.

DESIKACHARY, T.V., 1959. *Cyanophyta*, I.C.A.R. New Delhi, pp. 686.

IQBAL, M., 1986. Preliminary studies on limnology of Hub lake. *Kar. Univ. J. Sci.*, **14**:53-61.

JAHANGIR, T.M., KHUHAWAR, M. Y. AND LEGHARI, S. M., 2000. Some studies on water quality and biological life at Kinghar and Haleji lakes of District Thatta, Sindh, Pakistan. *Pakistan J. biol. Sci*., **3**: 1965 – 1972.

LEGHARI. S. M, JAFRI, S. I. H. AND KHUHAWAR, M. Y., 2000. Limnological study of Sonharo, Mehro, Pateji and Cholari Lakes of District Badin. Sindh, Pakistan. *Pakistan J. biol. Sci*., **3**: 1904 – 1909.

LEGHARI, S. M. AND KHUHAWAR, M. Y., 1999. Limnological studies of Chotiari reservoir District Sanghar, Sindh, Pakistan. *Proceedings of impact of environmental pollution in lakes of Sindh,* organized by Institute of Environmental Engineering and Management, MUET, Jamshoro, Sindh, Pakistan, pp. 22 – 27.

MUZINO, T., 1964. *Illustration of the fresh water plankton of Japan*. Hoikusha, Higashiku,

Osaka, Japan, pp. 351.

OSBORE, P. L. AND McLACLAN, A. J, 1985. The effect of tadpoles on algae growth in temporary rain – filled rock pools. *Freshw. Biol*., **15**: 77–88.

PRESCOTT, G.W., 1962. *Algae of the western great lakes area*, W.M.C. Brown Publishers, Dubuque, Iowa, pp. 977.

VINER, A. B., 1973. Responses of mixed phytoplankton population to nutrient enrichments of ammonia, phosphate and some associated ecological implication. *Proc. R. Soc. London (B)*, **183**: 351-370.

WARD, H.B. AND WHIPPLE, G.C., 1959. *Freshwater biology* (ed. W.T. Edmondson), pp. 1248. John Wiley and Sons, New York.

WHITTON, B.A.(Ed.)., 1975. *River ecology.* University of California, Berkeley and Los Angeles, pp. 1241.

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A NEW SPECIES OF GENUS *LEIONOTACRIS* JAGO (GOMPHOCERINAE: ACRIDIDAE: ORTHOPTERA) FROM PAKISTAN

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Abstract .- *Leionotacris beshamensis* is described as new species from Pakistan.

Key words: Orthoptera, Acrididae, NWFP.

The genus *Leionotacris* was erected by Jago (1996) for two species namely *Donopherula gilloni* Hollis (1966), an African species and *Auiocobothrus bolivari* Uvarov (1921), an Indian species. Presently a new species, *Leionotacris beshamensis,* is described from Pakistan. The addition of *L. beshamensis* makes a total of three species in this genus.

Leionotacris beshamensis, new species

Diagnosis

Very closely related to *Leionotacris bolivari* (Uvarov) but 3 mm smaller. In this new species the lateral carina of pronotum is strongly incurved in prozona, hind tibia is red color, epiphallus with wider inter lophal space and lateral plates are extended outwardly while in *L. bolivari* the lateral carinae of pronotum is smoothly incurved in prozona, hind tibia is orange yellow, epiphallus is with narrow inter lophal space and lateral plate are not outwardly extended.

Description of holotype male

Size small, dark brownish in color. Antenna 24-26 segmented, slightly longer than head and pronotum together. Fastigium of vertex with an strong ovoidal impression, transverse sulcus clearly visible crossing fastigium at or just infront of middle, fastigial foveolae weak elongate rhomboidal.

Pronotum (Fig. 1A) with well developed median carina, crossed by posterior sulcus at its middle, lateral carina being entire and strongly incurved in prozona. Tegmina extend, upto the hind knees; median and cubital areas equal in width, former bearing a median longitudinal intercalary vein. Hind tibia red in color with 11 spines on either side. Epiphallus (Fig. 1B) with paired lophal sclerotisation of roughly equal size. Ancorae slender slightly curved, anterior process robust slightly shorter than ancorae, thick and broad, bridge narrow with inner margin curved upwards.

Fig. 1. *Leionotacris beshamensis* new species male holotype: A, pronotum dorsal aspect; B, epiphallus, dorsal aspect. Scale Bar = 1 mm.

Allotype

As the holotype, but larger. Antenna shorter than head and pronotum together, fastigial foveolae very weak.

Paratype

In every respect like holotype and allotype.

Measurements (mm)

	Holotype male	Allotype female	Paratype male	Paratype female
Antennal length	5.6	5.7	6.0	6.3
Pronotal length	2.8	4.2	3.0	4.9
Tegminal length	12.8	17.0	12.6	17.0
Hind femur length	9.1	13.0	9.1	14.0
Hind tibia length	7.1	10.7	7.0	11.5
Total length	15.0	20.5	15.5	22.0

Material examined NWFP Besham, 2 males, including holotype; 2 females, 19.iv.2002 (M.S. Wagan)

Etymology

This new species is named after Besham town the locality of type material.

Repository

The type material is deposited in the Museum, Department of Zoology, University of the Sindh, Jamshoro, Pakistan.

Habitat

This species has been collected from the river bank having the mixed vegetation of grasses and herbs.

REFERENCES

HOLLIS, D., 1966. A revision of the genus *Dnopherula* Karsch. *EOS*., **51**: 267-329.

JAGO, N.D., 1996. Review of western and eastern African genera of the *Dnopherula* complex (Orthoptera, Acridoidea, Gomphocerinae) with description of new genera and species. *J. Orth. Res*., **5**: 67-124.

UVAROV, B.P., 1921. On records and description of Indian Acrididae. *Ann. Mag. nat Hist*., **9**: 480-509.

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SOME OBSERVATIONS ON THE SUBFAMILY EYPREPOCNEMIDINAE (ACRIDIDAE: ACRIDOIDEA: ORTHOPTERA) OF PAKISTAN

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Abstract .- Ten species and subspecies namely *Eyprepocenemis alacris* (Serville), *E. al. impicta* (Uvarov), *E. shirakii* (Bolivar), *Heteracris littoralis* (Rambur), *H. adspersa* (Redtenbacher), *H. persa* (Uvarov), *H. notabilis* (Uvarov), *Choroedocus illustris* (Walker), *Cataloipus cognatus* (Walker) and *Tylotropidius varicornis* (Walker) of subfamily Eyprepocnemidinae occur in Pakistan. *Cataloipus cognatus* is recorded for the first time from Paksitan. *H. persa* is restricted to Balochistan, while *Eyprepocnemis shirakii* to the NWFP of Pakistan.

Key words: Eyprepocenmidinae, Acrididae, Orthoptera, Pakistan.

INTRODUCTION

Until now the detailed study on the grasshopper species belonging to subfamily Eyprepocnemidinae of Pakistan has not been attempted, although several papers have made casual reference to it (Kirby, 1914; Uvarov, 1921; Bei-Beinko and Mishchenko, 1951; Dirsh, 1958; Ahmed, 1980; Wagan, 1990; Wagan and Solangi, 1990; Wagan and Naheed, 1997; Grunshaw, 1991; Mazhar, 1993; Tokhai, 1996; Yousuf, 1996).

The following study was carried out on the distribution and important host plants in order to update the knowledge of Eyprepocnemidinae of Pakistan.

MATERIALS AND METHODS

The present investigation was carried out on the material collected from Sindh, Baluchistan, Punjab and North-West Frontier Province (NWFP) of Pakistan by Dr. M.S. Wagan during 1983-2000 and housed in the Museum of Department of Zoology, University of Sindh, Jamshoro. Further material was recently collected from the various provinces of Pakistan by the present author.

The collected material was preserved by conventional method. Identification of the specimens was carried out with the help of available literature.

RESULTS AND DISCUSSION

A total of 10 species and subspecies were identified and their distributions at district level in the Sindh, Baluchistan, Punjab and NWFP of Pakistan are shown in Tables I-IV, respectively. Most of the species were collected from grasses, herbs and shrubs. However, some species, *Eyprepocnemis al alacris* (Serville), *Choroedocus illustris* (Walker) and *Heteracris littoralis* (Rambur) were collected from the cultivated sugarcane, maize and jowar fields, where they are regarded as minor pests.

It can be seen from the Table V that the species *Heteracris littoralis, H. notabilis, H. adspersa, Choroedocus illustris* and *Eyprepocnemidinae al. alacris* occur in all the provinces of Pakistan, while *H. persa* is restricted to Baluchistan whereas *Tylotropidius varicornis* and *E. shirakii* occur in the Punjab and NWFP, respectively.

Mazhar (1993) and Yousuf (1996) collected a single female of *Tylotropidius varicornis* from Rawalpindi. Presently, I have collected both male and female from Karor (at 3500 feet) near Murree and Islamabad and thus confirm its presence. The distribution of *H. persa* earlier reported from Quetta, Khuzdar and Nushki by Grunshaw (1991) has been extended to Loralai, Zhob districts of Baluchistan, *Cataloipus cognatus* was earlier recorded from Punjab by Wagan and Naheed (1997), respectively I have collected three specimens of this species, therefore I confirm the presence of this species and its distribution has been extended to Sindh. In addition, the distribution of many of the previously regarded species has been extended to the new localities. Ahmed (1980) recorded 6 species, while Yousuf (1996) collected 8 species from various provinces of Pakistan. Presently I have studied 10 species from various provinces of Pakistan.

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REFERENCES

AHMED, F.U., 1980. *Survey of grasshoppers in arid and semi-arid region of Pakistan*. Final Rep. PL-480 No. P.K-ARS-20 (FG-Pa-21), pp. 500.

BEI-BEINKO, G.Y. AND MISHCHENKO, L.L., 1951. *Locust and grasshoppers of U.S.S.R. and adjacent countries*. Vol. 2.

DIRSH, V.M., 1958. Revision of the genus *Eyprepocnemis* Fieber, 1853 (Orthoptera: Acrididae). *Proc. R. ent. Soc. Lond.* (B), **27**: 33-45.

GRUNSHAW, J.P., 1991. A revision of the grasshopper genus *Heteracris* (Orthoptera: Acrididae: Eyprepocnemidinae). *Nat. Res. Inst. Bull.*, **38**: 1-16.

KIRBY, W.F., 1914. *The fauna of British India including Ceylon and Burma Orthoptera (Acrididae)*, London, pp. 276.

MAZHAR, M.N., 1993. *Acrididae of the Punjab (Pakistan)*. M.Sc. thesis, University of Agriculture, Faisalabad, Pakistan.

TOKHAI, S., 1996. *Survey and taxonomy of Orthoptera of Zhob Division (Baluchistan) and adjoining areas*. M.Phil thesis, University of Sindh, Pakistan.

UVAROV, B.P., 1921. Notes on the Orthoptera in the British Museum. I. The group of Euprepocnemini. *Trans. R. ent. Soc. Lond.*, **1&2:** 106-143.

WAGAN, M.S., 1990. *Grasshoppers (Acrididae) of Sindh*. Final Technical Report. Pakistan Science Foundation, Islamabad, Pakistan.

WAGAN, M.S. AND SOLANGI, S.M., 1990. Distribution and incidence of grasshoppers (Acrididae) of Sindh. *Bol. Sang. Veg. Plagas (Fuera de Serie),* **20**: 125-129.

WAGAN, M.S. AND NAHEED, K., 1997. *Acrididae of the Punjab*. Pakistan Science Foundation, Final Technical Report, pp. 114 + 40 figures, 18 maps.

YOUSUF, M., 1996. *Taxonomic studies on grasshoppers and locusts (Acriloidea: Orthoptera) of Pakistan*. Pakistan Science Foundation, Final Report, pp. 158.

TABLE I.- DISTRIBUTION OF SPECIES AND SUBFAMILY EYPREPOCNEMIDINAE IN VARIOUS DISTRICTS OF SINDH.

Sindh Districts	***Eyprepocnemis alacris alacris***		***E. al. impicta***		***Heteracris littoralis***		***H. adspersa***		***H. notabilis***		***Chorocedocus illustris***		***Catalopius cognatus***	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Hyderabad	2	-	-	-	-	-	-	-	7	4	-	-	-	3
Dada	-	1	-	-	12	14	3	2	4	2	-	1	-	-
Badia	3	6	-	-	-	-	-	-	-	-	-	-	-	-
Nawabshah	-	-	-	-	5	-	-	-	3	-	-	-	-	-
Mirpurkhas	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Thar	-	-	-	-	-	1	2	-	-	-	-	-	-	-
Jacobabad	1	-	1	1	-	-	1	-	-	-	-	-	-	-
Shikarpur	-	2	-	-	-	1	1	-	-	-	-	-	-	-
Larkana	4	4	-	-	-	-	-	-	-	-	1	-	-	-
Thatta	1	1	-	-	-	-	-	-	-	-	-	-	-	-
Total	**11**	**14**	**1**	**1**	**17**	**16**	**8**	**2**	**14**	**6**	**1**	**1**	**-**	**3**

TABLE II.- DISTRIBUTION OF SPECIES AND SUBFAMILY EYPREPOCNEMIDINAE IN VARIOUS DISTRICTS OF BALUCHISTAN.

Balochistan Districts	***Eyprepocnemis alacris alacris***		***E. al. impicta***		***Heteracris littoralis***		***H. adspersa***		***H. persa***		***H. notabilis***		***Choroedocus illustris***	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Quetta	-	-	-	-	3	2	2	2	1	1	1	1	1	1
Sibi	1	1	1	1	2	2	-	-	-	-	1	1	-	-
Naseerabad	-	-	-	-	1	3	-	-	-	-	-	-	-	-
Bar Khan	-	-	-	-	3	3	-	-	1	2	-	-	-	-
Qila Saifullah	-	-	-	-	2	4	-	-	5	7	-	-	-	-
Munsakhail	-	-	-	-	1	3	-	-	1	2	-	-	-	-
Loralai	2	4	-	-	4	8	3	2	2	4	-	-	1	1
Zhob	1	1	-	-	6	10	1	4	2	3	-	-	2	2
Lasbella	2	2	-	-	2	2	-	-	1	1	-	-	-	-
Total	**6**	**8**	**1**	**1**	**24**	**37**	**6**	**8**	**13**	**20**	**2**	**2**	**4**	**4**

TABLE V.- DISTRIBUTION OF SPECIES AND SUBFAMILY EYPREPOCNEMIDINAE IN VARIOUS PROVINCES OF PAKISTAN.

Species & Subspecies	**Sindh**	**Punjab**	**NWFP**	**Balochistan**	**Total**
Eyprepocnemis alacris alacris (Serville)	25	15	4	14	58
E. al. impicta (Uvarov)	2	-	-	2	4
E. shirakii (Bolivar)	-	-	4	-	4
Heteracris littoralis (Rambur)	33	138	11	61	243
H. adspersa (Redtenbacher)	10	45	4	14	73
H. persa (Uvarov)	-	-	-	33	33
H. notabilis (Uvarov)	20	2	1	4	27
Choroedocus illustris (Walker)	2	2	1	8	13
Cataloipus cognatus (Walker)	3	2	-	-	5
Tylotropidius varicornis (Walker)	-	3	-	-	3

TABLE III.- DISTRIBUTION OF SPECIES AND SUBFAMILY EYPREPOCNEMIDINAE IN VARIOUS DISTRICTS OF PUNJAB.

Punjab District	*Eyprepocnemis alacris alacris*		*Heteracris littoralis*		*H. adspersa*		*H. notabilis*		*Chorocedocus illustris*		*Catalopius cognatus*		*Tylotropidius varicornis*	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
R. Y. Khan	-	-	2	2	2	3	-	-	-	-	-	-	-	-
Sargodha	1	-	3	1	-	1	-	-	-	-	-	-	-	-
Bahawalnagar	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Attock	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Bahawalpur	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lodhrain	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rawalpindi	1	2	2	2	1	2	-	-	-	-	-	-	-	1
Vehari	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mianwali	-	-	-	1	-	1	-	-	-	-	-	-	-	-
Sahiwal	-	-	-	2	-	-	-	-	-	-	-	-	-	-
Islamabad	1	1	1	1	-	-	-	-	-	-	-	-	1	1
Khanewal	-	-	-	1	-	1	-	-	-	-	-	-	-	-
Khushab	-	-	2	6	-	1	-	-	-	-	-	-	-	-
Multan	-	-	7	70	7	12	-	-	-	-	-	-	-	-
Gujrat	-	1	-	1	-	1	-	-	-	-	-	-	-	-
D.G. Khan	-	-	1	2	-	-	-	-	-	-	-	-	-	-
Muzaffargarh	-	-	3	6	-	1	-	-	-	-	-	-	-	-
Jhang	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Faisalabad	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Okara	-	-	2	3	-	-	-	-	-	-	-	-	-	-
Jhelum	1	-	3	4	-	-	1	1	-	-	-	-	-	-
Kasur	-	2	-	1	1	2	-	-	1	-	-	-	-	-
Chakwal	-	1	-	-	1	-	-	2	-	-	-	-	-	-
Sheikhupura	-	-	-	2	-	2	-	-	-	1	-	-	-	-
Lahore	1	1	2	6	1	1	-	-	-	-	-	2	-	-
Sialkot	-	1	-	-	-	1	-	-	-	-	-	-	-	-
Gujranwala	1	-	1	-	1	-	-	-	-	-	-	-	-	-
Total	**6**	**9**	**28**	**110**	**15**	**30**	**1**	**3**	**1**	**1**	**-**	**2**	**1**	**2**

TABLE IV.- DISTRIBUTION OF SPECIES AND SUBFAMILY EYPREPOCNEMIDINAE IN VARIOUS DISTRICTS OF NWFP.

NWFP Districts	*Eyprepocnemis alacris alacris*		*E. shirakii*		*Heteracris littoralis*		*H. adspersa*		*H. notabilis*		*Chorocedocus illustris*	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Peshawar	1	1	-	-	2	2	1	1	-	1	-	1
Sawat	-	-	2	2	-	1	-	-	-	-	-	1
Mansera	-	-	-	-	1	-	-	-	-	-	-	-
Abotabad	-	-	-	-	1	-	-	-	-	-	-	-
Mardan	-	-	-	-	-	1	-	-	-	-	-	-
Naushahra	1	1	-	2	1	4	1	2	-	-	-	1
Total	**2**	**2**	**2**	**2**	**4**	**7**	**2**	**2**	**-**	**1**	**-**	**1**

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THE REPRODUCTIVE BIOLOGY OF *LIMANDA LIMANDA* IN OXWICH BAY, SOUTH WALES, U.K.

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Abstract.- The reproductive biology of marine teleost, *Limanda limanda* has been studied. The fish fully mature in February – March had high. GSI value, which gradually decreased in April to July. Low temperature corresponded high value of GSI. Ovaries of larger females increased in weight earlier than those of smaller fish. Condition factor (k) showed no significant variation. Six development stages of eggs have been described: immature, developing immature, developing (early), developing late, ripe and spent. Monthly changes in size, frequency and distribution of oocytes were calculated. Primary oocytes were 30-70 µm and mature eggs were of 350-400 µm. Primary oocytes were present throughout the year.

Key words: Marine teleost, GSI value, condition factor, egg development stages, spawning season.

INTRODUCTION

The susceptibility of animals to pollution varies with their physiological state. Changes in reproductive cycle of aquatic animals due to pollution have been observed mostly in their breeding season (Vernberg *et al*., 1974). To understand the effects of pollution on reproductive fishes, the knowledge of fish state at different times of the year is necessary. Study of the seasonal reproductive cycle involve the spawning period, developmental changes in the gonads, changes in the weight of gonads (GSI) in both sexes, condition factor (K) and egg size frequency distributes (Hyslop, 1987). Barr (1963) determined GSI of male and female in plaice, *Pleuronectes platessa*. Htun-Han (1978a,b) reported the seasonal changes in GSI and HIS in *Limanda limanda* in the North Sea. Ortega-Salas (1980) studied the reproductive biology of *Limanda limanda* around the Isle of Man. The condition factor of the marine teleosts has been studied by several workers (Lecren, 1951; Bagenal, 1957; Lee, 1972; Htun-Han, 1978a,b; Cooper, 1983). Seasonal changes in breeding were observed in *Limanda limanda* and long rough dab, *Hippoglosoids platessoides* in the North-Sea (Bagenal, 1957; Lee, 1972).

Reproductive biology of some other marine teleost has also been reported by some workers. Shackley and King (1977) studied the seasonal changes in the size – frequency distribution of eggs in the ovaries of *Blennius pholis* and in bass, *Dicentrarchus labrax* by Mayer (1987). Abbasi (1999) studied the reproductive biology of marine teleost, *Liza ramada* in Bristle channel England.

Limanda limanda has feeding stocks in estuarine waters of Oxwich Bay where pollution may occur. The present study examines the reproductive cycle of *Limanda limanda* by determining the spawning period, gonadosomatic index (GSI), condition factor (K), egg size-frequency distribution and developmental stages of eggs. This work can be the base for the further study of pollution effects on reproductive cycle of fish.

MATERIALS AND METHODS

Monthly samples of females of *Limanda limanda*, ranging in length from 24 to 35 cm were collected from Oxwich Bay. Total length, body weight, and ovary weight were noted for each fish and gonadosomatic index (GSI) and condition factors (K) calculated. Otoliths were removed to determine the year classes. The fish samples were subdivided into three groups, small, medium and large to find out if there is a relationship between ovary weight and body length.

Gonadosomatic index [GSI = (wt. of gonads / body wt. of fish) x 100] is the weight of the gonads expressed as a percentage of the total body weight. The condition factors (K= Body wt./L^3) is an indicator of the changes in the amount of food reserved stored in the muscles, defined as the ratio of the weight of the whole fish, without the gonads, to the cube of its length. This will vary throughout the reproduction cycle and induced when the fish has energy available for reproduction.

Histological and histochemical procedure

At monthly intervals ovaries were removed from random samples and fixed in sea water Bouin's fixature for 48 h, then dehydrated in graded alcohol series, cleared with xylene and then embedded in paraffin wax. They sectioned at 8 µm and mounted on glass slides using D.P.X. the sections were stained with haematoxylin and eosin for general morphological studies

for periodic acid Schiff (PAS) and mercuric bromophenol blue for carbohydrates and proteins, respectively.

Measurements and counts of oocytes

The size frequency distribution of oocytes was measured from histological section of ovaries taken from five individual fish each month selected from the largest fish caught during the study period October 1986 to October 1988. Sea temperature noted at the end of the depth of the sampling collection.

RESULTS

Fish, *Limanda limanda,* collected from feeding ground ranging in size between 24 – 33 cm, with age between 2-8 years. The GSI value was calculated by forming three groups, small, medium and large size individuals. These groups were of size 24-26.9 cm, 27-29.9 cm and 30-33 cm, with age classes 2-4 years, 4-6 years and 6-8 years, respectively (Table I). The monthly GSI values over the study period is present in Table I and Figure 1. GSI starts increasing in December and was high in February and March, then decreases gradually in April to July. This suggests that spawning season took place during the month of April. The average GSI values of different groups are plotted in Figure 1. All these groups show cyclical changes in ovary weight. The ovaries in larger females increase in weight earlier than those of smaller fish. Monthly condition factor (K) indicates the general physiological state of fish. Condition factor (Table I) shows no significant variation throughout the year of the study.

Figure 2 shows sea water temperature (°C). Minimum temperature recorded during the maturing season in February until the end of March was between 6.5-7.5°C. The minimum water temperature corresponds with maximum GSI values.

Seven stages of egg development have been described by microscopic observation. Oogonia and primary oocytes with diameters between 30-70 µm are immature which occur during April to July (Table II). Oocytes with diameter between 70 and 150 µm are at developing immature stages and the majority of those occur between September and November. Oocytes with diameters between 151 and 250 µm are called early developing stages (Table II) and occur mostly during December and January. Eggs with diameters

Fig. 1. Monthly changes in the gonadosomatic index of female, dab, *Limanda limanda*, in Oxwich Bay, South Wales.

Fig. 2. Monthly variation in mean sea temperature in Oxwich Bay, South Wales.

from 251 to 400 µm, called late developing stage, occur between January, February and March. Fish have fully ripe eggs characterized by the full ovary with mature eggs which run from vent on a slight pressure. Fishes of this stage move to deeper water for spawning. The spent stages are characterized by the presence of large population of primary oocytes (with diameter 70 µm) and the presence of atretic oocytes which occur in April, May and June (Table II).

TABLE II.- MICROSCOPIC AND HISTOLOGICAL CHARACTERISTICS OF OVARY STAGES OF *LIMANDA LIMANDA*.

Maturity stage	External appearance of ovary	Histological appearance
1 and 2 stage (immature) 30-70 µm	Small, slender and conical; each ovary is pinkish in color and translucent, enveloped in a coat of slivery epithelium	Ovary containing oogonia 30 µm and primary oocytes of stage 1 and 2. Oocytes are stained dense cytoplasm
3 stages developing 71-150 µm	Larger, reddish and translucent	Stage 3 are predominant, with vacuolated cytoplasm and are larger in size. Deposition of a thick layer occurs is the zona radiate formation. Oocytes upto 150 µm.
4 developing (early) 151-250 µm	Pink, full, completely opaque, eggs clearly visible.	Oocytes of stage 4 are upto 250 µm, contain small yolk droplets and vesicles. Stage 5 (with densely staining yolk globules) are beginning to appear.
5 developing (late) 251-400 µm	Pink, full and heavy ovary; opaque eggs clearly visible.	Stage 4 and 5 oocytes with dense stained yolk globules. Oocytes 251-400 µm. The lipid stained around the protein yolk.
6 Ripe and/or running*	Ripe or running with eggs; most of the eggs are hyline, translucent, hyline, speckled appearance, full ovary, eggs run from vent on slight pressure.	Stage 5 oocytes are mature predominate, irregular in shape. In running fish, mature eggs are in lumen, ovarian wall is thinner due to distension in ovary.
7 spent	Fully spent and resembling an empty bag; very thick ovary wall.	Stages oocytes of stage 1 and 2 predominate; atretic follicles in various of absorption are evident.

Monthly changes in size – frequency distribution of oocytes are presented in Figure 3. The eggs of diameter 70 to 150 µm are in the previtellogenic stage, and those of diameter 150-400 µm are in the vitellogenic stage. Histograms of the seasonal changes in the size – frequency distribution of oocytes with the stages numbered from 1 to 5 are presented in Figure 3. In April oocytes of stage 1 were highest in number.

Fig. 3. Size-frequency distribution of oocytes for January to December, the separate development groups are numbered from stage 1 to stage 5.

From May to September, there is a sign of gonad development with an increase in the numbers of smallest oocytes of stage 2 and 3, but there is no recruitment of oocytes of large size classes. In October a further stage 4 oocytes have been added. The small oocytes now have been recruited to consecutively larger size oocytes. This development continues and in December all 5 stages of the oocytes have appeared, which remain in ovary upto March. In March the highest number are of stage 5 which are characterized as mature eggs (late development stage).

DISCUSSION

The spawning season for females starts when the gonadosomatic index is at its maximum. Htun-Han (1978b) recorded high GSI values for *L. limanda* in the southern North Sea where fish spawn between February and March. Ortega-Salas (1980) reported a high GSI value for *L. limanda* around the Isle of Man during February to April. Compared with present study, GSI of *L. limanda* in Oxwich Bay were high during February and March. The GSI ranged between 6.17 and 6.71 in the different length groups. This range was less than that found by Htun-Han (1978b) in the North Sea between (8.4-15.7) to be considered in ripe fish. Oxwich Bay is the feeding ground and the fish migrate to the deeper water for spawning (Edwards and Steele, 1968), can be the reason of lower value of GSI in the present study. Examination of the size frequency distribution of oocytes in the ovaries of selected large fish showed a steady increase in eggs undergoing vitellogenesis during October – January with a marked increase in February – March in oocytes diameter from 251 to 400 µm, this supports the idea that the GSI has high values during February – March. Htun-Han (1978b) suggested that in stages 5 and 6 most of the eggs are transluscent and some may be completely transparent (hyaline) which are called ripe or running eggs. In the present study the ripe eggs were not available due to migration of fish to deep water for spawning.

In the present study, the relationship between GSI values and total length was not significant especially between the length ranges of 27-33 cm. This may be due to the limited size range of female fish obtained during these periods. But, the GSI values increased significantly within the fish with body length of 24-30 cm. Delahunty and DeVlaming (1980) suggested that the relationship between gonad weights to body weight may change from year to year depending on environmental factors such as food availability and temperature. Tyler and Dunn (1976) fond that the percentage of females of

the winter flounder, *Pseudopleuronectes americanus*, with ovaries that develop yolk increased with meal frequency so that fish on higher rations had heavier ovaries.

Changes in condition factor have been observed in many teleost. The high values of `K' for seawater perch, *Perca fluviatilis* were obtained at the spawning time, or in the autumn when both the `K' factor and GSI weight were undergoing rapid changes (Le-Cren, 1951). In *Blennius pava* the high values of `K' were obtained during the spawning season (Pazner, 1983). Bagenal (1957) recorded the best condition factor `K' in long rough dab, *Hippoglossoides platessoides* during November and December followed by a decline to the spawning time in March and April, after which there was a slow recovery. Jones (1970) reported that in the turbot, *Scophthalmus maximus*, `K' was lowest in September after the fish had spawned. In *L. limanda*, `K' reached its lowest point during April – May, the months immediately after the spawning period (Lee, 1972; Htun-Han, 1978b). In the present study, the condition factor `K' was slightly high in the pre-spawning period (October – January) and lower in April, the month immediately after spawning period, but the overall `K' values show no significant change throughout the study period.

There is a direct relationship between the water temperature and the time of spawning. Ortega-Salas (1980) found that spawning of *L. limanda* in Isle of Man waters occurred when the sea temperature was around 8°C in February and the middle of April. Wheeler (1969) stated that in *L. limanda*, spawning took place during February and March off the south and west coast of England and in April in the North Sea and Icelandic waters. In sole, *Solea solea*, natural spawning occurs at sea temperatures between 7.5 and 13.8°C during April to May (Irvin, 1974). Tong (1967) suggested that the temperature is an important factor in the control of gonad development in *Blennius pholis*. The spawning period of *B. pholis* is between March and July when the average temperature is 15°C (Shackley and King, 1977). Mayer (1987) reported that bass, *Dicentrachus labrax* does not spawn until sea water reaches a minimum temperature of about 9°C. In the present study, the maturing time as indicated by maximum GSI values, closely related with the decrease in sea water temperature *i.e.* 5.5°C to 7.0°C.

The results obtained from the size – frequency oocytes study shows that there is a seasonal trend for maximum and minimum values of oocytes size,

indicate that the spawning occurs during February to March. There is a protracted period of slow oocytes growth during which the ovaries contain primary oocytes (>150 µm). Gradually oocytes are recruited into the secondary growth phase which accelerates from October–December onwards, and by January oocytes recruited were upto 250 µm. These results are consistent with ultrastructural studies on the oocytes development of *L. limanda*, when oocytes of diameter 150 µm are in previtellogenic stages, those > 150 µm are in the vitellogenic stage. The results obtained by sampling the population of Oxwich Bay, South Wales, are similar to those of other workers. Ortega-Salas (1980) recorded the maturing eggs in the feeding grounds in the Isle of Man is between February to April and in the southern North Sea from February to March by Htun-Han (1978a).

REFERENCES

ABBASI, AR., 1999. Reproductive biology of a marine teleost, thin-lipped grey mullet, *Liza ramada* R. with special reference to oogenesis. *Pakistan J. Zool.*, **31**: 249-254.

BAGENAL, T.B., 1957. The breeding and fecundity of the long rough Dab, *Hippoglooides platessoides* (FABR.) and the associated cycle in condition. *J. mar. biol. Assoc. UK*, **36**: 339-375.

BARR, W.A., 1963. The endocrine control of the sexual cycle in the plaice, *Pleuronectes platessa* L. I. cyclical changes in the normal ovary. *Gen. comp. Endocr.*, **3**: 197-204.

COOPER, A., 1983. The reproductive biology of poor-cod, *Trisopterus minutus* L. whiting, *Merlangus merlangus* L. and Norway pont, *Trisopterus esmarkii* Nilsson off the west coast of Scotland. *J. Fish Biol.*, **22**: 317-334.

DELAHUNTY, D. AND DeVLAMING, V.L., 1980. Seasonal relationships of ovary weight, liver weight and fat stores with body weight in the gold Fish, *Carassius auratus*. *J. Fish Biol.*, **16**: 5-14.

EDWARDS, R. AND STEELE, J.H., 1968. The ecology of o-group plaice and common dabs at Loch Eve. i, population and food. *J. exp. mar. Biol.*, **2**: 215-238.

HTUN-HAN, M., 1978a. The reproductive biology of the dab, *Limanda limanda* L. in the North Sea: seasonal changes in the ovary. *J. Fish Biol.*, **13**: 351-359.

HTUN-HAN, M., 1978b. The reproduction biology of the dab, *Limanda limanda* L. in the North Sea: gonadosomatic index, hepatosomatic index and condition factor. *J. Fish Biol.*, **13**: 369-378.

HYSLOP, E.J., 1987. The growth and feeding habits of *Clarias anguillaris*, during their first season in the flood plain pools of the sokoto-Rima river basin, Nigeria. *J. Fish Biol.*, **30**: 183-192.

IRVIN, D.N., 1974. Temperature tolerance of early development stages of Dove, *Sole solea* L. In: *The early life history of fish* (ed. J.H.S. Blaxter), pp. 465-487.

JONES, A., 1970. *Some aspects of the biology of the turbot,* Scophthalmus maximus *L. with special reference to feeding and growth in the juvenile stage.* Ph.D. thesis, University East

Anglia, UK, pp. 145.

LE-CREN, E.P., 1951. The length weight relationship and seasonal cycle in gonad weight and condition in the perch, *Perca fluviatilis. J. Anim. Ecol.*, **20**: 201-219.

LEE, K.C., 1972. *The biology and population dynamics of the common dab*, Limanda limanda *L. in the North Sea*. Ph.D. thesis, University of East Angia, UK, pp. 105.

MAYER, I., 1987. *Reproductive biology of the bass*, Dicentrarchus labrax *L.* Ph.D. thesis, University of Wales, pp. 144.

ORTEGA-SALAS, A.A., 1980. Seasonal changes in common dab, *Limanda limanda* L. in Isle of man waters. *J. Fish Biol.*, **16**: 75-82.

PAZNER, R.A., 1983. The reproductive of *Blennius pava*, Teleostei, Blenniidae. I. Ovarial cycle, environmental factors and feeding. *J. Helgol. Meeresunl.*, **36**: 105-117.

SHACKLEY, S.E. AND KING, P.E., 1977. Oogenesis in a marine teleost, *Blennius pholis*. *Cell. Tiss. Res*., **181**: 105-125.

TONG, L.J., 1967. *Endocrine studies on* Blennius pholis *L.* Ph.D. thesis, University of Wales, pp. 115.

TYLER, A.V. AND DUNN, R.S., 1976. Ration, growth and measures of somatic and organ condition in relation to meal frequency in winter flounder, *Pseudopleuronectes americanus*, with hypothesis regarding population homeostasis. *J. Fish. Res. Bd. Can.*, **33**: 63-75.

VERNBERG, W.B., DECOURSEY, P.J. AND O'HARA, J., 1974. Multiple environmental factor effects on physiology and behaviour of the fiddler crab, *Uca pugilator*. In: *Pollution and physiology of marine organism* (eds. F.J. Vernberg and W.B. Vemberg), pp. 381-425.

WHEELER, A. (Ed.), 1969. *The fishes of the British Isles and North West Europe*. MacMillan and Co. pp. 603.

TABLE I.- THE MEAN GONADOSOMATIC INDEX (GSI) AND CONDITION FACTOR (K) CALCULATED IN FEMALE OF *LIMANDA LIMANDA* IN DIFFERENT SIZE GROUP AND AGE CLASSES.

Months	**24 – 26.9 (2 – 4 Years)**			**27 – 29.9 (4 – 6 Years)**			**30 – 32.99 (6 – 8 Years)**		
	n	**GSI**	**K**	**n**	**GSI**	**K**	**n**	**GSI**	**K**
December	9	2.46±0.69	0.75±0.57	9	2.69±1.03	0.87±0.49	7	2.38±0.15	1.00±0.06
January	10	2.87±1.06	1.14±0.07	11	3.29±1.16	1.14±0.08	6	3.16±1.05	1.13±0.04
February	7	6.31±1.85	1.09±0.09	10	6.71±1.03	1.08±0.11	8	6.12±1.02	1.09±0.09
March	8	6.17±1.93	1.08±0.54	8	6.35±1.86	1.02±0.13	9	6.91±1.23	1.18±0.05
April	12	2.16±0.67	0.92±0.09	11	2.25±0.36	0.89±0.04	7	2.36±0.22	0.94±0.08
May	7	0.81±0.12	0.93±0.09	7	1.31±0.67	0.85±0.04	6	1.50±0.70	1.23±0.10
June	10	0.67±0.51	1.05±0.11	5	0.65±0.18	1.00±0.02	10	0.48±0.10	1.20±0.12
July	9	0.28±0.05	1.13±0.11	10	0.54±0.23	1.16±0.06	8	0.60±0.05	1.12±0.01
August	11	0.31±0.07	1.13±0.70	8	0.60±0.13	1.10±0.11	10	0.84±0.08	1.10±0.03
September	8	0.39±0.11	1.17±0.13	6	0.45±0.14	1.18±0.03	5	0.80±0.09	1.90±0.09
October	9	0.72±0.09	1.14±0.08	9	1.11±0.13	1.17±0.10	8	0.96±0.12	1.18±0.07
November	10	0.86±0.25	1.04±0.12	7	1.16±0.37	1.06±0.03	6	1.34±0.01	0.97±0.03

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MATURATIONAL CHANGES IN GONADS IN RELATION TO PITUITARY GLAND OF A TELEOST FISH, *CYPRINUS CARPIO*

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Abstract.- *Cyprinus carpio*, commonly called as Gulfam, is a commercial carp and presumably known as a group spawner fish. The present study was carried out in order to understand the sequence of the reproductive cycle and its correlation with the pituitary gonadotrophs especially because of its different breeding nature. The gonads (testes and ovaries) and the pituitaries were dissected out from at least 10 males and 10 females during each month throughout the year. The results are based on the mean values of the percentage of gonadosomatic indices and the histological studies. The annual reproductive cycle of *Cyprinus carpio* is summarized as preparatory period from November to January, prespawning in February, first spawning in March, second spawning in June which is followed by the post- spawning or quiscent phase from July to October. New batch of the cycle was also noted during postspawning period. Correlative changes in the pituitary GTH cells were also studied throughout the year which indicates that during preparatory season of the gonad the pituitary cells were small granulated/ chromophilic. Increasing number of cytoplasmic granules during preceding months coincide with the advancement of the gonadal development. GTH cells become degranulated/ chromophobic as the breeding starts and remain degranulated throughout the quiescent phase of the reproductive cycle.

Key words: Gonads, pituitary gland, teleost fish.

INTRODUCTION

Annual reproductive cycles for many species of Teleosts have most frequently been described in terms of seasonal changes in the gonadosomatic index and/ or histological changes in the ovary or testis (de Vlaming, 1972, 1974, 1975; Egami, 1954; Sundararaj and Vasal, 1976; Jalali and Haider, 1985; Shaikh and Jalali, 1989, 1991). Generally all fresh water temperate zone fishes spawning in the spring or early summer have gonadal recrudescence in the winter or spring in response to long photoperiods and warm temperatures (de Vlaming, 1972, 1974; Egami, 1954; Gillet *et al.*,

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1977; Harrington, 1957; Sundararaj and Vasal, 1976; Jalali and Haider, 1985; Shaikh and Jalali, 1989-91).

Considerable information is available pertaining to morphology, histology, cytology and physiology of the pituitary gland of teleost fishes (Pickford and Atz, 1957, Sundararraj, 1959; Olivereau and Ball, 1964; Ball and Baker, 1969). It is generally known that in vertebrates the pars distalis undergo seasonal changes which can be correlated with the seasonal cycle of the gonads. Among the tropical fishes only in a few species such correlative studies were made (Sathyanesan, 1958, 1963; Lal, 1964; Sundararaj, 1959; Van Overbeeke and McBride, 1967; Peter and Crim, 1979).

Reproductive functions and annual maturational cycles in fishes are governed by a variety of external and internal factors. These encompass environmental and hormonal agencies. The environmental factors trigger the hormonal outputs at the hypothalamic (GnRH), hypophyseal (GTH) and gonadal (steroid) levels. The gonads respond by producing various steroid harmones which guide local developmental events as well as peripheral physiological and behavioral processes. Earlier literature in the field is exhaustive and information has been reviewed by de Vlaming (1974). More recent information has been critically analyzed by Liley (1980). Baggerman (1980), Peter (1983), Lam (1983), de Vlaming (1983), Foster *et al.* (1983) and Bye (1987).

Vast body of information is also available on the steroid hormones and their biological significance in Various species, neither the relationship of trophic factors with specific ovarian and testicular stages nor the sites of steroid hormones synthesis have yet to be fully sorted out (Van den Hurk and Peute, 1979; Lang, 1981; Kagawa *et al.*, 1981; Nagahama, 1983; Pundey and Callard, 1984; Selman *et al.*, 1986; Wallace *et al.*, 1987). Both histochemical and histological methodologies have been considered fruitful in this context and have been applied as an attempt for a better understanding of these aspects (Kagawa *et al.*, 1981; Van den Hurk and Peute, 1979; Lang, 1981). As regards the endocrine mechanisms controlling reproduction and breeding cycles, no work has so far been done on any of the local species of fishes, freshwater or marine. Such information is essential in order to gain a better understanding of regulation of reproductive functions.

The present program of work was envisaged in the context of current understanding of reproductive mechanisms correlative to trophic hormones known in the literature and the fact that hardly any work has been done in Pakistan. *Cyprinus carpio* is a common carp available throughout the world and is a group spawner but whether spawning of the fish at different intervals is correlative to the pituitary GTH cells is not known. In this connection an attempt has been made to investigate the correlation of gonadal cycle with the pituitary GTH cells.

MATERIALS AND METHODS

About 250 fishes (male and female) mean weight 557±26.78 g and size 34±0.24 cm, were kept in a separate pond at the Maqbool Aijaz Hatchery at Ver Road, Thatta. The pond was filled with the tubewell water and continuous inlet and outlet of water was maintained according to the requirement of fish health. The routine food for the fish was water weeds but they were also fed choker daily. The objective of rearing this fish in the natural pond was to study the maturational changes in the gonadal cycle in relation to the pituitary gonadotrophs. Exactly on the 21st day of every month, 10 male and 10 female fishes were taken out from the pond with the help of cast net. The fishes were measured and weighed live and then killed with the help of chloroform. The pituitary gland and gonads were isolated from the fish and entire pituitary gland was fixed in the Bouin's fluid for further histological process. The gonads were weighed and then a middle piece of each male and female gonad was also fixed in the Bouin's fluid for histological examination.

Histological examination

The tissues (pituitary gland, testes and the ovaries) were washed in tap water and then processed through a routine procedure of dehydration and hydration. The tissues were embeded in the parafin wax and the sections were cut at the thickness of 6 µm using rotary microtome. Thin sections were stretched on the albumenized glass slide by using slide warmer. The histological section of the pituitary and gonads were studied under the light microscope.

RESULTS

Reproductive cycle of the male and female Cyprinus carpio

The reproductive cycle was studied in *C. carpio* and the results are based on the values of Gonadosomatic index and the histological studies. According to the data shown in Table I, the reproductive cycle of male and female is divided into four seasons *i.e.* preparatory or recrudescent, prespawning, spawning and postspawning.

Gonado-somatic index in male fish

Lowest values of GSI were noted in the month of October *i.e.* 6.21±0.36, the values of GSI abruptlly increased in the month of November 9.04±0.95 indicating the active proliferation of sparmatogenic cells. The highest percentage of GSI was achieved in the month of march *i.e.* 12.1±1.2 (Table I) which indicates that the testes are fully matured and ready to spawn. The values dropped abruptly in the subsequent months (April and May) *i.e.* 9.54±0.61 and 7.4±0.72, respectively and the GSI again elevated (12.06±0.72) in the month of June indicating the second breading. From the month of July to October the values remained much lower as compared to the breading season indicating the fish in the quiscent period (Table I).

Gonado-somatic index in female fish

The data of the GSI values in female indicates that lowest mean GSI (3.10 ±0.5) was observed in the month of October and increased abruptly in November (10.07 ±1.0), and increased gradually during the preceeding months and reach at the maximum during the month of March *i.e.* 23.61±2.67 (Table I) indicates the presence of full grown ovaries in the fish. The values were dropped immediately in the month of April (8.50±0.87), and again started increasing in the subsequent month and reaches to another peak in the month of June *i.e* 22.7±2.32 indicating the second spawning batch. The decline was noted in July and continued till October. The fish remained in the quiscent phase during this period.

Histological examination of testis

The histological picture of the male gonad (testis) of the fish *carpio* indicates that the recrudescence of testes occur in two phases. In the early

Fig. 1. Histological structure of pituitary gland and gonads of *Cyprinus carpio* during early preparatory season (November to January); a, shows GTH cells granulated but small in size (X 100); b, few spermatogonia (Sg) and mainly spermatocytes (Sc) (X 100), and section of maturing ovary shows almost all types of oocytes (Oct.) (X 40).

Fig. 2. Histological structure of pituitary gland and gonads of *Cyprinus carpio* during pre-spawning season (February); a, shows GTH cells granulated but small in size (X 100); b, shows seminiferous lobules (SL) filled with very large cysts (cy) containing spermatocytes (Sc) and spermatids (St) (X 100); c, shows the oocytes (Oct.) of different sizes and maturing follicles (f) (X 40).

Fig. 3. Histological structure of pituitary gland and gonads of *Cyprinus carpio* during first spawning season (March); a, shows large and fully granulated GTH cells (X 100); b, section of the testis shows the cysts (cy) filled with spermatozoa (sz) and few spermatogonia (sg) (X 100); c, section of the ovary shows matured Graffian follicles (Gf) ovulated or ready to ovulate and many maturing oocytes (Oct.) are also visible (X 40).

preparatory season (November-January) the recrudescence shown by the proliferation of primary spermatocytes from sparmatogonia as shown in (Fig. 1b). Large seminiferous lobules containing secondary spermatocytes and sparmatids were seen in the late prepartory season (February) which can be nominated as the prespawning season of the cycle (Fig. 2b). In the month of March (Fig. 3b) the histological sections indicate that all the lobules were filled with the spartmatozoa. However, few sparmatogonia were also seen indicating the first breeding season of the fish. The testicular section (Fig. 4b) of the month of June revealed that the lobules were partially filled with the sparmatozoa indicating the second spawning of the season. The histological sections of subsequent months from (August to October) revealed that the testes were only filled with remaining sparmatogenic material along with picnotic cells (Fig. 5b).

Histological examination of ovary

During early preparatory season (November to January) the ovary shows almost all the types of oocytes alongwith many immature follicles (Fig. 1c). As the ovarian cycle progresses the number of oocytes is reduced and the number of maturing follicles increased which is cleary visible by the presence of intact nuclear membrane of many oocytes (Fig. 2c). The ovaries of the fish during March were filled either with ovulated follicles or the ova ready to ovulate alongwith many maturing oocytes(Fig. 3c). After two months time in June it was noted that many matured follicles were available (Fig. 4c) that indicated the maturation of the left over maturing oocytes of last spawning season (March). Histological sections of the ovaries during the period of August to October revealed that only residual atretic follicles were present (Fig. 5c) indicating the fish were in postspawning season or in a quiscent phase.

Histological studies of gonadotroph

The gonadotrophs (GTH) during the early preparatory season were granulated but small in size and heavily present in the anterior pituitary (Fig. 1a). The size of the GTH cells gradually increased with countable number of cytoplasmic granules during prespawning season (Fig. 2a). The granulatuon in large sized GTH cells during first spawning season (Fig. 3a) was increased heavily. Degranulation was noted with large sized nuclei during second breeding (in June) (Fig. 4a). In the postspawning season the GTH cells were chromophobic (Fig. 4a) and this condition continued till the next recrudescence.

Fig. 4. Histological structure of pituitary gland and gonads of *Cyprinus carpio* during second spawning season (June); a, shows large sized degranulating (GTH) cells (X 100); b, section of the testis shows the large cysts (cy) filled with spermatozoa (Sz) (X 100); c, section of the ovary showing follicles with disintegrated nuclear envelope (ne) (X 40).

Fig. 5. Histological structure of pituitary gland and gonads of *Cyprinus carpio* during post-spawning season (August to September); a, shows empty GTH cells (Chronophobes) (X 100); b, section of testis showing the remaining spermatozoa (Sz) of the season (X 100); c, section of the ovary showing the atretic follicles (at) and few oocytes (Oct.) (X 40).

DISCUSSION

It has been possible in the present investigation on *Cyprinus carpio* to bring out some interesting morphological features of the gonadal cycle with relevance to the pituitary GTH cells. The results of the studies carried out will be an addition to the currently available information on the reproductive biology of *Cyprinus carpio.* The normal cycle of *Cyprinus carpio* comprises gonadal recrudescence (preparatory season) in November onward, first spawning in spring (March) and second spawning in summer (June) followed by regression and quiescence in the succeeding months (July-October). The results reported here show that the study of seasonal changes in the reproductive cycle of male and female *Cyprinus carpio* reveals that this fish is serial spawner. The reproductive cycle in male and female shows three main periods recrudescent, breeding and quiescent.

Both group and single spawning patterns have been described among the teleosts. A group spawner, *Chrysophrys auratus* (Crossland, 1977) and the Red progy, *Pagrus pagrus* (Manooch, 1976), *Acanthopagrus latus* and *Acanthopagrus cuvieri* (Hussain and Abdullah, 1977), *Barilius vagra* Ham. (Jalali and Haider, 1985), and *Cyprinion watsoni* (Shaikh and Jalali, 1989, 1991). Considerable information is available on the reproductive cycle of teleost fishes (Hoar, 1969; Polder, 1971; Cyrus and Blaber, 1984; Grier *et al*., 1980; Grier, 1981), however, very little work has been done on the reproductive cycle of cyprinid fish (Jalali and Haider, 1985; Shaikh and Jalali, 1989, 1991).

Gonado-somatic indices (GSI) in both the sexes were maximum during breeding period (March and June). The values declined after first breeding in March and again increased as a second peak in the month of June. Other studies showed variation in the maximum GSI during breeding period. It maximum in Feb-March in *Acanthopagrus* species (Abu Hakima, 1984); May and July and October to December, in *Ilishiya africana* males (Marcus and Kusemiju, 1980) and May to October and December in its females and March-April in *Cyprinion watsoni* (Shaikh and Jalali, 1989, 1991).

During the prespawning period, the basophils undergo the process of degranulation and granulation. The spawning period is characterized by granulated and fewer number of degranulated basophils. The granulated basophils start degranulating during the postspawning period and continue

till the early recrudescent. The earlier studies (Sundararaj, 1959; Peter and Crim, 1979; Kobayashi *et al*., 1988) have shown the similar finding about the GTH cells/basophils/Cytoplasmic granulation during the male and female reproductive cycles.

The pituitary GTH cells and the gonadal cycles of *Cyprinus carpio* exhibit marked seasonal changes. Cytoplasmic granulation of the GTH cells correspond to the presence of spermatocyctes alongwith few spermatogonia in the testis and few maturing follicles alongwith many oocytes of variable stages. It is well known that presence of cytoplasmic granules responsible for the release of gonadotrophin may induce the Leydigs cells in the testes and the granulosa cells and the theca cells of the ovarian follicles to release the androgens and estrogens respectively. They seem to be evolved in the development of spermatocytes and oocytes. Further proliferation of GTH cells and increased number of cytoplasmic granules during prespawning (February) implies to the advanced stages of the developing male and female gonads.

Sundararaj (1959) is of the opinion that during the prespawning period the basophils (GTH Cells) will be in the process of degranulation and granulation. The degranulation according to him is probably associated with the release of large quantity of FSH and the traces of LH for the development of the smaller oocytes. The results of the present study are in agreement with the findings of the author cited above.

Cyprinus carpio has been identified as a group spawner. It has been noted that the first spawning occurs in the month of March followed by a second spawning with month of June. Proliferation of GTH cells during first breeding in March coincides with the presence of spermatozoa in a very large sized cysts and the matured ova in the follicles indicates the probable role of GTH cells in the maturation, but it was not possible to sort out in the present study that what particular GTH cell (LH or FSH) is responsible for the existing maturational stage in both the gonads as has been claimed by the workers in the past studies (Burrows, 1949; Turner, 1952; Williams, 1955). It was also impossible to mention in this study about the possible role of GTH cells (FSH or LH) alone or in synergism as has been reported by (Burrows, 1949; Turner, 1952).

Overwhelming number of granulated basophils and few degranulated basophils during the first spawning reflects the status of gonadal maturation

in the final stage. This observation has already been referred by Sundararaj in 1959. The appearance of few degranulated basophils were also noted which might have involved in the production of LH required for the process of ovulation.

During postspawning most of the basophils were degranulated probably because the hormones FSH or LH are no more required as the spermiation and ovulation has been completed. This degranulation was noted throughout post spawning (July-October) and during early preparatory season (November) because of resting phase. Such degranulation of basophils during the post spawning and early recrudescence has also been shown by Sundararaj (1959).

CONCLUSIONS

During preparatory and prespawning periods (Nov-Feb) the testes and ovaries were preparing, cysts filled with all the stages of development including spermatozoa and gravid follicles. First spawning takes place in the month of March followed by second spawning in the month of June. During post-spawning period (July-Oct) development of new crop of the spermatocytes and oocytes was noted immediately after the spawning. The transitional lobe of the pituitary gland is the only region to exhibit marked seasonal changes. The variation in the pituitary GTH cells show a close correlation with the seasonal changes in the gonads. During the pre-spawning period the basophils undergo the process of degranulation and granulation. The spawning period is characterized by granulated and fewer number of degranulated basophils. The granulated basophils start degranulating during the postspawning period and continue till the early recrudescence. Finally it was observed that in *Cyprinus carpio* the seasonal changes in the GTH cells are correlative to the reproductive cycle.

REFERENCES

ABU-HAKIMA, R., 1984. Some aspects of reproductive biology of *Acanthopagrus* spp. (Family: Sparidae). *J. Fish Biol.*, **25**: 515-526.

BAGGERMAN, B., 1980. Photoperiodic and endogenous control of the annual reproductive cycle in teleost. In: *Environment physiology of fishes* (ed. M.A. Ali), pp. 533-567. Plenum Press, New York.

BALL, J.N. AND BARKER, B.I., 1969. The pituitary gland: Anatomy and high physiology. In: *Fish physiology* (eds. W.S. Near and D.J Randwell), vol. 2, p. 193. The Academic Press, New York.

BURROWS, H., 1949. *Biological action of sex hormones*. Cambridge University Press, Cambridge, UK.

BYE, V.J., 1987. Enviornmental management of marine fish rerpoduction in Europe. In: *Proceedings of the third international symposium on reproductive physiology of fish* (eds. D.R. Idler, L.W. Crim and J.M. Walsh), pp. 289-298. St. John's Newfoundland, Canada, 2-7 August, 1987.

CROSSLAND, J., 1977. Seasonal reproductive cycle of snapper *Chrysophrys auratus* (Forster) in the Hauraki Gulf. *N.Z.J. Mar. Freshw. Res.,* **11**: 37-60.

CYRUS, D. M. AND BLABER, S. J. M., 1984. The reproductive biology of *Gerres* in Natal estuaries . *J. Fish Biol*., **24**: 491-504.

DE VLAMING, V.L., 1972. Envoirnment control of teleost reproductive cycles: A brief review. *J. Fish Biol.,* **4**: 131-140.

DE VLAMING , V.L., 1974. *Control of sex in fishes* (ed. C.B. Schreck), pp. 13-83. Virginia Polytechnic Institute and State University, Blacksburg.

DE VLAMING, V.L., 1975. Effects of photoperiod and temperature on gonadal activity in the cyprinid teleost, *Notemigonus crysoleuces. Biol. Bull.* (WoodsHole, Mass), **148**: 402-415.

DE VLAMING, V.L., 1983. Oocyte development patterns and hormonal involvement among teleosts. In: *Control processes in fish physiology* (eds. J.C. Rankin, T.J. Pitcher and R.T. Duggan), pp. 176-199. Croom Helm, London.

EGAMI, 1954. Effects of pituarity substance and estrogen on the level purent of ovaries of adult female of *Oxyzias latipas,* in the sexually inactive seasons. *Annot. Zool. Japan,* **27**: 13.

EKENGREN, B., LINDAHL, K. AND FRIDBERG, G., 1978. Immunocytology and inneration of the gonadotropic cells in the teleost fish, *Rutilus rutilus. Acta Zool. (Stockholm)*, **59**: 125-133.

FOSTER, A., JALABERT, B., BILLAR, R., BRETON, B. AND ZOHAR, Y., 1983.The gonadol steroids. In: *Fish physiology* (eds. W.S. Hoar, D.J. Randall and E.M. Donaldson), vol. 9 A, pp. 277-371. Academic Press, New York.

GILLET, C. AND BILLORD, R., 1977. Stimulation of gonadotropin secretion in goldfish by elevation of rearing temperature. *Ann. Biol. Anim., Biochem. Biophys*., **17**: 673-678.

GRIER, H.J., 1981. Cellular organization of the testis and spermatogenesis in fishes. *Am. Zool*., **21**: 345-347.

GRIER, H.J., LINTON J.R., LEALTHERLAND, J.F. AND DE VLAMING, V.L., 1980. Structural evidence for two testicular types in teleost fishes. *Am. J. Anat*., **159**: 331-345.

HARRINGTON, R.W. JR., 1957. Sexual photoperiodicity of the cyprinid fish, *Notropis bifrenatus* (Cope) in relation to the phases of its annual reproductive cycle. *J. exp. Zool*., **135**: 529-555.

HOAR, W.S., 1969. Reproduction. In: *Fish physiology* (eds. W.S. Hoar and D. J. Randall), vol. 3, pp. 1-72. Academic Press, New York.

HUSSAIN, N. A. AND ABDULLAH, M.A.S., 1977. The length weight relationship, spawning season and food habits of six commercial fishes in Kuwaiti waters. *Ind. J. Fish*., **24**: 181-194.

JALALI, S. AND HAIDER, G., 1985. Seasonal variation in testicular androgen and histology of fish, *Barilius vagra* Ham. *Biologia,* **31**: 129-146.

KAGAWA, H., TAKANO, K. AND NAGAHAMA, Y., 1981. Correlation of plasma estradiol-17 and progesterone levels with ultrastructure and histochemistry of ovarian follicles in the white-spotted char, *Salvelinus leucomaenis*. *Cell Tiss. Res*., **218**: 315-329.

KOBAYASHI, *ET AL*., 1988: Hormones changes during the ovulatory cycle in gold fish. *Gen. Comp. Endocr.,* **69**: 301-307

KOBAYASHI, H., ISHII, S., AND GORBMAN, A., 1959. The hypothalamic neurosecretory apparatus and the pituitary gland of a teleost, *Lepidogobius lepidus*. *Gunma J. med. Sci*., **8**: 301-321.

LAL, B., 1964. Morphological and histochemical studies of the pituitary gland of *Cirrhina mrigala* (Hamilton). *Proc. Ind. Acad. Sci*., **59**: 297-317.

LAM, T.J., 1983. Environmental influences on gonadal activity in fish. In: *Fish physiology* (eds. W.S. Hour, D.J. Randall and E.M. Donaldson), Vol. 9B, pp. 65-115. Academic Press, New York.

LANG, I., 1981. Electron microscopic and histochemical investigations of the atretic oocyotes of *Perca fluviatilis* L. (Teleostei). *Cell Tiss. Res*., **220**: 201-212.

LILEY, N.R., 1980. Patterns of hormonal control in the reproductive behavior of fish, and their relevance to fish management and culture programs. In: *Fish behavior and its use in the capture of fishes* (eds. J. E. Bardach., J.J. Marnuson., R.C. May and J.M. Reinhart), ICLARM Conf. Proc. 5, pp. 210-246. Int. Cent. Living Aquat. Resour. Manage. Manila, Philippines.

MANOOCH, C.S., 1976. Reproductive cycle, fecundity and sex ratios of the red porgy, *Pagrus pagrus* (Pisces: Sparidae) in North Carolina. *Fish Bull*., **74**: 775-781.

MARCUS, O. AND KUSEMIJU, K., 1984. Some aspects of the reproductive biology of the clupeid, *Ilisha africana* (Bloch.) off the Lagos Coast, Nigeria. *J. Fish Biol*., **25**: 679-689.

NAGAHAMA, Y., 1983. The functional morphology of teleost gonads. In: *Fish physiology* (eds. W.S. Hour, D.J. Randall and E.M. Donaldson), Vol. 9 A, pp. 223-275.Academic Press, New York,

OLIVEREAU, M., 1964a. L' hematoxyline au plomb, permetelle 1' identification descellules corticotropes de 1'hypophyse des Teleosteens? *Z. Zellforsch. microscop. Anat*., **63**: 496-505.

PETER, R. E., 1983. The brain and neurohormones in teleost reproduction. In: *Fish physiology* (eds. W.S. Hoar, D.J. Randall and E.N. Donaldson), Vol. 9A, pp. 97-135. Academic Press, New York.

PETER, R. E. AND CRIM. L.W., 1979. Gonadal secretion during reproductive cycle in teleosts: Influence of environmental factors. *Gen. Comp. Endocr*., **45**: 294-305.

PICKFORD, G.E. AND ATZ, J.W., 1957. *The physiology of the pituitary gland of fishes*. New York Zoological Society, New York.

POLDER J.J.W, 1971: On gonals and reproductive behavior in the Cichliid fish, *Acquidens portalegrensis* Heurel. *Netherland J. Zool*., 265-365

PUNDEY, J. AND CALLARD, G. V., 1984. Development of agranular reticulum in Sertoli cell of the testis of the dogfish *Squalus acanthias* during spermatogenesis. *Anat. Rec*., **209**: 311-321.

SATHYANESAN, A.G., 1958. Studies on the morphology and seasonal histological changes in the pituitary gland of the fresh water teleost *Cirrhina reba* (Hamilton). *Indian J. Qet. Sci*., **26**: 13-20

SATHYANESAN, A. G., 1963. Histological changes in the pituitary and their correlation with the gonadal cycle in some teleosts. *Cellule,* **63**: 281-290.

SELMAN, K., WALLACE, R.A. AND BARR. V., 1986. Oogenesis in *Fundulus heteroclitus*. IV. Yold-vesicle formation. *J. exp. Zool*., **239**: 277-288.

SHAIKH, S.A. AND JALALI, S., 1989. Seasonal changes in the testes of the fiva, *Cyprinion watsoni. Proc. Pakistan Congr. Zool*., **9**: 47-59.

SHAIKH, S. A. AND JALALI, S., 1991. Seasonal changes in the ovary of the cyprinid fish *Cyprinion watsoni. Proc. Pakistan Congr. Zool*., **23**: 19-25.

SUNDARARAJ, B. I., 1959. A study on the correlation between the structure of the pituitary gland of the india cat fish, *Heteropneustes* and the seasonal changes in the ovary. *Acta Anat.,* **37**: 47-80.

SUNDARARAJ, B. I. AND VASAL, S., 1976. Photoperiod and temperature in the regulation of reproduction in the female catfish *Heteropneustes fossilis*. *J. Fish. Res. Bd. Can.,* **33**: 959-973.

TURNER, E.D., 1952. *General endocrinology.* W.B. Saunders, Philadelphia and London.

UEATEHRLAND, J.F. AND SONSTEGARD, R.A., 1978. Structure of normal testis and testicular tumor in cyprinids from Lake Ontario. *Cancer Res*., **38**: 3164-3173.

VAN DEN HURK, R. AND PEUTE, J., 1979. Cyclic changes in the ovary of the rainbow trout, *Salmo gairdneri*, with special reference to sites of steroidogenesis. *Cell Tiss. Res.*, **199**: 289-306.

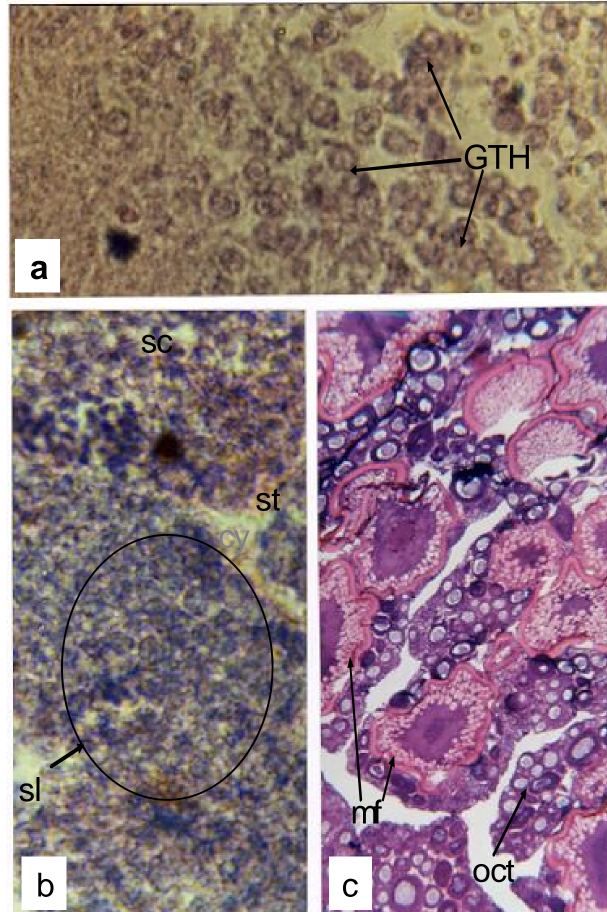
VAN OVERBEEKE. A. P. AND MCBRIDE, J.R., 1967. The pituitary gland of the sockeye (*Oncorhynchus nerka*) during sexual maturation and spawning. *J. Fish. Res. Bd. Can.*, **24**: 1791-1810.

WALLACE, R. A., SELMAN, K. AND PETRINO, T. R., 1987. Current status of the oocyte growth. In : *Proceedings of the third international symposium on the reproductive physiology of fish* (eds. D.R. Idler, L.W. Crim and J.M. Walsh), pp. 167-177. St. John's Newfoundland, Canada, 2-7 August, 1987.

WILLIAMS, R.H., 1955. *Textbook of endocrinology*, W.B. Saunders, Philadelphia and London.

TABLE I.- TOTAL LENGTH, STANDARD LENGTH, BODY WEIGHT, GONADAL WEIGHT AND GONAODSOMATIC INDEX (GSI) OF MALE AND FEMALE FISH *CYPRINUS CARPIO.*

S.No.	**Month**	**Total length (Cms)± SEM**	**Standard length (cms)**	**Body wt. (gm)**	**Gonadal wt. (gm)**	**G.S.I ± S.E.**
Males						6.21 ± 0.36
1	21-10-2000	34.4 ± 0.24	28.4 ± 0.48	557.7 ± 26.78	35 ± 3.95	
2	21-11-2000	31.91 ± 0.31	25.71 ± 0.96	419.± 12.04	44.16 ± 3.44	9.0 ± 0.95
3	21-12-2000	36.38 ± 0.90	32.12 ± 0.81	644 ± 44.95	60 ± 5.09	9.55 ± 0.23
4	21-01-2001	30.03 ± 0.81	26.03 ± 0.78	468.3 ± 25.18	44.16 ± 3.8	10.09 ± 0.78
5	21-02-2001	37.7± 0.46	31.1 ± 0.15	713 ± 52.60	80 ± 4.0	11.61 ± 1.0
6	21-03-2001	37.5±1.22	31.5 ± 1.13	758 ± 42.78	95 ± 3.11	12.1 ± 1.2
7	21-04-2001	34+ 0.48	27.4 ± 0.26	490 ± 26.07	47 ± 3.89	9.54 ± 1.0
8	21-05-2001	41.8± 1.29	37.8 ± 1.57	893.3 ± 31.32	68.33 ± 3.22	7.45 ± 0.91
9	21-06-2001	41.16± 1.16	34 ± 0.81	1020 ± 69.84	123.33 ± 8.94	12.06 ± 0.72
10	21-07-2001	38.1 ± 0.89	31.5 ± 0.83	725 ± 70.47	55 ± 6.01	7.55 ± 1.00
11	21-08-2001	36.9 ± 0.35	30.04 ± 0.81	658 ± 35.38	52 ± 3.04	7.95 ± 0.87
12	21-09-2001	37.9 ± 0.35	31.8 ± 0.22	848 ± 58.76	69.8 ± 18.03	7.98 ± 0.23
Females						
1	21-10-2000	35.2 ± 3.51	27.26 + 0.50	472 ± 30.19	14.4 ± 1.51	3.10 ± 05
2	21-11-2000	31.3 ± 0.60	25.24 ± 0.63	418 ± 22.16	42.6 ± 3.76	10.07 ± 1.0
3	21-12-2000	39.37 ± 0.40	32.12 ± 0.89	955 ± 40.38	120 ± 7.90	12.74 ± 1.02
4	21-01-2001	31.46 ± 1.16	26.5 ± 1.05	534 ± 36.16	90 ± 7.54	18.00 ± 1.78
5	21-02-2001	34.1 ± 0.79	28.0 ± 0.81	543 ± 24.31	113 ± 12.45	20.68 ± 1.90
6	21-03-2001	38.9 ± 0.87	32.36 ± 0.85	908 ± 62.12	214 ± 22.9	23.61 ± 267
7	21-04-2001	37.25 ± 1.15	30.87 ± 0.97	710 ± 47.56	60 ± 7.07	8.5 ± 0.87
8	21-05-2001	35.5 ± 1.21	31.12 ± 1.07	630 ± 46.77	99.5 ± 9.36	15.99 ± 1.20
9	21-06-2001	39.0 ± 3.97	35.5 ± 2.86	953.3 ± 160.76	221.66 ± 93.39	22.7 ± 2.32
10	21-07-2001	39.1 ± 1.31	33 ± 1.85	791 ± 41.25	115 ± 18.26	14.58 ± 1.23
11	21-08-2001	36.7 ± 1.36	29.6 ± 1.26	701 ± 46.65	101 ± 9.63	14.93 ± 1.26
12	21-09-2001	37.75 ± 1.13	30.75 ± 1.13	873.75 ± 86.01	83.75 ± 7.78	9.81 ± 0.96



Pre-spawning Season (February)

Fig. 4 (a). Shows GTH cells granulated and increased in size (100 X) , **(b).** Shows the seminiferous lobules (SL) filled with very large cysts(cy) containing spermatocytes (Sc) and spermatids(St) (100 X). (c). Shows the oocytes (oct) of different size and maturing follicles(f) (40 X).

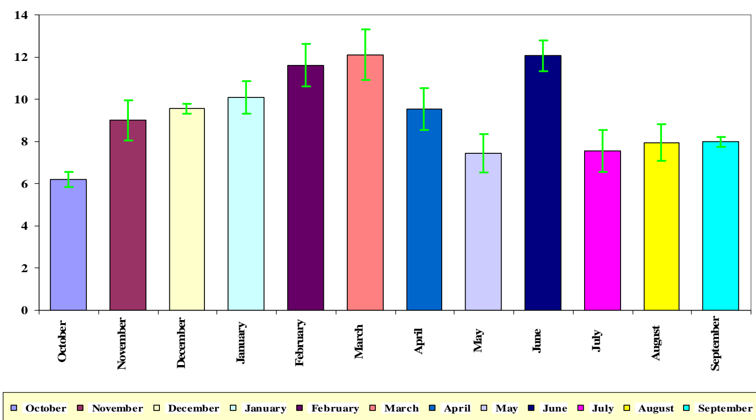


Fig: 1. Mean values of gonad somatic index for male fish *(Cyprinus carpio)*

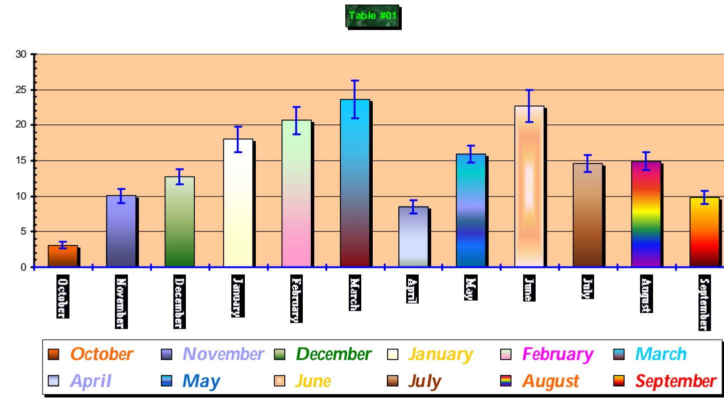


Fig: 2. Shows mean values of gonad somatic index for female fish *(Cyprinus carpio)*

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TOXICITY OF PHOSPHINE AGAINST 4TH INSTAR LARVAE OF *TROGODERMA GRANARIUM* COLLECTED FROM DIFFERENT GODOWNS OF PUNJAB

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Abstract.- Six populations of Khapra beetle, *Trogoderm granarium,* were collected from different godowns of the Punjab and were exposed to different doses of phosphine to determine its LC_{50} against stored grain pest. The phosphine concentrations in the range of 2-15 mg/l were used for LC_{50} determination. Mortality counts were made after 24 hours. The results were subjected to probit analysis and regression line for each population, from where the LC_{50} values for 268 Haroonabad, Chishtian, Lahore, Faqeerwali, 107 Haroonabad and Khanewal were estimated as 8.00, 8.00, 6.78, 6.60, 6.20 and 4.20mg/l, respectivly. So, the most resistant populations were those from Haroonabad and Chishtian and the most susceptible population was that of Khanewal.

Key words: Khapra beetle, phosphine, insecticide resistance.

INTRODUCTION

Trogoderma granarium, commonly called khapra beetle, is one of the cosmopolitan and serious pests of stored grains in Pakistan and elsewhere. The agro-ecological conditions of the country are also suitable for this purpose (Alam and Ahmad, 1989). The preferred food of most of these insects is wheat. During storage, wheat suffers a loss of about 3.5% for 5.4 months in public sector storage (Balock *et al*., 1986). To overcome this problem control measures of different nature are being adapted at farm, market and public sector storages. The most important is the use of contact insecticides and fumigants. The indiscriminate use of insecticides over a period of time has resulted in the development of resistance among the insects including *T. granarium* (Herron, 1990; Udeaan, 1992; Irshad and Iqbal, 1994; Bell and Wilson, 1995; Dong *et al*., 1998; Guillemaud *et al*., 1998; Scott, 1999).

The development of the resistance to phosphine in Khapra beetle was first reported from Punjab by Borah and Chahal in 1979. But Udeaan (1990)

studied susceptibility status of *T. granarium* population to phosphine in Indian Punjab and reported that it shows greater resistance as compared to the other pests.

Due to the development of multi-insecticide resistance strains of the insect, the problem of insect control has become more complicated and severe. The resistance against pesticides has gradually increased in insects over the years with increase in pesticide use (Saleem *et al*., 2000). It has therefore, become imperative to evolve reliable data to explain the resistance status in our local populations of this insect. The aim of the present study is to evaluate the development of resistance to phosphine in *T. granarium,* which is being extensively used to control the stored grain insect pests in Pakistan.

MATERIALS AND METHODS

Different populations of *T. granarium* were collected from different stores of Punjab like 268 Haroonabad, Chishtian, Lahore, Faqeerwali, 107 Haroonabad and Khanewal. These were tested for their susceptibility against phosphine. The resistance tests were conducted in the laboratory maintained at 35±1°C and 60±5% relative humidity. For this purpose FAO recommended procedure was adopted (FAO, 1969).

Preparation of phosphine gas

Commercially available pellets containing a phosphine equivalent of approximately 0.2 g were used as most suitable source of phosphine. The apparatus (Fig. 1) for generating phosphine was prepared by filling the gas jar collecting tube and rinsing tube with water. Approximately half the water in the gas jar was siphoned out and replaced by the 10% (v/v) H_2SO_4. A pellet containing aluminium phoshphide was then placed in the gas jar with the aid of stainless wire; the funnel was lifted slightly and placed over the pellet. Liberation of the gas came immediately. The gas mixture obtained in this manner from a fresh pellet contains approximately 86% phosphine.

After measuring the volume of desiccators insects were prepared for the test. For each population, in a desiccator three glass vials, each with 10 larvae were tested. The gas was sucked out through a rubber septum, fixed on the top of the glass tube of gas source, by a microsyringe (Hamilton).

Phosphine gas in known volume was sucked and pushed in to the sealed desiccators of the measured volumes. The larvae were tested at different doses. At the end of 24 hours of exposure the lid of the desiccator was removed. Mortality was monitored up to two weeks, and the data were subjected to the computerized Probit analysis and then regression lines for each population was drawn for the estimation of LC_{50} values.

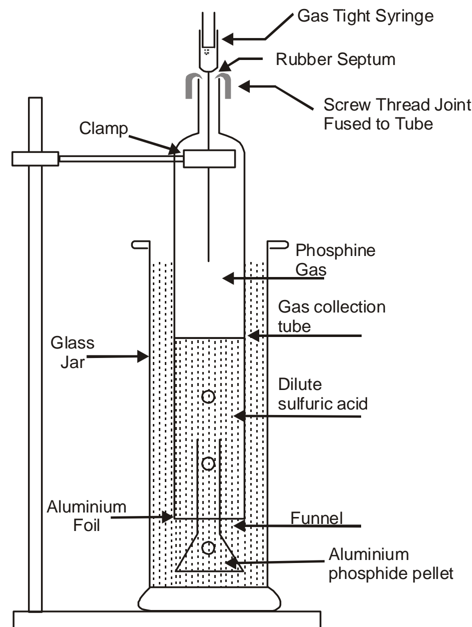

Fig. 1. The apparatus for generating phosphine gas.

Calculation of dose volumes and concentration

The volume (µl) of the 86% phosphine gas source at 35°C required to obtain a concentration of gas (mg/l) in a desiccator of specific volume (l) was calculated according to the FAO, Plant Protection Bulletin (Anonymous, 1975).

RESULTS AND DISCUSSION

According to the regression lines obtained by subjecting the original results to the Probit analysis, 268 Haroonabad and Chistian populations

showed maximum resistance against phosphine, and Khanewal population was the most susceptible among the various population groups of *Trogoderma granarium* under study. Although the LC_{50} values of both 268 Haroonabad and Chistian populations are equal to 8mg/l for 24 hours exposure period, however the complete kill of Chistian population required a dose of 15.45 mg/l which is 1.21 times greater as compared to the dose for complete kill of 268 Haroonabad population (Figs. 2,3).

The populations from Lahore, Faqeewali and 107 Haroonabad showed almost similar resistance level with LC_{50} values 6.78 mg/l, 6.60mg/l and 6.20 mg/l, respectively (Figs. 4-6). The R^2 value (relationship between phosphine dose and % age mortality) was strongest (0.991) for Lahore population and the frailest (0.861) for 107 Haroonabad population among the range of groups under study. Similarly the R^2 values calculated for 268 Haroonabad, Chistian and Khanewal are greater than 0.90, which means that the relationship between phosphine dose and percentage mortality is also stronger in these populations.

The population from Khanewal was most susceptible with 4.2mg/l LC_{50} value. It can be estimated from regression line that the complete kill of this population can be achieved only at 8.20mg/l (Fig. 7) From LC_{50} values it can be concluded as well, that Khanewal population is 1.9 times susceptible as compared with the most resistant populations from 268 Haroonabad and Chistian.

The results of this study support previous reports that phosphine resistant populations of the *Trogoderma granarium* exist world wide including Pakistan (Borah and Chahal, 1979; Winks *et al.,* 1980; Udeaan, 1992; Irshad and Iqbal, 1994). All populations tested showed high resistance against phosphine. It is obvious that control failure with phosphine is becoming the rule rather than the exception. Each population required different dose for effective control. Borah and Chahal (1979) tested two strains of khapra beetle from India and reported that strain P required higher dose of phosphine than strain N for the same level of kill. Irshad and Iqbal (1994) found that there is no recommended discriminating dose for *T. granarium* in the lab. It was not killed by the general fumigation dose. Such relationships are also noted in the present study. For example populations from 268 Haroonabad and Chistian (Farm level) have LC_{50} value 8.00mg/l for 24 hours exposure, while the LC_{50} of the Khanewal population is 4.20 mg/l for the same exposure period.

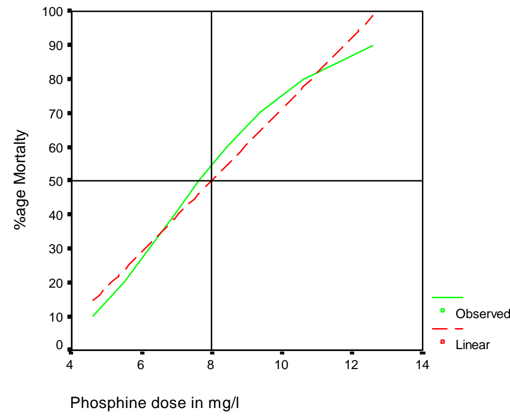

Fig. 2. Regression line of different concentrations of phosphine and mortality of *Trogoderma granarium* collected from 268 Haroonabad.

Y = %age Mortality

X = Phosphine dose in mg/I

R^2 = measure of relationship of dose and % age mortality

Y = -34.277 + 10.559 X

$R^2 = 0.968$

LC_{50} = 8 mg/l

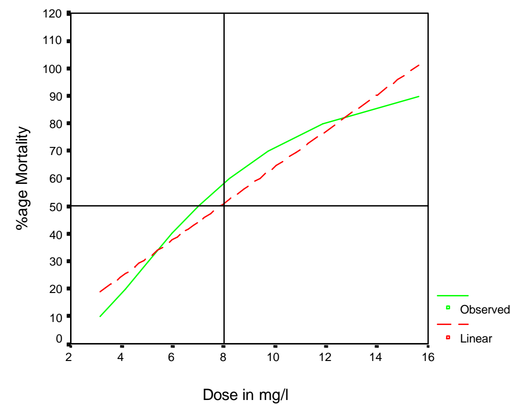

Fig. 3. Regression line concentrations of phosphine and mortality of *Trogoderma granarium* collected from Chishtian.

Y = %age Mortality

X = Phosphine dose in mg/I

R^2 = measure of relationship of dose and % age mortality

Y = -1.831 + 6.591 X

$R^2 = 0.932$

LC_{50} = 8.00mg/l

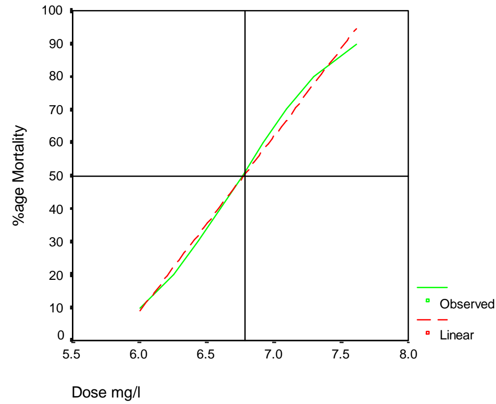

Fig. 4. Regression line concentrations of phosphine and mortality of *Trogoderma granarium* collected from Gulberg, Lahore:

Y = %age Mortality

X = Phosphine dose in mg/I

R^2 = measure of relationship of dose and % age mortality

Y = -309.5 + 53.067 X

R^2 = .991

LC_{50} = 6.78 mg/l

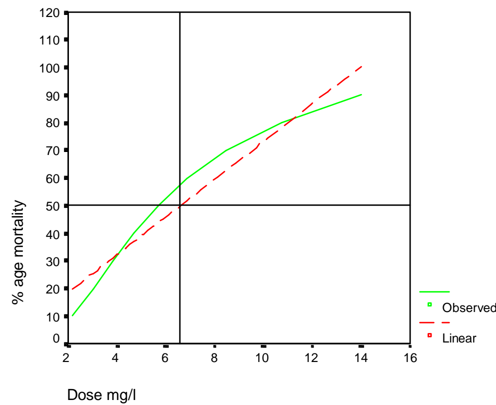

Fig. 5. Regression line concentrations of phosphine and mortality of *Trogoderma granarium* collected from Faqeerwali.

Y = %age Mortality

X = Phosphine dose in mg/I

R^2 = measure of relationship of dose and % age mortality

Y = 4.9064 + 6.826 X

R^2 = 0.932

LC_{50} = 6.60 mg/l

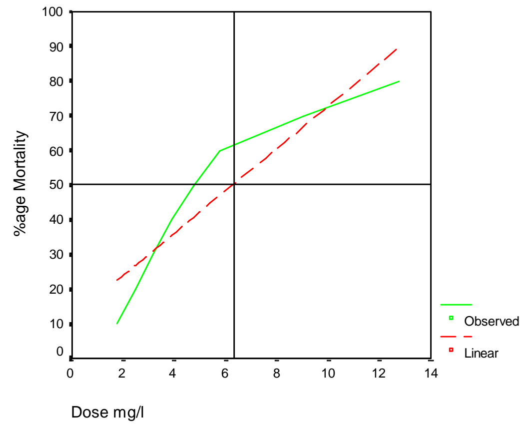

Fig. 6. Regression line concentrations of phosphine and mortality of *Trogoderma granarium* collected from 107 Haroonabad.

Y = %age Mortality

X = Phosphine dose in mg/I

R^2 = measure of relationship of dose and % age mortality

Y = 11.813 + 6.1048 X

$R^2 = 0.861$

LC_{50} = 6.2 mg/l

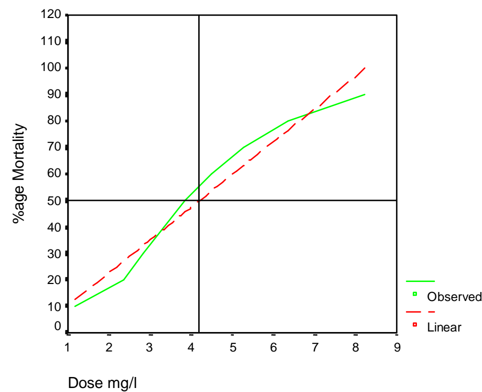

Fig. 7. Regression line concentrations of phosphine and mortality of *Trogoderma granarium* collected from Khanewal.

Y = %age Mortality

X = Phosphine dose in mg/I

R^2 = measure of relationship of dose and % age mortality

Y = -2.049 + 12.3698X

$R^2 = 0.953$

LC_{50} = 4.20 mg/l

The results also showed that the resistance level in these insects is going to increase at much faster rate. For example during 1988 different strains of the Khapra beetle were collected from the country *i.e.* Mamunkanjan, Fort Abbas, Bahawalnagar etc. The complete kill of the strains ranged from 0.4 to 3.2mg/l of phosphine for 20 hours exposure period (Irshad and Iqbal, 1994), while according to present results it can be guesstimated from the regression lines of most tolerant populations (268 Haroonabad and Chistian) that 100% kill can be achieved approximately at 12.71 mg/l of 268 Haroonabad and at 15.45mg/l for Chistian population for 24 hours exposure period. Similarly the 100 % kill of most susceptible population (Khanewal) among the group under present study, is observed at 8.24mg/l for 24 hours exposure. By considering most tolerant population *i.e.* Chistian (100 % mortality at 15.45mg/l for 24 hours exposure period) in the present study (2001) and Mamunkanjan strain (most tolerant strain of the previous studies) studied by Irshad and Iqbal during 1988 (100% mortality at 3.2mg/l for 20 hours exposure) it is found that there is approximately 4.82 times increase in the resistance level during thirteen years of continuous use of phosphine, which means control on these insects by using phosphine is going to fail.

Taylor (1986) reported that in many instances the reduced susceptibility to phosphine can be associated with poor techniques of fumigation. In public sector stores in Pakistan the dose of phosphine is not applied properly as during the process of fumigation the godowns are not properly sealed. Lethal concentrations hence are not maintained which offer opportunity to insects to develop resistance.

REFERENCES

ANONYMOUS, 1975. Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides. Tentative method for adults of some major pests species of stored cereals, with methyl bromide and phosphine. FAO Method No. 16. *FAO/Plant Prot. Bull*., **23**: 12-25.

ALAM, M.S. AND AHMAD, M., 1989. *Development of resistance in beetle, pests of stored grain against phosphine and contact insecticides in Pakistan*. Grain Quality Preservation group, Grain Storage Research Laboratory, Pest Management Research Institute, Pakistan Agricultural Research Council, Karachi University Campus, Karachi, Pakistan, pp.31.

BALOCK, U.K., AHMAD, H., BAJOL, I.M., KALIL, S.. AND QAYYUM, H.A., 1986. *Losses in public sector storage in Pakistan: Results of loss assessment survey 1984-1985*. Crop Science Division, PARC, Islamabad, pp.129.

BELL, C.H. AND WILSON, S.M., 1995. Phosphine tolerance and resistance in *Trogoderma granarium* (Everts). *J. stored Prod. Res*., **31**: 199-205.

BORAH, B. AND CHAHAL, B.S., 1979. Development of resistance in *Trogoderma granarium* (Everts) to phospine in Punjab. *FAO Plant Prot. Bull*., **27**: 77-80.

BUSVINE, J.R., 1971. *A critical review of techniques for testing insecticides*. The Common Wealth Institute of Entomology, London.

DONG, K., VALLES, S.M., SCHARF, M.E., ZEICHNER, B. AND BENNETT, G.W., 1998. The knockdown resistance mutation in pyrethroid resistant German cockroaches. *Pestic. Biochem. Physiol*., **60**: 195-204.

FINNEY, D.J; 1971 *Probit analysis,* 3rd edition. Cambridge University Press, London .

FAO, 1969. *Plant Prot. Bull*., **17**: 76-82.

GUILLEMAUD, T., LENORMAND, T., DOURGUET, D., CHEVILLON, C., PASTEUR, N. AND RAYMOND, M., 1998. Evolution of resistance in *Culex pipiens*: allele replacement and changing environment. *Evolution*, **52**: 443-453.

HERRON, G.A., 1990. Resistance to grain protectants and phosphine in coleopterous pests of grain stored on farms in New South Wales. *J. Australian entomol. Soc*., **29**: 183-189.

ICI TECHNICAL BULLETIN, 1993. *Actelic*. ICI Plant Protection Division, England.

IRSHAD, M. AND IQBAL, J., 1994. Phosphine resistance in important stored grain insects in Pakistan. *Pakistan J. Zool*., **26**: 347-350.

SCOTT, J.G., 1999. Cytochromes-P450 and insecticides resistance. *Insect Biochem. Mol. Biol*., **29**: 757-777.

SALEEM, M.A., SHAKOORI, A.R., RICHARD, M., WILKIN, R.M. AND DAVID, M.D., 2000. *In vitro* effect of deltamethrin, pirimiphos-methyl and gamma – HCH on proteases in insecticide resistant and susceptible strains of *Tribolium castaneum. Pakistan J. Zool.,* **30**: 53-59.

TAYLOR, R.W.P., 1986. Responses to phosphine of field strains of some insect pests of stored products. *GASGA seminar on the fumigation technology in developing countries*. March 18-21, TDRI SLOUG

UDEAAN, A.S., 1990. Susceptibility status of *Trogoderma granarium* (Everts) population to phosphine in Punjab. *Indian J. Ecol*., **17**: 195-196.

UDEAAN, A.S., 1992. Susceptibility status of *Trogoderma granarium* (Everts) to phosphine in North India. *J. Insect Sci*., **5**: 217-218.

WINKS, R.G., FANKS, H.J., WILLIAMS, P., BENGSTON, M. AND GREETING, H, G., 1980. Dosed recommendation for the fumigation of grain with phosphine. *Stand. Committee, Agric. Tech. Rep. Ser*., **8**: 1-9.

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EVALUATION OF BACTERIAL DIVERSITY IN SOIL UNDER SALT STRESS

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Abstract.- Soil microbial communities are the most diverse communities on earth and the factors that determine this extraordinarily high diversity are not well understood. The number of species currently cultivatable from soil is thought to represent 1 % or less of the total population. The present study was initiated to investigate microbial diversity in soils with different electrical conductivity (EC) levels using cultivation method. The aim of this preliminary study was to examine the influence of soil salinity on microbial community in rice rhizosphere, enumerate the total bacterial population, detect numerically important organisms, assess cultivation techniques, isolate and collect such bacterial strains that could play a role in biochemical processes in the stressed environment. Forty-eight soil samples were collected from 16 different varieties of rice grown in pots. Serial dilutions and spread plate method were used to enumerate soil microbial density and isolate bacterial strains. A total of 112 bacterial strains were selected, isolated and maintained by conventional culture methods for ongoing studies on bacterial diversity. Bacterial density recorded in normal soil fell in the range of 5.8×10^6 - 2.06×10^7 organisms per gram of soil. The influence of salinity on microbial diversity revealed that soil with EC 4.0 and 8.0 dSm^{-1} significantly reduced the diversity of bacteria. Bacterial diversity was significantly higher under normal soil conditions than under different EC levels. These results indicate that soil environment supports diversity of soil bacterial communities and may have impact on the sustainability of agricultural ecosystems.

Key words: Bacteria, diversity, soil, salt stress.

INTRODUCTION

Soil microorganisms mediate many processes that are essential to agricultural productivity of soil. These processes include recycling of plant nutrients, maintenance of soil structure, degradation of agro-chemicals and pollutants and the control of plant and animal pests (Parkinson and Coleman, 1991). Soils typically contain billions of microbial cells at several thousands of different genomes. The activity of soil microorganisms is crucial for the

degradation of dead organic matter, thus recycling carbon, nitrogen and minerals. However, very little is known about the functioning of individual groups of soil microorganisms in the environment, and about the role of microbial diversity for the functioning of the entire system. Since microbial diversity can be quickly affected by changes in ecosystem processes, temporal patterns of microbial diversity may be a sensitive indicator of ecosystem functioning (Kennedy and Smith, 1995).

Although the relationship between soil microbial diversity and the functioning and sustainability of agricultural ecosystems is unclear, examples which show that diversity of soil biota is important in key functions of agro-ecosystems have been documented (Swift and Anderson, 1993; Fragosa *et al*., 1997). Bacterial communities have traditionally been compared by analyzing isolates cultivated on plates. The fraction of the bacterial community in soil that can be cultured is estimated to range from 0.1 to 1% of the total; using molecular methods, a substantially large diversity of the bacterial community can be detected (Hugenholtz *et al*., 1998; Dunbar *et al*., 1999). Molecular techniques used in phylogenetic and biodiversity studies are: Restriction fragment length polymorphism (RFLP) coupled to the use of specific probes; Polymerase chain reaction (PCR) methods using random or specific primers 16S rRNA sequence analysis. The comparisons of the three elements of diversity in a sample *i.e.*, the types of bacteria present (composition), the number of types (richness), and the frequency distribution of relative abundance of types (structure) can be made by clustering isolates into operational taxonomic units based on phenotypic or genotypic characteristics (Dunbar *et al*., 1999). Evaluating these elements for collections of cultivated isolates provides relative measures of community diversity but not accurate descriptions of community diversity *in situ*.

Numerous studies have investigated the phylogenetic overlap between organisms obtained by cultivation and organisms identified by direct amplification and cloning of 16S rDNA (Stackebrandt *et al*., 1993; Chandler *et al*., 1997; Suzuki *et al*., 1997). These studies have consistently demonstrated that the two methods generally sample different fractions of bacterial communities. Major advances in molecular ecology over the past decade have given us a new perspective on the bacterial flora of soils (Liesack *et al*., 1997). The soil microbial community of a wheat field has been studied by using both cultivation-based and molecular methods (Smit *et al*., 2001).

It has usually been found that only a small proportion (typically 1%) of the microbial population of a soil can be cultivated, almost certainly a consequence of using inappropriate media and methods. Most studies to date have concentrated on fast growing, easily-isolated bacteria, rather than on dominant soil bacteria. Recent insights into the isolation of microorganisms from soil and the resuscitation of "viable but non-culturable" bacteria have allowed better recoveries of microorganism from soils, and allowed the isolation and identification of numerically-significant representatives of the microbial community (Grobkopf *et al.*, 1998). Soil salinity plays a major role in the microbial selection process as environmental stress has been known to reduce the bacterial diversity (Borneman, *et al.,* 1996). It has been observed that increasing salinity leads to decreasing diversity and soil salinity affects rhizosphere *Pseudomonas* populations (Rangarajan *et al.*, 2002).

The present study was initiated to investigate bacterial diversity in soils under salt stress. The aim of this preliminary study was to examine the influence of soil salinity on bacterial community in rice rhizosphere, enumerate the total bacterial population, detect numerically important organisms, assess cultivation techniques, isolate and collect such bacterial strains that could play a role in biogeochemical processes in the stressed environment.

MATERIALS AND METHODS

Soil samples

Soil samples were collected from the rhizosphere of different rice varieties grown in plastic pots under varied levels of salinity, *i.e.* normal, medium (EC 4.0 dSm^{-1}) and high (EC 8.0 dSm^{-1}). Plants were grown in silty clay soil and NaCl was added to soil in the irrigation water to give an EC of 4.0 and 8.0 dSm^{-1}. Soil closely adhering to the roots of two-weeks old seedling was removed. Sixteen samples were taken from each salt level. Fresh soil samples were processed for studying bacterial diversity. Each sample unit was analyzed individually immediately after receipt in the laboratory. Bacterial population was detected by cultivation method.

Cultivation media

Two media nutrient agar (NA) and peptone-yeast extract-dextrose agar (PYDA) were used for studying soil bacterial diversity.

Serial dilutions

For each soil sample one gram of soil was weighed out in a test tube, vortexed with 10 ml of sterile, distilled water for 2-3 minutes, mixed well, and then serially diluted upto 10^{-7}. One ml of each of the diluted soil and water samples was transferred into an appropriately labeled tube (10^{-1}). Approximately 9 ml of sterile water was added to each tube aseptically and swirled gently to allow thorough mixing.

Enumeration of bacterial diversity

Viable counts by spread plate method

The technique employed was viable counts on solid medium using spread plate method. From dilution series (10^{-1} to 10^{-7}) of a soil sample as made above, 0.1 ml of the dilutions was taken using pour plates. The three dilutions (10^{-5}, 10^{-6} and 10^{-7}) for each sample were plated out in duplicate, by transferring 0.1 ml aseptically to the surface of each plate. Soil suspension was spread evenly over the agar surface using a sterilized glass spreader. Plates were incubated at 37ºC for 18-24 hr. and colonies developing after incubation were counted. Plates containing between 20 and 200 colonies were examined. Colonies were counted promptly after the incubation period. From the whole count plates, plates of any dilution with between 20-200 colonies were selected and scored accurately the number of colonies. The count per g of organisms was calculated as follows:-

$$\text{Organisms per g of sample} = \frac{\text{Number of colonies}}{\text{Volume of sample plated x Dilution}}$$

Direct measurement by plate count

Soil sample (100 mg) was taken in microfuge tube and few drops of distilled water were added to it. Soil was mixed well with water. Inoculating loop was used to spread soil sample onto peptone-yeast extract-dextrose agar (PYDA) plate and was incubated at 30ºC for 24 hr. After incubation various bacterial colonies were examined and calculated through colony counter.

Characterization of colonies

Individual colonies were characterized on the basis of size, type of margin, colony elevation, colony texture, light transmission, colony pigmentation.

Isolation and cultivation of pure cultures

In order to obtain single, pure, colonies the streak plate method was used. Well-isolated colonies were picked, re-streaked onto a fresh plate and incubated. Purified isolates were maintained on nutrient agar slants. Each isolate was designated NIAB-RS, followed by the isolate number.

RESULTS AND DISCUSSION

Serial liquid dilutions of different soil samples plated on nutrient agar plates provided an estimation of the density of soil bacteria. Viable cell counts were made from 48 soil samples. Data of cell counts are shown in Table I. Bacterial density recorded in one gram of normal soil fell in the range of 5.8×10^6 - 2.06×10^7 organisms per gram of soil. The number of organisms determined in different soil samples revealed population sizes of up to 2.06×10^7 cells per g of soil able to grow on nutrient agar. Counts of soil with EC 4.0 dSm^{-1} showed a population size up to 1.74×10^7 organisms per g of soil. Counts of organisms able to grow with EC 8 dSm^{-1} soil revealed total population size of upto 1.36×10^7 cells per g of soil. Among 16 rice varieties observed under normal soil conditions (Table I), LP-7 was dominant variety showing the highest bacterial population (2.06×10^7), whereas, IR-6 manifested the lowest bacterial density (5.8×10^6). Bacterial diversity was significantly higher under normal soil conditions than under different levels of salt stress. The influence of salt stress on bacterial diversity revealed that soil with EC 4.0 and 8.0 dSm^{-1} gradually reduced the diversity of microorganisms. The high degree of diversity encountered in the normal soil is probably because of its non-saline nature. Torsvick *et al.* (1990) have provided evidence that in one gram of soil there are billions of individual organisms and thousands of species.

Since the average population size in saline soil (EC 4.0 and 8.0 dSm^{-1}) as determined by the viable count (3.77×10^6 and 1.97×10^6 organisms per g of soil) was lower than that of the normal soil (1.22×10^7 organisms per g of soil), low bacterial population in saline soil could be attributed to the high

salt concentration, particularly in EC 8.0 dSm^{-1} (Table II). Thus our data indicated that stressed environment retards the bacterial population (Fig. 1).

TABLE I.- COUNTS OF BACTERIAL POPULATION FROM SOIL OF DIFFERENT RICE VARIETIES GROWING UNDER SALT STRESS.

Sample #	Variety	Cell count (per g of soil)		
		Normal	**EC 4.0 dSm^{-1}**	**EC 8.0 dSm^{-1}**
1	IR-6	5.80×10^6	1.84×10^6	1.82×10^6
2	LP-8	1.36×10^7	1.92×10^6	1.16×10^6
3	WAB-56-125	1.46×10^7	2.00×10^6	1.94×10^6
4	WAB-56-104	8.60×10^6	1.58×10^6	1.30×10^6
5	3-J104-G3-291-4-2	1.08×10^7	1.98×10^6	6.20×10^5
6	IA cuba-25	9.80×10^6	1.88×10^6	1.44×10^6
7	LP-9	1.22×10^7	1.68×10^6	1.18×10^6
8	WAB100-B-B-B-B-2-1-H2	1.12×10^7	1.80×10^6	1.46×10^6
9	4-J104-N2-59-5	1.68×10^7	1.94×10^6	1.04×10^6
10	WAB-450-11-2-BL1-DR2	1.34×10^7	1.82×10^6	1.48×10^6
11	WAB450-5-1-BL1-DV6	8.80×10^6	1.92×10^6	1.96×10^5
12	LP-7	2.06×10^7	1.74×10^7	1.10×10^6
13	WAB272-B-B-2-H3	9.60×10^6	1.86×10^6	1.08×10^6
14	2-J104-N2-C53-2	1.44×10^7	1.96×10^6	1.30×10^6
15	WAB56-50	1.82×10^7	1.70×10^7	1.36×10^7
16	WAB450-I-B-P-157-2-1	7.80×10^6	1.78×10^6	8.20×10^5

Earlier reports also claim soil salinity to play a major role in the microbial selection process as environmental stress reduces bacterial diversity (Borneman, *et al.,* 1996). Growth of mycorrhizal fungi under varied levels of salt stress has been reported in the literature. Mycorrhizal colonization is higher in the control than in saline soil with medium salt stress and high salt stress (Al-Karaki, 2000; Al-Karaki *et al.*, 2001). Saline soil did not support the fungal population. There is a huge diversity of microbial species in soil and only a small proportion (typically 1 %) of the microbial population of a soil can be calculated. Our results indicate that soil environment supports diversity of soil microbial communities and may have impact on the sustainability of agricultural ecosystems. According to our observations one gram of soil contains a maximum of 2.06×10^7 organisms of diverse nature (Table I). At colony densities above 200/plate there may be underestimates of the true colony numbers due to competition for and depletion of nutrients; below colony densities of about 20/plate there are problems associated with the statistical errors present in counting such small numbers. Ideally all

viable counts are done in triplicate or more. The great advantage of this method is that only viable cells are counted. The great disadvantage lies in the 2-3 days that must elapse before colonies have developed to countable size. A further problem is that associated with counting cells which naturally occur in clumps or chains. This method is, however, reasonably reliable.

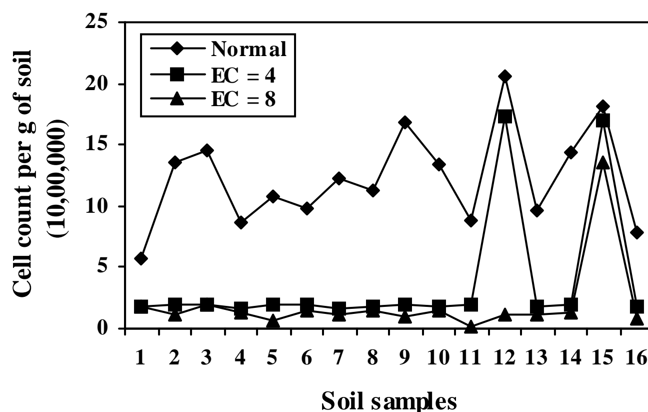

Fig. 1. Viable cell counts determined from various soil samples with different EC levels; numbers indicate rice varieties: 1, IR-6; 2, LP-8; 3, WAB-56-125; 4, WAB-56-104; 5, 3-J104-G3-291-4-2; 6, IA cuba-25; 7, LP-9; 8, WAB100-B-B-B-B-2-1-H2; 9, 4-J104-N2-59-5; 10, WAB-450-11-2-BL1-DR2; 11, WAB450-5-1-BL1-DV6; 12, LP-7; 13, WAB272-B-B-2-H3; 14, 2-J104-N2-C53-2; 15, WAB56-50; 16, WAB450-I-B-P-157-2-1.

TABLE II.- BACTERIAL DENSITY IN SOIL SAMPLES WITH VARIED LEVELS OF SALT CONCENTRATIONS DETERMINED BY SPREAD PLATE METHOD.

Soil	Cell count (per g of soil)		
	Sample size (n)	Mean ($\bar{X}$)	95% Confidence Interval
Normal	16	1.22×10^7	1.03×10^7 - 1.41×10^7
EC 4.0 dSm^{-1}	16	3.77×10^6	1.21×10^6 - 6.32×10^6
EC 8.0 dSm^{-1}	16	1.97×10^6	4.37×10^5 - 3.5×10^6

Bacterial isolates with different colony morphologies were examined on nutrient agar plates. Since colony morphology gives important clues as to the

identity of their constituent microorganisms, therefore, individual colonies were characterized on the basis of size, type of margin, colony elevation, colony texture, light transmission, colony pigmentation. Small, translucent colonies were numerous, whereas, medium, creamy and wrinkled colonies were comparatively less in number. Few large, creamy and feathery colonies forming a mat on agar plates, were also noted. Some bacterial mucoid and rugose colonies were observed which looked like fungi. The fungi had visible hyphae and indistinct colony edges. Though on agar plates, they did appear but did not grow too quickly. Isolation of some large colonies (which appeared to be one colony) showed mixed cultures and eventually were separated into a fungus and a bacteria that had been growing commensally. Soil bacteria are inter-related and rarely fit well into the classification systems that work so well for clinical isolates. Another problem is that in the initial dilution plates (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) symbiotic or otherwise close growth association between species and genera is dominant. It is estimated that media culturing only supports growth of ~1% of all soil microbes.

Community diversity in three soil environments was further studied by determining viable cell count and morphological variations in colonies on two different media. Eight soil samples from eight different rice varieties *i.e.* Bas 370, NR-1, WAB-450-IB-P-163, Kas Bas, WAB-450-IBP-20-HB, WAB-450-16-2-BLI-DR2, WAB-450-IBP-135-HB, O-Glaberrima-103929 were selected for evaluating bacterial diversity. Colony morphologies and counts were examined on both NA and PYDA plates. No fungal population was observed on PYDA medium. Most of the colonies on this medium were white occurring in chains, with distinctly mottled appearance (Fig. 2); some were mucoid and large (Fig. 2). PYDA medium supports the growth of *Bacillus* at 30ºC. Thermophilic and anaerobic bacteria will not grow at this temperature. *Bacillus* species are mesophilic, anaerobic heterotrophs. The colonies appearing on this medium were presumed as *Bacillus* and would be characterized biochemically. With two media, the proportion of cultivated colonies decreased from NA to PYDA in a collection obtained after 24 h of incubation (Fig. 3). Among eight different rice varieties, the maximum number of colonies was obtained in soil sample from “Kas Bas”; the proportion being 149 on NA and 130 on PYDA. Well-isolated colonies with diverse morphologies were picked at random and purified for further identification. Selection of isolates was made on the basis of size, colour and surface appearance of colonies on both culture media.

Fig. 2. Bacterial colonies on peptone yeast extract dextrose agar: (A) white colonies occurring in chains, with distinctly mottled appearance; (B) mucoid and large colonies.

Ninety-six isolates were obtained from soil samples collected from the rhizosphere of 16 rice varieties (Table I) grown under three varied levels of

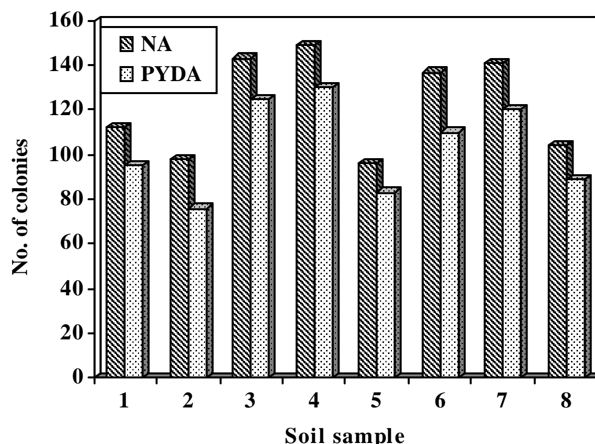

Fig. 3. Relationship between bacterial density and culture media; numbers indicate rice varieties: 1, Bas 370; 2, NR-1; 3, WAB-450-IB-P-163; 4, Kas Bas; 5, WAB-450-IBP-20-HB; 6, WAB-450-16-2-BLI-DR2; 7, WAB-450-IBP-135-HB; 8, O-Glaberrima-103929.

salt stress, *i.e.* normal, medium (EC 4.0 dSm^{-1}) and high (EC 8.0 dSm^{-1}). Two bacterial colonies were selected from each soil sample. In media comparison study, a total of 16 isolates were obtained from eight rice varieties shown in Figure 3. Overall 112 isolates were obtained from 56 soil samples. These isolates were proceeded from different varieties of rice grown under stress in soil with different EC levels. Hence these isolates showed clear morphological differences amongst them, therefore, would further be identified and characterized biochemically.

In the present study, bacterial community in the rhizosphere of different rice varieties grown under varied levels of salt stress, was enumerated by using cultivation method. The diversity and complexity of soil bacterial communities present a major challenge to our efforts to understand how biological processes can be managed in agricultural systems. Although more detailed knowledge of functional relationships among microorganisms is required to establish the effects of diversity on ecosystem functioning and stability, it is probably safer to adopt agricultural practices that preserve or restore microbial functional diversity than to adopt practices that diminish this component of total diversity. Thus, using new methods, many thousands

of microorganisms will be discovered and will provide a vast untapped reservoir of genetic and metabolic diversity. This will have far-reaching implications for society in enhanced food production, global environmental protection, antibiotic discovery, bioremediation of waste materials, and biotechnology.

REFERENCES

AL-KARAKI, G.N., 2000. Growth of mycorrhizal tomato and mineral acquisition under salt stress. *Mycorrhiza*, **10**: 51-54.

AL-KARAKI, G.N., HAMMAD, R. AND RUSAN, M., 2001. Response of two tomato cultivars differing in salt tolerance to inoculation with mycorrhizal fungi under salt stress. *Mycorrhiza*, **11**: 43-47.

BORNEMAN, J., SKROCH, P.W., O'SULLIVAN, K.M., PALUS, J.A., RUMJANEK, N.G., JANSEN, J.L., NIENHUIS, J. AND TRIPLETT, E.W., 1996. Molecular microbial diversity of an agricultural soil in Wisconsin. *Appl. environ. Microbiol*., **62**: 1935-1943.

CHANDLER, D.P., LI, S., SPADONI, C.M., DRAKE, G.R., BALKWILL, D.L., FREDRICKSON, J.K. AND BROCKMAN, F.J., 1997. A molecular comparison of culturable aerobic heterotrophic bacteria and 16S rDNA clones derived from a deep subsurface sediment. *FEMS Microbiol. Ecol.,* **23**: 131-144.

DUNBAR, J., TAKALA, S., BARNS, S.M., DAVIS, J.A. AND KUSKE, C.R., 1999. Levels of bacterial community diversity in four arid soils compared by cultivation and 16S rRNA gene cloning. *Appl. environ. Microbiol*., **65**: 1662-1669.

FRAGOSO, C., BROWN, G.G., PARTON, J.C., BLANCHART, E., LAVELLE, P., PASHANASI, B., SENAPATI, B. AND KUMAR, T., 1997. Agricultural intensification, soil biodiversity and agroecosystem function in the tropics: the role of earthworms. *Appl. Soil Ecol.,* **6**: 17-35.

GROBKOPF, R., JANSSEN, P.H. AND LIESACK, W., 1998. Diversity and structure of the methanogenic community in anoxic rice paddy soil microcosms as examined by cultivation and direct 16S rRNA gene sequence retrieval. *Appl. environ. Microbiol*., **64**: 960-969.

HUGENHOLTZ, P., GOEBEL, B.M. AND PACE, N.R., 1998. Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *J. Bact*., **180**: 4765-4774.

KENNEDY, A.C. AND SMITH, K.L., 1995. Soil microbial diversity and the sustainability of agricultural soils. *Plant Soil*, **170**: 75-86.

LIESACK, W., JANSSEN, P.H., RAINEY, F.A., WARD-RAINEY, N.L. AND STACKEBRANDT, E., 1997. Microbial diversity in soil: the need for a combined approach using molecular and cultivation techniques. In: *Modern soil microbiology* (eds. J.D. van Elsas, J.T. Trevors and E.M. Wellington), pp. 375-439. Marcel Dekker, New York, N.Y.

PARKINSON, D. AND COLEMAN, D.C., 1991. Microbial communities, activity and biomass. *Agric. Ecosyst. Environ.,* **34**: 3-33.

RANGARAJAN, S., SALEENA, L.M. AND NAIR, S., 2002. Diversity of *Pseudomonas* spp. isolated from rice rhizosphere populations grown along a salinity gradient. *Microb. Ecol*., **43**: 280-289.

STACKEBRANDT, E., LIESACK, W. AND GOEBEL, B.M., 1993. Bacterial diversity in soil sample from a subtropical Australian environment as determined by 16S rDNA analysis. *FASEB J.,* **7**: 232-236.

SUZUKI, M.T., RAPPE, M.S., HAIMBERGER, Z.W., WINEFIELD, H., ADAIR, N., STROBEL, J. AND GIOVANNONI, S.J., 1997. Bacterial diversity among small-subunit rRNA gene clones and cellular isolates from the same seawater sample. *Appl. environ. Microbiol*., **63**: 983-989.

SWIFT, M. J. AND ANDERSON, J. M., 1993. Biodiversity and ecosystem function in agricultural systems. In: *Biodiversity and ecosystem function* (eds. E.D. Schulze and H.A. Mooney), pp.15-41. Springer-Verlag, Berlin.

TORSVIK, V., SALTE, K., SORHEIM, R. AND GOKSOYR, J., 1990. Comparison of phenotypic diversity and DNA heterogeneity in a population of soil bacteria. *Appl. environ. Microbiol*., **56**: 782–787.

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SEASONAL VARIATION IN PROTEIN CONTENTS IN DIFFERENT ORGANS OF THREE SPECIES OF *LABEO* FOUND IN KALRI AND HALEJI LAKES DURING 3 YEARS' PERIOD

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Abstract .- Kalri (area 142.5 sq.km) and Haleji (16.9 sq.km) lakes have been the source of water supply to the Karachi city. Water from Kalri lake is still being supplied to Karachi. The object of the present study was to assess the seasonal variation of protein contents in three species of *Labeo* in Kalri and Haleji lakes. Total protein contents of different tissues were taken as growth parameter. This will provide us the base line information about effect of pollutants on fish present in two lakes.

Key words: Protein contents, *Labeo* spp., Kalri and Haleji lakes.

INTRODUCTION

Kalri lake (142.5 sq.km) and Haljei lake (16.9 sq.km) have been source of water supply to Karachi, a city of 1.5 million population. However, they are surrounded by agriculture fields and are being polluted by chemicals and also face eutrophication (Saqib *et al*., 1991).

Saqib and Siddiqui (1994a,b) studied the fresh water plankton population throughout the year in both these lakes. The planktons are used as food by the fishes, which may be a source of xenobiotics accumulation in their body. Siddiqui (1997) reported presence of pesticides in Haleji and Kalri lake water. The water supplies will have serious health problem for Karachi population, especially the health of people using Haleji lake water.

Mustafa and Zofair (1985) reported seasonal variation of protein during various months while Laha *et al*. (1990) reported increase in protein by poultry feed. Shakoori *et al*. (1992), Ara *et al*. (1994) and Anwar *et al*. (1998) reported the variation of proteins in various fishes either under normal environment or due to exposure of xenobiotics. Fish has been selected as biological indicator which will be evaluated by monitoring growth of the fish, as indicated by the total protein content of different organs of the fish.

In view of this, the study on total proteins in various tissues of three species of *Labeo* present in Kalri and Haljei lake were undertaken. Human population of lower Sindh area consumes these fishes, but no study was done for their nutritional value and xenobiotic (pollutants) effect.

MATERIALS AND METHODS

Samples for the study were collected from Kalri and Haleji lake at an interval of one month, for 3 years. Live *Labeo rohita*, *L. calbasu* and *L. sindensis* (five of each species) were collected in a controlled weight range of 600 to 850 gms and size from 10" to 14" with the help of net having mesh size of two inches thus making the number of fishes 15 per month. One gram tissue samples of stomach, liver, kidney, gills and edible muscles were crushed in 9 ml distilled water with pestle and mortar and then homogenized in Teflon tissue homogenizer at 1000 rpm for 5 minutes. Later the supernatant was centrifuged in Labofuge 15000 at a speed of 5000 rpm for 20 minutes placed in cold chamber. The supernatant was mixed with 1.0 ml TCA (5%) and the precipitate was obtained which was dissolved in 3 ml of IN NaOH. This was used for total protein estimation. Total protein was estimated according to Henry *et al*. (1974) using kit of Randox, Ardmore, UK. For this purpose biuret reagent was used.

RESULTS

Proteins were estimated in five organs of each fish species (*Labeo rohita, Labeo calbasu, Labeo sindensis*) *i.e.,* stomach, liver, kidney, gills, and edible muscles. Statistically analyzed results were recorded in Tables I-VI.

In *Labeo rohita* the sequence of protein content was muscles > gills > stomach > kidney and least in liver. In *Labeo calbasu* the sequence of protein contents is muscles > stomach > gills > kidneys and least in liver, in fishes from both lakes. In *Labeo sindensis* again the sequence of protein contents is similar to *L. rohita i.e.* muscles > gills > stomach = kidneys and least in liver, in the fishes from both lakes.

The protein contents ranged between 15-20 mg/100 gm in *L. rohita*, between 12-15 in *L. calbasu* and 15-20 mg/100 gm in *L. sindensis* in

muscles. In gills it ranged between 13-16 gm/100 gm in *L. rohita*, 12-14 and mg/100 gm in *L. calbasu* and 12-16 mg/100 gm in *L. sindensis*. In stomach and kidneys it varies between 10-12 mg/100 gm in *L. rohita*, 8-11 mg/100 gm in *L. calbasu* and 18-12 mg/100 gm in *L. sindensis*. While in liver it remained least in all the cases *i.e.* 5-7 mg/100 gm in *L. rohita*, 4-7 mg/100 gm in *L. calbasu* and 4-7 mg/100 g in *L. sindensis*.

As far as the seasonal variation in protein contents is concerned, it generally remained high between April to July / August, and low in other months. This coincides with breeding season of the fish.

DISCUSSION

The protein content peaks have been found during March to April to July / August and then there is reduction in protein contents in all the cases. This is related with breeding season. During this period growth development (maturity) takes place and the higher protein content is probably for the preparation of breeding and the juveniles appear in August / September. After that there is a tendency of low protein content upto January. However, Mustafa and Zofair (1985) reported the effect of seasonal variation in liver protein content in *L. rohita* and *Catla catla* but they reported higher contents of protein in December and June and decline in January and July. This difference may be due to ecological factors of that region, while Anwar *et al*. (1998) reported that higher protein contents are found in liver and muscles of *Schizothorax plagiostomatus* present in Neelam river as compared to the fish of Jehlum river. They correlated the temperature with higher protein content. Effect of mercury on protein content was found in present work. Similarly Medda *et al*. (1993) reported the effect of pesticides in the protein content. Although these reports are from the same region but have no correlation with the present work.

Laha *et al*. (1990) reported inrease in protein contents in *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* by providing poultry manure as feed. Decrease in protein content was reported by Shakoori *et al*. (1992) in *Cirrhina mrigala* liver and muscles by exposing them to higher concentration of lead acetate for 8 weeks. However, this is not comparable. Ara *et al*. (1994) reported higher protein contents (16%) than fat (1.4%) in *Mola mola*. By SDS-PAGE they found more bands of protein indicating higher contents of proteins in fish muscles which has been confirmed by present work. Most

of these reports are not comparable with the present results except that of Mustafa and Zofair (1985). However, a correlation has been found between protein contents and breeding phenomenon in all the 3 species of *Labeo* present in Kalri and Haleji lakes (The effect of pesticide pollutants has been discussed elsewhere).

REFERENCES

ANWAR, K.K., MINHAS, F., HUSSAIN, S. AND SHAKOORI, A.R., 1998. Comparative analysis of biochemical components of liver and muscles of a fish *Schizothorax plagiostomus* Heckel, from warm and cold water rivers near Muzaffarabad, Azad Kashmir, Pakistan. *Proc. Pakistan Congr. Zool*., **8**: 217-224.

ARA, J., SIDDIQUI, N.A., SULTANA, V. AND QASIM, R., 1994. Biochemical composition of rare fish *Mola mola* from Arabian Sea. *Proc. Pakistan Congr. Zool*., **14**: 301-305.

HENRY, R.J., CANNON, D.C. AND WINKELMAN, J.W., 1974. *Clinical chemistry, principles and techniques*. Harper and Row, 2nd Ed.

LAHA, U.K., BISWAS, S. AND MITRA, B., 1990. Effectiveness of some organic manures on the growth, production, total protein and total lipid contents of major carps in ponds of Burdwan District. *Environ. Ecol*., **8**: 374-379.

MEDDA, C., SARKAR, S.K., GANJULY, S. AND BHATTACHARAYYA, B., 1993. Effect of phosphamidon on three important bioindicators and their recovery in two freshwater carps, *Labeo rohita* and *Cirrhinus mrigala. Pollut. Res*., **12**: 151-157.

MUSTAFA, S. AND ZOFAIR, S.M., 1985. Seasonal variation in protein, RNA and DNA concentration in major carps *Catla catla, Labeo rohita* and *Cirrhina mrigala. Jap. J. Ichthyol*., **32**: 258-262.

SAQIB, T.A., 2000. *Study of nutrients in* Labeo *spp. and effect of environmental pollutants on them in Haleji and Kalri (Kinjhar) lakes*. Ph.D. thesis, Department of Zoology, University of Karachi.

SAQIB, T.A. AND SIDDIQUI, P.A., 1994a. Seasonal fluctuation and species composition of freshwater plankton in Haleji Lake. *Zoologica Pakistan*, **4**: 7-18.

SAQIB, T.A. AND SIDDIQUI, P.A., 1994b. Seasonal fluctuation and species composition of freshwater plankton in Kinjher Lake. *Zoologica Pakistan*, **4**: 31-42.

SAQIB, T.A., SIDDIQUI, P.A. AND QURESHI, W.M., 1990. Study on the productivity of bottom fauna in Kinjher (Kalri) lake as index of eutrophy (Sindh Pakistan). *Zoologica Pakistan*, **2**: 43-49.

SAQIB, T.A., SIDDIQUI, P.A. AND QURESHI, W.M., 1991. Study on the productivity of bottom fauna in Haleji lake, Sindh, Pakistan. *Zoologica Pakistan*, **2**: 51-58.

SHAKOORI, A.R., ILYAS, M. AND AZIZ, F., 1992. Toxicity of sublethal doses of trebon (Ethofenprox) on total blood serum proteins, acetylcholinesterase activity and SDS-PAGE pattern of blood serum proteins of *Cirrhinus mrigala. Pakistan J. Zool*., **24**: 253-241.

SIDDIQUI, P.A., 1997. *Bioecology of wetlands of lower Sindh with reference to Arifauna and organophosphates pollutants*. Ph.D. thesis, Department of Zoology, University of Karachi.

TABLE I.- TOTAL PROTEIN CONTENTS (mg/100 gm) IN DIFFERENT ORGANS OF *LABEO ROHITA* (Ham.) IN KALRI LAKE.

Organs period	**Stomach**		**Liver**		**Kidney**		**Gills**		**Edible muscles**	
	Mean	**Range***	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
January	12.183	12.096 to 12.269	6.7	6.529 to 6.871	11.857	11.798 to 11.915	15.33	15.266 to 15.393	16.236	16.178 to 16.294
February	12.343	12.319 to 12.367	6.68	6.626 to 6.736	11.867	11.745 to 11.989	16.16	15.884 to 16.436	16.6256	16.527 to 16.724
March	13.386	13.446 to 13.526	7.133	6.990 to 7.276	12.173	12.116 to 12.23	16.123	15.995 to 16.25	18.34	18.146 to 18.533
April	13.710	13.613 to 13.807	7.127	6.995 to 7.259	13.37	13.346 to 13.394	16.58	16.528 to 16.632	18.05	17.845 to 18.254
May	13.373	13.648 to 13.812	8.36	7.794 to 8.926	12.887	12.559 to 13.214	17.267	17.180 to 17.354	19.29	19.260 to 19.32
June	14.320	14.275 to 14.365	8.6	8.371 to 8.829	13.453	13.360 to 13.545	17.276	17.191 to 17.361	20.273	20.151 to 20.395
July	10.130	10.107 to 10.153	7.543	7.161 to 7.925	12.23	0.079 to 12.309	15.233	15.194 to 15.272	17.283	17.180 to 17.386
August	10.390	10.210 to 10.567	6.253	6.166 to 6.32	11.217	11.132 to 11.302	13.25	13.193 to 13.306	15.096	14.537 to 15.654
September	10.636	10.594 to 10.787	6.557	6.317 to 6.797	10.25	10.205 to 10.295	13.657	13.528 to 13.785	14.883	14.744 to 15.021
October	11.187	11.152 to 11.232	6.737	6.323 to 7.151	11.843	11.741 to 11.945	14.05	13.750 to 14.349	15.236	15.154 to 15.317
November	11.443	11.320 to 11.65	6.774	6.649 to 6.898	11.803	11.786 to 11.82	14.28	14.166 to 14.393	15.686	15.612 to 15.759
December	11.710	11.687 to 11.733	6.753	6.724 to 6.782	12.917	12.877 to 12.956	14.756	14.721 to 14.791	15.716	15.613 to 15.819

*Range at 95% confidence limit.

TABLE II.- TOTAL PROTEIN CONTENTS (mg/100 gm) IN DIFFERENT ORGANS OF *LABEO ROHITA* (Ham.) IN HALEJI LAKE.

Organs period	Stomach		Liver		Kidney		Gills		Edible muscles	
	Mean	**Range***	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
January	11.606	11.158 to 12.054	6.833	6.786 to 6.880	10.680	10.616 to 10.743	14.156	14.053 to 14.258	15.856	15.821 to 15.890
February	11.733	11.665 to 11.901	7.040	6.804 to 7.276	10.633	10.593 to 10.672	14.100	14.051 to 14.149	15.433	14.918 to 15.947
March	12.500	12.330 to 12.669	7.623	7.396 to 7.850	11.150	11.036 to 11.263	15.256	15.188 to 15.323	16.280	16.149 to 16.411
April	11.753	11.838 to 11.838	7.300	7.231 to 7.369	11.403	11.285 to 11.521	15.250	15.191 to 15.309	16.033	14.116 to 17.949
May	13.520	13.384 to 13.656	7.673	7.758 to 7.758	12.053	11.612 to 12.494	15.523	15.313 to 15.732	19.256	19.131 to 19.380
June	12.766	12.567 to 12.965	8.120	7.986 to 8.254	12.746	12.604 to 12.880	16.753	16.679 to 16.826	18.353	18.211 to 18.495
July	11.740	11.609 to 11.871	7.343	7.297 to 7.389	10.740	10.643 to 10.837	13.563	13.293 to 13.832	14.000	13.886 to 14.113
August	10.173	10.091 to 10.255	6.306	6.260 to 6.352	9.766	9.198 to 10.333	12.750	12.670 to 12.829	14.266	13.943 to 14.588
September	10.733	10.534 to 10.932	6.733	6.534 to 6.932	10.253	10.111 to 10.395	12.726	12.505 to 12.947	15.746	15.649 to 15.842
October	11.250	11.080 to 11.420	6.523	6.509 to 6.536	10.353	10.267 to 10.438	12.723	12.615 to 12.831	15.256	15.131 to 15.381
November	11.173	11.096 to 11.249	6.57	6.518 to 6.621	10.393	10.369 to 10.417	13.313	2.714 to 3.911	14.743	14.640 to 14.845
December	11.560	11.348 to 11.772	6.483	6.409 to 6.556	10.473	10.400 to 10.546	13.900	13.814 to 13.985	15.246	15.194 to 15297

*Range at 95% confidence limit.

TABLE III.- TOTAL PROTEIN CONTENTS (mg/100 gm) IN DIFFERENT ORGANS OF *LABEO CALBASU* (Ham.) IN KALRI LAKE.

Organs period	Stomach		Liver		Kidney		Gills		Edible muscles	
	Mean	**Range***	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
January	12.703	12.522 to 12.883	5.393	5.168 to 5.618	9.866	9.743 to 9.988	11.560	11.157 to 11.963	13.583	13.709 to 13.792
February	12.836	12.652 to 13.020	5.583	5.469 to 5.696	10.620	10.243 to 10.996	11.726	11.331 to 12.120	14.376	14.024 to 14.727
March	13.976	13.788 to 14.164	5.970	5.880 to 6.139	11.030	10.659 to 11.400	12.900	12.649 to 13.151	14.700	14.255 to 15.144
April	13.553	13.098 to 14.007	5.413	5.169 to 5.656	10.570	10.103 to 11.037	11.980	11.085 to 12.875	13.753	13.203 to 10.302
May	14.600	14.327 to 14.872	5.600	5.420 to 5.780	11.550	11.493 to 11.607	12.980	12.810 to 13.149	15.800	15.721 to 15.879
June	12.273	11.930 to 12.616	6.930	6.851 to 7.009	10.540	10.403 to 10.677	14.750	14.534 to 14.965	12.780	12.624 to 12.936
July	11.330	11.296 to 11.363	5.603	4.091 to 7.114	8.320	8.274 to 8.365	12.616	11.938 to 13.293	11.750	11.503 to 11.997
August	11.696	11.513 to 11.879	4.320	4.195 to 4.445	9.083	8.369 to 9.796	10.250	10.216 to 10.283	12.110	11.924 to 12.296
September	12.076	12.026 to 12.126	4.676	4.392 to 4.959	9.636	9.506 to 9.765	10.443	10.221 to 10.664	12.373	12.237 to 12.509
October	12.486	12.067 to 12.904	4.723	4.496 to 4.950	9.733	9.616 to 9.849	10.816	10.659 to 10.972	12.753	12.632 to 12.874
November	12.210	11.903 to 12.516	4.580	4.420 to 4.740	9.856	9.721 to 9.991	11.330	11.211 to 11.449	12.820	12.706 to 12.933
December	12.510	12.345 to 12.674	5.187	4.456 to 5.917	10.046	9.753 to 10.338	11.173	10.908 to 11.438	13.020	12.823 to 13.216

*Range at 95% confidence limit.

TABLE IV.- TOTAL PROTEIN CONTENTS (mg/100 gm) IN DIFFERENT ORGANS OF *LABEO CALBASU* (Ham.) IN HALEJI LAKE.

Organs period	Stomach		Liver		Kidney		Gills		Edible muscles	
	Mean	**Range***	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
January	12.313	11.989 to 12.636	5.183	4.925 to 5.440	9.473	9.121 to 9.824	11.370	10.852 to 11.887	12.163	11.877 to 12.449
February	12.740	12.371 to 13.108	5.386	5.346 to 5.425	9.693	9.269 to 10.116	11.556	11.067 to 12.044	12.496	12.112 to 12.879
March	13.543	12.857 to 14.228	6.440	5.830 to 7.049	10.250	9.756 to 10.743	11.986	10.731 to 13.240	13.603	13.053 to 14.152
April	12.603	12.304 to 12.902	5.676	5.623 to 5.728	10.760	10.701 to 10.818	11.380	11.305 to 11.454	14.266	14.107 to 14.385
May	14.300	14.198 to 14.402	5.983	5.654 to 6.312	10.820	10.718 to 10.922	12.553	11.839 to 13.266	14.950	14.853 to 15.047
June	14.183	13.829 to 14.536	6.750	6.716 to 6.783	10.810	10.628 to 10.991	13.320	13.252 to 13.387	14.013	13.135 to 14.891
July	11.313	11.246 to 11.379	6.210	5.265 to 7.154	7.440	7.365 to 7.514	11.110	12.089 to 12.089	11.730	11.628 to 11.832
August	11.320	11.241 to 11.399	4.250	4.216 to 4.284	8.540	8.144 to 8.935	10.150	10.042 to 10.258	11.876	11.635 to 12.116
September	11.480	11.270 to 11.689	4.623	4.185 to 5.060	7.776	7.376 to 8.175	10.670	10.245 to 11.094	12.176	11.938 to 12.413
October	11.413	11.321 to 11.505	4.606	4.190 to 5.021	8.430	8.208 to 8.659	10.610	10.211 to 11.008	11.890	11.838 to 11.941
November	11.673	11.649 to 11.697	4.980	4.738 to 5.221	9.143	8.919 to 9.366	10.610	10.324 to 10.895	12.147	11.851 to 12.442
December	11.630	11.539 to 11.721	5.116	4.927 to 5.304	9.353	9.256 to 9.449	10.703	10.248 to 11.157	11.993	11.741 to 12.245

*Range at 95% confidence limit.

TABLE V.- TOTAL PROTEIN CONTENTS (mg/100 gm) IN DIFFERENT ORGANS OF *LABEO SINDENSIS* (Day) IN KALRI LAKE.

Organs period	Stomach		Liver		Kidney		Gills		Edible muscles	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
January	11.34	10.728 to 11.952	6.74	60647 to 6.832	10.923	10.579 to 11.26	13.000	12.753 to 13.246	16.426	16.108 to 16.743
February	11.83	11.263 to 12.396	6.91	6.850 to 6.969	11.23	10.876 to 11.583	13.470	13.366 to 13.574	16.816	16.750 to 16.881
March	12.396	12.331 to 12.46	7.333	7.125 to 7.45	12.33	12.177 to 12.485	15.596	15.177 to 16.014	17.466	16.847 to 18.084
April	11.88	11.243 to 12.517	6.83	6.784 to 6.875	11.67	11.330 to 12.009	14.320	14.257 to 14.382	17.443	16.853 to 18.032
May	12.53	12.451 to 12.609	7.113	6.794 to 7.432	11.926	11.318 to 12.533	14.540	14.165 to 14.914	19.850	19.793 to 19.906
June	11.216	10.818 to 11.613	7.900	7.843 to 7.956	13.000	12.753 to 13.246	16.350	16.253 to 16.446	17.673	16.867 to 18.478
July	9.35	9.248 to 9.452	6.440	5.745 to 7.134	11.700	10.961 to 12.438	13.866	13.227 to 14.504	14.25	14.198 to 14.302
August	9.416	8.987 to 9.844	5.750	5.659 to 5.841	9.250	9.171 to 9.239	11.750	11.523 to 11.976	14.766	14.227 to 15.304
September	9.91	9.870 to 9.949	5.910	5.828 to 5.992	9.883	9.588 to 10.177	11.980	11.599 to 12.360	14.993	14.610 to 15.376
October	10.52	10.111 to 10.928	6.090	5.923 to 6.257	10.460	10.187 to 10.732	12.366	11.800 to 12.931	15.680	15.364 to 15.955
November	10.433	10.023 to 10.842	5.986	5.844 to 6.128	10.490	9.876 to 11.103	11.910	11.522 to 12.297	16.033	15.712 to 16.354
December	10.87	10.809 to 10.93	6.426	6.324 to 6.528	1055	10.272 to 10.828	12.453	12.220 to 12.685	15.526	15.149 to 15.902

*Range at 95% confidence limit.

TABLE VI.- TOTAL PROTEIN CONTENTS (mg/100 gm) IN DIFFERENT ORGANS OF *LABEO SINDENSIS* (Day) IN HALEJI LAKE.

Organs period	Stomach		Liver		Kidney		Gills		Edible muscles	
	Mean	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
January	9.980	9.815 to 10.145	5.936	5.818 to 6.053	9.686	9.543 to 9.829	12.083	11.862 to 12.303	14.316	14.250 to 14.381
February	9.863	9.783 to 9.942	5.976	5.812 to 6.139	9.833	9.612 to 10.054	12.510	11.965 to 13.054	14.536	14.428 to 14.643
March	10.330	10.231 to 10.429	6.240	6.143 to 6.336	9.910	9.670 to 10.149	12.373	11.839 to 12.906	14.723	14.628 to 14.817
April	14.403	11.133 to 11.672	6.233	6.080 to 6.386	11.496	11.206 to 11.785	13.143	12.721 to 13.564	17.580	16.859 to 18.300
May	11.523	11.139 to 11.907	6.926	6.703 to 7.148	11.996	11.835 to 12.157	13.956	13.841 to 14.070	18.236	17.368 to 19.103
June	10.730	10.637 to 8.22	7.513	7.481 to 7.542	12.530	12.463 to 12.596	15.306	15.181 to 15.431	17.883	17.597 to 18.168
July	9.143	9.083 to 9.208	6.670	5.745 to 7.594	9.940	9.633 to 10.247	13.626	12.977 to 14.274	13.246	12.957 to 13.534
August	9.200	9.120 to 9.279	5.523	5.409 to 5.636	8.323	8.261 to 8.385	11.546	11.517 to 11.574	13.350	13.214 to 13.486
September	9.576	9.524 to 9.627	5.596	5.548 to 5.643	8.346	8.247 to 8.445	11.666	11.513 to 11.818	13.310	13.100 to 13.519
October	9.613	9.596 to 9.629	5.6726	5.580 to 5.672	8.303	8.170 to 8.435	11.603	11.508 to 11.697	13.876	13.716 to 14.035
November	9.593	9.569 to 9.617	5.900	5.807 to 5.993	9.000	8.779 to 9.220	11.866	11.666 to 12.065	13.890	13.799 to 13.981
December	9.790	9.691 to 9.888	5.850	5.746 to 5.953	9.253	9.166 to 9.339	12.040	11.870 to 12.209	13.883	13.768 to 13.997

*Range at 95% confidence limit.

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BIOLOGICAL ANALYSIS OF MEKRAN COASTAL WETLANDS COMPLEX, PAKISTAN

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Abstract.- The survey was undertaken in the Mekran Coastal Wetlands Complex to assess the diversity and population density of reptiles, birds and mammals. The published records show that there are 105 bird species recorded from that area, 30 marine and terrestrial reptile species and 43 marine and terrestrial mammals. A range of widely used research methods was applied in the field to accumulate the maximum information and diversity. The methods included, point counts, random marine survey, specimen collection, group questionnaires, informal meetings and habitat classification and mapping. Observations included 125 birds, 11 mammals both marine and terrestrial, and 12 reptiles and amphibian species. Significant sightings included those of 27 Indian hump-backed dolphins with two calves and three stranded black finless porpoises. Among the birds, four species of international significance were documented including the Marbled Teal recorded at one of the freshwater dams. A mature marsh crocodile was observed at one of the ponds of the River Dasht, while footprints of three other sub-adults were also observed in the same area. The data collected was subjected to simple statistical regression analysis showing regression co-efficient (r^2) as high as 0.9601. The Shannon-Weiner Diversity Index was calculated for each habitat type and showed that towns, villages and cultivation were the most diverse, followed by freshwater lakes and reservoirs. Simple graphical presentation of results includes population numbers and diversity, population density trends and food preferences. The food pyramid showed that majority of the birds in the study area were piscivorous and insectivorous by habit covering 21% and 16% respectively. These prefer fish, insects, worms and arachnids as their food. After detailed analyses of the results, short and long-term recommendations are being proposed in order to carry out future work.

Key words: Wetlands, Mekran Coast, biological analysis, Shannon-Weiner Diversity Index.

INTRODUCTION

Pakistan's coastline is approximately 1,050km long starting from Sir Creek (Indian Border) to Jiwani near the Iranian Border. The major portion of this coastline, a 800km long stretch, falls within Balochistan (Saifullah,

1995) while the rest (250km) lies in Sindh (Fig. 1). These two areas along the coastline are rich in biological resources, support a large number of adjacent communities and ultimately contribute significantly to the gross domestic product (GDP) of the country.

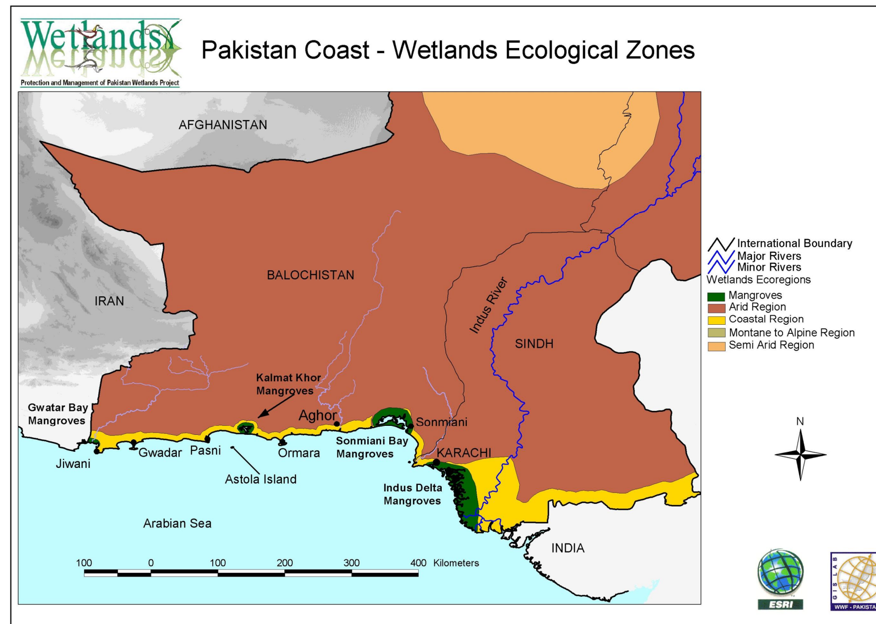

Fig. 1. Map showing Pakistan's coastline in Sindh and Balochistan

Balochistan Coastal Zone

The Balochistan Coast covers about 800km shore line and lies west of Karachi between longitudes 61^0 37'E and 68^0 12'E and Latitudes 25^0 00' and 25^0 30'N (Fig. 2). Balochistan is also the largest (approx. 50%) and least populated province of Pakistan. The coastline is rugged and irregular and runs in a generally east-west direction bordering the productive North Arabian Sea. It possesses a number of landforms which include bays, lagoons, alluvial plains, deltas, mudflats, rocky shores, sandy beaches, sea cliffs, beach ridges, marine terraces etc. (UNESCO, 1994). The continental shelf of Balochistan is about 40km wide and is relatively narrow, by global standards (Saifullah, 1995). The coastline extends from Hub River to the Iranian Border near Jiwani and is geometrically divided into two segments; Lasbela (66° 45' – 68° 12'E) and Makran Coast (61° 37' – 66° 45'E).

Lasbela Coast

The Lasbela Coast is situated between the Hub River to the east and the Hingol River to the west. It is characterised by sandy beaches and the relatively large Sonmiani Bay. Two offshore islands, Churna and Kiou, are located in an area off the Hub River and Miani Hor. A group of submarine mud volcanoes is located just offshore. The presence of mud volcanoes, formed by gas-charged water escaping to the surface, indicates an active geotectonic setting that is characteristic of the entire Balochistan Coast.

Mekran Coast

The Mekran coast stretches along a 550km segment of the province's southern border, largely arid, mountainous terrain. It is tectonically relatively active being situated in a subduction zone where the Indian Ocean continental plate moves northwards under continental crusts (Stone, 1990). The coastal terrain is an accretionary wedge of deformed sediment, ranging in age from late Cretaceous Period to the present. The coastline is punctuated by a number of small rivers that flood following short periods of heavy rainfall. The entire coast, lacking embayments and significant land promontories, is vulnerable to heavy erosion and loss of materials offshore.

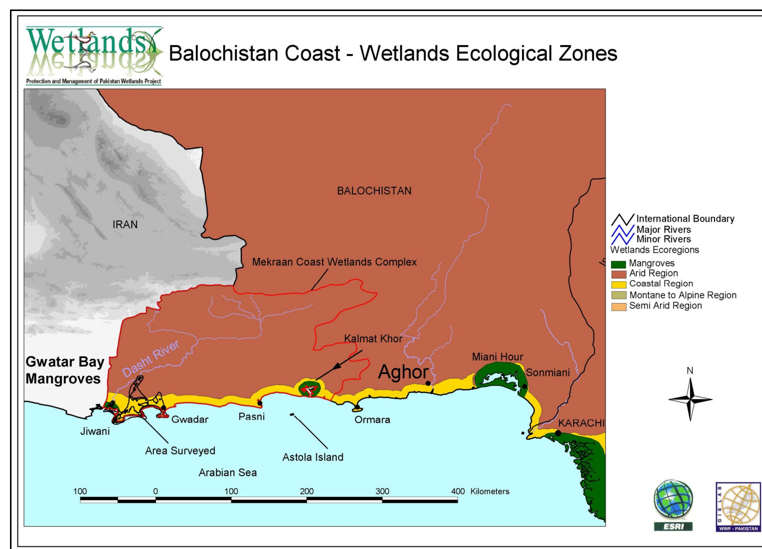

Fig. 2. Map showing Balochistan's coastline

Ecologically rich marine areas of Balochistan

Three small mangrove areas exist on the Balochistan coast, namely, Miani Hor near Sonmiani/Damb in Lasbela District, Kalmat Khor/Chundi near Pasni and Gwater Bay/Dasht Hor near Jiwani in Gwadar District (Fig. 2). These thickets are the only significant vegetation on Balochistan Coast and, therefore, the presence of these forests has a valuable role in the maintenance of biodiversity (Saifullah, 1995). The area of mangrove cover is only 7,285 ha (Saifullah, 1995). In addition, this relatively small area is not concentrated in one place but is separated into three separate patches that are located apart. The first two are lagoons whereas the last one is embayment.

Miani Hor (25^0 31'N, 66^0 20'E)

Miani Hor is an extensive lagoon that is situated some 90km west of away from Karachi on the easternmost part of the Balochistan Coast. It is approximately 60km long and 7km wide tortuous and contorted body of water, which is connected to the sea by a 4km wide mouth. The lagoon takes an immediate westerly direction, a short distance from its mouth. It then runs parallel to the shoreline in the shape of an arc, the extreme end of which lies almost in the same line as the mouth itself, except that a strip of land intervenes in between.

Two seasonal rivers enter into the bay. Porali River drains through the *Bela region* and empties into the central part whereas *Windor River* enters it near the mouth of the bay. The total area of the bay is 125.25km^2 and the shelf adjacent to it is 80km wide.

Kalmat Khor (25^0 27'N, 64^0 05'N)

This lagoon is located in the central part of the Balochistan Coast in between Miani Hor on the eastern side and Gwater Bay on the western side. It is only 220km away from the former and 255km from the latter locality. Pasni is c. 60km east of the Khor (Fig. 2).

Kalmat Khor resembles the shape of a tree (Fig. 2) and the entrance is a narrow 7km long and 2km wide canal and only 12m deep. It widens abruptly into a 19km long and 27km wide enclosed body of water with irregular contour. The total inundated area is c. 100 km^2.

Mud flats are widely developed in almost the entire lagoon, which are covered with shallow water at high tides. There is not a single river discharging into it. The Basol River falls into the sea 15km east of the Khor. The continental shelf facing the Khor is narrow and only 40km wide.

Astola Island is situated some 30km offshore on its southwestern side. It is the largest single island along the coast of Pakistan and is famous for upwelling, which is continuous throughout the year in the sea around it. It is without perennial fresh water and serves as a breeding ground for sea birds. It can be developed into a picnic resort and an ideal sanctuary for birds.

Gwater Bay (25° 10’N, 61° 31’E)

Gwater Bay is not a lagoon but a bay because its mouth is almost as wide as its length. It penetrates 30km inside the land and its sea entrance is 28km wide. Its area is c. 250 km^2, half of which lies in the Iranian territory and the remaining half belongs to Pakistan. It is the largest embayment along the entire coast and is also fed by the largest river of Balochistan, the Dasht River. The continental shelf bordering the bay is relatively narrow and measures only 30km in width.

Gwater Bay – the Western Mekran, Balochistan

In the west, pouch shaped Gwater bay lies between the headlands of Iran and the rocky platform of Jiwani, bordered by a swampy region which is the delta of Dasht Kaur, one of the largest rivers in Balochistan (Fig.3). This river is intermittent stream and floods only once 2-3 years. There is a wide beach along the bay, behind which are barrier bars, islets, mudflats and tidal lagoons, with clump of mangroves. There are approximately 2000 ha of mangrove forests in Gwater Bay in Jiwani (Saifullah and Rasool, 2001). These forests extend to Iran. Jiwani wetlands resources form a complex comprising of freshwater habitat, desert, marine habitat, tropical thorn forests and open scrub zone harbouring a large diversity of flora and fauna. The stretch of coast from Ras Jiwani to Ras Pishukan is comprised of cliffs, straight, sandy beaches backed by ridges or sand dunes or large shallow bays (WWF, 1998).

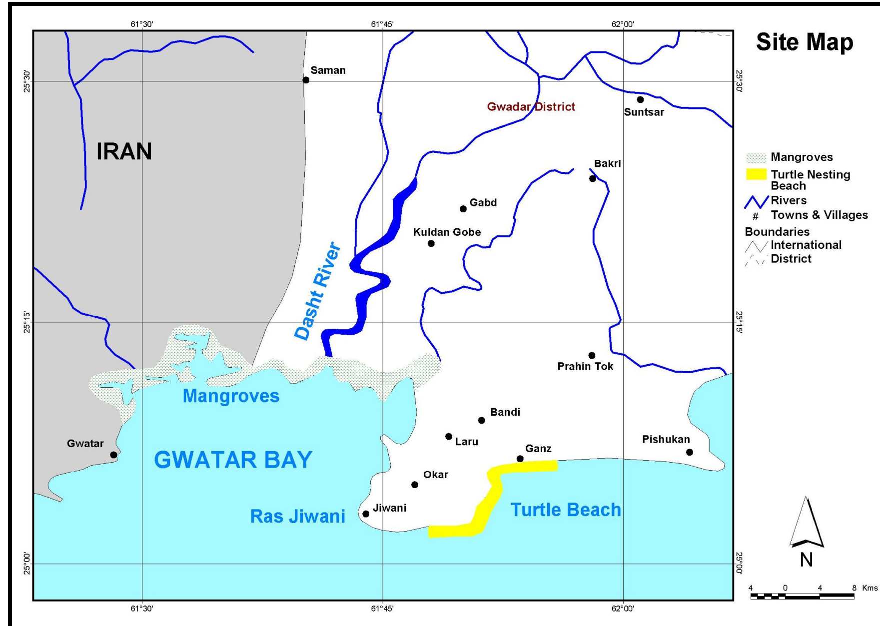

Fig. 3. Map of Balochistan showing Gwater Bay and the River Dasht.

Protection and Management of Pakistan Wetlands Project

The Protection and Management of Pakistan Wetlands Project is a UNDP/GEF funded initiative under the PDF-B phase (UNDP, 2000). The executing agency is the Government of Pakistan, which has delegated the implementation responsibility of this project to WWF-Pakistan.

The Mekran Coastal Wetlands Complex is globally significant and one of the four areas for wetlands intervention under this project. This complex is the outcome of a series of successful workshops conducted in collaboration with all the major stakeholders. A detailed biological inventory along with the socio-economic situation of the area is a pre-requisite to the next phase of the long-term GEF project and the present survey is the result of such effort.

Needs and objectives of the present survey

With specific regard to this GEF PDF-B, the objectives of this survey were to determine and assess the importance of Western Mekran Coastal

Wetlands Complex, which harbours a great diversity of animal and plant species. The basic aim was to determine the initial list of biological resources and their status, assess the existing potential in terms of species diversity and abundance and recommend short-term (bridging) and long-term future actions. In addition, group questionnaires were also conducted in order to assess the awareness level of the local community regarding the wise use of natural resources.

STUDY AREA

Area, location and access

The Mekran Coastal region lies between 26º to 26º 00 30 latitudes (North-South) and 61º 30' to 66º 45' longitudes (East-West). Administratively, the region includes Gwadar and Lasbela districts and covers an area of 55,000 km^2. It borders Iran in the West, Kalat in the North and Arabian Sea in the South.

The human component of the Mekran Coastal Wetlands Complex consists of more than 100 small and large villages and towns. Jiwani, a major town located in the western most part of the study area (Fig. 4), is a sub-division, situated at a distance of 90km northwest of District Gwadar (WWF, 1996, 1997). This complex has a very diverse ecosystem containing a wide range of habitat types, which were later grouped together in seven different habitats (Fig. 4).

Biological resources: their availability and conservation status

Roberts (1991, 1992) gave a detailed account of the birds of the area extending from Western Ormara to the extreme end of the Iranian border close to the River Dasht. He recorded 105 species of birds from this area; three of which are internationally threatened and categorized as endangered and vulnerable. The status of the rest of the category is not known.

The birds observed in the complex were mapped by GIS Lab of WWF based on the Roberts (1991, 1992) classification (Fig. 5). Roberts (1997) recorded 33 different species of terrestrial mammals from the study area; three of which are of global significance and have been assigned vulnerable, endangered and lower risk categories, three are data deficient (DD) while the

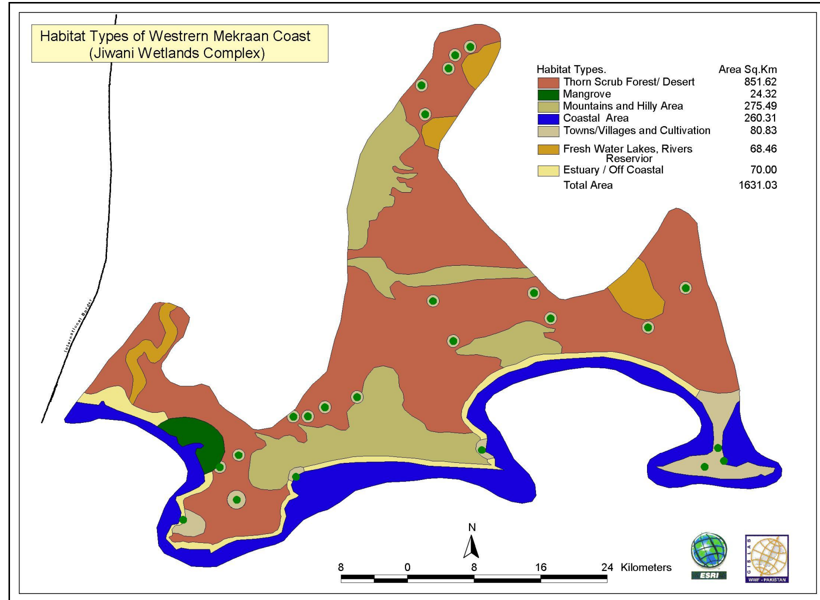

Fig. 4. Map showing a superficial classification of different habitat types in Western Mekran coast (Jiwani Wetlands Complex).

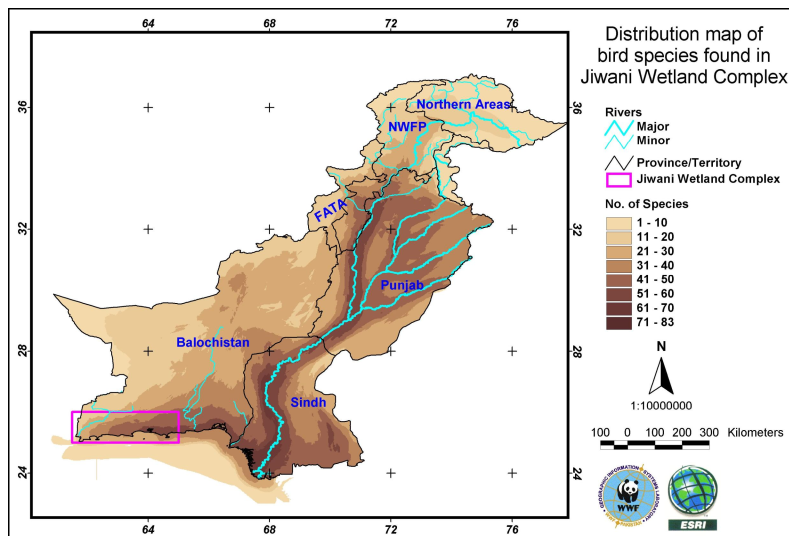

Fig. 5: Map of Pakistan showing bird density with special focus on Jiwani Wetland Complex.

status of the rest (27 species) is not known. Data deficient means that there is inadequate information to make a direct or indirect assessment of its risk of extinction based on its distribution and/or population status. This further implies that the species in this category may be well studied, and its biology is well known, but appropriate data on abundance and/or distribution is lacking (IUCN, 2000). In addition, Roberts (1997) recorded ten marine mammals from the area; three of them are internationally threatened classified as endangered and one vulnerable; two are data deficient and the status of the rest is not known. Ghalib *et al*. (1979) recorded a total of 30 reptile species from the area. Although their conservation status is not known, they can be classified as data deficient because there are no published reports and records available for them.

Ahmad *et al*. (1997a) conducted studies on the distribution, population dynamics and status of the vertebrate mangrove fauna of Balochistan coast and recorded 108 species of fish, six species of reptiles, eighty-three species of birds and two species of mammals. Four different categories of fish were recorded *i.e*., permanent or true residents, partial residents, tidal visitors and seasonal visitors. Six species of snakes belonging to five genera and eighty-three species of birds belonging to eight orders and twenty families were recorded. Two species of dolphins: cape dolphin and plumbeous dolphin were also recorded from the area.

A project proposal by WWF (1998) mentioned that Jiwani is one of the few beaches in Pakistan where nesting of marine turtles occur. The green turtle, *Chelonia mydas,* and olive ridley, Lepidochelys olivacea, are two endangered species found in the Mekran Coastal Wetlands Complex, Balochistan. Moazzam (1991) reported that there are forty species of mammals that exist in the area but their population is very thin. He further recorded green turtle and olive ridley turtle along the Mekran Coast. Ahmad *et al*. (1997b) have so far recorded ninety one species of waterbirds belonging to eight orders and twenty families from the area. Among them seventy are migratory and twenty one resident.

MATERIALS AND METHODS

Habitat classification and mapping

Field maps of the area were prepared by Geographic Information System (GIS) Laboratory of WWF-Pakistan. Keeping in view the diversity of

habitat ranging from marine ecosystem to the hilly areas and to freshwater dams, the area was surveyed and mapped according to habitat types. Observations were categorized according to habitat types and were later mapped to show the relative abundance and density in each habitat category.

Direct physical counts/point counts

It is one of the most widely used methods for determining the species diversity and its abundance (Haldin and Ulfvens, 1987). Direct counting is very useful when conducting surveys in open lands, oceans and coasts, they are easy to observe and identify. This is done by taking random points and recording species diversity and abundance. During the survey, mammals, reptiles and birds were directly identified in the field based on the field identification guides, professional experience, the animal's habitat and its behavioral ecology. Actual field surveys were conducted over a period of 9 days extending from December 28, 2001 to January 05, 2002. Counting the number of animals accurately in the field was made possible with the use of binoculars and spotting scopes.

Random sampling

In order to estimate marine species diversity and abundance (especially for marine mammals and birds) a marine survey, using a boat was conducted from the Old Marine Base of Jiwani to the River Dasht Estuary. This was done by taking random points at different places within the sea.

Indirect observations

Specimen collection

During the survey period, remains of dead birds and animals were found in the study area. Photographs were taken and specimen especially bones and skulls were collected for further examination. Measurements of these specimens were taken to know the actual size of the animal. In addition, coordinates using the Global Positioning System (GPS III Plus) were recorded in order to show the occurrence and distribution of the species in question. This will further help in mapping the biodiversity of the area.

Group questionnaire surveys

Surveys were conducted with the local community through group

questionnaires. A group normally consisted of 8-15 people of different ages with different experiences representing the whole community from a town. A participatory approach was followed and each and every member of the group was encouraged to participate in the discussion. During the interviews and useful discussions, the occurrence and distribution of certain species was confirmed by the group, which they came across in their daily lives during their fishing activities. In addition, field identification guides prepared by the team before hand served as a useful tool in understanding and identifying the species. Various techniques especially the size of a certain boat was used to measure the length of marine species.

Informal meetings with the locals

The purpose of this information was to collect anecdotal evidences of different species of the study area. Dialogues with the local community other than the fishermen were held in order to know the general status of wildlife of the area and especially for the species that are easily identified. Pictures of the species were shown to the community for species occurrence and distribution in the area. These meetings were held with the community during boat and field surveys and informal meetings with various other members of the community *i.e.* rest house staff, drivers, coast guards, local Nazim, Tehsildar and local shopkeepers were also held.

List of acronyms and abbreviations

DD, Data Deficient. EIA, Environmental Impact Assessment. GDP, Gross Domestic Product. GEF, Global Environment Facility. GIS, Geographic Information System. GoP, Government of Pakistan. GPS, Global Positional System. Ha, Hectares. ICZM, Integrated Coastal Zone Management. IUCN, World Conservation Union. Km, Kilometer. $Km^{2,}$ Square kilometer. NGOs, Non-Governmental Organisations. OSP, Ornithological Society of Pakistan. PDF, Project Development Fund. PTC, Pakistan Telecommunication. SWD, Sindh Wildlife Department. UNDP, United Nations Development Programme. UNESCO, United Nations Educational, Scientific and Cultural Organization. WAPDA, Water and Power Development Authority. WWF, World Wide Fund for Nature. ZSD, Zoological Survey Department.

List of vernacular terms

Kacha, unpaved or earthen. Lakh, Hundred thousand in numbers.

Nazim, Local elected leader by the community. Urdu, Pakistan's national and official language. Tehsildar, Local government employee who maintains revenue records.

RESULTS AND DISCUSSION

Avi-fauna of the study area

A total of 125 birds were recorded from the study area belonging to 17 orders and 42 families and sub-families (Table I). Passeriformes being well represented in the area, contain 14 families and sub-families with 36 species followed by Scolopacidae with 25 species, Anseriformes with 14 species and Acciptriformes together with Falconiformes with 14 species.

Mammalian fauna of the study area

A total of 11 mammal species were observed during the survey. These include one species of dolphin and one species of finless porpoise, two species of cats, four species of rodents, one jackal and a fox, and one bat species. The details of their occurrence, distribution and abundance are mentioned in Table II.

Marine mammals

Two species of marine mammals were recorded from the study area. These are Indian Hump-backed or Plumbeous dolphin and the Black Finless porpoise. A total of 27 Indian Hump-backed dolphins including two calves were sighted at the mouth of River Dasht, where it falls in the open sea. Three dead specimens of finless porpoises were sighted along the coastline near Gwadar.

Terrestrial mammals

During the survey period, 9 different species of terrestrial mammals were recorded from the study area. Most of these were found close to the coastline in the hilly areas. Some were found close to freshwater dams and in the Dasht desert. Four species of rodents (small mammals) were recorded. These included: small Indian mongoose, house rat, house mouse and five-striped palm squirrel. Two species of cat family i.e., jungle or swamp cat and Indian desert cat were recorded close to the freshwater dam. Two species

belonging to wolf family were observed in the Dasht desert. These included: Asiatic jackal and white-footed desert fox. One species of bat family comprising of six individuals was observed at Jiwani town.

In addition, the presence of Indian crested porcupine was confirmed from the study area because of the quills collected from different places and observed footprints at Saji Dam.

Reptiles and amphibians of the study area

Twelve species of reptiles and amphibians were observed at Jiwani Wetlands Complex. These included two species of turtles, one marsh crocodile, 2 species of agama, 2 lacerta, one lizard, one gecko, 2 snakes and a skittering frog. Their occurrence, distribution and habitat details are mentioned in Table III.

Marine reptiles

According to the Rahman *et al*. (1988), fourteen species of sea snakes have been recorded so far from the part of Arabian Sea that surrounds Pakistan. During this survey, one species i.e., annulated/chittul sea snake *Hydrophis cyanocinctus* was observed and identified (Minton, 1966) in the survey area.

Two species of marine turtles were recorded in the study area. One live green turtle (female) was recorded laying eggs at Daran beach close to Jiwani town. One dead specimen was recorded at the same beach. Three other dead specimens were recorded during the team's visit to Gwadar area along the coastline. In addition, one dead specimen of Pacific hawks-bill turtle was also recorded from the same area along the coastline close to Gwadar town.

Freshwater reptiles

One marsh crocodile (also known as swamp, muggar or snub-nosed crocodile) was observed from one of the reservoirs of River Dasht. Footprints of three other crocodiles were also observed along the bank of the river in the muddy area. This confirmed the presence of crocodiles in the area including one breeding pair and two juveniles. This shows that the area contains four animals and is also the last remaining habitat of mugger.

Terrestrial reptiles

Two species of agama *i.e.*, yellow-headed agama and brilliant agama, were observed along the coastline in the nearby hilly cum sandy area of the Pishkan village. Two species of lacerta; long-tailed desert lacerta and Punjab snake-eyed lacerta, were also observed in the same area. Two Mekran fringe-toed sand lizards were observed at the sandy bank of the River Dasht Estuary. One Mediterranean house gecko was observed close to the Jiwani rest house. This species is commonly found in towns and villages especially on walls. One species of land snake i.e., cliff racer was observed on the coastal hill, southwest of Gwadar town.

Amphibians

One species of frog *i.e.*, skittering frog *Rana cyanophlyctis* was observed during the survey. A total of 12 individuals were recorded at different places in the study area. Four were observed at River Dasht close to marsh crocodile habitat. Six were observed at Saji Dam, a freshwater reservoir and two were observed at Akara Dam, another freshwater reservoir. They are most commonly found in the springs of mountainous areas, in perennial water, freshwater reservoirs/lakes and rivers.

Flora of the study area

A major portion of the land is devoid of natural vegetation cover and plants are mainly found in depressions and along the river beds. There are 11 tree species that have been found in the area, 13 shrubs, 15 herbs, and seven grass species in the area. In addition, there are 5 plant species that have been planted by the community on their farm lands (Table IV).

Indirect field observations

A questionnaire survey was conducted to assess different marine species and their numbers along the Mekran coast. In order to carry out the study, interviews were arranged with fifteen different groups of local fishermen selected randomly either in the villages or on the coast. Questions were asked from a pre-prepared questionnaire and interviews were arranged mostly in groups for obtaining information. Interpreters were used on several occasions when the respondents were not familiar with ‘Urdu’. Some information needs to be cross-checked from other sources.

TABLE IV.- FLORA OF MEKRAN COASTAL WETLANDS COMPLEX.

Scientific name	Local name	Habitat	Tree/Shrub/ Hurbs/Grass
Acacia nilotica	Babul, Kiker	Plains	Tree
Acacia senegal	--------	Ravine	Tree
Acanthodium spicatum	--------	Plains	Tree
Aelorous lagopoides	-----	Plains	Hurb
Aerva javanica	------	Plains	Shrub
Aerva pseudotomentosa	-----	Plains	Shrub
Aloe spp.	------	Grave yards	Tree
Arthocnemum indicum	Lanna	Plains	Hurb
Asafoetida spp.	-----	Plains and Hills	Hurb
Avicennia marina	Timmer	Estuaries	Tree
Azadrichta indica	Neem	Villages	Tree
Bienartia cycloptera	----	Plains	Hurb
Calotropis procera	Ak	Plains	Shrub
Campylantha ramosissima	Kaller	Plains	Shrub
Capparis aphylla	-------	Plains	Tree
Capparis cartilaginea	Keerap	Hanging on hills from the cervices	Shrub
Casuarina equisetifolia	-----	Plains	Shrub
Cenchrus spp.	-----	Plains	Grass
Commiphora wightii	------	Plains	Shrub
Cressa cretica	-----	Plains	Hurb
Cynodon dactylon	------	Moist Places	Grass
Cyperus spp.	----	Plains	Hurb
Eleusine flagellifera	Grandil	Plains	Hurb
Eucaluptus spp.	Safeeda	Villages	Tree
Euphorbia caducifolia	Deedarr	Plains	Shrub
Ficus benghalensis	Barr	Villages	Tree
Halopaplus perfoliata	-----	Plains	Hurb
Haloxylon griffithii	Larrg	Plains	Shrub
Haloxylon recurvum	Khur	Plains	Shrub
Haloyrum mucronatum	-----	Plains	Shrub
Indigofera oblongifolia	Cheel	Moist places in river beds	Shrub
Ischoemum angustifolium	Gorkh	Plains and foot hills	Hurb
Lycium makranicum	-----	Plains	Hurb
Phoenix dactyliformes	Khajoor	Plains	Tree
Phragmites karka	------	River Banks	Grass
Plannicum turgidum	----	Plains	Grass
Prosopis cinerira	Kandi	Plains	Tree

Continued

Scientific name	Local name	Habitat	Tree/Shrub/ Hurbs/Grass
Prosopis juliflora	Kahur	Plains	Tree
Sacchrum spontaneum	Kashh	Plains	Hurb
Sacchrum spp.	Munj	River beds	Grass
Salsola baryosma	----	Plains	Hurb
Salvadora oleoides	Khabbar	Plains	Tree
Sesbania sesban	------	Plains and around the river bank	Shrub
Sporobolus arabicus	-----	Plains	Grass
Suaeda monica	Lanna	Plains	Hurb
Tamarix aphylla	Say gazz	Plains	Tree
Tamarix articulata	Say gazz	River Beds	Tree
Trianthenia pentandra	Indar kah	Plains and hills	Hurb
Typha spp.	-------	River banks	Grass
Urochondra setulosa	-----	Plains	Hurb
Zizyphus nummularia	Bair	Plains	Tree

Species account

The blue whale, *Balaenoptera musculus,* was reportedly seen with calves in the open sea and stranded twice, once at Pasni in 1967 and the other stranded at the Gunz beach 9 years ago. It is reported to effect the fishing boats by turning them upside down.

The bottle-nose dolphin, *Tursiops truncates,* was reported to be commonly found near the coast and in the open sea. It has mostly been seen in groups of 5 - 15 of different sizes and often gets entangled in fishing nets. Its meat is used as bait to catch whale shark. Along with this, its blubber is used to extract oil for lubrication of speed boats.

The bryde’s whale, *Balaenoptera edeni,* was not known to be very common and has been seen in groups of five with calves in the open sea.

The dwarf sperm whale, *Kogia simus,* was reported uncommon by the fishing community. Information revealed that it has been found near coast and in open sea in small groups of 3 - 6. Out of three reported strandings, one was stranded at Pishukan beach near Gwadar.

The black finless porpoise, *Neophocaena phocaenoides,* was reported relatively common. It is found near the coast and in the open sea in large

groups of 18 - 40 of more than one size. It is subjected to high levels of incidental mortality due to their entanglement in fishing nets. As a result, their population is likely to decline in the near future. Its meat is being used as bait by local fishermen to catch big fish like the whale shark. Fourteen strandings of this porpoise have been reported by the local fishermen so far. The survey team recorded three strandings on the Gwadar coast.

The Indian hump-backed dolphin, *Sousa plumbea,* is commonly found dolphin near the coast and in the open sea. It has reportedly been seen in groups of 4 - 20 of more than one size. During the team's survey, 27 Indian hump-backed dolphins of more than one size were observed at the mouth of River Dasht.

The long-beaked dolphin, *Delphinus tropicalus,* was not reported commonly by the fishing community. According to the local fishermen, it has been sighted near the coast and in the open sea in groups of 4 - 15 of more than one size. Out of the twelve reported strandings, two were reported near Daran beach and two at Gunz (1 year ago) and Pishukan beach (4 years ago).

The electra dolphin or melon-headed whale, *Peponocephala electra,* is also uncommon. It is usually sighted in the open sea and near the coast and in groups of 3 - 15 of more than one size. Out of the seven reported strandings, two were reported at Jiwani beach (3 years ago) and at Gunz beach (15 years ago), respectively.

The whale shark, *Rhincodon typus,* is the largest known fish. It reaches 15 meters (m) in length, and may reach 18 meters or more. Its weight can exceed 10 tonnes. It is found near the equator both along the coasts and in the open seas mostly near the surface. It feeds chiefly on plankton but also eats bigger fish such as tuna. Eighty-nine whale sharks have been reported so far by the different fishermen in the open sea and near the coast. These have been sited in groups of 3 - 14 of more than one size. They are caught by local fishermen and sold for Rs. 9 - 20,000 depending upon the size. These are sent to Karachi where oil is extracted from them and used for lubrication of boats and ships.

Manta ray, *Manta hamiltoni,* is a large beautiful sea creature that lives in warm temperate waters. It is wider than long; its wingspan can reach up to 7 m, and it glides through the water like birds. Its pectoral fins project from the

front of its head resembling devil's horns. These sweep plankton and small fish into their mouth. The long whip like tail may have one or more stinging spines. These fish have been commonly reported. They are found near the coast and in the open sea in groups of 5 - 25 of more than one size. They are caught and sold for Rs. 5 – 1,500 depending upon their size. A manta ray weighing 50kg is sold for Rs. 500. It is eaten by local community of Mekran coast while the rest are sold in Karachi. It has been reported to turn the boats upside down with its sharp pectoral fins that get entangled in nets.

There has been no report on the occurrence of Dugong or Sea Cow (*Dugong dugon)* by any fisherman. Occurrence of sea grass on which the dugongs feed is also not reported from the area.

The coastal area from Jiwani to Gwadar provides an important habitat for several globally important species including marine mammals such as whales, dolphins and finless porpoises. There are more than six hundred boats in Jiwani used for fishing by the local community. These are directly or indirectly engaged in fishing, due to which the coastal habitat is being degraded by over-fishing, pollution and entanglement of marine mammals and reptiles in fishing nets. Mangroves are ecologically important areas that provide nesting and breeding place for shrimps and finless porpoises that come to breed and feed in such an ecosystem. Accidental by-catch of these porpoises in fishing nets is leading to a continuous depletion or extirpation of these internationally endangered species.

Avian diversity analysis

Different analyses were carried out based on the data collected from the field. A simple regression analysis was carried out (MS Excel 2000) in order to know the population trends over the next few days, thus showing the best fit to the actual data. Shannon-weiner biodiversity index was calculated in order to know the species diversity in different habitats. It was calculated based on the abundance of the species by the following formula $H = - [\sum P_I \ln P_I]$, where H represents the symbol for the amount of diversity in an ecosystem, Pi represents the relative abundance to the total and the $\ln P_i$ represents the natural logarithm of it. Similar other analyses were carried out like the density of the birds based on the area searched, relative abundance based on the actual abundance and the dominant and sub-dominant birds again based on the abundance and distribution.

Simple regression analysis

Linear regression is a statistical tool used to predict future values from past values. Trends in the bird data were estimated using the number of species (diversity) as a parameter against the effort *i.e.* number of days (Fig. 6).

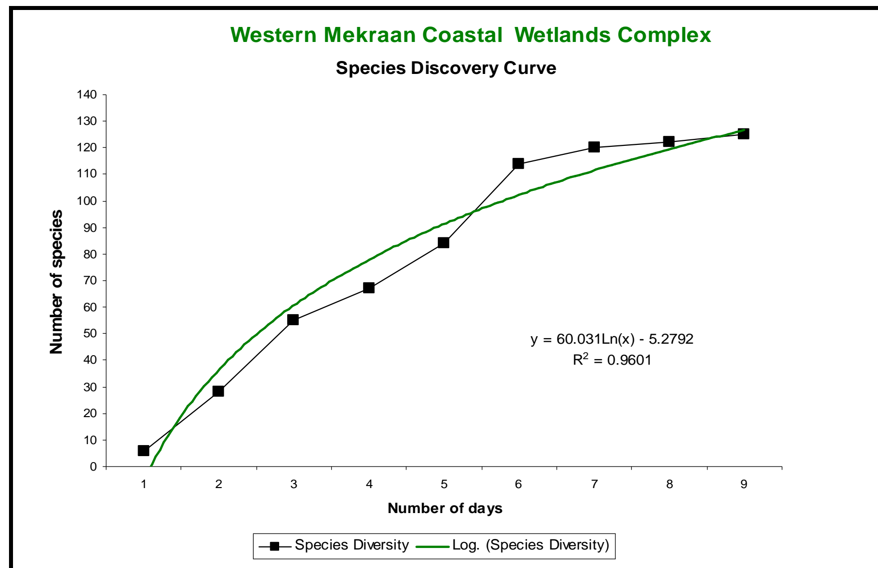

Fig. 6. Species curve with number of days and simple regression analysis

The initial six days showed a sharp increase in the diversity of bird species that were observed. With an increase in the number of days and effort, the diversity started decreasing and ultimately an approximate straight line trend was observed. A best fitted curve i.e. regression line, was drawn on the actual data and the regression co-efficient value r^2 was calculated (r^2 = 0.9601) thus showing the best fit to the data.

Shannon-Weiner biodiversity index

The Shannon-Weiner Index is a widely employed species diversity index (Hutchinson 1970). As mentioned before, the abundance of species is important, too. The presence of one individual of a species is not necessarily indicative of the species being present in a large number. When measuring

diversity, there is also a need to take the abundance of species into account. There are numerous mathematical expressions for diversity that take both into account and this index is one such example.

The Shannon Index (H) is appropriate when there is a random sample of species abundance's from a larger community as has been observed during this survey. Such a sample (unless extremely large) may not contain representatives for each species in the entire community. However, the lack of data on rare species has little effect on the value of H.

TABLE V.- SHANNON-WEINER BIODIVERSITY INDEX FOR AVI-FAUNA AT DIFFERENT HABITATS.

Habitat types	Abundance	Shannon Index (H)
Mangroves	3,096	2.20
Open Scrub, Thorn Forest and deserts	242	2.49
Hilly and Inland Mountains	120	2.74
Estuary and Off-coastal	326	0.68
Freshwater Lakes, Rivers and Reservoirs	7661	2.81
Towns, Villages and Cultivations	167	3.08
Coastline	5564	2.61
Total	8,862	3.599

Shannon-Weiner Biodiversity Index value for encountered birds was estimated to be 3.599. Towns, villages and cultivations were found to be the most diverse, because it has a higher index (3.08) than the others (Table V), indicating better environment and rich diversity of avi-fauna. Despite the fact that other habitats have higher species diversity, the even distribution and abundance of the species found in this habitat makes it more diverse.

Census (density) index and relative abundance

The area covered by the survey team under each habitat category was calculated using GIS techniques to calculate avi-fauna density. Table VI shows that the mangroves host the densest populations of birds with a density of 127 birds/km^2 followed by freshwater lakes and reservoirs with a density of 112 birds/km^2.

The least populated habitat was open scrub and thorn forest with a value of 0.28 birds/km^2. It was also observed that the terrestrial Passeriformes were less in numbers probably due to scarcity of water in the study area.

TABLE VI.- CENSUS INDEX AND RELATIVE ABUNDANCE OF AVI-FAUNA IN DIFFERENT HABITAT TYPES.

Habitat types	**Total population**	**Area** (km^2)	**Census index (density)**	**Relative abundance**
Mangroves	3,096	24.32	127.30	18.03
Open Scrub, Thorn Forest and deserts	242	851.62	0.28	1.41
Hilly and Inland Mountains	120	275.49	0.44	0.70
Estuary and Off-coastal	326	70.00	4.66	1.90
Freshwater Lakes, Rivers and Reservoirs	7661	68.46	111.90	44.60
Towns, Villages and Cultivations	167	80.83	2.07	0.97
Coastline	5564	260.31	21.37	32.39
Mekran Coastal Wetlands Complex	8,862	1631.03	5.43	

A total of 125 bird species were observed during the study period at seven different locations. This shows that 44.60% were inhabitants of freshwater lakes, rivers and reservoirs, followed by 32.39% and 18.03% of coastline and mangroves respectively (Table VI). The results show that the mangroves and freshwater ecosystems provide an ideal habitat for the avi-fauna of the study area having the maximum density of birds.

Dominant and sub-dominant index

Dominant and sub-dominant index described in Table VII revealed that few species in each habitat are abundantly found. The most dominant species was Dalmatian Pelican and Greater Flamingo with a dominant index value of 0.762 and 0.761 respectively. The Dalmatian Pelican has also been mentioned the most dominant in the area by Rose and Scott (1997) and ICBP (1992).

TABLE VII.- DOMINANT AND SUB-DOMINANT INDEX OF AVI-FAUNA.

Habitat types	Dominant		Sub-dominant	
	Species	**Index value**	**Species**	**Index value**
Mangroves	Herring Gull	41.86	Common black-headed Gull	29.72
Open Scrub, Thorn Forest and Deserts	Barn Swallow	0.058	Oriental Sky Lark	0.058
Hilly and Inland Mountains	Osprey	0.09	Pale Crag Martin & See see partridge	0.039
Estuary and Off-Coastal	Eurasian Oystercatcher	3.85	Stone Plover	0.27
Freshwater Lakes, Rivers and Reservoirs	Dalmatian Pelican	18.17	Greater Flamingo	18.14
Towns, Villages and Cultivations	Green Bee-eater	0.27	Crested Lark	0.18
Mekran Coastal Wetlands Complex	Dalmatian Pelican	0.762	Greater Flamingo	0.761

Diet analysis

Feeding habits of birds were assessed on the basis of food item preferences, as recorded by Roberts (1991, 1992). A total of 11 different groups of birds preferring different food items were documented. Depending on its feeding habits, each bird species was placed in its respective group.

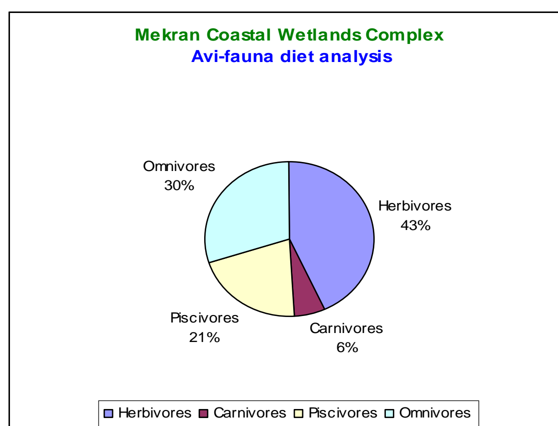

Fig. 7. Avi-fauna diet analysis of Mekran Coastal Wetlands Complex.

For a species preferring more than two groups of food items, due weightage was given to each type and their percentage was derived (Kontangale and Ghosh, 1997). Four broad categories were recognized *i.e.* carnivores, piscivores, omnivores and herbivores (Fig. 7). Piscivores are the ones who are pure fish eaters and therefore have been classified as a separate category.

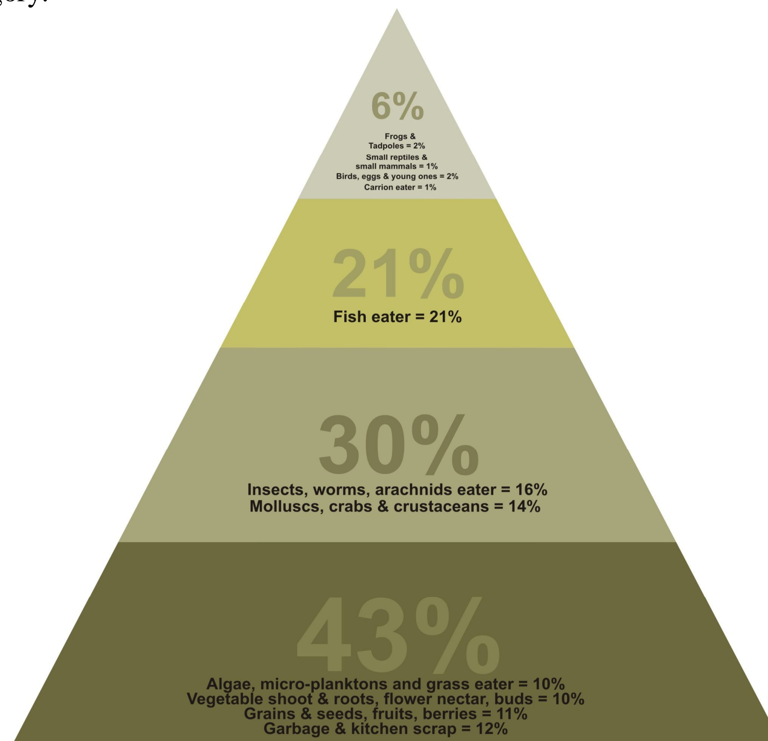

Fig. 8. Food pyramid showing different trophic levels

Based on the above information, a food pyramid showing different trophic levels within an ecosystem was developed (Fig. 8). The results revealed that majority of the birds in the study area were piscivorous (21%) and insectivorus (16%) in habit, preferring fish, insects, worms, arachnids as their food. These were followed by molluscs, crabs and crustaceans (14%), garbage and kitchen scrap eaters 12%, grainivores 11%, vegetable shoots, roots, flower buds, nectar eaters 10%, and algae, micro-planktons, grass eaters 10%. Carnivores lying at the top of the trophic pyramid consumed only 6% (Fig. 8).

Insects are an important part of a bird's diet. Though some birds are strictly herbivorous in habit, they also prefer animal food which is vital for their breeding and egg-laying.

Socio-economic set-up of the study area

Different areas of Mekran share many socioeconomic characteristics but there are major differences in the nature of district economies. Coastal Balochistan has lagged in economic development and suffers from a general lack of industrial infrastructure. Sixteen percent of the total population of Balochistan Province *i.e*., 5.82 million lives in Mekran division, which has a density of 30 persons/km^2 (1981 census). Fishing is the major profession of the local community and almost 70,000 fishermen are living and involved in this business.

The coastal water of Mekran offers a relatively great potential for development of fisheries. During 1984 total catch of fish was 72,000 tonnes out of which 87.32% was exported to Ceylon, U.S.A, Britain and other European countries. It brought foreign exchange earning of Rs. 214 million. A joint Pakistan-USSR survey conducted in 1969 located five fishing grounds between Ras Milan and Gwadar having potential for an estimated annual yield of 170,000 metric tonnes. The primitive methods used in catching fish yield poor return.

Skilled and technical manpower is relatively limited in Mekran, as industrial development is low. Out of a total of 5,464,583 ha of Mekran, 64,142 ha of land is presently cultivated because of shortage of irrigation water. Average rain in the coastal areas is 150mm yearly. Agriculture engages 80% of the population of this area. Water availability is a primary factor and is absolutely necessary for undertaking any development work in Mekran Division.

There are 268 primary, 42 middle and 21 high schools recorded for the Mekran Division. Only two colleges have been established at Panjgur and Kech respectively. The literacy rate of the area is 6% and female literacy is very low. Students of age groups ranging up to 15 years represent less than three percent of population.

There are inadequate heath facilities as there are 36 hospital beds and 43 dispensaries for a population of 6.53 lakhs (653,000). The existing road

network in Mekran is relatively poor. All towns and villages are connected through kacha fair weather roads and link roads. There is little no interaction between the economic growth of various zones due to the absence of appropriate and direct road links. In addition, Mekran has two air fields. Gwadar has an international airport, while Jiwani has a small kacha air field. Gwadar and Jiwani are connected by air with Karachi.

Electricity is supplied by WAPDA only to Gwadar. Jiwani receives a supply for only 8 – 12 hours/day. Alternators are also used to provide electricity to these areas. There are relatively limited repair facilities for these power alternators. In Mekran, promotion of agriculture, exploration of mineral resources, telecommunication, water and industry are hampered by the lack of electric power. Solar power stations are expensive and diesel generators are inefficient due to lack of repair facilities.

Telecommunication facilities are generally inadequate. The main difficulties faced by Pakistan Telecommunication Corporation (PTC) are difficult terrain with poor road communications, long distances, relatively low density human population resulting in low revenue returns and non availability of electric power. The Civil Aviation Authority (CAA) and Defense Force elements in the area are mostly served by their own radio communications and telephones wherever available.

The supply of drinking water to the coastal population is a primary problem. Moreover, water supply is dependent on rainfall which is stockpiled in dams and later supplied from these points to Gwadar district throughout the year. Gwadar sub-division is supplied water from Akara Kaur Dam that was completed last year and is situated about 28 km away from the town.

There are few industries of note except for date packing in Kech. The industrial sector in Balochistan, including Mekran, has remained neglected in the past. Balochistan is relatively rich in minerals that provide a good economic base for development. Copper and Iron ore deposits are present close to Mekran. it is locally believed that production can meet 40-50% requirement of Pakistan Steel Mills. Gypsum and Limestone are also available.

Threats to the biodiversity due to human interaction

It is widely recognized today that the future of most ecologically

important areas depends largely on the support of the surrounding local communities. As a result, there is an added emphasis on sustainable resource use and a broader approach involving land users in bioregional management at an ecosystem level. In a given assemblage of biological resources, there is a need to assess the current human actions that may adversely affect such resources.

Despite the area's location in the arid zone, the area has well diversified terrestrial and aquatic flora and fauna. If human use of these biological resources and human impacts on biodiversity generally are to be made sustainable, it is necessary to address a number of challenging issues.

Mangroves destruction

The mangrove ecosystem is rich in biodiversity and plays an important role in the marine ecosystem as most tropical marine species pass at least one stage of their life cycle in such forests. Over the past decade, there has been concern over the ways in which human activities have altered the mangrove ecosystem of Pakistan. In a preliminary survey of the mangroves of Balochistan (Saifullah, 1995) conducted by WWF-Pakistan in collaboration with the Department of Botany, University of Karachi, showed clear signs of mangrove destruction at all the three mangrove sites mentioned above. The most serious threat was indiscriminate cutting and lopping of mangroves for fuel and fodder, which has not only resulted in the loss of mangroves but the loss of important breeding places for fish and shrimps. The present study also revealed direct impacts of local communities on the mangrove forest for fuel and fodder.

Camel browsing has been another important factor associated with the loss of mangrove forests, which is extremely harmful for their regeneration.

Hunting wildlife for subsistence and recreation

Hunting has been a major factor that is responsible for losses among some terrestrial mammals, once seen commonly in this area. Moazzam (1991) mentioned that because of hunting pressure, human settlement and developmental activities (e.g. roads), the population of mammals is being further reduced. The population of large mammals such as panther, wolf, striped hyena, urial, Sindh wild goat and chinkara is most effected and their population is being wiped out from most parts of the coastal area.

Subsistence hunting and for recreation by the adjacent communities is another major conservation issue being faced by waterfowl. Although no direct incidences of hunting were observed in the study area, empty gun cartridges were collected from the banks of the two freshwater dams (Saji and Akara) and River Dasht, thus showing clear signs of extensive duck shooting and hunting. Falcon poaching for hunting houbara bustard is common and is another major concern for conservation where one is caught to kill the other. Although during our survey, we could not spot any houbara bustard in the Dasht area, it provides an ideal habitat for this species. Likewise, Marine turtles have been killed in the recent past for their blood, which was used to cure joint aches. In addition, turtle eggs are poached during the nesting season and are given as food to sick goats for strength and energy. Majority of the nests are lost due to animal predation especially by feral dogs. These eggs also get trampled easily by local communities living along the coastline.

A series of different wetlands in the main stream of River Dasht supports the remaining population of marsh crocodiles that were once common in the entire river extending upto Iran. River Dasht has been effected due to a long natural drought in the area and the main stream has been fragmented into small ponds. Drought has effected their numbers as has hunting. The species has been hunted ruthlessly for two reasons: 1) the crocodiles attack livestock, that use freshwater for drinking purposes; and 2) as a recreation especially during their migration from one pond to another.

Over-exploitation of fish resources

Fishing is the main profession of the community living along the Mekran coast line. The adjacent local communities are directly and indirectly engaged in fishing as a result of lack of viable alternate livelihoods (Majid and Moazzam, 1991). Over-harvesting of fish in the coastal areas has not only led to a substantial decrease in fish resources but has also posed a threat to the globally significant fishes, whale shark and manta ray. These are caught and sold at reasonably high prices resulting in a continuous decline in their numbers, reported by the local fishermen. In addition, the fishermen have to go to open sea now for a big catch, presenting an evidence of over-fishing along coastal areas. Coastal ecosystems have already lost much of their capacity to produce fish resources because of over-fishing, destructive trawling techniques, and destruction of nursery habitats near mangrove areas.

Marine mammals and reptiles caught in fishing nets

Marine mammals are naturally vulnerable because of their distribution near the shore. Populations of such mammals have been depleted because of habitat degradation, fishery by-catches or a combination of these factors. Strandings of Indian hump-backed dolphins, long-beaked dolphins, electra dolphins, bottle-nose dolphins and finless porpoises have been reported by local fishermen. The three observed strandings of finless porpoises during the survey revealed that there is a substantial by-catch of finless porpoise in the fishing nets. In addition, marine turtles and snakes have been reportedly entangled in fishing nets.

Habitat destruction

There are two kinds of threats to the biodiversity of the area i.e. natural and anthropogenic. Prolonged drought in the area has caused scarcity of water and food shortage for wildlife while the vegetative or nesting cover has also been reduced in open scrub forest. Wood cutting by the guardian community has resulted in loss of nesting cover for birds and the general habitat of other wildlife species. In addition, there is a network of unpaved tracks all over the Dasht area thus showing a large-scale disturbance in the habitat of important wildlife species. Habitat conversion and degradation for the purposes of growing agricultural crops especially vegetables, fruit orchards and other cash crops, is generally considered to be a significant impact and disturbance in the Dasht area for terrestrial life forms.

Overgrazing by free-ranging livestock

Livestock of the area depends entirely on their food from the desert ecosystem i.e., Dasht area, open scrub country and thorn forests. Due to a relatively large number of free-ranging domestic livestock in the area, there are clear signs of overgrazing, which have led to the shortage of fodder and forage for them. The prevailing drought conditions have further aggravated the situation resulting into livestock casualties (a dead cow was observed near Samati village). Extensive overgrazing and uprooting of vegetation for fuel wood by locals has led to substantial degradation of the resources. This is characterized not only by a low diversity of species but also in their frequency of occurrence.

Inland sand dune movement

One of the most pernicious forms of desertification in Pakistan is sand dune formation along the coast and the movement of these dunes inland. Dune movement occurs mostly in the lightly wooded areas during dry windy periods. Coastal sand dune formation and inland movement of dunes threatens animal forage and range lands, agricultural lands, roads and villages. Moving sands also affect the health of humans and livestock by irritating eyes, throat, skin and in some cases causing severe respiratory dysfunction.

Lack of implementation of policies

UNESCO (1994) conducted an international workshop on Integrated Coastal Zone Management (ICZM). The objectives were to evaluate the impact of socio-economic development in coastal areas of Pakistan and to draft guidelines for an integrated coastal zone management planning process so as to ensure sustainable development and protection of marine resources. The workshop identified ICZM as the most imperative and immediate need for Pakistan. It further recognized that the coastal zone is a complex environment, characterized by dynamic relationships amongst the natural environment and its ecosystems, and the societal demands for space and natural resources, and thus subject to both natural and human influences. Due to planning and implementation failures, the problems of extensive resource use by the community are on the rise resulting in the disturbance of the unique marine ecosystem. In addition, policies at the local and national level are not being implemented in their true sense.

In addition, with special reference to a brief socio-economic survey of Jiwani and Gwadar conducted by WWF-Pakistan (WWF, 1997; 2000) and Zuberi (1991) shows that all the civic amenities including basic education, health, electricity, water supply, sanitation and communication are inadequate in order to meet the needs of the community. These basic amenities and socio-economic problems are due to the lack of proper management policies and infrastructure.

RECOMMENDATIONS

Mitigation measures

Despite the location of the study area in the arid zone with harsh environmental conditions, the complex is rich in terrestrial and aquatic flora

and fauna. The different habitat types show that the area is also diverse in supporting large numbers of birds, mammals, reptiles and amphibian species. The biodiversity rich area is facing severe threats of wildlife poaching and habitat degradation coupled with weak law enforcement by the government and the lack of viable alternate livelihoods. In order to sustain the unique ecosystem, mitigation measures are essential primarily at the local level. The recommendations by the survey team have been broadly classified into two major groups: short-term (bridging) and the long-term measures. The short-term measures will be covered under the bridging phase of the Wetlands project before the initiation of the long-term full GEF project, while the long term activities will be carried out under the full project.

Short-term (bridging) measures

River Dasht is the only remaining habitat in that area for marsh crocodiles. The habitat is being degraded by the prolonged natural drought but the animals are facing severe threats of poaching and extensive killing by the local community. Thus there is a dire need to promote conservation and awareness programme for the long-term survival of this species in the region. Waterfowl forms the major avi-fauna diversity in the region. Their illegal hunting and poaching should be stopped and the relevant departments should help in protecting the rare birds of the area. Hunting big game for food and sport is an activity carried out by the locals in the area. Conservation and education awareness activities should be launched for the protection and management of wild mammals.

A Wetland Information Centre is being established in the Mekran Coastal Wetlands Complex. This centre should be used for enhancing the awareness of the local community regarding the wetlands and its resources. Small dams and ephemeral streams for freshwater supply and biodiversity should be conserved for the stability of the local ecosystem. Wildlife monitoring programmes should be conducted on regular basis to determine the population trends. Over fishing particularly of Whale Shark and Manta Rays should be prohibited during their breeding season.

Long-term measures

1. Mangrove forest protection and their plantation should be promoted in most degraded areas.

2. Efforts to promote wildlife conservation in the region should be prioritized as a tool to generate revenue for the local community.

3. Measures like Turtle Excluder Device (TED) should be promoted. This should be adopted by the fishing trawlers to avoid entanglement of the marine mammals and reptiles in the fishing nets.

4. Environmental education and building capacity be promoted to enable people to sustainably manage their natural resources.

5. Coordination amongst various departments like Fisheries, Forest, wildlife, Civil Aviation Authority (CAA), local administration, Irrigation and Pakistan Coast Guards should be facilitated and promoted.

6. Mekran Coastal Eco-tourism Development should be promoted in such areas for international recognition of the area in terms of its beauty and biodiversity.

7. EIA should be carried out for new developmental activities in the area.

8. Rare sea shells and fish species should be protected.

9. Effective measures should be undertaken to prevent coastal pollution especially due to land-based sources. Community solid waste management programmes should be initiated to effectively manage and protect coastal areas from degradation.

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REFERENCES

AHMAD, M.F., GHALIB, S.A., HASSAN, A., AND HASNAIN, S. A., 1997a. *Studies on the distribution, population dynamics and status of the vertebrate mangrove fauna of Balochistan coast.* Zoological Survey Department, Government of Pakistan. Pp. 68.

AHMAD, M.F., GHALIB, S.A., HASSAN, A., AND HASNAIN, S. A., 1997b. The waterfowl of Makran coast. In: *Proceedings of three-day national conference on problems and resources of Makran coast and plan of action for its development*. Pakistan Council for Science and Technology, Islamabad. pp. 113-123.

GHALIB, S. A., RAHMAN, H., IFFAT, F AND HASNAIN, S. A. 1979. A checklist of reptiles of Pakistan. *Rec. Zool. Surv. Dep*., **8**: 37-60.

HALDIN, M AND ULFVENS, J. 1987. On the efficiency of censusing waterbirds by boat. *Ornis Fennica*, **64:**74-75.

HUTCHESON, K., 1970. A test for comparing diversity based on the Shannon formula. *J. Theor. Biol*., **29**: 151-154

ICBP, 1992. *Handbook of the birds of the world.* Volume 1: *Ostrich to ducks* (eds. J. del Hoyo, A. Elliot and J. Sargatet), Lynx Edition.

IUCN, 2000. *Red list categories*. World Conservation Union, Switzerland

KOTANGALE, J. P. AND GHOSH, T. K. 1997. Diversity of Avi-fuana in and around Ranapratupsagar, Rajasthan. *Ecology*, **12**: 29-37.

MINTON, S. A. Jr., 1966. Contribution to the herpetology of West Pakistan. *Bull. Am. Mus. Nat. Hist. N.Y*., 134, Article 2.

MAJID, A., AND MOAZZAM, M., 1991**.** An overview of the fisheries resources of Mekran. In: *Proceedings of three-day national conference on problems and resources of Makran coast and plan of action for its development.* Pakistan Council for Science and Technology, Islamabad. pp.103-112

MOAZZAM, M., 1991. Conservation of fragile ecosystem of Makran. In: *Proceedings of three-day national conference on problems and resources of Makran coast and plan of action for its development*. Pakistan Council for Science and Technology, Islamabad. pp. 69-75.

RAHMAN, H., IFFAT, F., AND PARVEEN, Z., 1988**.** Marine snakes of Pakistan. In: *Marine Science of the Arabian Sea: Proceedings of an international Conference* (eds. M.F. Thompson and N. M. Trimzi). American Institute of Biological Sciences. Printed at Alnoor Enterprsies, Karachi, Pakistan.

ROBERTS, T. J., 1991. *The birds of Pakistan*. Volume 1. Oxford University Press, Karachi. pp. 598.

ROBERTS, T. J., 1992. *The birds of Pakistan*. Volume 2. Oxford University Press, Karachi. pp. 617.

ROBERTS, T. J., 1997. *The mammals of Pakistan*. Oxford University Press, Karachi. pp. 525.

ROSE, P. M., AND SCOTT, D. A., 1997. *Waterfowl population estimates*. Second Edition. *Wetlands. Internat. Newsl*., 44.

SAIFULLAH, S. M., 1995. Future prospects of mangroves in development of Mekran coast. In: *Proceedings of three-day national conference on problems and resources of Makran coast and plan of action for its development*. Pakistan Council for Science and Technology, Islamabad. pp. 63-68.

SAIFULLAH, S. M., 1995. *A preliminary survey of mangroves of Balochistan*. Scientific Committee Funded Project Final Report. pp. 99.

SAIFULLAH, S. M. AND RASOOL, F., 2001. Mangroves of Gwater Bay, Mekran Coast, near the Gulf of Oman. In: *Mangrove ecosystems: Natural distribution, biology and management. Proceedings of the international symposium on mangrove ecology and biology* (eds. N.R. Bhat, F.K. Taha and A.Y. A-Nasser), Kuwait Institute for Scientific Research.

STONE, S., 1990. *Oman's geological heritage*. Petroleum development Oman. Stacey International Publisher, London.

UNDP, 2000. *Protection and management of Pakistan Wetlands Project – Project document*. pp21.

UNESCO, 1994. *International workshop on integrated coastal zone management (ICZM)*. Organised in cooperation with the National Institute of Oceanography, Ministry of Science and Technology, Government of Pakistan. Intergovernmental Oceanographic Commission, Workshop Report No. 114. Pp. 35.

WWF, 1996. *Conservation of mangrove forests at the coastal areas of Sindh and Balochistan*. WWF-Pakistan's Project Document.

WWF, 1997. *Reconnaissance survey of Jiwani and Gwadar.* A survey report submitted to WWF-Pakistan. pp.19

WWF, 1998. *Conservation of biodiversity in Gwater Bay (Jiwani) on Balochistan coast*. WWF-Pakistan Project proposal.

WWF, 2000. Pakistan: Mangroves. In: *The root causes of biodiversity loss* (eds. A. Wood, P.S.-Edwards and J. Mang), pp. 255-281. Earthscan Publications Ltd.

ZUBERI, S. J., 1991. Problems and resources of Mekran. In: *Proceedings of three-day national conference on problems and resources of Makran coast and plan of action for its development.* Pakistan Council for Science and Technology, Islamabad. pp 21-29

TABLE I.- AVIAN DIVERSITY ANALYSIS AT MEKRAN WETLANDS COMPLEX, PAKISTAN.

Ser . #	Common Name	Scientific name	Total	Relative abundance	Census index/ km²	Shannon-Weiner Diversity Index (H)
1	Little Grebe or Dabchick	*Tachybaptus ruficollis*	28	0.31595577	0.01716707	-0.0474052
2	Great Crested Grebe	*Podiceps cristatus*	41	0.46264951	0.02513749	-0.061752
3	Black-necked Grebe	*Podiceps nigricollis*	36	0.40622884	0.02207194	-0.0565157
4	Great Cormorant	*Phalaerocorax carbosinensis*	785	8.85804559	0.48129096	-0.0466483
5	Dalmation Pelican	*Pelicanus crispus*	1244	14.0374633	0.76270823	0.2067559
6	Indian Pond Heron	*Ardeola grayii*	2	0.02256827	0.00122622	-0.0059727
7	Western Reef Heron	*Egrette gularis*	28	0.31595577	0.01716707	-0.0474052
8	Little White Egret	*Egretta garzetta*	20	0.22568269	0.01226219	-0.0371587
9	Large Egret	*Egretta alba*	9	0.10155721	0.00551799	-0.0202433
10	Grey Heron	*Ardea cinerea*	35	0.39494471	0.02145883	-0.0554291
11	Glossy Ibis	*Plegadis falcinellus*	4	0.04513654	0.00245244	-0.0105866
12	White Spoonbill	*Platalea leucordia*	17	0.19183029	0.01042286	-0.0329388
13	Greater Flamingo	*Phoenicopterus ruber*	1242	14.0148951	0.76148201	0.2054441
14	Common Shellduck	*Tadorna tadorna*	33	0.37237644	0.02023261	-0.0532133
15	Wigeon	*Anas penelope*	4	0.04513654	0.00245244	-0.0105866
16	Gadwal	*Anas strepera*	95	1.07199278	0.0582454	-0.1039627
17	Common Teal	*Anas crecca*	18	0.20311442	0.01103597	-0.0343722
18	Mallard	*Anas platyrhynchos*	79	0.89144663	0.04843565	-0.0935934
19	Northern Pintail	*Anas acuta*	17	0.19183029	0.01042286	-0.0329388
20	Shoveler	*Anas clypeata*	186	2.09884902	0.11403837	-0.1423059
21	Marbled Teal	*Marmaronetta angustirostris*	15	0.16926202	0.00919664	-0.0299838

Continued

Ser . #	Common Name	Scientific name	Total	Relative abundance	Census index/ km²	Shannon-Weiner Diversity Index (H)
22	Red-crested Pochard	*Netta rufina*	2	0.02256827	0.00122622	-0.0059727
23	Common Pochard	*Aythya ferina*	297	3.35138795	0.18209352	-0.1591156
24	Ferruginous Duck	*Aythya nyroca*	2	0.02256827	0.00122622	-0.0059727
25	Tufted Duck	*Aythya fuligula*	31	0.34980817	0.01900639	-0.050938
26	Red-breasted Merganser	*Mergus serrator*	10	0.11284135	0.0061311	-0.0219762
27	Common Merganser	*Mergus merganser*	7	0.07898894	0.00429177	-0.0166069
28	Pallas's Fish Eagle	*Haliaeetus leucoryphus*	1	0.01128413	0.00061311	-0.003326
29	Marsh Harrier	*Circus aeruginosus*	1	0.01128413	0.00061311	-0.003326
30	Palllid Harrier	*Circus macrourus*	2	0.02256827	0.00122622	-0.0059727
31	Sparrow Hawk	*Accipiter nisus*	2	0.02256827	0.00122622	-0.0059727
32	Common Buzzard	*Buteo buteo*	1	0.01128413	0.00061311	-0.003326
33	Long -legged Buzzard	*Buteo rufinos*	3	0.0338524	0.00183933	-0.0083629
34	Greater Spotted Eagle	*Aquila clanga*	6	0.06770481	0.00367866	-0.0146877
35	Tawny Eagle	*Aquila rapax*	1	0.01128413	0.00061311	-0.003326
36	Steppe Eagle	*Aquila nipalensis*	2	0.02256827	0.00122622	-0.0059727
37	Imperial Eagle	*Aquila heliaca*	5	0.05642067	0.00306555	-0.0126865
38	Osprey	*Pandion haliatus*	37	0.41751298	0.02268505	-0.0575888
39	Eurasian Kestrel	*Falco tinnunculus*	3	0.0338524	0.00183933	-0.0083629
40	Sooty Falcon	*Falco concolor*	1	0.01128413	0.00061311	-0.003326
41	Red-napped Shaheen	*Falco pelegrinoides babylonicus*	1	0.01128413	0.00061311	-0.003326
42	See See Partridge	*Ammoperdix griseogularis*	11	0.12412548	0.0067442	-0.02366

Continued

Ser . #	Common Name	Scientific name	Total	Relative abundance	Census index/ km²	Shannon-Weiner Diversity Index (H)
43	Grey Partridge	*Francolinus pondicerianus*	1	0.01128413	0.00061311	-0.003326
44	Black or Eurasian Coot	*Fulica atra*	457	5.15684947	0.28019104	-0.148319
45	Common Moorhen	*Gallinula chloropus*	1	0.01128413	0.00061311	-0.003326
46	Eurasian Oyster Catcher	*Haematopus ostralegus*	270	3.04671632	0.16553957	-0.1572617
47	Black-winged Stilt	*Himantopus himantopus*	5	0.05642067	0.00306555	-0.0126865
48	Greater Thicknee or Stone Plover	*Esacus recurvirostris*	30	0.33852404	0.01839329	-0.0497769
49	Cream-coloured Courser	*Cursorius cursor*	25	0.28210336	0.01532774	-0.0437145
50	Little-ringed Plover	*Charadrius dubius*	2	0.02256827	0.00122622	-0.0059727
51	Kentish or Snowy Plover	*Charadrius alexandrinus*	41	0.46264951	0.02513749	-0.061752
52	Lesser Sand Plover	*Charadrius mongolus*	19	0.21439856	0.01164908	-0.0357784
53	Greater Sand Plover	*Charadrius leschenaultil*	40	0.45136538	0.02452438	-0.0607299
54	Red-wattled Lapwing	*Hoplopterus indicus*	2	0.02256827	0.00122622	-0.0059727
55	White-tailed Lapwing	*Chettusia leucura*	4	0.04513654	0.00245244	-0.0105866
56	Sanderling	*Calidris alba*	16	0.18054615	0.00980975	-0.0314766
57	Curlew Sandpiper	*Calidris ferruginea*	76	0.85759422	0.04659632	-0.0914811
58	Little Stint	*Calidris minuta*	4	0.04513654	0.00245244	-0.0105866
59	Dunlin	*Calidris alpina*	15	0.16926202	0.00919664	-0.0299838
60	Common Snipe	*Gallinago gallinago*	1	0.01128413	0.00061311	-0.003326
61	Black-tailed Godwit	*Limosa limosa*	5	0.05642067	0.00306555	-0.0126865
62	Bar-tailed Godwit	*Limosa lapponica*	29	0.3272399	0.01778018	-0.0485995
63	Whimbril	*Numenius phaeopus*	6	0.06770481	0.00367866	-0.0146877

Continued

Ser . #	Common Name	Scientific name	Total	Relative abundance	Census index/ km²	Shannon-Weiner Diversity Index (H)
64	Eurasian Curlew	*Numenius arquata*	88	0.99300384	0.05395364	-0.0996032
65	Common Redshank	*Tringa totanus*	9	0.10155721	0.00551799	-0.0202433
66	Common Greenshank	*Tringa nebularia*	4	0.04513654	0.00245244	-0.0105866
67	Green Sandpiper	*Tringa octiropus*	2	0.02256827	0.00122622	-0.0059727
68	Terek Sandpiper	*Xenus cinereus*	17	0.19183029	0.01042286	-0.0329388
69	Common Sandpiper	*Actitis hypoleucos*	14	0.15797788	0.00858353	-0.0284582
70	Sooty Gull	*Larus hemprichii*	94	1.06070864	0.05763229	-0.1033559
71	Common Black-headed Gull	*Larus ridibundus*	723	8.15842925	0.44327817	-0.0721152
72	Great Black-headed Gull	*Larus ichthyaetus*	365	4.1187091	0.22378497	-0.1586687
73	Slender-billed Gull	*Larus genei*	81	0.9140149	0.04966187	-0.0949704
74	Herring Gull	*Larus argentatus*	1018	11.4872489	0.62414548	0.0691717
75	Lesser Black-headed Gull	*Larus fuscus*	386	4.35567592	0.23666027	-0.1572157
76	Caspian Tern	*Sterna caspia*	61	0.6883322	0.03739968	-0.0799981
77	Great Crested Tern	*Sterna bergii*	6	0.06770481	0.00367866	-0.0146877
78	Sandwitch	*Sterna asandvicensis*	10	0.11284135	0.0061311	-0.0219762
79	Gull billed Tern	*Gelochelidon nilotica*	21	0.23696682	0.0128753	-0.0385145
80	Imperial Sandgrouse	*Plerocles orientalis*	8	0.09027308	0.00490488	-0.0184558
81	Blue Rock Pigeon	*Columba livia*	13	0.14669375	0.00797042	-0.0268976
82	Indian Ring Dove	*Streptopelia decaocta*	12	0.13540961	0.00735731	-0.0252993
83	Little Brown Dove	*Streptopelia senegalensis*	8	0.09027308	0.00490488	-0.0184558
84	Common Koel	*Eudynamys scolopacea*	1	0.01128413	0.00061311	-0.003326
85	Pallid Swift	*Apus pallidus*	12	0.13540961	0.00735731	-0.0252993

Continued

Ser . #	Common Name	Scientific name	Total	Relative abundance	Census index/ km²	Shannon-Weiner Diversity Index (H)
86	White-breasted Kingfisher	*Halcyon smyrnensis*	2	0.02256827	0.00122622	-0.0059727
87	Common Kingfisher	*Alcedo atthis*	2	0.02256827	0.00122622	-0.0059727
88	Little Green Bea-eater	*Merops orientalis*	22	0.24825096	0.01348841	-0.039847
89	Indian Roller or Blue Jay	*Coracias benghalensis*	7	0.07898894	0.00429177	-0.0166069
90	Black-tailed Lark	*Ammomanes cincturus*	14	0.15797788	0.00858353	-0.0284582
91	Desert Lark	*Ammomanes deserti*	7	0.07898894	0.00429177	-0.0166069
92	Hoopoe Lark	*Alaemon alaudipes*	4	0.04513654	0.00245244	-0.0105866
93	Crested Lark	*Galerida cristata*	15	0.16926202	0.00919664	-0.0299838
94	Oriental Skylark	*Alauda gulgula*	50	0.56420673	0.03065548	-0.0704447
95	Hume's short-toed Lark	*Calandrella acutirostris*	16	0.18054615	0.00980975	-0.0314766
96	Indian Sand Martin	*Riparia paludicola*	6	0.06770481	0.00367866	-0.0146877
97	Pali crag Martin	*Ptyonoprogne fuligula*	11	0.12412548	0.0067442	-0.02366
98	Barn or common swallow	*Hirundo rustica*	115	1.29767547	0.07050759	-0.1150823
99	Tawny Pipit	*Anthus campestris*	2	0.02256827	0.00122622	-0.0059727
100	Brown Rock Pipit	*Anthus similis*	4	0.04513654	0.00245244	-0.0105866
101	White or Pied Wagtail	*Motacila alba*	7	0.07898894	0.00429177	-0.0166069
102	White Cheeked Bulbul	*Pycnonotus leucogenys*	6	0.06770481	0.00367866	-0.0146877
103	Blue-throat	*Luscinia svecica*	2	0.02256827	0.00122622	-0.0059727
104	Black Redstart	*Phoenicurus ochruros*	12	0.13540961	0.00735731	-0.0252993
105	Pied Bush Chat	*Saxicola caprata*	1	0.01128413	0.00061311	-0.003326
106	Desert Wheatear	*Oenanthe deserti*	6	0.06770481	0.00367866	-0.0146877
107	Red-tailed Wheatear	*Oenanthe xanthoprymna*	2	0.02256827	0.00122622	-0.0059727

Continued

Ser . #	Common Name	Scientific name	Total	Relative abundance	Census index/ km²	Shannon-Weiner Diversity Index (H)
108	Variable Wheatear	*Oenanthe picata*	8	0.09027308	0.00490488	-0.0184558
109	Hooded Wheatear	*Oenanthe monacha*	3	0.0338524	0.00183933	-0.0083629
110	Cetti's Warbler	*Cettia cetti*	2	0.02256827	0.00122622	-0.0059727
111	Graceful Prinia	*Prinia gracillis*	10	0.11284135	0.0061311	-0.0219762
112	Rofous-fronted Prinia	*Prinia buchanani*	4	0.04513654	0.00245244	-0.0105866
113	Blyth’s Reed Warbler	*Acrocephalus dumetorum*	4	0.04513654	0.00245244	-0.0105866
114	Desert Warbler	*Sylvia nana*	1	0.01128413	0.00061311	-0.003326
115	Lesser Whitethroat	*Sylvia curruca*	6	0.06770481	0.00367866	-0.0146877
116	Eurasian Chiff Chaf	*Phylloscopic collybita*	3	0.0338524	0.00183933	-0.0083629
117	Common Babbler	*Turdoides caudatus*	25	0.28210336	0.01532774	-0.0437145
118	Bay-backed Shrike	*Lanius vittatus*	1	0.01128413	0.00061311	-0.003326
119	Great Grey Shrike	*Lanius excubitor*	8	0.09027308	0.00490488	-0.0184558
120	House Crow	*Corvus splendens*	4	0.04513654	0.00245244	-0.0105866
121	Desert Raven	*Corvus ruficollis*	6	0.06770481	0.00367866	-0.0146877
122	Common Myna	*Acridotheres tristris*	6	0.06770481	0.00367866	-0.0146877
123	House Sparrow	*Passer domesticus indicus*	86	0.97043557	0.05272742	-0.0983084
124	Purple Sunbird	*Nectarinia asiatica*	4	0.04513654	0.00245244	-0.0105866
125	Trumpter Bulfinch	*Bucanetes mongolicus*	2	0.02256827	0.00122622	-0.0059727
Total Number of Birds					**8862**	
Total Number of Species					**125**	
Census (Density) Index km²					**5.43**	
Shannon-Weiner Diversity Index (H)					**3.599**	

TABLE II.- MARINE AND LAND MAMMALS OBSERVED AT MEKRAN COASTAL WETLANDS COMPLEX.

#	Common name	Scientific name	Nos.	Remarks
1	Indian Hump-backed or Plumbeous Dolphin	*Sousa plumbea*	27	Between old Naval Base and Estuary of Dasht River, in the sea, during our visit by boat to Dasht River Estuary. 30-12-2001
2	Black Finless or Little Indian Porpoise	*Neophocaena phocaenoides*	3	These dead specimen were seen on Sandy beach (western side) of Gwader dated 04-01-2002
3	Asiatic Jackal	*Canis aureus*	2	Wandering on road side near Bindri village (Jiwani) dated 01-01-2002.
4	White-footed Desert Fox	*Vulpes vulpes pusillus*	1	Crossed our vehicle near Samati village (Saji Dam) Jiwani, dated 02-01-2002
5	Jungle Cat or Swamp Cat	Felis chaus	2	At Saji Dam, in mesquite trees, dated 02-01-2002
6	Desert Cat	*Felis Libyea ornata*	1	At Saji Dam, in mesquite trees, dated 02-01-2002
7	Small Indian or Small Asian Mongoose	*Herpestes javanicus*	1	At the rocky beach of Jiwani near Jiwani Rest House, dated 02-01-2002
8	Black Rat or Roof Rat	*Rattus rattus*	5	At the rocky beach of Pishukan near Pishukan village dated 30-12-2001
9	House Mouse	*Mus musculus*	1	Road side at Gwader town (South western coastal hill), dated 04-01-2002
10	Northern Palm Squirrel or Five Striped Palm Squirrel	*Funambulus pennantii*	2	These both squirrel were observed on tree planted at Gwader Airport, dated 05-01-2002 on our way back to Karachi
11	Kuhl's Pipistrelle (Bat)	*Pipistrellus kuhlii*	6	These are commonest bats of Jiwani and its surrounding. Dated 31-12-2001

TABLE III.- AMPHIBIANS AND REPTILES OBSERVED AT MEKRAN COASTAL WETLANDS COMPLEX.

#	Common Name	Scientific Name	Nos.	Remarks
1	Indian Ocean Green Turtle	*Chelonia mydas*	4	One alive female was observed at Dharan beach with egg laying, near Jiwani; date: 28-12-2001. Three dead specimens were observed at Gwadar western beach. Dated: 4-1-2001.
2	Pacific Hawks Bill Turtle	*Eretmochely imbricata squamata*	1	This dead specimen was observed, noted and photographs were taken at Western Beach of Gwader
3	Snub-nosed / Muggar / Marsh Crocodile	*Crocodylus palustris palustris*	4	Remaining population of this isolated group is in Dasht River, at least one breeding pair and two juvenile. Observed dated 01-01-2002 between Suntsar and Mitting Village in the Dast River
4	Yellow-headed Agama	*Agama nupta fusca*	1	On the hill at Pishukan coast near Pishukan Village. Dated 31-12-2001
5	Brilliant Agama	*Agama agilis*	1	On the hill at Pishukan coast near Pishukan Village. Dated 31-12-2001
6	Long-tailed Desert Lacerta	*Eremius guttulata watsonana*	3	Observed on the hill top and sandy area of Pishukan village situated at the coast line. Observed dated 31-12-2001
7	Mekran Fringe-toed Sand Lizard	*Acanthodactylus cantonis blandfordi*	2	Observed on the sandy bank of Dasht River Estuary. Dated 30-12-2001
8	Punjab Snake-eyed Lacerta	*Ophisops jerdoni*	1	On the hill-top at the coast of Pishukan village. Observation date 31-12-2001

Continued

#	Common Name	Scientific Name	Nos.	Remarks
9	Mediterranean Gecko	*Hemidactylus turcicus turcicus*	1	This lizard was seen in Jiwani Rest House, Dated 31-12-2001
10	Cliff Racer	*Coluber rhodorachis*	1	This alive snake was seen on the coastal hill (southwest of Gwader). Observation date 04-01-2002
11	Annulated or Chittul Sea Snake	*Hydrophis cyanocinctus*	1	This snake was observed during visit to Dasht River Estuary, dated 30-12-2001
12	Skittering Frog	*Rana cyanophlyctis*	12	Four frogs were seen at crocodile ponds at Dasht River (01-01-2002). Six frogs were seen at Saji Dam dated 02-01-2002. Two frogs were seen at Akara Dam. Dated 04-01-2002.

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CHANGES IN BLOOD PARAMETERS DUE TO BLADDER WORM (CESTODA) INFECTION IN LIVER OF *RATTUS NORVEGICUS*

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Abstract.- A total of 200 brown rats, *Rattus norvegicus,* were examined from Hyderabad and its adjoining areas. The haematological values of the rats harboring bladder worm (Cestoda) in liver were determined and compared with those of the non-infected rats (Control). The haematological values of total erythrocyte count (TEC), haemoglobin (Hb) and packed cell volume (PCV) recorded for infected male and female rats were found significantly lower than those of the control male and female rats. There was no significant change in erythrocyte sedimentation rate (ESR) values obtained during bladder worm infection in liver of both male and female rats. Infected male and female rats showed significant rise in total leucocyte count (TLC) as compared to the control rats of both sexes. During differential leucocyte count (DLC) the values of monocytes for infected male and female rats were found significantly lower than those of their control male and female rats. Lymphocyte value was lower in infected male rats, while infected female rats showed higher value than those of the control rats. Neutrophils in infected male and female rats were significantly higher than their control rats of both sexes. Furthermore, there was no significant change in the values of eosinophil and basophil recorded from the control and infected male rats, however, the females showed significantly high values in these two parameters. Present work provides evidence that the bladder worm infection in liver of rats causes changes, not in all, but in most of the haematological parameters suggesting host-parasite interaction.

Key words: Brown rats, *Rattus norvegicus*, endoparasites, bladder worm (Cestoda), haematological parameters.

INTRODUCTION

Rats are most widely distributed rodents of the world and have always followed human civilization. The brown rat, *Rattus norvegicus* causes much damage to merchandise, foodstuffs, and attacks domestic animals, poultry, and occasionally humans (Fatima, 1991). Rats are also carrier of a number of diseases (plague, murine typhus, leptospirosis, trichinosis, salmonellosis, tape worm infections and rickettsial pox) transmissible to humans (Cheng, 1986).

The liver is main organ for metabolic activities. Several important proteins (albumin, globulin, fibrinogen and prothrombin) found in the blood are produced in the liver (Frisell, 1982). It also serves as the principal source and site of reserve for the fat-soluble vitamins-A, D, E and K, whereas the water soluble vitamins-B_{12} and folic acid can be stored to any significant degree in the liver (Frisell, 1982; Green *et al*., 1986). It also helps to clear the toxic substances from the bloodstream (Crawford, 1997). The pathogenic mechanisms during parasitic infection include toxic effects, growth effects, mechanical injury, effects on the host's reproductive system and various metabolic changes (Chappell, 1980).

The values of haematological parameters under normal conditions in animals are constant within certain limits, thus any variation in the haematological indices may represent impaired metabolic activities. It has also been demonstrated that parasitic infection brings about biochemical and haematological changes in their hosts (Kameswari *et al*., 1975; Joshi, 1979; Mohsin *et al*., 1991; Kolb *et al*., 1993; Romaniuk *et al*., 1993). Most of the studies conducted in Sindh region were centered upon the morphotaxonomy of the endoparasites of the rats (Frooq and Yousuf, 1986; Noor-un-Nisa and Ghazi, 1993). It is evident from the literature search that haematological studies in relation to bladder worm infection in the liver of *R. norvegicus* have not yet been conducted in Pakistan. Present investigation was, therefore, undertaken to observe changes in the haematological parameters due to bladder worm (Cestoda) infection in liver of *R. norvegicus*.

MATERIALS AND METHODS

During present investigation 200 (65 males and 135 females) brown rats, *Rattus norvegicus* (85.29 ± 2.53g body weight) were collected at random from houses of Hyderabad and its adjoining areas and kept in laboratory (4 rats/cage). They were fed with vegetables, fruits, grains, bread, insects and water *ad libitum.* Autopsy of all rats was done to know the natural parasitic infection in these hosts. In the text the term "Infected rats" refers those which harbour bladder worm in the cysts, where as non-infected rats are control rats.

Collection of parasites from the rats

Rats were anaesthetized with chloroform. Viscera were completely exposed by transverse cuts in the body. Liver was gently removed and

transferred to the Petri dish (6” in diameter) containing normal saline solution (0.9% NaCl). Thorough examination of the liver revealed whitish cysts which were gently removed from the tissue. Each cyst was then cut with the fine scissor under the microscope to remove the bladder worm from the cyst and then preservation, fixation and staining of the bladder worm was carried out by the method described by Fatima (1991). The bladder worms were identified with the help of the description previously given by Soulsby (1978) and Cable (1985).

Blood sampling and procedure

Blood was collected directly from the heart by the syringe and immediately transferred to the vial containing a mixture of ammonium and potassium oxalate as anticoagulant (Wintrobe, 1961). The haematological parameters were determined by the methods described by Wintrobe (1961) and Swarup *et al*. (1989).

Statistical analysis

Data obtained were subjected to Student's t-test to determine the significant difference between non-infected (control) and infected rats (Christian, 1986). Statistical significance was accepted at $P \leq 0.05$ (Rohlf and Sokal, 1981). Data are expressed as Mean ± SEM.

RESULTS AND DISCUSSION

Present study reports the changes in haematological parameters due to bladder worm infection in the liver of rats. The bladder worm stages of *Hydatigera taeniaeformis* were recovered from the liver of the brown rats, *Rattus norvegicus*. These bladder worms were identified as *Cysticercus fasciolaris* (Soulsby, 1978; Cable, 1985). The same has also earlier been reported by Faiyaz-ul-Haque *et al*. (1990) and Fatima (1991) from the *Rattus rattus* of Rawalpindi–Islamabad and Multan, respectively. Table I shows the incidence of natural parasitic infection in rats. Bladder worm infection in rats was found to be 16% (6% in male and 10% in female), whereas the infection caused by other parasites was 64% (19% male and 45% female). The bladder worm infection in rats was much higher than that of Fatima (1991) who reported 0.88% of such infection. However, it was lower than that of Faiyaz-ul-Haque *et al*. (1990) who observed 35% of *Cysticercus fasciolaris* infection in *Rattus rattus* of Rawalpindi - Islamabad.

TABLE I.- INCIDENCE OF NATURAL PARASITIC INFECTION IN RATS. (VALUES IN PARENTHESIS INDICATE THE % OF INFECTION).

Nature of infection	**Rats**		**Total**
	Male	**Female**	
Non-infected (Control)	15	25	40
Bladder worm infection	12 (6)	20 (10)	32 (16)
Other parasitic infection	38 (19)	90 (45)	128 (64)
Total	65	135	200

Erythrocyte indices observed during present study are shown in Table II. The results indicate a significant fall (P< 0.001) in TEC and Hb concentration of male and female rats during bladder worm infection as compared to the control rats of both sexes. This observation is in agreement to those reported by Ogunrinade and Bomgboxe (1980), Haroun (1986), Chaudri *et al*. (1988) and Mohsin *et al*. (1991) for cattle and buffaloes naturally infected with *Fasciola gigantica*. Reddy *et al*. (1989) also observed significant fall in the erythrocyte count and subsequent decrease in Hb content in fish infected with haemoflagellate.

TABLE II.- ERYTHROCYTE INDICES OF BLADDER WORM INFECTION IN LIVER.

Parameters	**Male**		**Female**	
	Control (n=15)	**Infected (n=12)**	**Control (n=25)**	**Infected (n=20)**
TEC / cmm	5.83 ± 0.11	4.52 ± 0.15**	4.45 ± 0.06	3.5 ± 0.08**
Hb %	14.82 ± 0.13	9.06 ± 0.17**	11.36 ± 0.11	6.83 ± 0.11**
ESR mm /1 h	0.11 ± 0.01	0.11± 0.00	0.11 ± 0.00	0.12 ± 0.01
PCV %	4.41 ± 0.08	3.65 ± 0.07**	3.90 ± 0.05	3.00 ± 0.14**

Significant: **p < 0.001

It is well established that different metabolic pathways take place in liver cells. Due to growth of cyst, compression develops in the liver tissue and the surrounding cyst wall. Under such condition some toxic end-products of metabolism (Chappell, 1980) might be released from the cyst and do come in extra-cellular fluid resulting in toxic effects. Such toxic effects cause abnormal functioning of the liver, which in turn depress the haemopoietic system. The significant fall in the values of TEC, as revealed in Table II can therefore be attributed to the toxic effect on the production of the erythrocytes. Significantly lower (P<0.001) Hb level, as recorded in

bladder worm infected male and female rats (Table II), may be the result of decreased value of TEC. The reduction in Hb level is an important factor, which causes anemia in rats and may lead to its death.

The parameter of ESR was also investigated. The results revealed no significant change in ESR values recorded for the infected and control male and female rats (Table II). The PCV value recorded for the infected male and female rats was significantly lower (P<0.001) than that of the control rats of both sexes. Similar observations were made by Ogunrinade and Bomgboxe (1980), Haroun (1986), Chaudri *et al*. (1988) and Mohsin *et al*. (1991) for cattle and buffaloes naturally infected with *Fasciola gigantica*.

The values of TLC recorded from infected male and female rats was significantly higher (P<0.001) than that of the control male and female rats. Cestodes are endoparasitic, living as adult in the lumen of the vertebrate intestine and as larvae in the tissues of either invertebrates or vertebrates (Chappell, 1980; Cheng, 1986). Bladder worm stages (*Cysticercus fasciolaris*) were recorded from the liver of *R. norvegicus*. Although some larger parasites (*e.g*., the larvae of tape worms) form cysts in host tissues that are covered by a dense fibrous capsule that walls them off from host immune responses (Samuelson, 1997). Yet these stages, unlike the lumen-dwelling cestodes, are in more intimate contact with host defence mechanisms (Chappell, 1980; Cheng, 1986). The tissue stages, as indicated above, are antigenically intimate with the host. Under such condition, perhaps the toxic end-products of metabolism (Chappell, 1980) and inorganic materials (Salisbury and Anderson, 1939) come in contact with the blood which consequently trigger the immune response resulting in the increased TLC. Such significant changes have also been observed during *Fasciola* infection in cattle (Mohsin *et al*., 1991) and haemoflagellate infection in fish (Reddy *et al*., 1989).

During DLC, the values of monocyte recorded for the infected male and female rats, were found significantly lower (P<0.001) than that of the control rats of both sexes (Table III). This result is in contradictory to Mohsin *et al*. (1991). The difference in the results of present study and that of Mohsin *et al*. (1991) may be due to different types of parasitic infection and hosts as well. However, the fall in the values of monocyte could not be explained now, however, this might be associated with the metabolic activity and the defense mechanism of the host. Lymphocytes, a type of leucocytes that produce antibodies, are main constituents of immune system and regulate this

mechanism which is also a defense against the attack of pathogens (Tagliasacchi and Carboni, 1997; Anonymous, 2001). Monitoring the lymphocyte counts is one way to examine the deficiency of the immune system (Anonymous, 2001). In the present study lymphocyte counts were found lower than normal in male infected rats and greater than normal in female infected rats (Table III). Such variations in lymphocyte counts may be associated with the intensity of immune responses in rats harboring the bladder worm infection in liver.

TABLE III.- LEUCOCYTE COUNTS OF BLADDER WORM INFECTION IN LIVER.

Parameters	**Male**		**Female**	
	Control (n=15)	**Infected (n=12)**	**Control (n=25)**	**Infected (n=20)**
TLC / cmm	2823.26 ± 17.89	3502.25 ± 78.58**	1981.80 ± 49.49	4098.15 ± 132.49**
DLC/ cmm				
Monocytes	103.67 ± 3.53	67.08 ± 6.75**	103.00 ± 3.46	63.55 ± 5.54**
Lymphocytes	1998.87 ± 7.45	1453.50 ± 55.76**	1223.40 ± 2.95	1620.65 ± 48.44**
Neutrophils	651.20 ± 7.06	1902.67 ± 55.73**	603.32 ± 3.92	2290.90 ± 49.67**
Eosinophils	54..27 ± 4.36	55.42 ± 8.01	35.08 ± 3.06	92.25 ± 7.21**
Basophils	16.80 ± 3.67	23.33 ± 4.97	16.00 ± 2.00	28.70 ± 5.24*

Significant: $^{*}p < 0.02$, $^{**}p < 0.001$.

Neutrophil count recorded for the infected male and female rats was found significantly higher than that of the control male and female rats (Table III). Similar observation was made by Mohsin *et al*. (1991) in infected adult cattle. It seems that the neutrophils become more active during bladder worm infection, thus resulting in the increase of the neutrophil counts. It indicates the triggering of the immunological phenomenon in the infected host (*Rattus norvegicus*).

Eosinophil values showed significant difference between the infected and control female rats, but male rats did not show any significant change as compared to their control. Mohsin *et al*. (1991) recorded eosinophilia in cattle infected with *Fasciola gigantica*, whereas Reddy *et al*. (1989) observed eosinophilia in fish infected with haemoflagellate. Their observations were not based on sexes of the hosts, hence can't be compared with the present results. Eosinophilia is caused due to allergic disorder, parasitic infestations, miscellaneous disorders and Loeffler's syndrome (Wintrobe, 1961). It appears that there exists no allergic condition in male harboring bladder worm, or there may be some other factor not affecting the eosinophil count.

Basophil count like that of eosinophil, showed significant change in female rats as compared to the control female rats but male rats did not show significant difference between infected and their control male rats. In contrast to the present result a slight increase in the number of basophils was noticed in the infected fish (Reddy *et al*., 1989).

In conclusion, bladder worm infection in the liver causes changes in most of the haematological parameters, suggesting physiological state and general health of the rats. The observed alterations in blood picture may be attributed to the metabolic activities, toxic end-products of bladder worm stages and defense mechanism of the host as well. More over, this study also provides information to understand the host-parasite interaction.

The results obtained during present investigation are reported for the first time from the brown rats *Rattus norvegicus* collected from the areas understudy.

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REFERENCES

ANONYMOUS, 2001. *Blood work: A useful tool for monitoring HIV*. Project Inform, Inc., San Francisco, CA.

BILQEES, F. M., 1985. Cestodes of vertebrates in Pakistan. *Proc. Parasitol*., **1**: 39-144.

BILQEES, F. M. AND SIDDIQUI, I. H., 1981. Two cestodes from rats in Karachi. *Pakistan J. agric. Res*., **2**: 68-70.

CABLE, M. R., 1985. *An illustrated laboratory manual of parasitology*. 5th ed. Surajeet Publications, Delhi.

CHAPPELL, L. H., 1980. *Physiology of parasites.* 1st ed. Blackie & son Ltd., Glasgow.

CHAUDRI, S. S., MANDOKHOT, V. M., GUPTA, R. P. AND YADAV, C. L., 1988. Haematological and biochemical observations in buffaloes naturally infected with *Fasciola gigantica*. *Indian Vet. J*., **65**: 23-27.

CHENG, T. C., 1986. *General parasitology*. 2nd ed. Academic Press, Inc. Orlando, Florida, USA.

CHRISTIAN, G. D., 1986. *Analytical chemistry.* 4th ed. John Wiley & Sons, New York.

CRAWFORD, J. M., 1997. The liver and biliary In: *Basic pathology* (eds. V. Kumar, R.S. Cotran and S.L. Robbins) 6th ed., pp. 517 - 555. W. B. Saunders Company, USA.

FAIYAZ -UL-HAQUE, M., QAYUM, M. AND PAL, R. A., 1990. Prevalence of helminth parasite in the digestive tract of house rat *Rattus rattus rufescens* in the twin cities of Rawalpindi -Islamabad. *J. Sci. Tech*., **14**: 65-70.

FATIMA, T., 1991. *Ecto and endoparasites of rat* (Rattus rattus) *of Multan and Khanewal (Punjab: Pakistan)*. M.Sc thesis, Institute of Pure and Applied Biology (Zoology Division). Bahauddin Zakria university, Multan.

FRISELL, W. R., 1982. *Human biochemistry*. Macmillan Publishing Co. Inc, New York.

FROOQ, M. AND YOUSUF, S., 1986. A new trematodes from the intestine of *Rattus Rattus* from campus, University of Karachi. *Pakistan J. Zool*., **18**: 341-343.

GREEN, N. P. O., SHOUT, G. W. AND TAYLOR, D. J., 1986. *Biological Science 1 Organisms, energy and environment* (ed. R. Soper), vol. 1, Cambridge University Press, Cambridge.

HAROUN, E. M., GADIR, H.A. AND GOMEL, A. A., 1986. Studies on naturally occurring ovine fascioliasis in Sudan. *J. Helminthol*., **60**: 47-53.

JOSHI, B. D., 1979. Biochemical changes in the liver and blood of a fresh water fish, *Rita rita*, infested with a trematode *Opisthorchis pedicellata. Folia Parasitol*., **26**: 143-144.

KOLB, E., REHBEIN, S., RIBBECK, R., ALAWAD, A., LEO, M. AND SIEBERT, P., 1993. The behavioure of haematological values (haemoglobin, haematocrit, leukocyte count) and of clinico chemical values in the plasma (glucose, total protein, alpha-amino-N, urea, pepsinogen, ascorbic acid, Fe, Cu, and Zn) as well as that of ascorbic acid in the liver, spleen and adrenal glands in healthy lambs and in lambs infected with *Haemonchus contortus* and *Trichostrongylus colubriformis*. *Berl. Munch. Tierarztl. Wochensch*., **106**: 411-418.

KAMESWARI, M., RAMULU, G. R. AND RAO, L. N., 1975. Effect of helminth infections on the macromolecuar contents of the liver of the *Rana tigrina. Ind. J. exp. Biol*., **17**: 976-979.

MOHSIN, M., RAHMAN, M. M., DAS, P. M. AND FAZLUL HUQUE, A. K. M., 1991. Haematological observations in cattle naturally infected with *Fasciola gigantica. Bangladesh Veterin*., **8**: 31-34.

NOOR - UN -NISA AND GHAZI, R. R., 1993. Parasitic infections of rats collected from commercial areas of Karachi city. *Abstract, Nat. Symp. Parasitol*. Dept. of Zool. University of Sindh , Jamshoro., (8-9th Dec, 1993), p. 3.

OGUNRINADE, A. E. AND BOMGBOXE, F.A., 1980. Haematological indices and their correlation with worm burden in chronic fascioliasis. *Br. Vet. J*., 136: 457- 462.

REDDY, Ch. PURUSHOTHAM. V., RAJENDRANATH, T., DAYAKAR, P. AND BANERJI, G., 1989. Some haematological changes in *Mystus vittatus* infected with a haemoflagellate. In: *Recent research in zoological science* (eds. C. B. Srivastava and K. C. Pandey), pp. 77–80. Shree Publishing House, New Delhi.

ROHLF, F. J. AND SOKAL, R. R., 1981. *Statistical tables.* 2nd ed. W. H. Freeman and Company, New York.

ROMANIUK, K., MICHALSKI, M., SOKOL, R. AND SZELAGIEWICZ, M., 1993. Influence of *Eimeria* spp. and round worms on the level of some haematological and biochemical indices in the blood of lambs. *Medycyna Waterynary*, **49**: 273-275.

SALISBURY, L. F. AND ANDERSON, R. J., 1939. Concerning the chemical composition of

Cysticercus fasciolaris. J. biol. Chem., **129**: 505-517.

SAMUELSON, J., 1997. General Pathology of infectious diseases In: *Basic pathology* (eds.V. Kumar, S. Ramzi, S. Cotran, and S.L. Robbins), 6th ed., pp. 274-275. W. B. Saunders Company, USA.

SOULSBY, E. J. L., 1978. *Helminththes, arthropods and protozoa of domesticated animals*. 6th ed. Bailliere, Tindall and Cassell, Ltd., London.

SWARUP, H., ARORA, S. AND PATHAK, S. C., 1989. *Laboratory techniques in modern biology*. 2nd ed. Kalyani Publishers, New Delhi, India.

TAGLIASACCHI, D. AND CARBONI, G., 1997. *Let's oberve the blood cells*. Giorgio Carboni.

WINTROBE, M. M., 1961. *Clinical hematology* 5th ed. Lea & Febiger, Philadelphia.

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STUDIES ON HABITATS AND LIFE CYCLE OF *POEKILOCERUS PICTUS* (FABRICIUS) (ORTHOPTERA: ACRIDOIDEA) IN THE DESERT AREA OF LASBELLA, BALOCHISTAN

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Abstract.- The studies on habitats and life cycle of 'Ak' grasshopper, *Poekilocerus pictus* (Fabricius), were conducted in the desert area of Lasbella, Balochistan, in field conditions. Both hoppers and adults cause damage to the plant `Ak', *Calotropis procera,* and other agricultural crops. Number of eggs per pod varies from 90 to 210. The non- diapause and diapause eggs hatch after 8-10 months. Duration of hopper stage lasts 2-7 months in winter season and 1-3 months in summer season. It complete one generation in a year.

Key words: 'Ak' grasshopper, *Poekilocerus pictus*, life cycle, egg laying, duration of hoppers, Orthoptera.

INTRODUCTION

The 'Ak' grasshopper, *Peokilocerus pictus* (Fabricius), is a serious pest of plant 'Ak', *Calotropis procera* (Wild), R.Br.I.C. in Pakistan. Moizuddin (1987, 2002) reported its damage apart from 'Ak' plants to other agricultural crops such as castor, papaya and water melon in the area of Lasbella, Balochistan. Similarly, Ghouri (1957) reported its attack on cotton seedlings, melon, chilli and vegetables from the area of Punjab, Pakistan. The damage caused by this pest has also been reported from different parts of India on bamboo, citrus plants, papaya, lady's finger, brinjal, tomato, castor and flowers (Pruthi and Nigam, 1939; Mathur, 1944; Pruthi, 1954; Batra, 1955; Atwal, 1993).

The life cycle, habitats, egg laying, diapause, non-diapause of eggs and hopper development period vary greatly from place to place. Taking into consideration its economic importance and gap in our knowledge on its habitats and life cycle in field conditions, the the present work was undertaken.

MATERIALS AND METHODS

Regular survey and studies were conducted in the desert area of Chachai, District Lasbella, Balochistan for five years. The collection of solitary adults was made atleast twice in a month. The average number of adults collected per man, per hour, per month was calculated from the collection of adults made by different collectors. The collection was made between 1000 hours to 1300 hours.

RESULTS

Location

The studies on emergence and development of `Ak' grasshopper *Poekilocerus pictus* (F) were conducted in the breeding place in the desert area of Chachai, district Lasbella, Balochistan. It is situated between 25 15°N and 66 45°E. It is about 5 kilometers away from the Coastal area of Gadani and the soil is sandy.

Climatic conditions

During summer season from April to October the maximum temperature ranges from 26°C to 46°C and the minimum temperature ranges from 12°C to 29°C and during winter season from November to March the maximum temperature ranges from 16°C to 35°C and the minimum temperature ranges from 1°C to 12°C. The rain may occur in summer season during July to September and in winter season during December to March. The average rainfall is 150 mm in summer season and 32 mm in winter season. The average rain fall is 183 mm per annum. Sometimes there is no rain for a year or two.

Life cycle

The life cycle comprises egg, hopper and adult stages and vary greatly in each stage in number of days. The temperature and the seasonal conditions appear to be the most important factors in each development stage.

Habitats

The 'Ak' grasshopper is fond of leaves and twigs of 'Ak' plant *Calotropic procera*. However, they also attack papaya, castor and water melon in the nearby field and damage leaves.

Egg laying

The egg laying occurs during the summer season from July to October in the light sandy soil near the roots of `Ak' plant. The egg pods were laid at the depth of 1-3 inches. The number of the eggs per pod varies from 90 to 210 with an average of 140 eggs.

Diapause and non-diapause eggs

The eggs usually remain in diapause till next summer season and hatch in April and May with the rise of temperature. The eggs remain in diapause for 8-10 months. However, some of the eggs hatch in the same summer season during September to November. In this the case the eggs remain in incubation period for 1-4 months. Thus both diapause and non-diapause eggs have been observed.

Hopper development

The hoppers which hatched in April and May from the diapause eggs become adult in June and July. The hopper development period lasts for 1-3 months. The hoppers which hatched during September to November from non-diapause eggs become adult during January to April. The hopper development period in this case lasts for 2-7 months. The duration of hopper development period is shorter in summer season and longer in winter season.

Maturation

The fledgling or immature adults become adult within a week and start copulation. The duration of copulation lasts from few minutes to several hours. It completes one generation in a year.

Emergence of adults

The adults usually appear on the 'Ak' plants in summer season in June and July. The population rapidly increase in August and become in abundance. The population gradually decreases in the following months from September to December. The fresh adults again appear in winter season in the month of January and onwards. The population increases in the month of March. The population gradually decreases from the month of April and become lowest in May.

Seasonal variation

Table I shows the average number of adults collected per man, per hour, per month from January to December. During the summer season the population was in peak in August and during the winter season the population was in peak in March. Figure 1 shows the line of graph of seasonal variation of adults in winter and summer season.

TABLE I.- AVERAGE NUMBER OF ADULTS COLLECTED PER MONTHS, PER HOUR, PER MONTH AT CHACHI, LASBELLA, BALOCHISTAN.

Months	**No. of adults**
January	2
February	3
March	6
April	3
May	1
June	4
July	8
August	11
September	7
October	5
November	3
December	1

Fig. 1. Average number of adults collected per man, per hour, per month at Chachi, Lasbella, Balochistan.

DISCUSSION

The number of the eggs per pod varies greatly. In the present study, 90 to 210 eggs with an average of 140 eggs has been noted. The number of the eggs varying from 60 to 241 per pod has been recorded by various authors (Pruthi and Nigam, 1939; Vanugopal, 1959; Sheri, 1976; Syed *et al*., 1993; Moizuddin, 2002), which is almost similar to the present findings.

Pruthi (1939) noted that the eggs usually undergo embryonic diapause, but sometimes there is no embryonic diapause and hatching occurs about one month after laying. Pruthi and Nigam (1939) noted that the egg laying occurs during the monsoon rains from June to September and the eggs over winter and hatch in the next spring during March to April. Mathur (1944) noted that the egg laying occurs in the beginning of the monsoon rains from May to July. Syed *et al*. (1993) noted incubation period as 59.78 days. Moizuddin (2002) noted both non-diapause and diapause eggs in controlled and in room temperature. He further notd that the eggs laid in the early summer are usually non-diapause eggs and the eggs laid in the late summer are usually diapause eggs. The incubation period has been recorded as 53 to 68 days in non diapause eggs and as 157 to 191 days in diapause eggs.

In the present study it has been noted that the eggs are laid during the summer season from July to October. Some of the eggs hatch during September to November after 1-4 months but most of the eggs hatch in the next summer season in April and May after remaining in diapause for to 8-10 months. The results correlate with those of the above authors.

Venugopal (1959) noted that the hopper development takes 55 to 60 days and the adults appear from May to July. Syed *et al*. (1993) noted that the duration of life cycle is 195.83. Moizudding (*op.cit.*) noted that hoppers which hatched from non-diapause eggs develop in winter season and become adult in 157 to 230 days, while the hoppers which hatch from diapause eggs develop in summer season and become adult in 50 to 88 days. In the present study it has been observed that the hoppers which hatch in April and May from the diapause eggs become adult in June and July after 1-3 months while the hoppers which hatch during September to November from non-diapause eggs become adult during January to April after 2-7 months. The findings slightly vary from each other.

REFERENCES

ATWAL, A.S., 1993. *Agricultural pests of India and South East Asia*. Kalyani publisher, New Delhi.

BATRA, H.N., 1955. The ‘Ak’ grasshopper *Poekilocerus pictus* (Fbr.) (Acrididae) as a pest of papaya and some other plants in Delhi. *Indian J. Ent*., **17**: 132.

GHOURI, A.S.K., 1975. *Poekilocerus pictus* in the Punjab. *Plant Prot. Bull. F.A.O.,* **23**: 52-53.

MATHUR, R.N., 1994. Bamboo defoliators. *Indian J. Ent.,* **5**: 117-128.

MOIZUDDIN, M., 1987. Aspects of external morphology of *Poekilocerus pictus* (Fabracius) (Orthoptera:Acriodoidea) with special reference to its genetalia and their bearing on classification. *Proc. Pakistan Congr. Zool*., **7**: 135-144.

MOIZUDDIN, M., 2002. Studies on habitats and life cycle of *Poekilocerus pictus* (Fabricius) (Orthoptera: Acridoidea) in laboratory conditions. *Proc. Pakistan Congr. Zool*., **22**: 37-42.

PRUTHI, H.S. 1939. Report of the imperial entomologist. *Scient. Rep. Indian Agric. Res. Inst.,* 1937-1939: 113-128.

PRUTHI, H.S., 1954. The ‘Ak’ grasshopper *Poekilocerus pictus* (Fabr.) (Acrididae) attacking citrus in Delhi. *Indian J. Ent.,* **16**:196.

PRUTHI, H.S. AND NIGAM, L.N., 1939. The bionomics, life history and control of the grasshopper, *Poekilocerus pictus* (Fabr.). *Indian J. agric. Sci*., **9**: 629-641.

SHERI, A.N., 1976. Reproduction of a common grasshopper *Poekilocerus pictus* Fabricius. *Pakistan J. agric. Sci*., **13**: 37-40.

SYED, T.S., AWAN, M.S., WAGAN., M.S. AND SHAH, A.A., 1993: Effect of food plant on the biology of *Poekilocerus pictus* (Pyrogomorphidae: Acridoidea: Orthoptera). *Proc. Pakistan Congr. Zool*., **13**: 449 – 456.

VENUGOPAL, S. 1959. *Poekilocerus pictus* Fabr. A painted grasshopper pest on *Calotropis gigentea* in South India. *J. Madras Univ.* (B), **29**: 1-8.

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DIEL PATTERN OF FEEDING OF SOME CATFISHES FROM CHENAB RIVER, PUNJAB, PAKISTAN*

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Abstract.- Diurnal feeding of seven species of catfishes *viz*., *Sperata sarwari*, *Mystus bleekeri*, *Mystus vittatus, Wallago attu, Ompok bimaculatus, Bagarius bagarius* and *Heteropneustes fossilis* were studied during the first week of the month of October 1993 in Chenab River, Punjab, Pakistan. The stomachs of catfishes contained food almost throughout the 24 hours period. The index of stomach fullness showed two peaks in the feeding cycles of catfish at the twilight period of dusk and dawn. Seven kinds of food group organisms were identified in the stomachs of catfishes and their feeding periodicities during the 24-hour period were established.

Key words: Catfish, Diel pattern of feeding, dusk and dawn, feeding chronology.

INTRODUCTION

A knowledge of the food of fish and its feeding behaviour can help in understanding ecological relationships and therefore, in management. Many contributions to the food and feeding habits of freshwater catfishes (Sandhu, 2000) are available, but little work has been done on the daily feeding chronology of freshwater catfishes. The valuable works in this regard are those of Javaid (1970, 1971) and Shrivastava *et al*. (1992). The present work, therefore, was undertaken to ascertain the feeding periodicity, diversity of food organisms and the relative amount of ingested food by the seven catfishes of the Punjab, Pakistan, during 24 hours.

MATERIALS AND METHODS

The seven catfishes (*S. sarwari, M. bleekeri, M. vittatus, W. attu, O. bimaculatus, B. bagarius* and *H. fossilis*) were collected every two hours over a period of 24 hours from two sampling stations *viz*., Khanki Headworks and Qadirabad Barrage in Chenab River, District Gujranwala,

* Part of Ph.D. thesis of first author.

Punjab, Pakistan, by cast net and gill netting during the first week of the month of October, 1993. Attempts were made to have at least 5 fishes for each of twelve samples as far as possible. All catfishes were injected with 10% formalin to prevent any digestion due to enzymatic activity in the digestive tract, immediately after catching. The body cavity was opened and the gut was removed in its entirety. The food contents of the maximum number of available specimens were analyzed to avoid bias. The specimens were finally preserved in 10% formalin for later examination of stomach contents. The stomach contents were released and placed in specimen tubes for macroscopic and microscopic analysis. The food organisms were classified taxonomically according to Ward and Whipple (1959), Mellanby (1963) and Needham and Needham (1988). Fishes were classified taxonomically according to Mirza (1990), Mirza and Sharif (1996) and Jayaram (1999). The percentage composition of different food groups in the gut contents of the catfishes was studied.

RESULTS

The feeding spectrum of catfishes is extremely broad and were categorized into following groups: Algae, Crustacea (Cladocera, Copepoda, Decapoda, Amphipoda), Insecta (Coleoptera, Diptera, Hemiptera, Ephemeroptera, Odonata, Trichoptera, Plecoptera), eggs of invertebrates, Mollusca, teleosts, and debris (organic matter). The stomachs of all the catfishes studied contained food almost throughout the 24 hours period. *Sperata sarwari, W. attu, O. bimaculatus, B. bagarius* depended on fish as most important food, while insects and crustaceans came as second food category. These fishes fed on smaller fish in daylight and insect larvae and crustaceans mostly in darkness while *B. bagarius* and *W. attu* also feed on fishes in darkness along with insects and crustaceans. In *H. fossilis* and *Mystus* spp. the most dominant food were insect larvae and molluscs while crustaceans were the second most important food category. These fishes fed mainly on insect larvae in daylight and molluscs, crustaceans during night. The diel activity of feeding chronology shows that all the catfishes show maximum indices of stomach fullness after the periods of dawn and dusk *i.e.*, at twilight period (Fig.1, Table I). In the present study indices of stomach fullness showed that the peak feeding occurred at 06.00 and 18.00 hours in the month of October.

Fig. 1. Diurnal pattern of relative gut contents in percent of body weight in seven catfishes of the Punjab, Pakistan.

DISCUSSION

Forage fish were the most important food for *W. attu, B. bagarius, O. bimaculatus* and *S. sarwari*. The second most important food category was insect larvae. It was also noticed that *B. bagarius, W. attu, O. bimaculatus* and *S. sarwari* fed on smaller fish in daylight and insects larvae mostly in the darkness. Crustaceans and organic matter ranked third in order of importance as indicated by their percentage of occurrence of *B. bagarius, W. attu, S. sarwari* and *O. bimaculatus*. In *H. fossilis, Mystus bleekeri* and *M. vittatus* the most dominant food throughout the year was insect larvae and molluscs. Organic matter and other food groups (eggs of invertebrates, Cladocera, Copepoda and Decapoda) came as the second most important food category. *H. fossilis* fed mainly on insect larvae and organic matter in the light hours. In the present study similar food groups were encountered from the gut contents of all the catfishes as described by Qayyum and Qasim (1964), Bhatt (1968, 1970), Chaudhary (1971), Vinci (1986) and Shrivastava et al. (1992).

Among animals, the most common types expected to fall prey to the aggression of fish are those, who are the most sluggish, and therefore, incapable of darting away towards a shelter of thick vegetation etc. This is however, not the case. Molluscs, which among animal food of fish are the slowest, were found in much lesser number in fish guts, than were the adult insects and crustaceans. This seems to be because of their shells, which make them "hard nuts" for fish to crack. The rather repulsive odour, given out by

some molluscs, such as that by gastropods, seems to be another reason for lack of popularity of these animals as food for catfish. Also, their comparatively larger size makes them a much bigger "morsel" for small fish to swallow. Among Arthropoda, the immature stages, such as larvae, pupae, and nymphs were found in much greater number in catfish guts than were the adults, perhaps, because they have all the excellent qualities of becoming food for fish. They are sluggish or sedentary, and therefore, easy to catch; and they are small and soft, and therefore, easy to swallow. The comparatively more active and immature forms like nymphs, fall prey to fish, perhaps because of their unfamiliarity with the predators as a dangerous mate, and perhaps also because of lack of experience to escape to proper hide outs from predators.

The fluctuations and variations, in the amount and type of food items of the same fish, seem to be related with seasonal and daily abundance and availability of food items, locality, extent of availability of hiding places, such as aquatic vegetation and bottom contour of a body of water. Age and size of fish is another factor, which results in the observed variation in the gut contents of fish. At younger stages of life, fishes can utilize smaller food items. When the fish attains a larger size, they divert to larger food items, which can then not only be swallowed easily, but also satisfy the dietary needs of fish in much shorter time. It would take a very long time to satisfy the appetite, if smaller food items were utilized by larger fishes.

In the present study it was observed that *S. sarwari, W. attu, O. bimaculatus* and *B. bagarius* feed on surface and sub-surface zone, while *H. fossilis, Mystus* spp. fed on benthic zone. It was also observed that *O. bimaculatus* and *S. sarwari* fed mainly on smaller fish in daylight and insect larvae mostly during night, whereas *W. attu* and *B. bagarius* mainly feed on fishes throughout 24 hours. This may be due to the fact that majority of insect larvae commonly exhibit diel vertical migration moving down towards the bottom of the water body during the day (Peterson *et al*., 1982). The relevance of this behavior to the present study is that the vertical migrations make the fish food least available during the day. Thus, due to the restrictions of prey availability, surface and sub-surface feeder catfish, *S. sarwari* and *O. bimaclatus* fed mainly on forage fish in daylight and in darkness feed on insect larvae and other food groups. In *H. fossilis* and *Mystus* spp., reverse feeding pattern was recorded. The maximum percentage of insect larvae was reported in daylight and Mollusca, Decapoda and organic matter during the

night. Similar to insect larvae, one of the most striking phenomena manifested by plankton is the diurnal vertical migration of certain planktons. Of the zooplanktons, planktonic Crustacea (Cladocera and Copepoda) comprise very largely the species, which exhibit diurnal vertical movements. Certain rotifers also display diurnal movements. There occurs an upward movement of the algae, starting in early hours of the morning, climaxing in the late afternoon and a downward migration starting at dusk and culminating at pre-dawn hours (Jhingran, 1991).

Many field studies have dealt with diel variation in fish feeding activity. Some studies on plankton feeders and browsers indicate that they feed continuously throughout the day, *i.e.*, light hours (Elliott, 1976). However, the great majority of studies have shown that fish have distinct preferred feeding time (Hoar, 1942; Sarker, 1977; Noeske and Spieler, 1984; Shrivastava *et al*., 1992). In the present study maximum fullness of stomach indices was found during twilight periods *i.e.*, shortly before sunrise and after sunset. Indices of stomach fullness show that the peak feeding occurred at 06.00 and 18.00 hours during the month of October in all of the seven catfish species. More than three fourth fullness of stomachs occurred at 06.00 hours indicating the first peak in the feeding activity of all the catfish. At 16.00 hours stomach fullness was at lowest level and thereafter, feeding activity tended to increase. By 18.00 hours three fourth fullness of stomach occurred, indicating the second peak, after which the fullness of stomach showed a decline. The feeding activity of catfish mainly occurred during hours of darkness with two noticeable peaks, one at sunset and the other shortly before sunrise, but feeding evidently took place throughout the 24 hours period. This diel pattern, with the dusk and dawn peaks in stomach fullness, are similar to those reported by Javaid (1970, 1971), Daham *et al*. (1977) and Shrivastava *et al*. (1992) from aquatic bodies of Pakistan, Iraq and India.

Twilight is a time of transition between daytime and nighttime modes, when diurnal fishes cease activity and nocturnal fishes initiate feeding. Twilight, or crepuscular, periods occur during each diel cycle: at dusk and dawn. Evening twilight lasts from sunset until the sun is at a certain angle below the horizon (180 below for astronomical twilight). At tropical latitudes, twilight generally lasts between 70 and 85 minute, depending on the time of year. Taken together, dusk and dawn amount to only 5% of the 24 hour diel cycle, but ecologically, twilight may play a role in the lives of fishes out of proportion to the actual time involved. Two keys to

understanding this role are first, that twilight is a period of environmental, behavioural and ecological transition, and second, that predators exploit the transitional nature of these periods (Helfman, 1993). Thus catfishes, being of predatory nature, exploit this time for feeding to their maximum advantage. Environmental transitions primarily involve changes in light. Illumination levels at the surface fall from approximately 100 lux at sunset to about 0.01 lux 30 minute later. During this period, diurnal fishes 'change over' from their daytime activity mode to their nighttime inactivity mode, whereas nocturnal fishes initiate activity. During twilight, predators seem to be maximally active and successful (Helfman, 1993).

Catfishes are visual feeders and feed on benthic, midwater and surface organisms (Shrivastava *et al.*, 2001). Several fish species that feed on active prey adopt the strategy of attacking obliquely from below. This mode of attack is likely to be particularly effective under relatively low light conditions (*e.g.* at twilight), where only in the downward direction is there sufficient light for vision. Thus, a number of predatory fish species including catfishes appear to concentrate their hunting and feeding bouts to the periods around dusk and dawn (Jobling, 1995).

Catfishes live in hides, at the bottom, and frequently leave it to rise to surface to perform their daily chores of feeding, reproduction and social communication. Their locomotory activity is characterized by surfacing movement. Few catfishes have been studied for their diel activity pattern and invariably they have been found to be nocturnal, *i.e.*, resting by day and active by night, *viz. Mystus vittatus* (Devi, 1987) and *Heteropneustes fossilis* (Devi, 1987). The diel pattern of locomotor activity of catfishes is found to be light dependent. A clear circadian rhythm in locomotor activity of catfishes is discernible much in the same way as for air breathing and feeding (Boujard *et al*., 1990).

REFERENCES

BHATT, V.S., 1968. Studies on the biology of some freshwater fishes. Part VII. *Heteroponeustes fossilis* (Bloch). *Indian J. Fish*., **15**: 99-115.

BHATT, V.S., 1970. Studies on the biology of some freshwater fishes. Part IV. *Mystus seenghala* (Sykes). *J. Bombay Nat. Hist. Soc*., **67**: 194-211.

BOUJARD, T., KEITH, P. AND LUQUET, P., 1990. Diel cycle in *Hoplosternum littorale* (Teleostei): evidence for synchronization of locomotor, air-breathing and feeding activity by circadian alternation of light and dark. *J. Fish Biol*., **36**: 133-140.

CHAUDHARY, H., 1971. Culture of air breathing fishes. In: *First workshop report of all India coordinated research project on culture of air breathing fishes*, held at Cuttack, 6 p.

DAHAM, N.A.K., LATIF, S.A. AND BHATTI, M.N., 1977. Diel pattern of feeding in *Heteropneustes fossilis* from Southern Iraq. *Trans. Am. Fish. Soc*., **106**: 614-616.

DEVI, U., 1987. *Studies on the pseudobranchial neurosecretory system of certain teleosts with particular reference to its function*. D.Phil. Thesis, University of Allahabad, India.

ELLIOTT, G.V., 1976. Diel activity and feeding of schooled large mouth bass Fry. *Trans. Am. fish. Soc*., **105**: 624-627.

HELFMAN, G.S., 1993. Fish behaviour by day, night and twilight. In: *Behaviour of teleost fishes* (ed. T.J. Pitcher), pp.479-512. Chapman & Hall, London, New York.

HOAR, W.S., 1942. Diurnal variations in feeding activity of young Salmon and trout. *J. Fish. Res. Bd. Can*., **6**: 90-101.

JAVAID, M.Y., 1970. Diurnal periodicity in the feeding activity of some freshwater fishes of West Pakistan. I. Studies on *Channa punctatus* and *Mystus vittatus*. *Pakistan J. Zool*., **2**(1): 101-111.

JAVAID, M.Y., 1971. Diurnal periodicity in the feeding activity of some freshwater fishes of West Pakistan. II. Studies on *Heteropneustes fossilis* and *Puntius sophore*. *Nucleus* (Karachi), **8**: 131-144.

JAYARAM, K.C., 1999. *The freshwater fishes of the Indian region*. Narendra Publishing House Delhi-110006(India).

JHINGRAN, V.G., 1991. *Fish and fisheries of India*, 3rd ed. Hindustan Publishing Corporation (India), Delhi, pp.1-727.

JOBLING, M., 1995. *Environmental biology of fishes*. Fish and Fisheries Series 16. Chapman & Hall, London.

MELLANBY, H., 1963. *Animal life in freshwater*. *A guide to freshwater invertebrates*. 6th ed. 308 pp. Methven & Co. Ltd., London.

MIRZA, M.R., 1990. *Freshwater Fishes in Pakistan*. Urdu Science Board, Lahore (In Urdu).

MIRZA, M.R. AND SHARIF, H.M., 1996. *A key to the fishes of the Punjab*. Ilmi Kitab Khana, Lahore, pp.32.

NEEDHAM, J.G. AND NEEDHAM, P.R., 1988. *A Guide to the study of freshwater biology*, 5th ed., 108 pp. Holden-Day, Inc., Oakland.

NOESKE, T.A. AND SPIELER, R.E., 1984. Circadian feeding time affects growth of fish. *Trans. Am. Fish. Soc*., **113**: 540-544.

PETERSON, W.T., BRODEUR, R.D. AND PEARCY, W.G., 1982. Food habits of Juvenile Salmon in the Oregon Coastal Zone, June 1979. *Fish. Bull*., **80**: 841-851.

QAYYUM, A. AND QASIM, S.Z., 1964. Studies on the biology of some freshwater fishes, Part I-III. *J. Bombay Nat. Hist. Soc*., **61**: 627-650.

SANDHU, A.A., 2000. *Comparative anatomical and histological studies of the gastrointestinal tract of some freshwater catfishes in relation to their food (Pisces : Siluriformes)*. Ph.D. thesis, Punjab University, Lahore.

SARKER, A.L., 1977. Feeding ecology of the blue gill, *Lepomis macrochirus* in two heated reservoir of Taxas III. Time of the day and pattern of feeding. *Trans. Am. Fish. Soc*.,

106: 596-601.

SHRIVASTAVA, S., RAO, K.S. AND DUBEY, R., 1992. Diel feeding patterns of some freshwater catfish from Kshipra River, India. *Pakistan J. Zool*., **24**(1): 27-29.

SHRIVASTAVA, S., RAO, K.S. AND SHUKLA, A. N., 2001. Benthic invertebrate fauna and feeding relationship of catfish from a tropical river, Kshipra River (M. P.), India. *Pakistan J. Zool*., **33**(4): 299-306.

VINCI, G.K., 1986. Biology of *Mystus seenghala* (Sykes) from Nagarjuna Sarga reservoir, Andhra Pradesh. *Indian J. Anim. Sci*., **56**: 814-821.

WARD, H.B. AND WHIPPLE, G.C., 1959. *Freshwater biology* (ed. W.T. Edmondson), 2nd ed, pp. 1248. John Wiley & Sons, New York.

TABLE I.- DIURNAL CHANGES IN PERCENTAGE BY WEIGHT OF THE MAJOR FOOD ORGANISMS IN SEVEN CATIFHES OF THE PUNJAB, PAKISTAN.

Species	Food group	Hours of feeding											
		0	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**
S. sarwari	Algae	-	-	-	15	10	5	2	-	-	-	-	-
	Crustaceans	-	-	-	15	10	8	9	5	-	8	8	5
	Insects	10	5	5	60	55	40	41	20	-	10	12	10
	Fishes	35	15	5	10	7	-	-	-	-	80	65	45
	Undigested matter	55	80	90	-	18	37	42	75	100	-	15	40
M. bleekeri	Algae	-	-	-	32	26	18	15	8	3	5	3	-
	Crustaceans	7	6	2	20	12	13	11	9	3	12	8	6
	Insects	14	12	3	45	38	36	34	28	9	18	16	14
	Molluscs	-	-	-	2	1	1	-	-	-	-	-	-
	Fishes	35	22	5	-	-	-	-	-	-	65	60	55
	Worms	-	-	-	1	-	-	-	-	-	-	-	-
	Undigested matter	44	60	90	-	13	32	40	55	85	-	13	25
M. vittatus	Algae	-	-	-	35	27	18	15	6	3	1	-	-
	Crustaceans	6	5	3	23	13	12	10	8	3	13	8	6
	Insects	13	7	4	39	36	34	31	29	5	18	11	8
	Molluscs	-	-	-	1	1	1	1	-	-	-	-	-
	Fishes	36	23	4	-	-	-	-	-	-	68	55	47
	Worms	-	-	-	2	1	-	-	-	-	-	-	-
	Undigested matter	45	65	89	-	22	35	43	57	89	-	26	39

Continued

Species	Food group	Hours of feeding											
		0	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**
W. attu	Algae	-	-	-	3	1	-	-	-	-	-	-	-
	Crustaceans	4	3	1	9	7	5	8	5	3	1	3	2
	Insects	3	3	1	10	3	3	3	2	2	1	2	1
	Fishes	25	12	3	78	68	52	35	22	6	95	88	52
	Undigested matter	67	85	92	-	22	38	52	68	82	-	5	43
O. bimaculatus	Algae	3	1	-	5	3	2	1	-	-	-	3	5
	Crustaceans	7	3	-	35	27	17	16	10	6	2	13	14
	Insects	8	6	8	48	40	38	28	22	12	3	17	16
	Fishes	15	5	-	12	8	5	3	-	-	95	62	22
	Undigested matter	68	85	92	-	22	38	52	68	82	-	5	43
B. bagarius	Algae	-	-	-	5	3	2	2	1	1	-	-	-
	Crustaceans	3	2	1	3	3	3	3	2	1	3	2	1
	Insects	2	2	1	2	4	2	2	3	1	1	1	1
	Fishes	26	13	5	90	70	53	37	24	6	96	67	43
	Undigested matter	69	83	93	-	20	40	56	72	92	-	30	55
H. fossilis	Algae	14	12	6	-	-	-	-	-	-	45	22	18
	Crustaceans	6	1	1	26	19	14	9	5	5	17	19	14
	Insects	12	2	1	29	28	18	16	9	7	18	35	18
	Molluscs	4	1	-	-	-	-	-	-	-	15	9	8
	Fishes	-	-	-	45	38	30	20	12	6	3	-	-
	Worms	-	-	-	-	-	-	-	-	-	2	1	-
	Undigested matter	64	84	92	-	15	38	55	72	82	-	14	42

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DEVELOPMENT OF STR MULTIPLEX SYSTEM (D5S818, D7S820 AND D18S51) FOR FORENSIC CASEWORK

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Abstract.- The current method of forensic DNA typing utilizes a standard battery of 13 short tandem repeat (STR) loci. These loci are polymorphic in human population. STR loci with non-overlapping size ranges may be combined into a single multiplex PCR amplification system. STR multiplexes have proved to be indispensable for the efficient genotyping of biological samples for forensic purposes. The present study aims at the production of multiplex PCR system of three STR markers (D5S818, D7S820 and D18S51) out of 13 STR loci and validation studies of developed multiplex system using biological samples collected randomly from all the four provinces and federal capital of Pakistan. Multiplex PCR system composed of three STR loci (D5S818, D7S820 and D18S51) was successfully developed using unlabelled primers followed by resolving the PCR product in sequencing Polyacrylamide gel and visualized by silver staining. Developed multiplex PCR system was then validated on Pakistani population for forensic purposes. This study will not only help forensic scientists to generate DNA profile of biological sample in less time but also ensure speedy justice.

Key words: STR multiplex, DNA typing, Validation studies.

INTRODUCTION

Genomic DNA contains repetitive sequences within coding and non-coding- regions. The number of these repeats differs between individuals and thus gives rise to alleles of different length. This type of polymorphism is referred to as length polymorphism. There can also exist multiple single nucleotide variations at the same locus, denoted as sequence polymorphism (Wyman and White, 1980). Identity testing by DNA analysis became a practical reality when Jeffreys (1979) identified the hypervariable "minisatellite" or variable number of tandem repeat (VNTR) loci in the human genome. "Microsatellite" or short tandem repeats (STR), which

belong to the family of repetitive non-coding DNA sequences, provide another rich source of polymorphic markers resulting from variations in the number of copies of the repeated motif. PCR-based typing of VNTR/STR loci has become a technique of growing importance in forensic analysis and paternity testing (Evett *et al*. 1996).

Technologies used for performing forensic DNA analysis differ in their ability to differentiate two individuals and in the speed with which results can be obtained. The speed of analysis has dramatically improved for forensic DNA analysis. DNA testing that previously took 6 or 8 weeks can now be performed in a few hours (Butler, 2001). The current method of forensic DNA typing utilizes a standard battery of 13 STR loci. These 13 STR loci are also referred to as the FBI's core loci, composed of tetra repeats present in non-coding regions of the human genome. These loci are very polymorphic in human populations. In other words, they occur in different lengths in different individuals according to how many times the pattern of four bases are repeated. These loci are, therefore, well suited for the type of identity testing needed in the forensic cases (Becherer, 2000).

The loci are subjected to PCR (polymerase chain reaction), which amplifies specific DNA. This process involves the use of primers, which are short segments of DNA constructed so as to bind the target DNA by base pairing with specific complimentary nucleotide sequences. Following the binding of the primers to the denatured DNA, primer extension results in a copy of the DNA that is bracketed by the primers. The process of repetitively copying a selected DNA segment is referred to as PCR, and the resultant exponential increase in DNA segment copy number is called amplification (Becherer, 2000). STR loci with non-overlapping size ranges may be combined into a single multiplex PCR amplification system. Such multiplex PCR procedures streamline STR profiling and allow more markers to be typed per gel (Scherczinger *et al.* 2000). Multiplex STRs system is valuable because it can produce highly discriminating results and can successfully measure sample mixtures and biological materials containing degraded DNA molecules. STR multiplexes have been indispensable for the efficient genotyping of forensic samples (Butler, 2001). Use of multiplex PCR system in population studies has been reported by different workers.

The present study aims at the development of indigenous multiplex PCR system for three STR markers (D5S818, D7S820 and D18S51) and validation using samples collected randomly from all over Pakistan.

MATERIALS AND METHODS

Selection of STR Loci

Unlabelled primers for STR loci "D5S818,D7S820 and D18S51" were used to amplify these three loci separately according to the pre-optimized conditions (Hussain, 2001; Khanum, 2002) and then PCR conditions for multiplex of STR loci "D5S818, D7S820 and D18S51" were optimized after extensive experimentation using MJ PTC-100 thermal cycler. Conditions for multiplex amplification system were optimized either by changing concentration of different PCR components or the PCR cycling profile.Amphions were resolved on 2% agarose gel.

Sample collection and DNA extraction

DNA extraction was performed, using phenol chloroform extraction method (Singer *et al.* 1988) from all the samples. Extracted DNA was re-suspended in TE by incubating at 56ºC for 1 hour to get homogenous DNA solution. DNA concentration was estimated by spectrophotometery followed by agarose gel. PCR ready DNA dilutions (5ng/µl) of all the DNA samples were made and concentration was confirmed by agarose gel electrophoresis as the quantity of DNA as a template for PCR amplification is very critical.

For the validation studies of the developed multiplex 209 blood samples collected at random all over Pakistan. Whole blood was used as raw material to extract the DNA. Using Phenol Chloroform DNA extraction method (Signer *et al*. 1988). PCR ready dilutions of template DNA were prepared as 5ng/µl.

PCR amplification

Multiplex amplifications were performed in a reaction volumes of 25 µl with 1 unit of Taq DNA polymerase, 1X PCR buffer, 0.1 mM dNTP's, 1.5mM MgCl2, 1.25 µl primer mix (0.15 µM for D5S818, 0.15 µM for D7S820 and 0.2 µM for D18S51) and DNA template (5ng). Thermal cycling was conducted on MJ PTC-100 thermal cycler using following conditions: {95°C for 3 min, 94°C for 30 sec, [4 cycles: 60°C (-1°C/cycle) for 45 sec, 72°C for 1 min], [35 cycles: 90°C for 30 sec, 57°C for 45sec, 70°C for 1 min], 72°C for 10 min and 4°C for indefinite time}.

Resolution of PCR product

PCR conditions were optimized for the development of multiplex PCR amplification system for STR loci D5S818, D7S820 and D18S51. Conditions were optimized to amplify the three STR loci on single annealing temperature separately, first using same PCR profile and then in the same reaction. Amplicons were resolved on 2% agarose gel to observe the success of amplification.

The optimized multiplex conditions for STR loci "D5S818, D7S820 and D18S51" were validated by genotyping 209 collected DNA by resolving amplicons on 6% denaturing polyacrylamide gel electrophoresis (PAGE) including urea, 10X TBE, acrylamide, bisacrylamide and water. Plates were bound after thorough washing, salinization and applying sigmacoat. Gel solution was proured and 0.35mm thick gel was prepared after letting it to polymerize for 45-50 min. Gel was pre-run and then the amplicons were run. Bands were visualized by silver staining.

The conditions optimized for multiplex PCR amplification system were validated by genotyping 209 individuals for these three STR loci.

RESULTS AND DISCUSSION

Figure 1 shows the DNA amplification for STR loci STR loci D5S818, D7S820 and D18S51 according to conditions reported earlier by (Hussain, 2001) for D5S818 and D7S820 and Khanum (2002) for D18S51. All the three STR loci were amplified separately. Figure 2 displays the separate as well as multiplex amplification along with the 50bp marker.

The three STR loci selected for this study includes D5S818, D7S820 and D18S51, which are out of the 13 CODIS (Combined DNA Index System) loci identified by FBI (US. Department of Justice, 1996). Some work has already been done on these three loci on Pakistani population. Hussain (2001) has worked on D5S818 and D7S820 in Punjabi population, whereas Khanum (2002) has studied D18S51. These studies were through monoplex amplification of single STR locus in one PCR reaction, for which more resources are utilized for the same set of results. Development of multiplex PCR amplification system was carried out to overcome these difficulties and help forensic scientists to proceed more quickly towards dependable analytical results. Multiplex PCR amplification system is very

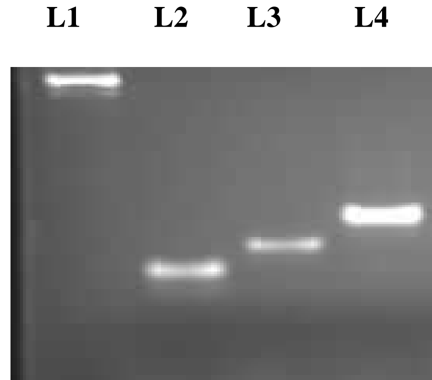

Fig. 1. Amplification of STR loci D5S818, D7S820 and D18S51; L1 = Genomic DNA, L2 = D5S818, L3 = D7S820, L4 = D18S51.

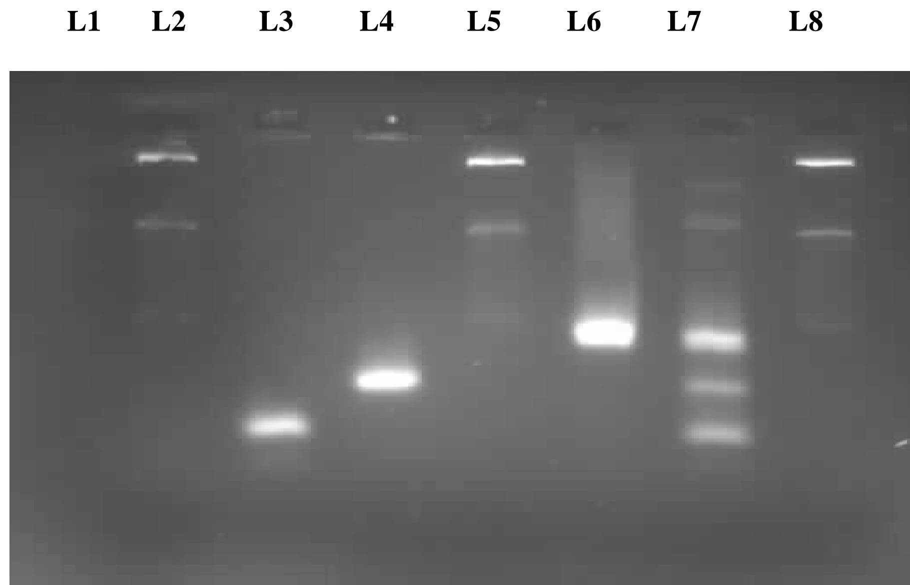

Fig. 2. Amplicons showing multiplex amplification; L1 = Negative Control, L2 = 50bp Ladder, L3 = D5S818, L4 = D5S820, L5 = 50bp Ladder, L6 = D18S51, L7 = Multiplex of D5S818, D7S820 and D18S51, L8 = 50bp Ladder.

helpful for efficient forensic case work (Hammond *etal*. 1994, Lins *et al*. 1996, Xiao *et al*. 1998, Gill *et al*. 1995, Miscicka-Silwka *et al*. 1996 and Young *et al*., 2001). Multiplex system can save time money and most important of all biological evidence (sample) which may be in very limited amount (Benecke *et al*. 1996). For this reason multiplex satisfies the requirements of most national data bases (Krenke *et al*. 2002). Multiplex PCR amplification system has enabled the forensic scientists to derive DNA profiles from minute amounts of biological samples, which may be as little as 100 picograms (Kloostermann and Kersbergen, 2002). In this study minute amount of DNA *i.e.* 5ng was amplified for three STR loci, even with degraded DNA template.

Hussain (2001) has reported that multiplex formation of STR loci D5S818, D7S820 and D13S317 is not possible due to different PCR conditions and close proximity of alleles. This claim was based on the experimental data obtained from population related to STR loci D5S818 (141-157 bp), D7S820 (194-234 bp) and D13S317 (165-197 bp). These observations show overlapping of STR locus D13S317 with STR loci D5S818 and D7S820 in size of fragments. It was thought to replace the interfering STR locus D13S317 with a suitable one. Khanum (2002) reported a highly polymorphic STR locus D18S51 (262-342 bp) showing 42 possible alleles. It was decided to replace STR locus D13S317 with D18S51 in this study to overcome the problem of matching alleles among the participating STR loci D5S818 (141-157 bp), D7S820 (194-234 bp) and D18S51 (262-342 bp) in multiplex PCR amplification system under development. PCR conditions were optimized to amplify all three STR loci in one reaction using the same reagents and PCR profile successfully.

This study by development of a multiplex PCR amplification system will help forensic scientists to work more efficiently with less inputs. It has also laid a milestone in the development of indigenous multiplex PCR amplification systems in the country which will reduce our dependence on imported kits saving a lot of foreign exchange.

REFERENCES

BECHERER, D., 2000. The Use of STRs in Forensic Science. http://www.wustl.edu/~jstader/becherer.html.

BENECKE, M., SCHMITT, C. AND STAAK, M., 1996. Development of a fast new STR triplex system for identification of urine samples. *Poster at the First European Symposium on Human Identification.* Toulouse, 29.-31.5. Promega Co.

BUTLER, J.M., 2001. *Forensic DNA typing: biology and technology behind STR markers*. Academic Press, New York.

EVETT, I.W., LAMBERT, J.A., KNIGHT, S.D., FAIRLEY, M. AND LEE, L.D. 1996. A study of independence between STR and conventional blood type loci. *Forensic Sci. Int.,* **79**:163-166.

GILL, P., KIMPTON, C.P., URQUHART, A., OLDROYD, N., MILLICAN, E.S., WATSON, S.K. AND DOWNES, T.J. 1995. Automated STR analysis in forensic casework-a strategy for the future. *Electrophoresis,* **16**: 1543-1552.

HAMMOND, H. A., JIN, L., ZHONG, Y., CASKEY, C.T. AND CHAKRABORTY, R. 1994. Evaluation of 13 short tandem repeat loci for use in personal identification applications. *Am. J. Hum. Gen*., **55**: 175-189.

HUSSAIN, A., 2001. *Allelic frequency distribution and genotyping at three STR loci (D5S818, D7S820 and D13S317) in Punjabi population*. M.Phil. thesis, National Centre of Excellence in Molecular Biology, University of the Punjab, Lahore.

JEFFREYS, A. J., 1979. DNA sequence variants in the G-gamma, delta and β- globbin genes of man. *Cell*, **18**: 1-10.

KHANUM, S., 2002. *Allelic frequency distribution of STR loci (D8S1179, D18S51 and D21S11) in Punjabi population of Pakistan*. M.Phil. thesis, National Centre of Excellence in Molecular Biology, University of the Punjab, Lahore.

KLOOSTERMAN, A. AND KERSBERGEN, P., 2002. Efficancy and limits of genotyping low copy number (LCN) DNA samples by multiplex PCR of STR loci. In: *Symposium chips in clinical and forensic analysis* (eds. S. Jitka, W. Verpoorte and C. Thormann), Published Online.

KRENKE, B.E., TEREBA, A., ANDERSON, S.J., BUEL, E., CULHANE. S., FINIS, C.J., TOMSEY, C.S., ZACHETTI, J.M. AND SPRECHER, C.J., 2002. Validation of a 16-locus fluorescent multiplex system. *J. Foren. Sci.,*. **47**:1-13.

LINS, A.M., SPRECHER, C.J., PUERS, C. AND SCHUMM, J.W., 1996. Multiplex sets for the amplification of polymorphic STR loci, silver stain and fluorescent detection. *BioTechnique*, **20**: 882-889.

MISCICKA-SILWKA, D., WOZNIAK, M. AND GRZYBOWSKI, T., 1996. A multiplex system useful for both manual and automated analysis of microsatellite polymorphisms. In: *Proceedings from the First European Symposium on Human Identification.* Toulouse (France).

SCHERCZINGER, C.A., HINTZ, J. L., PECK, B. J., ADAMOWICZ, M. S., BOURKE, M. T., COYLE, H. M., LADD, C., YANG, N. C., BUDOWLE, B. AND LEE, H. C., 2000. Allele frequencies for the CODIS core STR loci in Connecticut populations. *J. Foren. Sci.,* **45**: 938-40.

SINGER, E., KUENZLE, C. C., THOMANN, P. E. AND HUBSCHER, U., 1988. DNA fingerprinting: Improved DNA extraction from small blood samples. *Nucl. Acids Res.,* **16**: 7738.

STRbase, http://www.cstl.nist.gov/biotech/strbase/index.htm

U.S. DEPARTMENT OF JUSTICE, 1996. *State DNA database laws: Outline of provisions.*

WYMAN A.R. AND WHITE, R., 1980. A highly polymorphic locus in human DNA. *Proc. Natl. Acad. Sci.* USA., **77**:6754-6758.

XIAO, F.X., GILISSEN, A., GU, X.X., CASSIMAN, J.J. AND DECORTE, R., 1998. Genetic data obtained for two Chinese Han populations with a quadruplex fluorescent STR typing system (HUMvWA, HUMTHO1,D21S11 and HPRT). *Int. J. Legal Med.,* **111**: 343-345.

YOUNG, D.R., TUN, Z., HONDA, K. AND MATOBA, R., 2001. Identifying sex chromosome abnormalities in forensic DNA testing using amelogenin and sex chromosome short tandem repeats.. *J. Foren. Sci.,* **46**: 346-348.

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EFFICACY OF CONFIDOR 70 WSC AND TEMIK 15 G AGAINST EARLY SEASON SUCKING PESTS OF CIM 240 COTTON

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Abstract .- Seed treatment (10 g/kg seed) of Confidor 70 WSC (timidacloprid) and seed furrow application (3 kg/acre) of Temik 15G (aldicarb) was tested against early season sucking pests *viz.* cotton jassid, *Amrasca biguttula biguttula* (Ishida) and cotton whiteflies, *Bemisia* spp. on cotton CIM 240. Confidor 70 WSC suppressed the population of jassid effectively for 55 days after sowing and also enhanced yield though non-significantly. Both the insecticides proved ineffective in controlling whiteflies rather their population increased in the plots treated with Confidor 70 WSC followed by Temik 15G.

Key words: Confidor 70 WSC, Temik 15G, cotton pest.

INTRODUCTION

Cotton whiteflies, *Bemisia* spp. and cotton jassid, *Amrasca biguttula biguttula* destroy whole cotton crop during its early growth period. Heavy infestation of the both pests at early reproductive phase of the crop results premature fruit shedding that leads to delayed crop maturity and reduced yield of seed cotton. Control of several different early season insect pests of cotton contributes well in increasing yield (Scott *et al*., 1987), however, it destroys the beneficial fauna resulting in resurgence of the other insect pests. The seed treatment technology has greatly impacted the cotton industry by uniform crop stand and better insect pest management (Rushing, 1987). Elbert *et al*. (1990) reported imidacloprid, a broad spectrum seed dressing insecticide that gives satisfactory control of *Thrips tabaci* Lind. and *A. biguttula biguttula* (Ishida) in South Africa and Spain upto 36 days after sowing of cotton crop. Attique and Ghaffar (1996) and Dewar and Read (1991) also found imidacloprid as seed treatment effective against early season sucking pests on cotton and sugarbeet. Similarly Temik (aldicarb) when applied in seed furrows had also been found effective against sucking

pests of cotton and sunflower (Karar, 1990; Gupta *et al*., 1983; Wahid, 1970) and was safe for predators (Gupta *et al*., 1983).

Thus the present studies were planned to evaluate Confidor (imidacloprid) 70 WSC and Temik (aldicarb) 15G at their recommended doses aganst important early season sucking insect pests *viz*. *Bemisia* spp. and *A. biguttula biguttual* under local conditions at Hasilpr (Bahawalpur), Pakistan.

MATERIALS AND METHODS

The experiment was established at Mr. Anser Mahmood's Farm, Chak # 19/FW, Tehsil Hasilpur (Bahawalpur). Acid delinted seed of cotton variety CIM 240 was sown in June 26, 1996 in lines with the help of tractor mounted drill maintaining 75 cm row to row and 15 cm plant to plant distance. Randomized Complete Block Design was adopted with four replication. The experimental unit for each treatment was 17.5 x 98 ft. in size. The treatments were given as Confidor 70 WSC (imidacloprid), 10 g/kg seed, and Temik 15G (aldicarb) 3 kg/acre.

One kilogram delinted cotton seed was treated with 10g of Confidor 70 WSC mixed with 30 ml water. A polythene bag was used for thorough mixing. Temik 15G was applied at the time of sowing in furrows with the help of a plastic bottle having a hole in its lid for disposal of the granules. The parameters studied included the counting of adult and immature stages of jassid and whiteflies started after three weeks of planting till reaching the economic threshold level. The data were recorded on per leaf basis by randomly taking 10 leaves from central two rows of each treatment. The yield data were summarized as kilograms of seed cotton per acre.

The collected data were analyzed statistically and the treatment means were compared using DMR test at 5% level of significance (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Weekly data of mean populations of jassid and whiteflies recorded from 20 days to 62 days after sowing of cotton crop are presented in Table I. Confidor 70 WSC remained the best by keeping jassid population below economic injury level upto 55 days of sowing but its effects against the pest

were non-significant upto 41 days of sowing. Temik 15G suppressed the jassid population upto 41 days of sowing but its results remained non-significant. After 48 days of sowing its results became significant but the pest population could not be brought below economic injury level.

Both the insecticides proved ineffective against whiteflies. The pest population was statistically similar upto 62 days. On the contrary the whiteflies population remained more in the plots treated with Confidor 70 WSC followed by the plots treated with Temik 15G as compared to untreated plots (Table II).

A comparison of mean values of yield (Table II) for all treatments revealed non-significant results. However, numerically high yield (703.55 kg/acre) was obtained from the plots treated with Confidor 70 WSC.

TABLE I.- A COMPARISON OF THE MEAN VALUES OF SEED YIELD (kg per acre) OF CIM 240 VARIETY OF COTTON WITH CONFIDOR 70 WSC AND TEMIK 15G AT RECOMMENDED DOSES.

Treatments	Dose	Yield (kg per acre)
Confidor 70 WSC	10 g/ kg seed	703.55a
Temik 15 G	3 kg/acre	668a
Control	-	657a

DMR test, analyzed at 5% level of significance. Values with the same symbols are not significantly different from each other.

Elbert *et al*. (1990) obtained a satisfactory control of *T. tabaci* and *A. biguttula biguttula* with imidacloprid through seed dressing in South Africa and Spain upto 36 days after sowing of cotton crop. According to Attique and Ghaffar (1996) Promet (furathiocarb) and Confidor (imidacloprid) effectively controlled early season attack of *A. devastans* on cotton in Pakistan. Dewar and Read (1991) concluded that imidacloprid as seed treatment had a potential to be an alternative of aldicarb (Temik) for controlling sucking pests like aphid on sugarbeet in England.

According to Karar (1990) from Pakistan Temik 10G proved effective against whiteflies and jassid but in our experiment it controlled the jassid to some extent but remained ineffective against whiteflies. Our results also do not agree with Gupta *et al*. (1983) who found aldicarb more effective for insect pests. Other workers namely Ali and Attique (1987) and Ali *et al*.

(1988) reported that aldicarb did not play an important role in increasing yield of cotton but rather showed some phytotoxic effects. Taai and You (1963) were of the view that granular insecticide application after 3 to 6 weeks of sowing of cotton was more economical than seed furrow treatment. Patana and Ridgway (1967) and Ridgway *et al*. (1968) reported that efficacy of Temik increased after irrigation because it enhanced the uptake of side dressed Temik. The same was observed in our experiment when the results of Confidor and Temik became significant on 48th day after sowing due to first irrigation. The results of the experiment disagree with the conclusion of Courier (1999) that the early season sucking insect pests of cotton can be controlled effectively with imidacloprid as seed treatment.

It is concluded that Confidor 70 WSC can be recommended as seed treatment for the control of early season attack of jassid on cotton but its causation of increase in whiteflies population needs further investigation.

REFERENCES

ALI, M. AND ATTIQUE, M.R., 1987. Growth and development of cotton as affected by different groups of insecticides in Punjab, Pakistan. *Pakistan Cott.*, **31**: 251-221.

ALI, M., AHMAD, A., ATTIQUE, M.R. AND WAHEED, T., 1988. Effect of some insecticides on the growth, fruit development and yield of two cotton varieties in Punjab, Pakistan. *Pakistan Cott.*, **32**: 30-36.

ATTIQUE, M.R. AND GHAFFAR, A., 1996. Control of early season sucking pests of cotton with seed protectant insecticides and their impact on natural enemies and yield of seed cotton. *Pakistan J. Zool*., **28**: 253-255.

COURIER, 1999. Gaucho helps hybrid realize full potential. *Bayer Agrochem.*, **2**: 47-489.

DEWAR, A.M. AND READ, L.A., 1991. Evaluation of an insecticide seed treatment, imidacloprid for the control of aphid on sugarbeet. *Proc. Brighton Crop Prot. Conf. Pests Dis*., **2**: 721-726.

ELBERT, A., IWAYA, O.K. AND TRUBOIL, S., 1990. Imidacloprid, a novel synthetic nitromethylene analogue insecticide for crop protection. *Proc. Brighton Crop Prot. Conf. Pests Dis*., **1**: 21-28.

GUPTA, P.R., MISHRA, R.C. AND DOGRA, G.S., 1983. Control of the painted bug and the cabbage aphid infesting cauliflower seed crop with reference to safety to coccinellid predators. *Ind. J. agric. Sci*., **53**: 365-369.

KARAR, H., 1990. *The comparative effect of different pest control practices on the population of sucking pests and yield of sunflower*. M.Sc. thesis, Department of Agricultural Entomology, University of Agriculture, Faisalabad.

PATANA, R. AND RIDGWAY, R.L., 1967. Stem and soil application of systemic insecticides to cotton for control of *Lygus hesparus* Knight. *J. econ. Ent*., **60**: 1185-1160.

RIDGWAY, R.L., JONES, S.L., COPPEDGE, J.K. AND LINDQUIST, D.A., 1968. Systemic activity of U-C-01149 in the cotton plant with special reference to the boll weevil. *J.*

econ. Ent., **61**: 1705-1709.

RUSHING, K.W., 1987. Cottonseed treatment technology, development and application of chemical and biological control agents and industry view of research needs. *Proc. Beltw. Cott. Conf*., pp. 25-27.

SCOTT, W.P., SNODGRASS, G.L. AND SMITH, J.W., 1987. A two years study of the effect of early season insect control in cotton yields. *Proc. Beltw. Cott. Conf*., pp. 237-243.

STEEL, R.G.D. AND TORRIE, M., 1980. *Principles and procedures of statistics*. McGraw Hill, New York. pp. 633.

TAAI, Y.P. AND YOU, C.H., 1963. Field test with Thimet on cotton insect control in Taiwan. *Agric. Res*., **10**: 48-64.

WAHID, M., 1970. Evaluation of granular insecticides for the control of cotton pest. *Res. Stud. WPAU, Lyallpur*, **1157**: 42.

TABLE II.- A COMPARISON OF THE MEAN VALUES OF THE POPULATION OF JASSID AND WHITEFLIES (LEAF-1) ON CIM 240 COTTON TREATED WITH CONFIDOR 70 WSC AND TEMIK 15G AT RECOMMENDED DOSES.

Treatment	Dose	Interval after sowing (Days)						
		20	**27**	**34**	**41**	**48**	**55**	**62**
Jassid								
Confidor 70 WSC	10 g/kg seed	0.05a	0.15a	0.12a	0.25a	0.82c	0.72c	1.10c
Temik 15 G	3 kg/acre	0.12a	0.12a	0.25a	0.55a	1.85b	1.92b	2.60b
Control	-	0.12a	0.25a	0.75a	0.60a	2.17a	2.27a	2.72a
Whiteflies								
Confidor 70 WSC	10 g/kg seed	0.125a	0.20a	0.82a	0.62a	1.72a	6.75a	9.15a
Temik 15 G	3 kg/acre	0.10a	0.25a	0.55a	0.67a	0.62c	5.97a	6.12a
Control	-	0.15a	0.15a	0.42a	0.50a	0.80b	3.87a	5.40a

Analyzed at 5% level of significance. The values with the same symbols are not significantly different from each other.

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DETERMINATION OF SUITABLE MICROALGAL DIET DURING GONAD DEVELOPMENT AND LARVAL REARING OF *PLACENTA PLACENTA* (L.)

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Abstract.- For development of windowpane oyster industry in Pakistan it is imperative to develop hatchery techniques. To achieve this, present study was conducted to determine suitable algal diet for *Placenta placenta* (L.) during gonad development and larval rearing. Because of the scarcity of mature *P. placenta*, needed for spawning, a condition technique to produce sexually mature adults under controlled condition also needs to be worked out. Sexually immature individuals were reared in the well-aerated aquaria for four months. The experimental individuals were fed daily with a mixture of *Navicula* and *Nitzschia* at 100,000 cells/ml, 1:1 (100- Na:Ni) or 200,000cells/ml, 3:1 (200-3Na:Ni). The histological studies, performed regularly on monthly basis, showed that sexual maturity was achieved by animals fed 200- 3Na: Ni diet in four months but not in 100-Na: Ni. Thirty percent of the brood stock were sexually mature, as they reached at a shell length of 75 mm (males) 95 mm (females), and released unfertilized gametes after exposure to UV light radiation. Fertilization occurred in seawater. One-day-old larvae were reared in UV irradiated seawater until metamorphosis to plantigrade. During rearing the larvae were daily fed with monoalgal diet *Nitzschia*, *Navicula* and *Chlamydomonas*. Larval settlement is observed in all diets after 14 days. Highest survival rate (32%) is observed in *Navicula* fed larvae and lowest (15%) in *Chlamydomonas*. During the 15 days rearing period, the average shell length increment was 17.5, 16.9 and 12.38µm day^{-1} for *Navicula*, *Chlamydomonas* and *Nitzschia* respectively.

Key words: Windowpane oysters, algal diet, Korangi Creek.

INTRODUCTION

Windowpane oyster, *Placenta placenta* (Linne), commonly known as "Khappo" among Pakistani fishermen, is a bivalve mollusc which has potential for commercial utilization. The off- white translucent shells are raw materials in shell craft industry. Because of their transluscence and durability, the "khappo" shells are used as windowpane materials in building construction and decoration and manufacture of shell craft products such as lampshades, lantern, shields, screens, trays, tablemats, picture frames, pearl

essence for pearl beads and other novelties for homes and offices. In addition to the shells, the meat is also a good source of protein food for human consumption as well as for feeding shrimps at ponds. Analysis of *P. placenta* meat contents (FNRC-NSBD Handbook, 4th Revision, 1968, Manila, Philippines) revealed that it contains 23.3grams of protein per 100 grams of fresh meat and other minerals needed by the body. Magsuci *et al*. (1980) has documented that the protein content of *Placenta* is higher than those of mussels and oysters. The pearls produced by these oysters are used in local medicines (Hikmat) and cosmetic industry. Local fishermen community also eat the flesh (Personal communication).

The "Mirs" (Rulers of Sindh) established a substantially large windowpane oyster industry in Karachi especially around Gizri and Korangi creek waters during 1830 to 1906 (Rai, 1932). However continual mismanagement and gradual rise of pollution resulted in the depletion of stocks to such an extent that the industrial commercialization has ceased to exist (Moazzam and Ahmed, 1994). A recent study (Anonymous, 2001) has shown that the muddy bottoms of bays and creeks along Pakistani creeks still harbour good numbers of *P. placenta* (160-350 individuals/m^2), but stocks are not large enough to support any commercial endeavor. At present these populations are exploited by local fishermen folk for their shells and pearls and some times for meat as well (Anonymous, 2001), In order to revive the commercial exploitation natural population has to be augmented to suffice the requirement for mariculture. As suggested by Zehra (1996), a windowpane oyster fishery is feasible in Korangi creek waters but to relieve pressure on the existing wild stock population, there is a need to develop the hatchery techniques for windowpane oysters. Microalgae are used as live food for all developmental stages of crustacean, fish and molluscs. Studies have documented that numerous algal species not only support growth (Enright *et al*., 1986, Aldana-Aranda *et al*., 1989) but also improve the settlement rate of bivalve larvae (Utting, 1986) and other molluscs. *P. placenta* lives buried in muddy bottoms and creek system around Karachi. It is a primary and secondary consumer and feeds upon phytoplankton, zooplankton and detritus, swept into mantle cavity during filter feeding. Gallardo *et al*. (1992) has shown that the gonad maturation in *P. placenta* is accelerated when provided with mixed algal diet. In consideration of this documentation, the present probe was conducted to determine a suitable algal diet for *P. placenta* during gonad development and larval rearing. Because of the scarcity of mature *P. placenta*, needed for spawning and to rejuvenate the present stock for commercial utilization, a condition technique to produce

sexually mature adults under controlled condition is also the call of the time. The proper introduction and establishment of “Khappo” industry would help not only to improve the social status of the fishermen community but would also be a good source of foreign exchange.

MATERIALS AND METHODS

The present probe is a part of a research project “Development of a Fishery for window pane oyster *Placenta placenta* (L) from Korangi creek Karachi”. The studies were conducted from December 1999 to November 2002. *P. placenta* brood stock (30±4.5mm shell length) were collected from the Korangi creek (24.6°N 67.1°E). In the field adult *P. placenta* attain a shell height of 80- 140mm. Plankton population and the physico-chemical conditions of the selected area were also monitored. The individuals were kept in glass aquarium (2 x 1.5 x 1.2 m) for 4 days. No supplemental diet was provided for three days prior to use in the experiment. Histological analysis of gonad samples (n=10) was done to ascertain gonad stages (Rosell, 1979) at the start of the conditioning experiment.

The sampled individuals were separated in two groups: A, B. Each group was randomly stocked into a continuously aerated glass aquarium (1.0 x 0.6 x 0.8m) at a density of 70 individuals for four months. The aquaria were filled with 250 litre fibre glass wool filtered sea water. After 3 days, the brood stock group A was supplied with a mixture of *Navicula* and *Nitzschia* at 100,000 cells/ml, 1:1 (100- Na:Ni), while the group B with a mixture of the same micro algae at 200,000cells/ml, 3:1 (200-3Na:Ni). Prior to the feeding, algal density of the rearing water was checked daily using haemocytometer and the required algal cells were added to the water. This ensured that the appropriate algal density is provided to the rearing individuals. The rearing water was changed every 2-3 days.

Water temperature and salinity during the conditioning period ranged from 25-30 ^{o}C and 29-38 ppt respectively. Ten to fifteen individuals per treatment were taken monthly and induced to spawn by exposure to UV light-irradiated seawater (Madrones-Ladja and Milagros, 2000). Fertilization of gametes occurred in seawater. The histological studies were performed regularly on monthly basis using the gonad samples of individuals that did not spawn. Gonadal stages of the brood stock samples were evaluated monthly. For calculation of gonad index, five stages (0-5) were identified. These stages represented the changes that occurred in the gonad during

development: no gonad, o; immature, 1-2; early active, 3; late active, 4; ripe, 5; partially spawned, 3 and spent, 1. The sum of the products of the percentage of each stage and its ranking defines the gonad index (Gallardo *et al.*, 1992).

For larval feeding experiment, one-day-old larvae, obtained by induction of mature *P. placenta* with UV light irradiation, were stocked in 100 litre glass jars, at a density of 800 larvae per litre in 100 litre UV light irradiated sea water. Three replicates were prepared for experimentation. Immediately after stocking, the larvae were fed singly with *Nitzschia, Navicula* and *Chlamydomonas*, given once daily at initial density of 10000cell/ml. As the larvae grew the algal density was progressively increased to 30000 cells per ml. The experiment continued until the onset of larval settlement, which occurred within 15 days after spawning. During the culture period, water salinity ranged from 32-34 ppt; temperature from 25-28°C; pH from 6.9-7.2 and dissolved oxygen from 2.65-6.06 ml/L. Survival rate and the average shell length increment were also observed.

The microalgae, used in the larval settlement, were cultured in Conway medium (Walne, 1970) following Madrones-Ladja and Milagros (2000). Five batches of each alga were cultured in 10 litre borosilicate conical flasks. Throughout the experiment, the algae were exposed to continuous illumination supplied with 2 daylight (40 watt) florescent tubes. Continuous aeration was also provided to enhance the growth and prevent the algae from settling.

RESULTS

Rearing of individuals

Histological studies delineate that the *P. placenta* (L) fed with 200-3Na:Ni show better gonad development as compared to those fed with 100-Na:Ni (Table I). The analysis of the data (Fig. 1) further reveals that the gonad index increases consistently every month reaching 450 on the fourth month. Thirty percent of the brood stock were sexually mature, as they reached at a shell length of 75 mm (males); 95 mm (females), and released gametes after I h exposure to UV-irradiated seawater, fertilization of eggs took place in seawater. The fertilized eggs (42-45µm) developed to veligers.

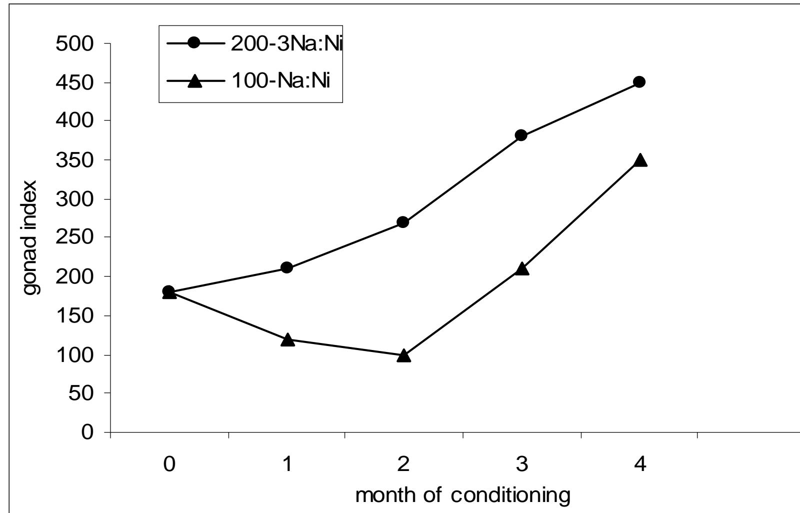

Fig. 1: Gonad development of *Placenta placenta* reared in aquarium fed with microalgae.

P. placenta (L) fed 100 Na:Ni diet exhibited decline in the gonad index one month after induction of the diet and none of the m reached sexual maturity. The batch attained gonad index of 350 by the end of the 4-month conditioning period.

Rearing of larvae

Immediately after completing the embryonic development, when straight –hinged stage is reached, the larvae began feeding on micro algae. This stage, as stated by Madrones-Ladja and Milagros (2000), is very crucial in larval development since food acceptance may not be immediate, thus resulting to high mortality in the succeeding larval stage. Studies on the survival rate and shell growth at metamorphosis (Table II) have shown that the larvae fed Navicula exhibit highest survival rate (32%), followed by larvae fed *Nitzschia* (22%) and *Chlamydomonas* fed larvae(15%). Shell length increments also followed similar pattern: Highest shell length increment 17.5µm day^{-1} being achieved by *Navicula* fed larvae, 16.917.5µm day^{1} by *Chlamydomonas* fed larvae and lowest, 2.38µm day^{-1} by *Nitzschia* fed larvae (Table II). However the settlement and metamorphosis of larvae occurred after 14 days following fertilization in all treatment diets, when the larvae attained a shell length of 250µm.

TABLE II.- SURVIVAL RATE (%) AND SHELL GROWTH (µm) OF *P. PLACENTA* LARVAE AT METAMORPHOSIS.

Microalgae	Survival rate	Shell length	Shell height	Increment (µm/d)	Length height
Navicula	32%	280 ±1.9	262 ± 4.7	17.5	17.5
Nitzschia	22%	248 ±1.9	226 ±4.7	12.38	12.38
Chlamydomonas	15%	260 ±	240 ± 6.5	16.9	16.9

DISCUSSION

The results of the present investigation show that the density of 200,000 cells per ml, 3:1 ratio of *Navicula* and *Nitzschia* is a suitable ration for *P. placenta* (L) as it enhanced the development of ripe gonads and production of gametes in comparison with the low density diet of 100,000cells per ml (Na: Ni). None of the individuals from the latter group attained sexual maturity. The gonad index of the later declined after one month following the induction of 100-Na: Ni. Higher food value is obtained from mixed diet (3Na:Ni) which is likely to contain the diversity of biochemical compounds needed not only for growth but also for gametogenesis. Sastry (1979) established that energy plays a vital role in the gonad development. According to Harrison (1990) maternal nutrition must be augmented. This is needed to provide sufficient energy and appropriate nutrients to meet, the metabolic costs of biosynthesis and metabolization of nutrients for the manufacture of gonads, oocytes and egg yolk. The results of this experiment show that provision of mixed diet enhances the gonad development in *P. placenta.* (L). Gallardo *et al*. (1992) and Madrones-Ladja and Milagros (2000) have also reported that a mixed diet poses a positive effect on the gonad development of *P. placenta* population from Philippines.

The larvae settled and metamorphosed after 14 days following fertilization in all treatment diets at a shell length of 250-260µm. Young (1980 observed the metamorphosis in *P. placenta* (L) at a shell length of 220-230µm, slightly earlier than Pakistani *P. placenta* larva*e.* A number of workers have shown that marine invertebrate larvae metamorphose only in response to a specific stimulus and are capable of delaying metamorphosis and entering an extended searching phase until such a stimulus is received (Nelson, 1928; Colman, 1933; Wilson, 1952; Bayne, 1965; Crisp, 1965; Young, 1980). Moreover, Scheltema (1961) has shown that metamorphosis-

including factors ("biologically-active substances') are probably water soluble and may be transferred from the substratum to the adjacent water, so that metamorphosing larvae can recognize a favourable site without actual contact with the substrate itself. As water used in the larval cultures was taken from an area where *P. placenta* (L) naturally occurs in abundance (therefore a favourable site), it does seem probable that the laboratory-reared larvae were stimulated by metamorphosis-including factors already present in the water.

The results of the experiments presented herein clearly state the requirements of *P. placenta* brood stock for a better gonad development as well as of the rearing larvae. However, the selection of microalgae as natural food for bivalves should be done with utmost care to produce nutritionally sound larvae and juveniles.

AKNOWLEDGEMENT

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REFERENCES

ALDANA ARANDA, D., LUCAS, A., BRULE, T., SALGUERO, E. AND RENDON, F., 1989. Effects of temperature, algal food, food feeding rate and density on the larval growth of the milk conch (*Strombus costatus*) in Mexico. *Aquaculture*, **76**: 361-371.

ANONYMOUS, 2001. *Development of a fishery for windowpane oyster* Placenta placenta *(L) from Korangi creek, Karachi.* Annual report, Project # CEMB-UGC-CRP-3.

BAYNE, B.L., 1965. Growth and the delay of metamorphosis of the larvae of *Mytilus edulis* (L). *Ophelia*, **2**: 1-47.

COLMAN, J., 1933. The nature of the intertidal zonation of plants and animals. *J. Mar. biol. Assoc. U.K,* **18**: 435-476.

CRISP, D.J., 1965. Surface chemistry, a factor in the settlement of marine invertebrate larvae. Bot. Gothoburg. III. *Proc. 5th Mar. Biol. Symp*., Goteborg, pp. 51-65.

ENRIGHT, C.T., NEWKIRK, G.F., CRAIGIE, J.S. AND CASTELL, J.D., 1986. Evaluation of phytoplankton as diet for juvenile *Ostrea edulis* L. *J. Mar. Biol. Ecol*., **96**: 1-13.

GALLARDO, W.G., DE CASTRO, M.T.R., BUENSUCESO, R.T., ESPEGADERA, C.C. AND BAYLON, C.C., 1992. Gonad development of *Placuna placenta* Linnaeus fed *Isochrysis galbana* Prke, *Tetraselmis tetrahele* (G.S. West) Butch, or their combination. *Aquaculture*, **102**: 357-361.

HARRISON, K.E., 1990. The role of nutrition in manufacture, reproduction, and embryonic development of decapod crustacean: A Review. *J. Shellfish Res*., **9**: 1-28.

MADRONES-LADJA, J.A. AND MILAGROS R., 2000. Hatchery management for the window-pane shell, Placuna placenta Linnaeus, 1758. *Phuket Mar. Biol. Cent. Sp. Publ.*, **21**: 189-194.

MAGSUCI, H., CONLU, A. AND MOYANO-AYPA, S., 1980. Windowpane oyster fishery of west Visayas. *Fish. Res. J. Philippines*, **5**: 74-80.

MOAZZAM, M. AND AHMED, J., 1994. Prospects of development of molluscan fisheries in Pakistan. *Proc. National seminar on fisheries policy and planning*. Marine Fisheries Department, Govt. of Pakistan: pp. 41-64

NELSON, T.C., 1928. Pelagic dissoconchs of the common mussel, *Mytillus edulis,* with observations on the behaviour of the larvae of allied species. *Biol. Bull. WoodsHole,* **55**: 180-192.

RAI, H..S., 1932. The shell-fisheries of the Bombay Presidency. Report of the Bombay Natural History Society's Survey. *J. Bomb. Nat. Hist. Soc*., **35**: 826-847.

ROSELL, N.C., 1979. A study on the biology and ecology of *Placenta placenta* Linne. *Natl. Appl*., **31**: 203-251.

SASTRY, A.N., 1979. Pelecypoda (excluding Ostriedae). In: *Reproduction of marine invertebrates,* Vol. 5, *Molluscs: Pelecypods and lesser classes* (eds. A.C. Giese and J.S. Pearse), pp. 113-292. Plenum Press, New York.

SCHELTEMA, R.S., 1961. Metamorphosis of the veliger larvae of *Nassarius obsoletus* (Gastropoda) in response to bottom sediment. *Biol. Bull*., **120**: 92-109.

WALNE, P.R., 1970. Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera *Ostrea, Crassostrea, Mercenaria* and *Mytilus.* Ministry of Agriculture, Fisheries and Food: Fishery Investigations, Series II, Vol. 26 (5). Her Majesty's Stationary Office, London, 61pp.

WILSON, D.P., 1952. The influence of the nature of the substratum on the metamorphosis of the larvae of marine animals, especially the larvae of *Ophelia bicornis* Savingy. *Ann. Ins. Oceanogr. (Monaco)*, **27**: 40-156.

UTTING, S.D., 1986. a preliminary study on the growth of *Crassostrea gigas* larvae and spat in relation to dietary protein. *Aquaculture*, **56**: 123-138.

YOUNG, A.L., 1980. Larval and post larval development of the window-pane shell, *Placuna placenta* Linnaeus, (Bivalvia: Placunidae) with a discussion on its natural settlement. *The Veliger,* **23**: 141-148.

ZEHRA, I., 1996. Case study: Development of a fishery for windowpane oyster *Placuna placenta* (Linne) in Pakistani waters. *Proceedings of the UNESCO workshop on coastal aquaculture*, pp. 147-150.

TABLE I.- PERCENTAGE DISTRIBUTION OF GONADAL STAGES OF *P. PLACENTA* FED WITH MICROALGAL DIET.

Stages	**Initial**	**1st Month**		**2nd Month**		**3rd Month**		**4th Month**	
		100-Na:Ni	**200-3Na:Ni**	**100-Na:Ni**	**100-3Na:Ni**	**100-Na:Ni**	**100-3Na:Ni**	**100-Na:Ni**	**100-3Na:Ni**
No gonad	0	0	0	20	0	0	0	0	0
Immature	80	75	30	55	10	35	0	30	0
Early active	20	25	70	25	90	60	62	55	40
Late active	0	0	0	0	0	5	38	15	30
Ripe	0	0	0		0	0	0	0	30
Partially spawn	0	0	0	0	0	0	0	0	0
Spent	10	0	0	0	0	0	0	0	0

Legend: 100Na:Ni=100,000 cells/ml, *Navicula*:*Nitzschia* 1:1; 200-3 Na:Ni =200,000 cells/ml, *Navicula:Nitzschia* 3:1

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ANALYSIS OF INSULIN GENE IN PATIENTS WITH TYPE 1 DIABETES MELLITUS FOR MUTATIONS

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Abstract.- Type 1 diabetes is a disorder of glucose homeostasis caused by insulin deficiency. There are more than fifteen loci identified which showed positive evidence of linkage to the disease, strongly suggesting that type 1 diabetes is inherited in a polygenic fashion. Mutational studies were performed in insulin gene (INS) of 36 children with type 1 diabetes. No mutation was found. Exon 1, 2 and 3 of insulin gene were sequenced with 100% homology to the normal sequence. Mutation may be in the promoter region of INS or it may be in loci other than INS. It is also possible that involvement of any environmental factor may cause the disease. Therefore, it is proposed that the promoter region of insulin gene and other regions involved in type 1 diabetes should be sequenced to identify the causative mutation.

Keywords: Mutation Analysis, Type 1 diabetes, IDDM, Sequencing.

INTRODUCTION

Type 1 diabetes is one of the most frequent chronic diseases in children caused by a combination of genetic and environmental factors (She and Marron, 1998). In Type 1 diabetes insulin deficiency causes impaired peripheral glucose utilization, which results in elevated blood glucose levels (hyperglycemia). The destruction of ß-cells leads to almost total insulin deficiency.

Type 1 diabetes accounts for about 10% of all diabetes, affecting approximately 1.4 million people in the U.S., and 10-20 million globally (Rewers, 1991; Libman *et al*., 1993). About 40% of persons with Type 1 diabetes develop disease before 20 years of age, thus making it one of the most common severe chronic disease of childhood. The peak incidence occurs at adolescence, with a smaller peak in preschool children. In addition, there are data to suggest that 5 -10% of all adults diagnosed with Type 2 diabetes may actually have Type 1 (latent autoimmune disease of adults),

suggesting that there may be more patients with the disease than previously thought (Krolewski *et al*., 1987).

Type 1 diabetes, also known as, insulin dependent diabetes mellitus (IDDM) is a disorder of glucose homeostasis that is characterized by susceptibility to ketoacidosis in the absence of insulin therapy. It involves a T- cell-mediated destruction of the pancreatic beta cells, the body's sole source for insulin (Eisenbarth, 1986). Type 1 diabetes has been shown to involve a genetic component and environmental factors (Todd, 1998). The genes that are known to play role in genetic susceptibility include those in the Human Leukocyte Antigen (HLA) complex on chromosome 6p21 and the insulin gene on chromosome 11p15 (She and Marron, 1998).

It has been estimated that 60% of the genetic susceptibility to Type 1 diabetes is conferred by HLA (Risch, 1989). It is speculated that the HLA DR or DQ class II molecules associated with Type 1 diabetes provide antigen presentations that generate T-helper cells that initiate an immune response to specific islet cell autoantigens. There is a genetic interaction between Type 1 and Type 2 diabetes mediated by HLA locus (Li e*t al*., 2001). The combination of major histocompatibility complex class I chain related A gene (MICA) alleles and HLA DR, DQ conferred increased risk for adult onset Type 1 diabetes (Gambelunghe *et al*., 2001). Currently, there are more than15 such candidate loci identified.

The role of insulin region in Type 1 diabetes has been identified by various laboratories using association studies (Lucassen e*t al*., 1993; Julier *et al*., 1994). Insulin is a major disease determinant in Type 1 diabetes and related disorders such as obesity. A highly polymorphic variable number of tandem repeat (VNTR) located in promoter region of the insulin gene 365 bp upstream of the transcription initiation site on chromosome 11p15 has been proposed to influence the level of expression of insulin in both the Islets of Langerhans and the thymus. Class-I alleles (26-63 repeats) predispose in a recessive way to Type 1 diabetes. Most recent studies have provided evidence that maternal enterovirus infections increase the risk of the offspring developing Type 1 diabetes (Hyoty *et al*., 1995).

Islet autoantibodies are very common at the time of onset of Type 1 diabetes and can differentiate Type 1 from Type 2 diabetes. The first islet autoantigen and β cell specific autoantigen reported was insulin (Palmer *et al*., 1983). DNA polymerase chain reaction can be a powerful tool for

amplifying selected segments of genomic DNA for investigation of point mutations. Heritable abnormalities in insulin gene have often been considered in terms of their potential for contributing to diabetes. Analysis of amino acid sequence of a mutant insulin purified from pancreas of diabetic patients with hyperinsulinemia suggested a substitution of leucine for valine at the third position of the A chain, A3 (Val → Leu) proinsulin gene exon 3 showed a heterozygous point mutation (CGT → CAT) resulting in the substitution of Arg → His in position 65 (Roder *et al*., 1996). Studies of mutant human insulin gene have provided insights into subjects as varied as insulin biosynthesis, structure – activity relationship in insulin recognition and physiology in insulin recognition and physiology of insulin action.

The purpose of present study is to carry out mutational analysis of insulin gene in children with Type 1 diabetes. Sequence analysis can determine different mutations in the same gene, if the patients are not related and separated geographically. It will permit a more accurate genetic counseling to be offered and may allow the development of new specific therapies.

MATERIALS AND METHODS

Blood samples were collected from 36 unrelated informed volunteers from the Punjab province (30 samples from the diabetic patients and 6 from the normal individuals). From each individual, 3ml blood was drawn after filling in the consent form of that individual. The drawn blood was mixed with 70µl of 0.5 M EDTA on the spot in a 15 ml culture tube and shifted to the Laboratory. Aliquots 700µl –800µl of the blood were made in 1.5 ml centrifuge tubes, and then frozen at -70°C. The DNA was extracted without using Proteinase K (Subbarayan *et al.,* 2002). The quantity of the extracted DNA in the T.E. solution was estimated first by Spectrophotometery followed by Yield Gel Electrophoresis.

Optimization of Exon 1, 2 and 3 of insulin gene (INS) was necessary to conduct the mutational analysis for the patients samples and to compare the results with the normal ones. First of all PCR conditions were optimized separately for Exon 1, Exon 2 and Exon 3. For this purpose PCR reactions were set up and reactions conditions optimized.

Amplification of 36 DNA samples of patients as well as normal (5ng/µl,

20ng/µl dilutions) was done for the Exon 1, 2 and 3 of insulin gene using region specific oligonucleotide.

PRIMER SEQUENCES OF INS EXONS FOR AMPLIFICATION

Exon		Primer sequence	Product size
INS. EXON-1	F	cggaaattgcagcctcag	294 bp
	R	ctcacccccacatgcttc	
INS. EXON-2	F	gaagcatgtgggggtgag	443 bp
	R	acttttaggacgtgaccaagagaac	
INS. EXON-3	F	cactgtgtctccctgactgtgt	400 bp
	R	cccagagagcgtggagag	

For optimization and amplification three thermal cyclers GeneAmp PCR system 2700 (Applied Biosystems), MJ Research Inc. PTC-100 and Hybaid Thermal Cycler were used. After amplification 2% agarose gel was run followed by purification of amplified Exon 1, 2 and 3 of insulin gene (INS). BigDye^{TM} Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) was used to sequence the PCR products of exons 1, 2 and 3 of INS. Finally after sequencing reaction, purification of amplified Exon 1, 2 and 3 of INS was done and then the samples were sequenced by Sequencer.

RESULTS AND DISCUSSION

Type1 diabetes is a complex disease caused by a combination of genetic and environmental factors. It is highly multigenic and multi factorial disease. Genome wide linkage studies in Type 1 diabetes have been performed with the aim of identifying novel susceptibility loci (Mein *et al.,* 1998). Genetic studies performed in human indicate the unexpected multiplicity of genetic markers linked with the disease (>15). In addition to verifying HLA and INS susceptibility loci, the study confirmed the locus IDDM1 on chromosome 6, IDDM2 on chromosome 2 and 11, GCK on chromosome 7 and IDDM10 on chromosome 10 (Cox *et al.,* 2001). In present study exon 1, 2 and 3 of INS of children with type1 diabetes were sequenced with 100% homology to the normal sequence. INS is a small gene having only 3 exons. Sequencing of all three exons showed entirely normal nucleotide sequence and no mutation was observed in any of three exons of INS. It is, therefore, concluded that mutation may be in the promoter region, so affecting the transcription of the gene, or it may be in loci other than INS that are involved in developing type

1 diabetes as it is highly multigenic. It is also possible that the disease developed due to the involvement of any environmental factor.

Results of this study revealed that the prevalence rate of insulin gene defect in type 1 diabetic patients is lower as compared to other genes causing type 1 diabetes. Although, evidence has demonstrated the insulin gene mutations in man and the secretion of abnormal human insulin in affected individuals with diabetes. Some of the reported insulin gene mutations are INS, PHE25LEU (Targer *et al.,* 1979), INS, PHE24SER (Haneda *et al.,* 1983), INS, HIS10ASP (Chan *et al.,* 1987), INS, ARG65HIS (Barbetti *et al.,* 1990), INS, VAL3LEU (Nanjo *et al.,* 1986), INS, ARG65LEU (Yano *et al.,* 1992) and INS, ARG65PRO (Warren-Perry *et al.,* 1979). Heritable abnormalities in insulin gene have often been considered in terms of their potential for contributing to diabetes.

It is considered that mutation may be in insulin gene promoter region, possibly reflecting impaired insulin gene promoter function. The promoter not only regulates the transcriptional response to glucose but also restricts expression of the insulin gene exclusively to beta cells. A highly polymorphic variable number of tandem repeat (VNTR) located in promoter region is associated with variation in the expression of INS (Le Stunff *et al.,* 2001).

In addition to INS on chromosome 11, Human Leukocyte Antigen (HLA) complex on chromosome 6 play strong role in genetic susceptibility to type1 diabetes (She and Marron, 1998). As no mutation was observed in INS exons, it is speculated that the disease may developed as a result of autoimmune disorder that is an abnormal immune response against beta cells of pancreatic islets of Langerhans. The well-documented loci in pediatric patients have been mapped to HLA-DR and DQ regions (Erlich *et al.,* 1991; Todd *et al.,* 1990). More than half of the inherited predisposition to type I diabetes maps to HLA class II genes. Possible genetic interaction between type I and type II diabetes was found mediated by HLA locus (Li *et al.,* 2001). HLA haplotype DQA1, DQB1 and DRB1 are associated with protection from type 1 diabetes (Greenbaum *et al.,* 2000; Redondo *et al.,* 2000).

Mutations in insulin receptor gene can render the cell resistant to the biological action of insulin by decreasing the level of insulin receptor mRNA which leads to a decrease in the rate of receptor biosynthesis and

consequently, a decrease in the number of receptors on the cell surface (Takashi *et al.,* 1990). In the light of central role of insulin resistance in predisposing to development of type1 diabetes, it is reasonable to inquire whether patient may have mutations in the insulin receptor gene. Type1 diabetes is a ‘complex trait’, which means that mutations in several genes likely contribute to the disease, as it is evident from the results of present study. Normal insulin gene of diabetic patients shows that there may be mutation in any other gene which may result in the impairment of normal gene function leading to the development of disease. This doesn’t mean that insulin gene is not involved is type 1 diabetes. It is proposed that more affected individuals should be enrolled and screened for insulin gene, perhaps some individuals might have mutated insulin gene which will give better insight into the understanding of the role of insulin gene region in developing type1 diabetes.

REFERENCES

BARBETTI, F., RABEN, N., KADOWAKI, T., CAMA, A., ACCILI, D., GABBAY, K.H., MERENICH, J.A., TAYLOR, S.I. AND ROTH, J., 1990. Two unrelated patients with familial hyperproinsulinemia due to a mutation substituting histamine for a arginine at position 65 in the proinsulin molecule: identification of the mutation by direct sequencing of genomic deoxyribonucleic acid amplified by polymerase chain reaction. *J. clin. Endocr. Metab.*, **71**: 164-169

CHAN, S.J., SEINO, S., GRUPPUSO, P.A., SCHWARTZ, R. AND STEINER, D.F., 1987. A mutation in the B chain coding region is associated with impaired prinsulin conversion in a family with hyperproinsulinemia. *Proc. Nat. Acad. Sci.*, **84**:2194-2197.

COX, N.J., WAPELHORST, B., MORRISON, V.A., JOHNSON, L., PINCHUK, L., SPIELMAN, R.S., TODD, J.A. AND CONCANNON, P., 2001. Seven regions of the genome show evidence of linkage to type 1 diabetes in a consensus analysis of 767 multiplex families. *Am. J. Hum. Genet.,* **69**: 820-830.

EISENBARTH, G.S., 1986. Type 1 diabetes mellitus. A chronic autoimmune disease. *N. Engl. J. Med.,* **314**: 1360-8.

ERLICH, H.A., GRIFFITH, R.L., BUGAWAN, T.L., ZIEGLER, R., ALPER, C. AND EISENBARTH, G., 1991. Implication of specific DQB1 alleles in genetic susceptibility and resistance by identification of IDDM siblings with novel HLA-DQB1 allele and unusual DR2 and DR1 haplotypes. *Diabetes*, **40**: 478-481.

GAMBELUNGHE, G., GHADERI, M., TORTOIOLI, C.A., SANTEUSANIO, F., BRUNETTI, P., SANJEEVI, C.B., FALORNI, A., 2001. Two distinct MICA gene markers discriminate major autoimmune diabetes types. *J. clin. Endocr. Metab.,* **86**: 3754-3760.

GREENBAUM, C.J., SCHATZ, D.A., CUTHERBERTSON, D., ZEIDLER, A., EISENBARTH, G.S. AND KRISCHER, J.P., 2000. Islets cell antibody-positive relatives with human leucocytes antigen DQA1 0102, DQB1 0602: identification by diabetes prevention trial type 1. *J. clin. Endocr. Metab.,* **85**: 1255-1260.

HANEDA, M., CHAN, S.J., KWOK, S.C.M., RUBENSTEIN, A.H. AND STEINER, D.F., 1983. Studies on mutant human insulin genes: identification and sequence analysis of a gene encoding (ser-B24) insulin. *Proc. Nat. Acad. Sci.*, **80**: 6366-6370.

HYOTY, H., HILTUNEN, M., KNIP, M., LAAKKONEN, M., VAHASALO, P., KARJALAINEN, J., 1995. A prospective study of the role of coxsackie B and other enterovirus infections in the pathogenesis of IDDM. Childhood Diabetes in Finland (DiMe) Study. *Diabetes,* **44**: 652-7.

JULIER, C., 1994. Multiple DNA variant association analysis: application to the insulin gene region in type 1 diabetes. *Am. J. Hum. Gen.,* **55**: 1247-54

KROLEWSKI, A.S., WARRAM, J.H., RAND, L.L. AND KAHN, C.R., 1987. Epidekmiologic approach to the etiology of type 1 diabetes mellitus and its complications. *N. Engl. J. Med.,* **317**: 1390-1398.

LI, H., LINDHOLM, E., ALMGREN, P., GUSTAFSSON, A., FORSBLOM, C. AND GROOP L, TUOMI, 2001. Possible human leukocyte antigen-mediated genetic interaction between type 1 and type 2 diabetes. *J. clin. Endocr, Metab.,* **86**: 874-582.

LIBMAN, I., SONGER, T. AND LAPORTE, R., 1993. How many people in the U.S. have IDDM? *Diabetes Care*, **16**: 841-842.

LUCASSEN, A.M., JULIER, C., BERESSI, J.P., BOITARD, C., FROGUEL, P., LATHROP, M. AND BELL, J.I., 1993. Susceptibility to insulin dependent diabetes mellitus maps to a 4.1 kb segment of DNA spanning the insulin gene and associated VNTR. *Nature Genet.,* **4**: 305-310.

MEIN, C.A., ESPOSITO, L. AND DUNN, M.G., 1998. A search for type 1 diabetes susceptibility genes in families from the United Kingdom. *Nature Genet*., **19**: 297-300.

NANJO, K., SANKE, T., MIYANO, M., OKAI, K., SOWA, R., KONDO, M., NISHIMURA, S., IWO, K., MIYAMURA, K., GIVEN, B.D., CHAN, S.J., TAGER, H.S., STEINER, D.F., RUBENSTEIN, A.H., 1986. Diabetes due to secretion of a structurally abnormal insulin (insulin wakayama): Clinical and functional characteristics of (Leu-A3) insulin. *J. clin. Invest.*, 77: 514-519.

PALMER, J.P., ASPLIN, C.M., CLEMONS, P., LYEN, K., TATPATI, O., RAGHU, P.K., PAQUETTE, T.L., 1983. Insulin antibodies in insulin-dependent diabetics before insulin treatment. *Science,* **222**: 1337-1339.

REDONDO, M.J., REWERS, M., YU, L., GARG, S., PILCHER, C.C., ELLIOTT, R.B., EISENBARTH, G.S., 2000. Genetic determination of islet cell autoimmunity in monozygotic twins. *Br. med. J.,* **318**: 698-702.

REWERS, M., 1991. The changing face of the epidemiology of insulin-dependent diabetes mellitus (IDDM): Research designs and models of disease causation. *Ann. Med.,* **23**: 419-426.

RISCH, N., 1989. Genetics of IDDM: Evidence for complex inheritance with HLA. *Genet. Epidemiol.,* **6**: 143-8.

RODER, M.E., VISSING, H. AND NAUCK, M.A., 1996. Hyperproinsulinemia in a three-generation Caucasian family due to mutant proinsulin (Arg 65 - >His) not associated with impaired glucose tolerance: The contribution of mutant proinsulin to insulin bioactivity. *J. clin. Endocr. Metab.*, **81**: 1634-1640.

SHE, J.X. AND MARRON, M.P., 1998. Genetic susceptibility factors in type 1 diabetes: lineage, disequilibrium and functional analyses. *Curr. Opin. Immunol.,* **10**: 682-9.

SUBBARAYAN, P.R., SARKER, M. AND ARDALAN, B., 2002. Isolation of genomic DNA from human whole blood. *Biotechniques*, **33**: 1231-1234.

TAKASHI, K., HIROKO, K. AND SIMEON, I.T., 1990. A non sense mutation causing

decreased levels of insulin receptor mRNA. *Proc. Natl. Acad. Sci. USA,* **87**: 658-662.

TARGER, H., GIVEN, B., BALDWIN, D., MAKO, M., MARKESE, J., RUBENSTEIN, A., OLEFSKY, J., KOBAYASHI, M., KOLTERMAN, O. AND POUCHER, R., 1979. A structurally abnormal insulin causing human diabetes. *Nature*, **281**: 122-125

TODD, J.A., 1990. The role of MHC class II genes in susceptibility to insulin-dependent diabetes mellitus. *Curr. Top. Microbiol. Immunol.*, **164**: 17-40.

TODD, J.A., 1998. From genome to aetiology in a multifactorial disease, type 1 diabetes. *Bioessays*, **21**: 164-74.

WARREN-PERRY, M.G., MANLEY, S.E., OSTREGA, D., POLONSKY, K., MUSSETT, S., BROWN, P., TURNER, R.C., WILBERZ, S., PARKE, H.J., DAGNAES-HANSEN, F. AND HERBERG, L., 1991. Persistent MHV (mouse hepatitis virus) infection reduces the incidence of diabetes mellitus in non-obese diabetic mice. *Diabetologia,* **34**: 2-5.

YANO, H., KITANO, N., MORIMOTO, M., POLONSKY, K., IMURA, H. AND SEINO, Y., 1992. A novel point mutation in the human insulin gene giving rise to hyper-proinsulinemia (proinsulin Kyoto). *J. clin. Invest.*, **89**: 1902-1907.

ORIGNAL SEQUENCE OF EXON 1 OF INSULIN GENE (INS).

```
****** target
>>>>>> left primer
<<<<<< right primer
1     caggtgagggctttgctctcctggagacatttgcccccagctgtgagcagggacaggtct
61    ggccaccgggcccctggttaagactctaatgacccgctggtcctgaggaagaggtgctga
121   cgaccaaggagatcttcccacagacccagcaccagggaaatggtccggaaattgcagcct
                                               >>>>>>>>>>>>>>>>>
181   cagcccccagccatctgccgacccccccacccaggccctaatgggccaggcggcagggg
      >>>
241   ttgagaggtaggggagatgggctctgagactataaagccagcgggggcccagcagccctc
      ******************************************************
301 AGCCCTCCAGGACAGGCTGCATCAGAAGAGGCCATCAAGCAGgtctgttccaagggcctt
    ********************************************************************
361   tgcgtcaggtgggctcaggattccagggtggctggaccccaggccccagctctgcagcag
      *********************************
421   ggaggacgtggctgggctcgtgaagcatgtgggggtgagcccaggggccccaaggcaggg
                         <<<<<<<<<<<<<<<<<<<<
481   cacctggccttcagcctgcctcagccctgcctgtctcccag
```

ORIGNAL SEQUENCE OF EXON 2 OF INSULIN GENE (INS)

```
1  gtctgttccaagggcctttgcgtcaggtgggctcaggattccagggtggctggaccccag
61 gccccagctctgcagcagggaggacgtggctgggctcgtgaagcatgtgggggtgagccc
   >>>>>>>>>>>>>>>>>>>>
121aggggccccaaggcagggcacctggccttcagcctgcctcagccctgcctgtctcccagA

***************************************************************
181
TCACTGTCCTTCTGCCATGGCCCTGTGGATGCGCCTCCTGCCCCTGCTGGCGCTGC
TGGC

************************************************************************
***
241CCTCTGGGGACCTGACCCAGCCGCAGCCTTTGTGAACCAACACCTGTGCGGCT
CACACCT

************************************************************************
*****
301GGTGGAAGCTCTCTACCTAGTGTGCGGGGAACGAGGCTTCTTCTACACACCCA
AGACCCG

************************************************************************
******
361CCGGGAGGCAGAGGACCTGCAGGgtgagccaactgcccattgctgcccctggccgccccc
```

****
421agccaccccctgctcctggcgctcccacccagcatgggcagaagggggcaggaggctgcc
481acccagcagggggtcaggtgcactttttaaaaagaagttctcttggtcacgtcctaaaa
<<<<<<<<<<<<<<<<<<<<<<<<<<<
541gtgaccagctccctgtggcccagtcagaatctcagcctgaggacggtgttggcttcggca
<<
601gccccgagatacatcagagggtgggcacgctcctccctccactcgcccctcaaacaaatg
661ccccgcagcccatttctccaccctcatttgatgaccgcagattcaagtgttttgttaagt
721aaagtcctgggtgacctggggtcacagggtgccccacgctgcctgcctctgggcgaacac
781cccatcacgcccggaggagggcg

Orignal Sequence of Exon 3 of Insulin Gene (INS).

1 acagggtgccccacgctgcctgcctctgggcgaacaccccatcacgcccggaggagggcg

61 tggctgcctgcctgagtgggccagacccctgtcgccaggcctcacggcagctccatagtc

121 aggagatggggaagatgctggggacaggccctggggagaagtactgggatcacctgttca

181 ggctcccactgtgacgctgccccggggcgggggaaggaggtgggacatgtgggcgttggg

241 gcctgtaggtccacacccagtgtgggtgaccctccctctaacctgggtccagcccggctg

301 gagatgggtgggagtgcgacctagggctggcgggcaggcgggcactgtgtctccctgact
>>>>>>>>>>>>>>>>>>>>>
361 gtgtcctcctgtgtccctctgcctcgccgctgttccggaacctgctctgcgcggcacgtc
>>>>***
421
ctggcagTGGGGCAGGTGGAGCTGGGCGGGGGCCCTGGTGCAGGCAGCCTGCAGCCC
TTG

****
481
GCCCTGGAGGGGTCCCTGCAGAAGCGTGGCATTGTGGAACAATGCTGTACCAGCA
TCTGC
****
541
TCCCTCTACCAGCTGGAGAACTACTGCAACTAGACGCAGCCCGCAGGCAGCCCCA
CACCC
****
601
GCCGCCTCCTGCACCGAGAGAGATGGAATAAAGCCCTTGAACCAGCcctgctgtgccgtc
****
661 tgtgtgtcttgggggccctgggccaagccccacttcccggcactgttgtgagcccctccc
****
721agctctctccacgctctctgggtgcccacaggtgccaacgccggccaggcccagcatgca
****<<<<<<<<<<<<<<<<<<<<<<
781 gtggctctccccaaagcggccatgcctgtcggctgcctgctgcccccaccctgtggctca

841 gggtccagtatgggagctgcgggggtctctgaggggccaggggtggtggggccactgaga

901agtgacttcttgttcagtagctctggactcttggagtccccagaga

Figure 1 shows the DNA extracted from different blood dsampled. Figure 2 is the gel photograph of Exons 1 in which a no. of amplicons are shown along with the 100 bp ladder marker. Figure 3 is the gel photograph of Exons 2 in which amplicons are shown. Figure 4 is the gel photograph of Exons 3 in which amplicons are shown with marker. Figure 5 shows the PCR products of amplified exons 1, 2 and 3 on agarose gel. Figure 6 shows the quantification of PCR products of insulin gene (INS) by agarose gel electrophoresis.

L1 L2 L3 L4 L5 L6 L7 L8 L9 L10 L11 L12 L13 L14

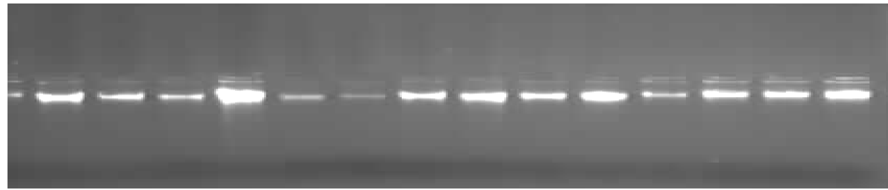

Fig. 1. DNA extraction from the whole blood samples.

L1 L2 L3 L4 L5 L6 L7 L8100bp L9 L10 L11 L12 L13 L14 L15
ladder

Fig. 2. Optimization of Exon 1 of insulin gene; L1-L8, amplified samples of Exon 1 of insulin gene; L9, 100 bp size marker; L10-L15, amplified samples of Exon 1.

L1 L2 L3 L4 L5 L6

Fig. 3: Optimization of Exon 2 of insulin gene; L1-L4 amplified samples of Exon 2; L5, negative control; L6, 100 bp size marker.

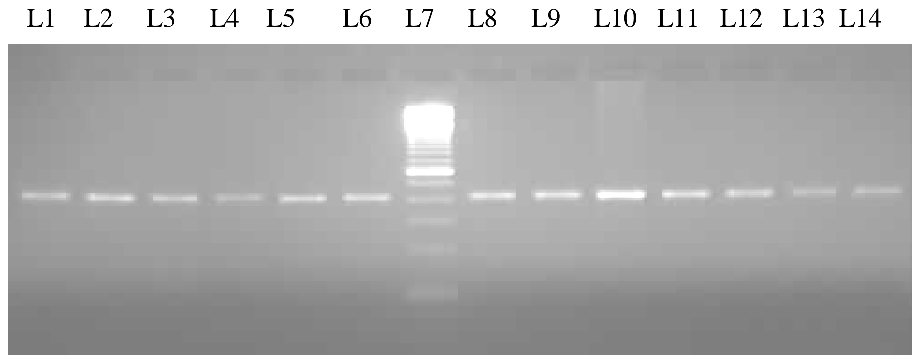

Fig. 2. Optimization of Exon 3 of insulin gene; L1-L6, amplified samples of Exon 2; L7, 100 bp size marker; L8-L14, amplified samples of Exon 2.

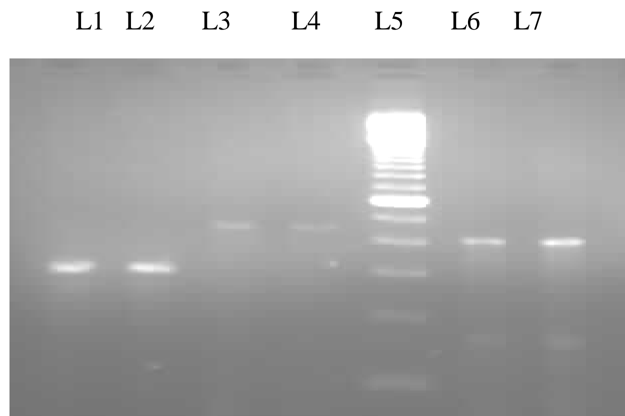

Fig. 5: PCR products of amplified Exons 1, 2 And 3 on 2% agarose gel; L1-L2, amplified samples of Exon 1 of insulin gene. Product size 294 bp; L3-L4, amplified samples of Exon 2; product size 443 bp; L5, 100 bp size marker; L6-L7, amplified samples of Exon 3 product size 400 bp

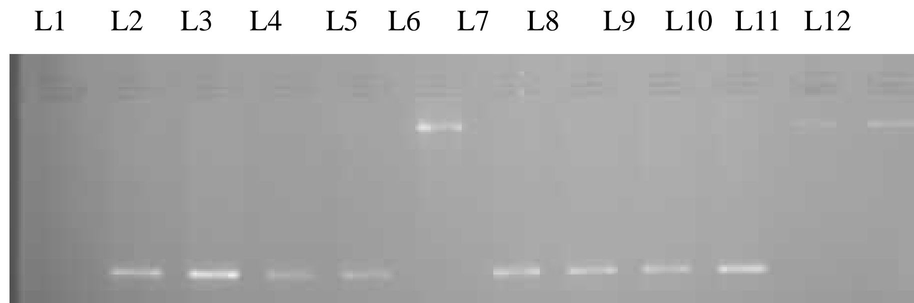

Fig. 6: Quantification of PCR products of insulin gene by agarose gel electrophoresis; L1, blank; L2-L5, PCR products of insulin gene; L6, 20ng standard marker; L7-L10, PCR products of insulin gene; L11, 5ng standard marker; L12, 10ng standard marker.

DISCUSSION

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SEASONAL SUCCESSION OF ALGAL SPECIES IN RAWAL DAM, ISLAMABAD

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Abstract.- An ecological survey of algal species from Rawal Dam was carried out during June 2000 to May 2001. Water samples were collected for determination of physico-chemical parameters such as temperature, pH, Free carbon dioxide, dissolved oxygen, ammonia nitrogen, humidity, total hardness, orthophaspate etc. A total of 257 algal species belonging to 97 genera of 7 algal classes were collected by phytoplankton net, slide cover, forceps, tooth brush, pipet, knife, hand picking etc from different zones like mixed reactors, too old branches, basins, pools, dead zones at the back of tree, groynes, stones etc. The classes of algae represented were: Cyanophyceae (62 species belonging to 18 genera), Chlorophyceae (120 species, 53 genera), Bacillariophyceae (55 species, 16 genera), Chrysophyceae (1 species, 1 genus), Xanthophyceae (6 species, 5 genera), Euglenophyceae (12 species, 3 genera) and Charophyceae (1 species, 1 genus).

Key words: Seasonal succession, Algae, Rawal Dam.

INTRODUCTION

Qualitative and quantitative determinations along with seasonal succession of algal species are essential for determining the aquatic productivity. They are the chief source of food for aquatic animals including fishes. Algal species fix nitrogen and can be benificial for crops like sugarcane and rice etc. Algal species are also a good indicator for pollution (Patrick and Reimer, 1966). Due to favourable climatic conditions, our country is quite rich in algal species. Algae is a rich source of protein and can be used as dietary ingredient both for animal and human diets.

The fresh water of Rawal Dam passes through siwalik rocks exposed in the area. The Siwalik rocks consist of Murree, Kamlial, Soan formation and Lai conglomerate. Murree formation is composed of a monotonous sequence of dark red and purple clay, purple grey and greenish grey sandstone with subordinate intraformational conglomerate. Murree formation

unconformably overlies of Eocene age. Its upper contact is broadly transitional with the Kamlial formation. Kamlial formation consists of purple grey and dark brick – red sandstone which is medium to coorse grained and contains interbeds of hard purple shale, yellow and purple intra formational conglomerate. Soan formation consists essentially of compact massive conglomerate with subordinate interbeds of varicolored sand stone, siltstone and clay. Lai Conglomerate formation is composed of coarse boulder and pebbple conglomerates, with minor coarse and cross-bedded sandstone.

Rawal dam is situated on the outskirts of Islamabad and is replenished by Chhatar Nulla. The collections were done from freshwater of Rawal Dam and its spillway upto near the bridge on the way of NARC (latitude is 33°.42' north, longitude 73°.07' east) at the altitude of 1800 ft. above the sea level. Ahmad *et al*. (1985) described seven physico-chemical parameter of Rawal Lake. Some other work on algal flora was undertaken by Baqai *et al*. (1974) on the limnological survey of Haleji Lake. Nazneen (1974) and Nazneen and Bari (1984) described the seasonal distribution of phytoplankton in Kalri Lake and Haleji Lake. Leghari and Leghari (1999), Leghari *et al*. (2000) and Leghari and Leghari (2001) described seasonal variations of phytoplankton of Bakar Lake, Tatta Pani, River Punch and Bakar and Phoosna Lakes. The present study is the first report from Rawal Dam.

The present work will deal with seasonal succession of algal species of Rawal dam where different physico-chemical properties and other parameters have been taken into consideration to study the Algal species.

MATERIALS AND METHODS

Sample collection

Algal species were collected monthly from June 2000 to May 2001 between 11 a.m. to 3 p.m with the help of a phytoplankton net of 5-10 μm mesh size. Filamentous algae were collected with the help of forceps, blue green algae were collected with slide cover, diatoms with tooth brush while algae that can be see easily with naked eye like *Chara, Cladophora, Spirogyra, Hydrodictyon* etc. was picked with hands from the different zones of Nulla Rawal Dam like mixed reactors, too old branches, basins, pools, dead zones at the back of tree groynes, stones etc.

Physicochemical features of sample

All the collected samples were brought to the herbarium for taxonomical studies of the algal species, the samples were preserved in 4% commercial formalin solution (Mason, 1967) other samples were stored at room temperature for chemical analysis.

Water samples were collected in Nansen bottles for studying physico-chemical features using standard methods (APHA, 1985). The procedures of determination of physico-chemical parameters are based on standard methods described below: (1) pH value: pH value of the water samples was determined by using pH meter Hanna Woonsocket No., RI 02895 Made in Portugal, (2) TDS value of the water samples was determined by using TDS Meter Hanna Woonsocket No., RI 02895 Made in Portugal, (3) Water and air temperature was determined by using mercury thermometer Made in England (minus – 20°C – 100°C), (4) Total hardness of water was determined by using T. Hardness Test Kit Code: HI-3812 Hanna Made in Italy, (5) Salinity of water was determined by using salinity test kit code/Hi 3835 Hanna Made in Italy, (6) Carbon dioxide of water was determined by using carbon dioxide test kit code HI-3818 Hanna Made in Italy, (7) Dissolve oxygen (DO) of water was determined by using dissolve oxygen test kit code HI-3810 Hanna Made in Italy, (8) Humidity in air was determined by using Hygrometer made in Japan, and (9) Light transparency was determined by Secchi disc (Meter) made in U.S.A.

Identification of algal species

The species composition was determined by utremohal method (Lund *et al*., 1958). Algal species identification and counts were done using inverted light microscope (BH-2 Olympus Japan 10x40) and identified with the help of available literature (Tilden, 1910; Hustedt, 1930; Majeed, 1935; Smith, 1950; Desikachary, 1959; Prescott, 1961; Siddiqi and Faridi, 1964; Patrick and Reimer, 1966; Philpose, 1967; Tiffany and Briton, 1971; Vinyard, 1979; Akiyama and Yamagishi, 1981).

RESULTS AND DISCUSSION

Algal species

A total of 257 species belonging to 97 genera of 7 algal classes were observed. Cyanophyceae (62 species; belonging to 28 genera; 24.12%),

Chlorophyceae (120 species; 53 genera; 46.7%), Bacillariophyceae (55 species; 16 genera; 21.4%), Chrysophyceae (1 species; 1 genus; 0.4%), Xanthophyceae (6 species; 5 genera; 2.33%), Euglenophyceae (12 species; 3 genera; 4.7%), Charophyceae (1 species; 1 genus; 0.4%) and their seasonal succession in Table I.

TABLE I.- SEASONAL SUCCESSION OF ALGAL SPECIES IN RAWAL DAM WATER, ISLAMABAD

	Name of Species	**Spring March – May**	**Summer June– Aug.**	**Autumn Sept.– Nov.**	**Winter Dec.–Feb.**
Division: Cyanophyta					
Class: Cyanophyceae					
Order: Chroococcales					
Family: Chroococcaceae					
1.	*Aphanocapsa elachista* West & West	c	c	vc	vc
2.	*A. montana* Cramer	a	a	r	r
3.	*A. virescens* (Hass.) Raben	c	r	c	c
4.	*Aphanothece nidulans* Richter	c	r	c	vc
5.	*A. saxicola* Naegeli	r	a	a	a
6.	*Chroococcus bituminosus* (Bory) Hansgirg	a	a	vr	vr
7.	*C. cohaerens* (Breb) Naegeli	a	a	a	vr
8.	*C. endophyticus* Copeland	c	c	c	c
9.	*C. giganteus* W. & West	a	vr	vr	a
10.	*C. lithophilus* Erceg.	a	a	vr	a
11.	*C. minutus* var. *thermalis* Copeland	a	vr	vr	a
12.	*C. turgidus* (Kuetz.) Näg.	vc	vc	vc	vc
13.	*C. turgidus* var. *tenax* Kirchin	a	a	vr	a
14.	*Gloeocapsa ambigua* var. *violacea* Naeg.	a	a	vr	vr
15.	*G. compacta* Kuetz.	c	c	C	c
16.	*G. gelatinosa* (carm.) Kuetz.	r	c	C	r
17.	*G. lithophila* (Ereeg.) Hollerb	a	a	vr	a
18.	*G. nigrescens* Naeg.	a	a	vr	a
19.	*Gloeothece confluens* Nag.	c	c	C	c
20.	*G. aponina* Kuetz.	vc	vc	vc	vc
21.	*G. aponina* var. *cordiformis* Elenk.	vc	vc	vc	vc

Continued

Name of Species		Spring March – May	Summer June– Aug.	Autumn Sept.– Nov.	Winter Dec.–Feb.
22.	*G. aponina* var. *delicatula* virieux.	vc	vc	vc	vc
23.	*G. lacustris* Chod.	vc	vc	vc	vc
24.	*Merismopedia convoluta* Breb.	c	c	c	c
25.	*M. glucum* (Ehr.) Naegeli	vc	vc	vc	vc
26.	*Microcystis aeruginosa* Breb.	c	c	c	c
27.	*M. flos-aquae* (Wittrock) Kirchner.	a	a	a	r
28.	*Pseudoholopedia convoluta* (Breb.) Elekin.	vr	vr	vr	vr
Order: Oscillatoriales					
Family: Oscillatoriaceae					
29.	*Lyngbya bornettii* Zukal	a	a	vr	vr
30.	*L. confervoides* Agardh	c	c	c	c
31.	*L. martensiana* Meneghini	r	c	c	r
32.	*Oscillatoria agardhii* Gomont	r	r	r	r
33.	*O. bornethii* Zukal	r	vr	vr	c
34.	*O. curvceps* Agardh	c	c	c	c
35.	*O. formosa* Bory	c	c	c	c
36.	*O. iwanoffiana* (Nyg.) Geitler	a	a	vr	vr
37.	*O. limosa* Ag.	a	vr	a	a
38.	*O. minnestensis* Tilden	a	a	vr	r
39.	*O. musicola* Woronich.	a	a	r	r
40.	*O. proboscidea* Gom.	a	r	r	a
41.	*O. quasiperforata* var. *crassa* Em.et Hirose	vr	vr	vr	vr
42.	*O. tenuis* Agardh.	c	c	c	c
43.	*Phormidium faveolarum* (Mont.) Gom.	a	a	vr	a
44.	*P. orientale* West	a	vr	vr	a
45.	*P. subfuscum* (Ag.) Kuetz.	a	vr	vr	a
46.	*P. tenue* (Menegh.) Gom.	a	vr	vr	a
47.	*P. uncinatum* (Agardh.) Goment.	a	r	r	a
Order: Nostocales					
Family: Nostocaceae					
48.	*Anabaena aequalis* Borge	a	vr	vr	a
49.	*A. inaequalis* (Kuetz.) Born	a	r	r	a
50.	*A iyengari* var. *tenuis*	a	vr	vr	a

Continued

	Name of Species	Spring March – May	Summer June– Aug.	Autumn Sept.– Nov.	Winter Dec.–Feb.
51.	*A. variabilis* Kuetz.	a	r	r	a
52.	*Anabaenopsis reciborskii* Wolos	a	a	vr	a
53.	*Nostoc commune* Vaucher	a	r	r	a
54.	*N. muscorum* Ag.	a	r	r	a
55.	*N. populorum* (Geit) Hollerb	a	a	vr	a
Family: Rivulariaceae					
56.	*Calothrix centarenii* (Zanard) Born.	a	a	vr	a
57.	*C. stagnalis* Gomont.	a	vr	vr	a
58.	*Rivularia atra* Roth	a	vr	vr	a
59.	*R. minutula* (Kuetz.) Born.	a	vr	vr	a
60.	*R. natans* (Hedw.) Welw.	a	vr	vr	a
Family: Scytonemataceae					
61.	*Sacconema rupestre* Borzi	a	vr	vr	a
62.	*Tolypothrix distorta* var. *penicillata* (Ag.) Lem.	a	vr	vr	a
Division: Chlorophyta					
Class: Chlorophyceae					
Order: Chlorococcales					
Family: Oocystaceae					
1.	*Ankistrodesmus falcatus* (Corda) Ralfs	vr	vr	vr	vr
2.	*A. falcatus* var. *acicularis* (A. Br.) West.	a	a	vr	vr
3.	*A. falcatus* var. *stipitatus* (Chod) Lemm.	a	a	vr	vr
4.	*Chlorella ellipsoidea* Gerneck	vc	vc	vc	vc
5.	*C. pyrenoidosa* Chick	vc	vc	vc	vc
6.	*C. vulgaris* Beyerinck	c	c	c	c
7.	*Gloeotaenium loitelsbergerianum* Hansgirg	a	a	vr	a
8.	*Kircheneriella lunaris* (Kirch.) Moebius.	c	c	c	c
9.	*K. subsolitoria* West.	a	a	vr	a
10.	*Nephrocytium agardhianum* Naegeli	a	a	vr	vr
11.	*Oocystis borgei* Snow	vc	vc	vc	vc
12.	*O. crassa* Wittrock	vc	vc	vc	vc
13.	*O. parva* West & West.	c	c	C	c
14.	*Quadrigula lacustris* (Chod.) Smith.	a	a	vr	a
15.	*Trochiscia hirta* West.	a	a	vr	vr

Continued

Name of Species		Spring March – May	Summer June– Aug.	Autumn Sept.– Nov.	Winter Dec.–Feb.
Family: Chlorococcaceae					
16.	*Chlorococcum humicola* (Naeg.) Rab.	c	c	C	c
Family: Radiococcaceae/Myurococcaceae					
17.	*Coenochloris pyrenoidosa* Korschikoff	c	r	C	c
Family: Dictyosphaeriaceae					
18.	*D ictyosphaerium ehrenbergianum* Naegali	c	c	C	c
19.	*D. pulchellum* var. *ovatum*	vr	vr	R	c
Family: Micractiniaceae					
20.	*Golenkinia paucispina* West & West.	a	a	Vr	vr
Family: Coelastraceae					
21.	*Coelastrum cambricum* Archer.	c	r	C	c
22.	*C. microporum* Naegeli	c	c	C	c
23.	*C. sphaericum* Naegeli	c	c	C	c
Family: Hydrodictyaceae					
24.	*Hydrodictyon reticulatum* (L.) Lagerheim.	vc	vc	Vc	vc
25.	*Pediastrum boryanum* (Turp.) Men.	c	c	C	c
26.	*P. duplex* Meyen	a	vr	Vr	a
27.	*P. duplex* var. *clathratum* (A. Braun.) Lagerheim	a	a	Vr	a
28.	*P. intergrum* Naegeli	a	a	Vr	a
29.	*P. tetras* (Ehr.) Ralfs.	c	c	C	c
Family: Scenedesmaceae					
30.	*Crucigenia lauterbornii* Schmidle	a	a	Vr	vr
31.	*Scenedesmus abundans* (Kirch.) Chodat	c	c	C	c
32.	*S. acuminatus* var. *acuminatus*	c	c	C	c
33.	*S. arcuatus* Lemm.	c	c	C	c
34.	*S. arcuatus* var. *arcuatus*	c	c	C	c
35.	*S. arcuatus* var. *platydisca* Smith	r	a	Vr	vr
36.	*S. aculeolatus* Lemm.	a	a	Vr	a
37.	*S. dimorphus* (Turp.) Kuetz.	a	a	Vr	A
38.	*S. acutiformis* Schroeder	a	a	Vr	Vr
39.	*S. circumfusus* Hortobagyi	a	a	Vr	Vr
40.	*S. longus* Meyen	a	a	Vr	A

Continued

Name of Species		Spring March – May	Summer June– Aug.	Autumn Sept.– Nov.	Winter Dec.–Feb.
41.	*S. minutus* (Smith) Chodat	a	a	Vr	a
42.	*S. opliquus* (Turp.) Kuetz.	a	a	Vr	a
43.	*S. quadricauda* var. *quadricauda*	c	c	C	c
44.	*S. tibiscensis* Uherkovich.	a	a	vr	a
Order: Cladophorales					
Family: Cladophoraceae					
45.	*Basicladia chelonum* (Collins) Hoffiman	a	a	a	vr
46.	*Cladophora glomerata* (L.) Kuetz.	vc	vc	vc	vc
47.	*Pithophora varia* Wille	a	a	vr	r
48.	*P. oedogonia* (Mon.) Wittrock.	a	a	r	r
Order: Chaetophorales					
Family: Chaetophoraceae					
49.	*Chaetophora elegans* (Roth) Agardh	a	a	vr	a
Family: Chaetosphaeridiaceae					
50.	*Chaetosphaeridium pringsheimii* Klebahn.	a	a	vr	a
Family: Coleochaetaceae					
51.	*Coleochaete orbicularis* Pringsheim	a	a	a	vr
Order: Sphaeropleales					
Family: Sphaeropleaceae					
52.	*Sphaeroplea annulina* (Roth) Agardh.	a	a	vr	a
Order: Oedogoniales					
Family: Oedogoniaceae					
53.	*Bulbochaete gigantea* Pringsheim	a	a	vr	a
54.	*Oedogonium olevaceum* Ehr.	c	c	c	c
55.	*O. angustissimum* W.&W.	c	c	c	c
Order: Tetrasporales					
Family: Palmellaceae					
56.	*Palmella mucosa* Kuetz.	a	a	vr	a
57.	*Sphaerocystis schroeteri* Chodat	r	r	r	r
Family: Cocomaxaceae					
58.	*Elakatothrix gelatinosa* Wille	r	r	r	r
Family: Tetrasporaceae					
59.	*Tetrospora lacustris* Lemm.	a	a	R	r

Continued

Name of Species		Spring March – May	Summer June– Aug.	Autumn Sept.– Nov.	Winter Dec.–Feb.
60.	*T. lubrica* (Roth.) Agardh	a	a	R	r
Family: Chlorangiaceae					
61.	*Chlorangium stentorinum* (Ehr.) Stein.	a	a	Vr	a
Order: Ulotrichales					
Family: Ulotrichaceae					
62.	*Koliella helvetica* (Kol.) Hindok	a	a	Vr	a
63.	*Ulothrix aequalis* Kuetz.	a	a	Vr	a
64.	*U. bacillaris* (Naeg.) Com	c	c	C	c
65.	*U. subconstricta* West.	c	c	C	c
66.	*U. tenuissima* Kuetz.	a	a	Vr	a
67.	*U. zonata* (Web. & Mohr.) Kuetz.	a	a	Vr	a
68.	*Uronema confervicola* Lagerheim	c	c	C	c
Family: Microsporaceae					
69.	*Microspora tumidula* Hazen.	r	r	R	r
Order: Volvocales					
Family: Chlamydomonadaceae					
70.	*Chlamydomonas pseudopertyi* Pascher.	vc	vc	Vc	vc
71.	*Platymonas elliptica* Smith	a	a	Vr	a
72.	*Sphaerellopsis fluviatile* (Stein) Pasch.	a	a	Vr	a
Family: Phacotaceae					
73.	*Pteromonas angulosa* (Cart) Lemm.	a	a	A	vr
Family: Volvocaceae					
74.	*Gonium quadratum* Pringsheim	a	a	R	r
75.	*Eudorina elegans* Ehr.	r	vr	R	c
76.	*Pandorina morum* Prins.	a	a	Vr	r
77.	*Pleodorina illinoisensis* Kafoid.	a	a	Vr	r
Order: Zygnematales					
Family: Desmidiaceae/Closterieae					
78.	*Closterium calosporium* var. *majus* W.& West	c	c	C	c
79.	*Cl. dianae* Ehr.	vc	vc	Vc	vc
80.	*Cl. ehrenbergii* Menegh	vc	vc	Vc	vc
81.	*Cl. leibleinii* Kuetz	vc	vc	Vc	vc
82.	*Cl. littorale* Gay	a	a	Vr	a

Continued

	Name of Species	Spring March – May	Summer June– Aug.	Autumn Sept.– Nov.	Winter Dec.–Feb.
83.	*Cl. moniliferum* (Bory) Ehr.	a	a	Vr	a
84.	*Cl. pusillum* var. *minus* Allorge.	a	a	vr	vr
Subfamily: Cosmarieae					
85.	*Cosmarium contractum* var. *ellipsoideum* W. & West	vc	vc	vc	vc
86.	*C. dichondrum* var. *subhexagonum* W. & West	vc	vc	vc	vc
87.	*C. gibberulum* Latkem.	vc	vc	vc	vc
88.	*C. innotum* n. sp.	vc	vc	vc	vc
89.	*C. impressulum* var. *suborthogonum* (Racib.) W.& West.	vc	vc	vc	vc
90.	*C. nitidulum* Denotaris	c	c	c	c
91.	*C. occidentale* (Turp.) Gerloff.	c	c	c	c
92.	*C. subtumidum* var. *rotundum* Hirano	c	c	c	c
93.	*C. oreniferum* sp.	c	c	c	c
94.	*C. palustren* sp.	a	a	vr	vr
95.	*C. reniforme* var. *alaskanum* Croasdole	a	a	vr	vr
96.	*C. phaseolus* var. *phaseolus*	a	a	vr	vr
97.	*C. pseudoconnatum* f. *major* Wille	a	a	vr	vr
98.	*C. ralfsii* var. *spinigerum* Scott & Gron.	a	a	vr	vr
99.	*C. speciosum* L.	c	c	c	c
100.	*C. supergranatum* f. *minor*	a	a	vr	a
101.	*C. tetrachondrum* var. *perornatum* Skuja	a	a	vr	vr
102.	*C. trachypleurum* var. *cornutum*	c	vr	c	c
103.	*C. undulatum* Corda.	a	a	vr	vr
104.	*C. punctulatum* Breb.	a	a	r	r
105.	*Dysphinctium retusum* n. sp.	r	r	r	r
106.	*Penium simplex* n. sp.	a	a	vr	a
107.	*Pleurotaenium ehrenbergii* (Breb.) Bory	a	a	vr	vr
108.	*Staurastrum dilatalum* Ehr.	a	a	vr	a
109.	*S. hexacerum* (Ehr.) Wittr.	a	a	vr	a
110.	*S. hexacerum* f. pentagona	a	a	vr	a
111.	*S. iotanum* Wolle	a	a	vr	a
112.	*S. margaritaceum* (Ehr.) Menghini	c	c	c	c
113.	*S. punctulatum* Breb	c	c	c	c

Continued

Name of Species		Spring March – May	Summer June– Aug.	Autumn Sept.– Nov.	Winter Dec.–Feb.
Family: Zygnemataceae					
114.	*Spirogyra crassa* Kuetz.	vc	vc	vc	vc
115.	*S. aequinoctialis* West				
116.	*M ougeotia gracillima* (Hassal) Wittrock	a	a	a	vr
117.	*M. virescens* (Hassal) Borge	a	a	a	vr
118.	*M. viridis* (Kuetz.) Wittrock	a	a	a	vr
119.	*Sirogonium sticticum* Kuetz.	a	a	a	vr
120.	*Zygnema sterile* Transeau	a	a	a	vr
Division: Bacillariophyta					
Class: Bacillariophyceae					
Order; Achnanthalales					
Family: Achnanthaceae					
1.	*Achnanthes inflata* Kuetz.	vc	vc	vc	vc
2.	*A. lanceolata* (Breb.) Grun.	vc	vc	vc	vc
3.	*Cocconeis placentula* var. *lineata* Ehr. Cleve	vc	vc	vc	vc
4.	*C. disculus* Schum	a	a	vr	vr
5.	*C. thumensis* Mayer	a	a	vr	vr
Order: Centrales					
Family: Coscinodiscaceae					
6.	*Cyclotella antiqua* Smith	c	c	c	c
7.	*C. planctonica* Hustedt.	c	c	c	c
Order: Epithemialales					
Family: Epithemiaceae					
8.	*Epithemia argus* Kuetz.	a	a	vr	a
9.	*E. ocellata* (Her.) Kuetz.	a	a	vr	a
10.	*E. zebra* (Ehr.) Kuetz.	c	c	c	c
11.	*Denticula tenuis* Kuetz.	a	a	vr	a
Order: Naviculales					
Family: Cymbellaceae					
12.	*Amphora ovalis* Kuetz.	vc	vc	vc	vc
13.	*Cymbella brehmii* Hust.	a	a	vr	vr
14.	*C. laevis* Naegeli	c	c	c	c
15.	*C. leptoceros* (Ehr.) Grunow.	a	a	a	vr

Continued

Name of Species		Spring March – May	Summer June– Aug.	Autumn Sept.– Nov.	Winter Dec.–Feb.
16.	*C. tumida* (Breb.) van Heurck.	a	a	vr	a
17.	*C. turgida* Gregory	a	a	vr	a
18.	*C. ventricosa* Kuetz.	c	c	c	c
Family: Gomphonemaceae					
19.	*Gomphonema abbreviatum* Agardh	vc	vc	vc	vc
20.	*G. affine* var. *insigne*	a	a	vr	r
21.	*G. augur* Ehr.	a	a	r	r
22.	*G. longiceps* f. *gracilis* Hust.	c	c	c	c
23.	*G. olivaceum* var. *calcarea* Cleve	c	c	c	c
24.	*G. parvulum* var. *subelliptica* Cleve	a	a	vr	a
25.	*G. ventricosum* Gregory.	a	a	vr	vr
26.	*G. ghosea* Grun.	vc	vc	vc	vc
Family: Naviculaceae					
27.	*Gyrosigma kuetzingii* (Grun.) Cleve	a	a	vr	vr
28.	*Navicula cryptocephala* Hust.	c	c	c	c
29.	*N. cryptocephala* var. intermedia Grun.	c	c	c	c
30.	*N. disjuncta* Hust.	a	a	a	vr
31.	*N. exilissima* Grun.	a	a	vr	vr
32.	*N. incerta* Grun.	c	c	c	c
33.	*N. protracta* (Grun.) Cleve.	c	c	c	c
34.	*N. pupula* var. *mutata* (Kr.) Hust.	a	a	vr	a
35.	*N. radiosa* var. *tenella* Grun.	vc	vc	vc	vc
36.	*N. salinarum* Grunow	c	c	c	c
37.	*N. simlex* Krabke	a	a	vr	a
38.	*N. sohrensis* Krabke	a	a	vr	a
39.	*Pinnularia gibba* (Van Heurk) Boyer	vc	vc	vc	vc
40.	*P. debesi* Schale	a	a	vr	a
41.	*Rhopaldia gibba* (Kuetz.) Mueller	vc	vc	vc	vc
Order: Fragilariales					
Family: Fragilariaceae					
42.	*Fragilaria capucina* Desmazieres	vc	vc	vc	vc
43.	*F. construens* (Ehr.) Grunow	vc	vc	vc	vc
44.	*F. virescens* Ralfs	a	a	vr	vr
45.	*Synedra accus* Kuetz.	a	a	vr	vr

Continued

Name of Species		Spring March – May	Summer June– Aug.	Autumn Sept.– Nov.	Winter Dec.–Feb.
46.	*S. affinis* (Kuetz.) Pascher	c	c	c	c
47.	*S. amphicephala* var. *austriaca*	a	a	vr	vr
48.	*S. rumpens* var. *meneghiniana* Grun.	a	a	vr	vr
49.	*S. ulna* (Nitzsch) Ehr.	vc	vc	vc	vc
50.	*S. ulna* var. *aequalis* (Kuetz.) Hust.	c	c	c	c
51.	*S. ulna* var. *oxyrhynchus*	vc	vc	vc	vc
52.	*S. ulna* var. *danica* (Kuetz.) Grunow	c	c	vc	vc
Order: Nitzschialales					
Family: Nitzschiaceae					
53.	*Nitzschia ignorata* Krabke	a	a	vr	a
54.	*N. filiformis* (Smith) Hust.	a	a	vr	a
Order: Surirellialales					
Family: Surirelliaceae					
55.	*Surirella elegans* Ehr.	a	a	vr	vr
Division: Chrysophyta					
Class: Chrysophyceae					
Order: Ochromonadales					
Family: Dinobryaceae					
1.	*Dinobryon sociale* Ehr.	a	a	vr	vr
Class: Xanthophyceae					
Order: Mischococcales					
Family: Chlorobotrydaceae					
1.	*Chlorellidiopsis separabilis* Pascher	a	a	vr	r
2.	*Chlorobotrys regularis* (W. & W.) Bahlin	a	a	vr	vr
3.	*Perone dimorpha* Pascher	a	a	vr	vr
Family: Pleurochloridaceae					
4.	*Diachros simplex* Pascher	a	a	a	vr
Order: Vaucheriales					
Family: Vaucheriaceae					
5.	*Vaucheria sessilis* DeCandolle	c	vc	vc	vc
6.	*V. geminata* DeCandolle	c	vc	vc	vc

Continued

Name of Species		Spring March – May	Summer June– Aug.	Autumn Sept.– Nov.	Winter Dec.–Feb.
Division: Euglenophyta					
Class: Euglenophyceae					
Order: Euglenales					
Family: Euglenaceae					
1.	*Euglena cyclopicola* Geik	a	a	vr	vr
2.	*E. desus* Ehr.	c	c	c	vc
3.	*E. intermedia* (Klebs) Schmitz.	c	c	c	vc
4.	*E. proxima* Dangeard	a	a	vr	vr
5.	*E. viridis* Ehr.	c	c	c	c
6.	*E. pascheri* Swirenko	c	c	c	c
7.	*Phacus caudatus* Hleeb	a	a	vr	vr
8.	*P. curvicauda* Swirenko	a	a	vr	vr
9.	*P. caudatus* var. *ovalis*	a	a	vr	vr
10.	*P. suecicus* Lemm.	a	a	vr	vr
11.	*P. longicauda* (Ehr.) Diyardin	a	a	vr	vr
12.	*Trachelomonas volvocina* Ehr.	a	a	vr	vr
Class: Charophyceae					
Order: Charales					
Family: Characeae					
1.	*Chara vulgare* L.	vc	vc	vc	vc

Vr = very rare, r = rare, c = common, vc = very common, a = absent

Physico-chemical feature of water

Physical and chemical parameters in Table II show the effect of temperature. High air temperature (39°C) change the climate, directly affecting glacier, snow, water form and water surface temperature (31°C). High temperature of water surface helps in dissolving the organic and inorganic matter and temperature often controls the horizontal distribution of many algae. The photosynthetic rate of phytoplankton has been found to increase with increase of temperature. pH (8.5) show that the water is alkaline. pH increase is due to high concentration of dissolved organic and inorganic matter, temperature and algal species, etc. T.D.S. (300 ppm) showing the concentration of total dissolved solids of the dam is quite productive aquatic life. T.S.S. (1.6 mg/L) shows the ratio of total suspended

TABLE II.- SEASONAL PHYSICO-CHEMICAL PROPERTIES OF RAWAL DAM WATER, ISLAMABAD.

		Spring March – May	**Summer June – August**	**Autumn Sept. – Nov.**	**Winter Dec. – Feb.**
1.	Air temperature °C	37	39	35	28
2.	Water surface temperature ^{0}C	28	31	26	14
3.	pH	8	8.5	7.7	8.2
4.	Turbidity in NTU Range on 100	2.5	4	2	2.5
5.	T.D.S (ppm)	200	300	200	200
6.	T.S.S. mg/L	2	1.2	1	1.6
7.	Conductivity (m.Ohms x^{10})	223	228	228	225
8.	Salinity (NaC1 ppt)	1.5	1.9	1.8	1.8
9.	Humidity (%)	55	75	63	50
10.	Light transparency by Secchi disc (meter)	3.5	3	4	3.5
11.	Dissolved oxygen (mg/l)	8	7	8.1	9.4
12.	CO_2 (ppm)	No free	180	140	105
13.	Ammonia Nitrogen (NH_4 N_2 ppm)	0.03	0.04	0.03	0.03
14.	Nitrate µg/L.	2.4	2.6	2.4	2.4
15.	Density (30^{0}C g/v)	1.006	1.006	1.006	1.006
16.	Phosphate (ug/l)	0.7	0.9	0.8	0.7
17.	Orthophosphate (ug/1)	0.02	0.02	0.02	0.02
18.	Total Hardness ($CaCO_3$ mg/l)	180	190	180	180
19.	Ca^{++} Hardness (mg/l)	110	120	110	110
20.	Mg^{++} Hardness (mg/l)	70	70	70	70
21.	Refractiv index (30°C)	1.33	1.33	1.33	1.33
22.	Wave (inch)	1	2	2	1
23.	Taste	Tasteless	Tasteless	Tasteless	Tasteless
24.	Water colour	Gray green	Bluish green, muddy	Gray, green, muddy	Bluish, green
25.	Odour	Aquatic vegetation	Odourless	Odourless	Fishy
26.	Wind	Dry	Dry hot, moist	Dry	Cold
27.	Day	Clear, shiny	Cloudy, clear	Clear	Clear, shiny
28.	Weather	Dry clear	Clear	Clear	Foggy
29.	Water	Shallow	Deep	Deep	Shallow

Vr, very rare; r, rare; c, common; vc, very common; a, absent.

solids in water, high T.S.S. value show that high concentration of non living particulates originate from catchment area's derived silts, clay, mud, organic matter, etc. This high value is due to rains and floods so flood episodes are the major disturbance in dam affecting composition and biomass of the plankton/algae. The high turbidity (4) value shows that water is too much turbid. High concentration of abiogenic turbidity, whether disturbance or not, water colum mixing as such or in combination with in organic turbidity cause concomitant changes in the light penetration field over time. Consequently, vertical mixing and suspended solids significantly affect phytoplankton photosynthesis and productivity in aquatic environment. Conductivity (228 M ohms x^{10}) shows the ions for production pointing that water is quite productive. Salinity (1.9 ppt) and Orthophosphate (0.02 µg/L) were low. The result show that low salinity, orthophosphate, low temperature provide chances for the presence of species of class Chrysophyceae. High Humidity (75%) value shows that Fungi, Bacteria, Cyanobacteria, Aerial algae etc. could be present as humidity directly affects light and temperature. Light transparency was 4 meter in turbid water low depth of transparent that indicates the water is too much turbid which affect the light limit and thus algae/phytoplankton photosynthesis and therefore restrict biomass production but this result is vice versa. Dissolved oxygen (9.4 mg/L) was in sufficient quantity for production of aquatic life like fish and other fauna etc. but this high concentration of D.O. was due to low temperature, outlet, inlet of water, qualitatively and quantitatively abundance growth of algae, aquatic vegetation, movement of water by boats, wind etc. Carbon dioxide (180 ppm) was in sufficient quantity for the growth of phytoplankton/algal species as increase in CO_2 causes increased algal species. Blue green algae were found in colonies like *Nostoc*, *Anabaena, Oscillatoria, Phormidium* etc. and green algae making mats like *Cladophora, Spirogyra, Hydrodictyon, Chara* etc. as well as layer of *Cosmarium*. Higher value of CO_2 shows availability of carbonaceous rock. Higher Nitrate (2.6 ug/L) value in summer season increased chlorophyll-a distribution. In cold/winter season low chlorophyll-a were observed which means that both nitrate and temperature play significant role in chlorophyll distribution. Phosphate (0.9 ug/L) plays significant role to control the algal growth. Total hardness (190 mg/L) concentration show that sufficient quantity of blue green algae was available in this water. Increase in calcium hardness (120 mg/L) increases the production of green algae. Increase in the concentration of magnesium hardness (70 mg/L) results in the production of colonies of Cyanophyceae. Higher wave (2 inch) reading was due to wind, outlet and inlet water. Taste, odour, and colour of the water of

Rawal dam showed that it was tasteless, odourless and sometimes feel aquatic vegetation, fishy smell, colour in winter season was gray bluish green, green but in summer and in monsoon season it was colourless, mixed with sand, mud, etc. The fresh water is rich in algal species due to which several fish species like *Barbus tor, B. ticto, B. sophore, Barilius, Discognathus, Botia, Sisor, Gagata, Mastacembelus, Clupisoma, Callichrous, Macrones, Mystus, Carassius, Tilapia, Rita rita* Ham., *Catla catla* Ham., *Tor tor, Notopterus chitala*, *Labeo gonius*, *L. dyocheilus, L. rohita* Ham. were commonly found. It is interesting to note that about 70% of the diatoms from these studies were recorded from the gut content of the fish caught from these waters.

TABLE III.- QUALITATIVE AND QUANTITATIVE SEASONAL SUCCESSION OF ALGAL SPECIES OF RAWAL DAM WATER, ISLAMABAD

Very Rare	4	22	119	59
Rare	11	15	21	22
Common	63	58	61	59
Very Common	36	38	39	43
Total	113	133	240	183

Vr, very rare; r, rare; c, common; vc, very common; a, absent.

CONCLUSIONS

1. The species of green algae were dominant as compared with other groups.
2. Flow, turbidity, rain factors affected the growth of the algae/plankton.
3. Cold temperatures helped in growth of diatoms and green algae.
4. Total hardness and magnesium hardness are indicators for growth of blue green algae.
5. Fish were commonly found due to richness of algal flora.
6. Water was alkaline.

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REFERENCES

AHMED, M., BARLAS, M. A. AND IQBAL, Q. J., 1985. Some physico-chemical variables of Rawal Lake, Islamabad. *Pakistan J. Zool.*, **17**: 310-313.

AKIYAMA, M. AND YAMAGISHI, T., 1981. *Illustrations of the Japanese freshwater algae*. Uchidarokokuho Publishing Co Ltd 1-2-1 Kudankita Chiyoda ker, Tokyo, Japan U.R. No. 200-2 p-1-933.

APHA, 1985. *Standard methods for the examination of water and waste water*. American Public Health Association, Washington D.C., pp. 1268.

BAQAI, I. U., SIDDIQUI, P. A. AND IQBAL, M., 1974. Limnological studies of Haleji Lake. *Agric. Pakistan,* **25**: 321-344.

DESIKACHARY, T.V., 1959. *Cyanophyta I.C.A.T. Monograph on Algae*. New Delhi, pp. 900.

HUSTEDT, F., 1930. Bacillariophyta (Diatomeae). In: *Diessusswasser Flora von Mitteleuropa* (ed. A. Pascher), Verlag G. Fischer, Jena, pp. 466.

LEGHARI, M. K. AND LEGHARI, M. Y., 1999. Seasonal variation of phytoplankton part-I in Fresh water Lake Bakar District Sanghar, Sindh. *Pak. J. Plant Sci*., **5**: 159-171.

LEGHARI, S.M., LEGHARI, M.K., KHUHAWAR, M. Y. AND JAHANGIR, T. M., 2000. Limnological study of Tatta Pani hot spring and river punch, Azad Kashmir. *Pak. Sci. Khyber,* **13**: 73-85.

LEGHARI, M.K. AND LEGHARI, M. Y., 2001. Comparative ecological study of Phytoplankton of Bakar and Phoosna Lakes. *Pakistan J. Sci. Tech. Dev*., **20**: 6-10.

LUND, J.W.G., KIPLING, C. AND LECREN, E. D., 1958. The inverted microscope method of estimating algal numbers and the statical basis of estimations of counting. *Hydrobiologia*, **11**: 143-170.

MAJEED, A., 1935. *The fresh water algae of the Punjab. Part-I Bacillariophyta (Diatomaceae),* vol. 1 (ed. S. L. Ghose), University of Punjab Lahore, pp. 1-45.

MASON, D.J., 1967. *Limnology of Monolake*. California University, California, pp. 102.

NAZNEEN, S., 1974. Seasonal distribution of phytoplankton in Kinjhar (Kalri) Lake. *Pak. J. Bot*. **6**: 69-82.

NAZNEEN, S. AND BARI, G.A., 1984. Seasonal distribution of phytoplankton in Haleji Lake. *Pakistan J. agric. Res.*, **5**: 183-189.

PATRICK, R. AND REIMER, C. W., 1966. *The diatoms of the United States V-I*. The Monograph, Academy of Natural Science of Philadelphia, USA.

PATRICK, R. AND REIMER, C. W., 1975. *The diatoms of the United States* vol.2, part I. ed. The Monograph, Academy Natural Science, Philadelphia, USA, **13**: 1-213.

PHILPOSE, M.T., 1967. *Chlorococcales*. I.C.A.R. Krishan Bhawan New Dheli, pp. 300.

PRESCOTT, G.W., 1961. *Algae of the western Great Lake Area Monograph,* Michigan State University, revised edition, WMC Brown Co. Inc., Dubuque, Iowa, USA, pp. 975.

SIDDIQI, I.I. AND FARIDI, M.A.F., 1964. The Chlorococcales of Peshawar valley. *Biologia*, **10**: 1-88.

SMITH, G.M., 1950. *Fresh water algae of United State of America*. McGraw Hill, New York. pp. 719.

TIFFANY, L.H. AND BRITTON, M. E., 1971. *The algae of Illinois*. Hapner P. Company, pp. 1-395.

TILDEN, J., 1910. *Minnesota algae*, Vol.1. Minneapolis, pp. 555.

VINYARD, W.C., 1979. *Diatoms of North America*. Mad River Press Inc, Ecireka, California, USA, pp. 119.

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ISOPOD PARASITES OF MARINE FISHES OF PAKISTAN

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Abstract.- Sixteen species of Isopods infesting the marine fishes of Karachi coast are being reported.

Key words: Crustaceans, Karachi coasts, parasitic isopods.

INTRODUCTION

Isopods are small sized crustaceans known as pill bugs, wood lices or snow bugs. They are cosmopolitan, found in all habitats: marine, fresh water and terrestrial. Some species burrow still others are parasitic, and have become structurally adapted to a parasitic life to varying degrees especially in the adult stage. The parasitic species of Bopyrids from prawns have been reported by Markham (1982). Chopra (1922) published preliminary work on the family Bopyridae from India and later in 1923, reported 13 genera and 32 species of Bopyrid parasites infesting decapod macruran Crustacea. Pillai (1964) reported the parasitic isopods of the family Cymothoidae from South Indian fishes. William and William (1961), gave description of nine new species of Isopoda, Cymothoidae, which were external parasites on the reef fishes from West Indian Ocean. Eight Cymothoid species from the fishes of the Arabian Gulf have been documented by Bowman and Tareen (1983). A valuable contribution to the knowledge of Indian ocean parasitic isopods is by Bruce (1990). The present study deals wit the Isopod parasites of marine bony fishes of Karachi coast, Pakistan.

MATERIALS AND METHODS

The specimens of parasitic isopods were collected from catch of small sized specimens including fish, prawns, carideans, crabs, mollusks etc. from Fish Harbour, Korangi Creek, Karachi during 1991-1993, on monthly basis. The specimens, which are attached to the body surface of fish, usually get dislodged during the capture, whereas some specimens were also separated from gills and mouths of fishes with the help of forceps. The specimens were

brought to the laboratory, sorted out and frozen in a deep freezer. The positions of attachment of parasites and size of infested fish were noted. The fishes were identified according to Munro (1955). The specimens were preserved in 7% formaldehyde then transferred to 70% alcohol for later studies.

RESULTS AND DISCUSSION

The present study has revealed the presence of sixteen parasitic isopods on marine fishes of Pakistan (Table I). The families represented are Cymothoidae, Corrallinidae and Bopyridae, of which the most common

TABLE I.-OCCURRENCE (%) OF PARASITES.

Name of parasites	Name of fishes	Total No. of fishes	No. of infected fishes	% of infection
Anilocra dimidiata	Found Unattached	-	-	-
Argathona muraenecae	*Argyrops spinifer*	2199	05	0.23
	Epinephelus chlorasitgma	951	01	0.10
Catoessa ambassae	*Chorinemus tala*	1469	39	2.65
	Carangoides malabaricus	1383	8	0.50
	Chorinemus lysan	1616	40	2.43
Cymothoa eremita	*Parastromateus niger*	1198	7	0.58
Elthusa rayanaudi	*Nematalosa nasus*	945	8	0.85
Joryma sawayah	*Sardinella albella*	940	4	0.43
	Sardinella fimbriata	990	3	0.30
Joryma engraulidis	*Sardinella albella*	940	2	0.21
	Sardinella fimbriata	990	3	0.30
Nerocila phaiopleura	*Chirocentrus nudus*	1325	10	0.75
Nerocila depressa	Found unattached	-	-	-
Nerocilla (Emphylia) kisra	*Johnius sina*	802	7	0.87
	Otolithus argenteus	2335	12	0.47
	Pomadasys maculates	1277	4	0.31
	Johnius axillaries	1654	4	0.24
	Johnius argentatus	1319	2	0.15
Nerocila sigani	*Netuma thalassinus*	697	1	0.14
Nerocila serra	*Pseudorius jella*	700	1	0.14
	Netuma thalassinus	697	2	0.28
	Hexanematichthy sona	1060	2	0.18
	Osteogeneiosus sthenocephalus	1346	1	0.07

Continued

Name of parasites	Name of fishes	Total No. of fishes	No. of infected fishes	% of infection
Nerocila barramundae	*Pseudarius jella*	700	11	1.57
	Aroides dussumieri	719	5	0.69
	Arius thalassinus	1005	3	029
Nerocila orbignyi	*Tachysurus maculates*	1349	3	0.22
	Pseudarius jella	700	21	3.00
	Netuma thalassinus	697	2	0.28
Norileca indica	*Decapterus russelli*	1376	85	6.17
	Rastrelliger kanagurta	1618	58	3.58
Norileca triangulata	*Rastrelliger kanagurta*	1618	3	0.18

family is Cymothoidae and the fish *Decapterus russelli* (Rupelle) is the most infested fish, *Norileca indica* (Milne-Edwards) is the most common species amog the parasitic Isopods of the region. The parasites are usually host specific. There can be several hosts for a single species of parasites as in the case of *Nerocila (Emphylia) kisra*, Bowman and Tareen (1983), it is found on six species of fishes. The body surface between pelvic and pectoral fin is the most preferred site for the attachment of body parasites (Table II). *Cymothoa eremite* (Brunnich) from mouth of *Parastromateus niger* (Bloch) has the largest size of 44.00 mm.

Throughout the study period the difficulty of identifying the species was main factor. It was because of the large number of variations in, the same species, Brusca (1978) is of the opinion, “the characters classically used to distinguished the species of *Nerocila* are known to be somewhat unreliable”. Taxonomically it seems very difficult to place the species with certainty. Most of the species are based on minor details. There is a need revise the classification of parasitic isopods.

Isopod parasites are present in the bronchial chambers, mouths and on the skin of fishes, where they suck blood. With the sucking of blood a fish becomes weak due to lack of oxygen and nutrients. A weak fish is more vulnerable to various fatal diseases. Moreover, the hook like attachments to the body (legs) cause lesions, thus making them susceptible to pathogens, bacterial growth and other diseases such as ulceration.

REFERENCES

BOWMAN, T.E. AND TAREEN, I.U., 1983. Cymothoidae of Kuwait (Arabian Gulf) (Crustacea: Isopoda). *Smithsonian Contr. Zool*., **382**: 1-30.

BRUCE, N.L., 1990. The genera *Catoessa, Enispa, Ichthyoxenus, Idusa, Livoneca* and *Norileca* n.gen., (Isopoda: Cymothoidae), crustacean parasites of marine fishes, with descriptions of eastern Australian species. *Rec. Australian Mus*., **42**: 247-300.

BRUSCA, R.C., 1981. A monograph on the Isopoda Cymothoidae (Crustacea) of the eastern Pacific. *Zool. J. Linn. Soc*., **73**: 117-199.

CHOPRA, B., 1923. Bopyrid isopods parasitic on Indian Decapoda Macrure. *Rec. Indian Mus*., **25**: 411-550.

MARKHAM, 1982. Bopyrid isopods parasitic on Decapod Crustaceans in Hong Kong and Southern China. In: *The marine flora and fauna of Hong Kong and Southern China* (eds. B.S. Morton and S.E. Tseng), pp. 325-391. Hong Kong University Press, Hong Kong.

MUNRO, 1955. *The marine and freshwater fishes of Ceylon.*

PILLLAI, N.K., 1964. Parasitic isopods of the family Cymothoidae from South Indian Fishes. *Parasitology*, 54: 211-223.

TABLE II.- PLACE OF ATTACHMENT OF THE PARASITES ON THE HOSTS.

Name of parasite	Name of fish	Place of attachment on body surface						
		On anal fin	**On pector al fin**	**On pelvic fin**	**Lower portion of mouth**	**B/W pelvic and pectoral fin**	**On caudal fin**	**On lateral fin**
Nerocila barramundae	*Pseudarius jella*	-	-	*	-	*	-	-
	Aroides dussumieri	-	-	*	*	-	-	-
	Arius thalassmus	-	-	*	-	*	-	-
Nerocila depressa		-	-	-	-	-	-	-
Nerocila (Emphylia) kisra	*Johnius sina*	*	-	-	-	-	-	-
	Otolithus argenteus	-	-	*	-	-	-	-
	Pomadasys maculates	-	-	-	*	-	-	-
	Johnius axillaries	*	-	-	-	-	-	-
	Johnius argentatus	-	*	-	-	-	-	-
Nerocila orbignyi	*Tachysurus maculates*	*	-	-	-	-	-	-
	Pseudarius jella	-	*	-	-	*	-	-
	Netuma thalassinus	-	-	*	-	*	-	-
Nerocila phatopleura	*Chirocentrus nudus*	-	-	-	-	-	*	*
Nerocila signai	*Pseudarius jella*	*	-	-	-	-	-	-
	Netuma thalassinus	*	-	-	-	*	-	-
	Hexanematichthy sona	*	-	-	-	*	-	-
	Osteogenelosus sthenacephalus	-	-	-	-	*	-	-
Nerocila serra	*Nerocila thalassinus*	-	-	-	-	*	-	-

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ASSESSMENT OF WATER QUALITY AND BIOLOGICAL INDICATORS IN THE FEEDING AND SPINAL DRAINS OF THE LEFT BANK OUTFALL DRAIN (LBOD), SINDH, PAKISTAN

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Abstract.- Water, algal and fish species were studied from spinal and feeding drains of Tando Adam, Sanghar, Kandyari, Sindhri, Mirpurkhs and Kadhan - Pateji Outfall Drain (KPOD). Water samples were analyzed for Physico – chemical and biological analysis. High flow of water occurred in feeding drains of Sanghar and Sindhri during summer season. In KPOD, tidal link and Cholri Lake have come under the influence of seawater. The water from the seawater moves back along tidal link and KPOD due to a number of breaches and extensive erosion in the region. The lower areas of KPOD toward tidal link have become highly saline (brackish water). Salinity was in between 44.6 – 51.5g/L. Some migratory seawater fishes and shrimps have been identified and recorded. Two main algal species *Microspora floccosa* and *Dichotomosiphon tuberosus* covered the moist part of the bank.

Key wards: Water quality, flora, fauna, Pateji outfall drain, Sindh.

INTRODUCTION

Left Bank Outfall Drain (LBOD) project was carried out to drain excess water from the irrigation canals and waterlogged lands to control salinity and water logging in Nawabshah, Nowshehro Feroz, Sanghar, Mirpurkhas and Badin districts of Sindh. A spinal drain has been constructed, which is connected with feeding drains from various sites of the irrigated parts of the regions. The spinal drain is connected with KPOD, which was eventually connected with tidal link canal to Shah Samdro creek of Arabian Sea. A weir connected the tidal link canal with a number of brackish lakes on the right of the canal (Fig.1). The exact description about LBOD and chemical assessment of water quality at KPOD Tidal Link Canal project has been already described (Jafri, 1997; Jahangir *et al.,* 1997). Leghari *et al.* (2000) and Jafri *et al.* (2000) studied brackish water algal and fish species in Cholri,

Fig. 1. Map of tidal link lakes showing study area of Badin and Sanghar Districts of Sindh. (Source: Final Fisheries Report of Tidal Link lakes, Department of Fresh Water Biology and Fisheries, University of Sindh, Jamshoro, 1997).

Pateji and Sonahro – Mehro lakes of coastal regions of Sindh. The present study was conducted to explore the physico – chemical and biological changes of water and occurrence of algal and fish species in outfall drains. The work was also compared with the water quality and biological life studied during 1997 in the drains.

MATERIALS AND METHODS

Water samples were collected from spinal drains between Tando Adam to Mirpurkhas, feeding drains and KPOD from Kadhan bridge upto Pateji Lake.

Three samples were collected from spinal drain (1) Tando Adam – Sanghar road bridge, (2) Tando Adam – Kandyari road bridge and (3) Khipro – Mirpurkhas road bridge. Six feeding drains were sampled (1) Kaloi drain from Tando Adam – Sanghar road, (2) Link drain before Naoabad, (3) Khagri link drain (SMD – I), (4) Sanghar link drain II (SMD – II), (5) Kandyari – Sindhri drain and (6) drain from Abu Bakar Junejo village. Six samples from KPOD were collected from Kadhan bridge near Singhari village up to Pateji lake at an interval of 5 to 10km, at, (1) Kadhan bridge, (2) after 10km from the Kadhan bridge, (3) after 20km from the Kadhan bridge, (4) after 26km from the Kadhan bridge, (5) after 30km from the Kadhan bridge and (6) after 40km from the Kadhan bridge near Pateji lake. A sample of Pateji Lake was also collected. Water samples were collected from the surface at the depth of about 3 to 9 inches. The water was collected in 1.5 L plastic bottle, which was rinsed several times with sampling water before collection of samples. The temperatures of water and air (1meter above the surface of water) were noted. The conductivity, salinity and total dissolved solids were recorded with WTW 320 conductivity meter at the sampling site. The pH was measured with Orion 420 A pH meter (APHA, 1976).

Chloride, alkalinity and hardness were determined by titration with standard silver nitrate, hydrochloric acid and EDTA, respectively. Dissolved oxygen was evaluated by Winkler method. Chemical oxygen demand (COD) was determined by acid dichromate oxidation method using silver sulphate as catalyst. Sodium, potassium, calcium and magnesium were determined by Varian Spectr AA – 20 atomic absorption spectrophotometer with air – acetylene flame using standard burner at the conditions recommended by the manufacturer. The sodium, potassium, calcium and magnesium were determined at 589.0 nm, 766.5 nm, 422.7 nm and 285.2 nm, respectively, with integration time 3 sec and delay time 3 sec.

Plankton samples were collected by plankton net (No. 25, # 0.55 mm mesh size). Algal and higher aquatic plants were collected by hand picking and were preserved in 3 % farmline. The fish samples were collected from fishermen at the site and preserved in 10 % farmline and brought to the laboratory for identification. The algal flora and fishes were identified after references of Prescott (1962), Desikachary (1959) and Fischer and Bianchi (1984).

RESULTS AND DISCUSSION

Three samples from the spinal drains were collected from Tando Adam – Sanghar, Tandoadam – Kandyari Road Bridge and Khipro – Mirpurkhas road. Six feeding drains were included, in the study where (Sanghar 1, Sanghar 2 and Kandyari at Sindhri drains) were draining their effluents into spinal drain. Kaloi drain; near Naoabad and drain near Abu Bakar Junejo village were dried before reaching to spinal drain. The results of chemical analysis are summarized in Table I. The Table indicates that the conductivity and TDS in the feeding drains throwing their effluents in spinal drains were in the range of 22 – 48 mS/cm and 19200 – 31200 mg/L respectively. The conductivity and TDS in the three drains, which failed to reach up to spinal drains, were in the range of 24 – 60 mS/cm and 15552 – 38784 mg/L respectively. The spinal drains indicated much lower values of conductivity and TDS than the feeding drains in the range of 6.3 – 14.46 mS/cm and 4007 – 9255 mg/L. The lower values may be due to the addition of fresh canal water. However along the spinal drain the conductivity and TDS gradually increased due to mixing (intrusion) of seawater with drain water.

The algal species were present in the small feeding and main drain from Tandoadam to Mirpurkhas. The water contained *Spirogyra fluviatilis, Spirogyra rhizobrachialis, Spirogyra nitida, spirogyra longata, Oocystis elliptica, Cosmarium reniforme, Cosmarium* sp., *Scenedesmus bijugatus, Scenedesmus quadricauda, Oedogonium* sp., *Closterium* sp., *Cladophora glomerata, Stigeoclonium attenuatum, Rhizoclonium hieroglyphicum, Enteromorpha salina, Enteromorpha prolifera* of Chlorophyta*, Chara zeylanica, Chara zeylanica, F. elegans* and *Lamprothamnium succinctum* belongs to Chlorophyta. In Cyanophyta – *Merismopedia glauca, Merismopedia elegans, Gomphosphaeria aponina* var. *cordiformis, Calothrix marchica, Calothrix epiphytica, Johannesbaptistia pellucida* were found along with *Typha domingensis* and *Typha elephantina, Phragmites communis* are emergent and also on waterlogged soil. *Najas minor, Najas major* were

submerged, *Lemna minor* were free floating along with fishes, *Oreochromis mossambicus, Channa marulla, Cirrhinus mirgala, Notopterus notopterus, Wallago attu and Puntius ticto.*

Kadhan Pateji outfall drain (KPOD) was examined from Kadhan Bridge to zero point of KPOD. Six samples were collected at an interval of 5 to 10 km. The chemical analyses are summarized in Table II. The conductivity and TDS observed at Kadhan Bridge was 16.7 mS/cm and 10713 mg/L, respectively. The conductivity and TDS raised steeply after 30 km from Kadhan Bridge. The conductivity and TDS reached 64.3 mS/cm and 41150 mg/L and nearly remained constant up to zero point of KPOD about 40 km from Kadhan Bridge. The results of study are considerably higher than observed during 1997 (Jahangir *et al*., 1997). The conductivity and TDS in1997 in the region were observed in the range of 5.52 – 6.22 mS/cm corresponding 3533 – 3981 mg/L (Table II). The results indicate that a significant movement of the seawater towards KPOD with definite effects on Kadhan Bridge was observed. The *Dichotomosiphon tuberosus, Microcoleus chthonoplastes, Microspora floccosa, Microspora* sp, *Rhizoclonium hieroglyphicum, Enteromorpha prolifera,* and *Enteromorpha compressa* were dominant flora on moist soil.

TABLE III.- FISH SPECIES PRESENT IN THE SPINAL AND FEEDING DRAINS.

Scientific name	**Local names**	**Feeding drain**	**Spinal drain**
Arius arius Hamilton	Khagga	+	+
Boleophthalmus dussumieri Cuvier.	Gullo	-	++
Cobius ocellatus Day	Vacho,Gullo	+	++
Dendrophysa russelli Cuvier	Goli	-	++
Eleutheronema tetradactytum	Sear	+	+
Liza subvirids	Chodi	-	+++
Liza carinata	Moor, Mullet	-	++
Lates calcarifer Bleeker	Dangri	-	++
Lutjanus lutjanus Park	Dandio	-	++
Tenualosa ilisha	Palla	+	+*
Valamugil speigleri Bleeker	Phar	-	+++*
Shrimps			+
Penaeus japonicus			+
Penaeus sp.			+
Parapenaeopsis stylifera			+

- Absent, + rarely present, ++ present, +++ abundant, * first time recorded

Some ploanktonic species were found floating in the drain water *Synechocystis aquatilis, Chroococcus minor, Chroococcus turgidus, Lyngbya lagerheimii, Lyngbya aestuarii, Merismopedia* sp*, Oscillatoria chlorina, O. limnetica, O. raoi, O. tenuis, O. earlei, O. fremigii, O. sancta, Phormidium anomala* Rao, *P. corium, Spirulina subsalsa, Spirulina major, Spirulina meneghiniana* of *Cyanophyta.*

Among the fish species *Oreochromis mossambicus* and *Wallago attu* occured in Spinal drain. *Tenualosa ilisha, Gobius oceallatus, Valamugil speigleri, Liza subviridis, Liza carinata, Dendropyysa russelli, Lates calcarifer* (Dangri)*, Arius arius,* and Shrimps (*Penaeus* sp, and *Parapenaeopsis* sp.) were present in the KPOD.

CONCLUSIONS

(1). During last five years of operation of LBOD, significant erosion has taken place in tidal link and KPOD area. The tidal link canal along with the Cholri wear have been destroyed.

(2). The bottom of the KPOD has deepened below the sea level with the results that there is back flow of seawater along the KPOD. There is also significant transfer of the bottom sediments and the soil from KPOD to the sea.

(3). The flora and fauna of the connecting lakes have changed to marine water.

(4). The KPOD contains a good mixture of brackish water fish communities and of migratory fishes. The canal provides a feeding as well as spanning area of fishes.

REFERENCE

APHA, 1976. *Standard methods for the examination of water and wastewater*, 14th Ed., American Publication Health Association, Washington D.C. pp: 143 – 628**.**

DESIKACHRY, T.V., 1959. *Cyanophyta*. I.C.A.R. New Delhi, pp. 1-686.

FISCHER, W. AND BIANCHI, S. G., 1984. *FAO Species identificaton sheets for fishery purpose. Western Indian Ocean*, vol. 1 – IV, FAO, Rome.

JAFRI. S. I. H., 1997. *Final report on fishries survey of Chotiary Reserviour (Sanghar) and Tidal Link Lake Badin*. Department of Freshwater Biology and Fisheries, University of Sindh, Jamshoro, pp. 129.

JAFRI, S. I. H., ALI, S.S., MAHAR, M. A., HUSSAIN, S. M. AND KHATOON, Z., 2000. Fisheries potential of Tidal Link Lakes (Distt: Badin) of Sindh coast (Northern Arabian Sea). *Pakistan J. Zool.*, **32**: 301 – 306

JAHANGIR, T. M., KHUHAWAR, M. Y. AND MASTOI, G. M., 1997. Chemical assessment of water quality at KPOD and Tidal Link of LBOD project. *Symposium on Left Bank Out Fall Drain (LBOD) Project and its Impact*. M. U. E. T., Jamshoro, Sindh 29th Nov. 1997; pp. 53 – 58.

LEGHARI, S. M., JAFRI, S. I. H. AND KHUHAWAR, M. Y. 2000. Limnological study of Sonhro, Mehro, pategi and Cholri lakes of Distt: Badin, Sindh Pakistan. *Pakistan J. biol. Sci*., **3**: 1904 – 1909.

PRESCOTT, G.W., 1962. *Algae of the Western Great lakes area*. W.M.C.Brown Company, Publishers, Dubuque, Iowa, U.S.A., pp: 977.

TABLE I.- ANALYSIS OF WATER OF SANGHAR AND MIRPURKHAS MAIN DRAIN AND ADJOINING LINK DRAINS, COLLECTION ON AUGUST 8, 2000.

S. No	Parameters with units	1	2	3	4	5	6	7	8	9
1.	Time	9:40	10:55	11:20	12:45	13:10	13:30	14:00	15:00	15:30
2.	Temperature of air (ºC)	33	33	34	37	37	36	36	33	33
3.	Temperature of water (ºC)	30	30	31	30	30	33	33	30	30
4.	Color	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid
5.	Visibility (inches)	-	10	2.5	10	8	10	10	10	8
6.	Depth of water (feet)	13	3.5	-	-	5.5	5.5	1.5	-	2
7.	pH,	8.13	7.92	8.14	8.11	8.19	7.88	8.11	8.17	8.12
8.	Conductivity (mS/cm)	24.3	6.26	19.48	22.00	13.36	48.8	30.0	14.46	60.6
9.	TDS (mg/L)	15552	4007	12467	14080	8550	31200	19200	9255	38784
10	Salinity (g/L)	14.8	3.4	11.6	13.3	7.7	32.0	18.7	8.4	40.9
11	COD (mg/L)	96	50	41	48	32	128	144	64	45
12	DO (mg/L)	4.2	8.4	3.2	7.1	2.7	6.5	6.9	7.1	3.2
13	Chloride (mg/L)	6203	1666	5743	6416	3864	16697	10989	5318	23042
14	M–alkalinity (as $CaCO_3$, mg/L)	350	200	300	250	200	250	200	200	200
15	P- alkalinity (as $CaCO_3$, mg/L)	50	-	25	50	50	-	50	50	50
16	Hardness (as $CaCO_3$, mg/L)	4040	1250	3280	3660	2500	9500	6100	2600	66000
17	Sodium (mg/L)	175	20	36	294	224	9772	5000	2270	15035
18	Calcium (mg/L)	360	144	299	360	260	858	1246	268	898
19	Magnesium (mg/L)	752	167	523	407	406	190	420	968	417
20	Potassium (mg/L)	36	22	32	58	37	85	51	3.5	71

Sampling stations. (1) Kai drain bridge, Tando Adam – Sanghar road, (2) Spinal drain, Tando Adam – Sanghar road, (3) Link drain, 3 km before Naoabad, (4) Khagri link drain (SMD – I), (5) Spinal drain at Tando Adam – Kandyari road bridge, (6) Sanghar link drain II at 688 RD (SMD – II), (7) Link drain, Kandyari – Sindhri road, (8) Spinal drain, Khipro – Mirpurkhas road and (9) Link drain from Haji Abu Bakar Junejo village.

TABLE II.- ANALYSIS OF WATER OF KADHAN PATEJI OUT FALL DRAIN (KPOD) COLLECTED ON AUGUST 11, 2000.

S. No	Parameters with units	1	2	3	4	5	6	7
1	Time	10:30	11:45	12:30	13:00	13:20	13:55	13:45
2	Temperature of air (ºC)	34	36	36	36	36	36	36
3	Temperature of water (ºC)	31	31	31	30	30	30	30
4	Color of water	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid
5	Visibility of water (inches)	3	3	3	4	4	5.5	3
6	PH	7.75	7.83	7.80	7.90	7.93	7.93	7.90
7	Conductivity (mS/cm)	16.74	22	22.9	25.7	64.3	68.6	74.2
8	TDS (mg/L)	10713	14080	14656	16448	41152	43904	47488
9	Salinity (mg/L)	9.8	13.2	13.9	15.7	43.6	47.2	51.5
10	DO (mg/L)	6.5	6.3	6.7	6.6	4.8	4.7	6.2
11	M. Alkalinity (mg/L)	267	233	200	250	233	235	250
12	Hardness (as $CaCO_3$, mg/L)	4000	4000	5333	5335	11000	11333	12000
13	Chloride (mg/L)	5908	7326	8035	9217	28537	28596	31905

Sampling stations. (1) Kadhan Pateji Out Fall Drain (KPOD), at Kadhan bridge, (2) After 10 km from Kadhan bridge, KPOD at Sheikhani village, (3) After 20 km from Kadhan bridge, KPOD beside 2 km from Dodo Tomb, (4) after 26 km from Kadhan bridge, KPOD at Sirani Drain near Rangers Post, (5) After 30 km from Kadhan bridge, KPOD, (6) After 40 km from Kadhan bridge, KPOD and (7) Pateji Lake from North of KPOD after 40 km from Kadhan bridge, KPOD.

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TRENDS AND PATTERNS OF BACTERIAL DISEASES OF POULTRY IN COMMERCIAL BROILER FARMS OF HYDERABAD DISTRICT

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Abstract.- The present study was designed to study the incidence, prevalence and trends of the most commonly occurring poultry bacterial diseases on the commercial broiler farms of Hyderabad district. A total of 799,750 birds were examined for two bacterial diseases *Salmonella pollorum* and fowl cholera in the Hyderabad district. Three-year data was collected from 25 commercial broiler farms of Hyderabad district. Out of which 398,150 were examined for *Salmonella pollorum* and 401,600 were for fowl cholera infection. It is also observed that 49% birds were infected with *Salmonella* and 51% with fowl cholera. The mortality rate due to *Salmonella pollorum* was comparatively higher than that of fowl cholera.

Key words: *Salmonella pollorum*, fowl cholera, broiler chicken, bacterial disease in poultry.

INTRODUCTION

The commercial broiler farming is widely practiced in Pakistan and has made considerable contribution in the economy of country. During the past 24 years the large-scale commercial poultry farming has grown. In Pakistan about 13,3000 commercial poultry farms and 450 breeding farms are in operation and produce 3,550,000 tones meat and 7,679 million eggs in a year (Economic Survey of Pakistan, 2001-2002). Commercial poultry farming in the country was started in 1963. Presently poultry production infrastructure comprises a fixed investment of nearly 50 billion rupees with an annual turn over of about 40 billion rupees at various stages (Economic Survey of Pakistan, 2001-2002). The poultry sub-sector employs one hundred and fifty thousand workers and is based on an estimated capacity of 350 million eggs setting in hatcheries.

The salmonella and fowl cholera are the most commonly occurring

bacterial diseases. Duitschaever (1977) reported 34.8% contamination of retail chicken products during a survey in Canada. A high rate of *Salmonella* contamination of carcasses was due to contamination at the poultry processing plants. The study was designed to study the incidence, prevalence patterns and trends of most commonly occurring poultry diseases in the commercially operating poultry farms. The results of this study are expected to help in formulating a meaningful diagnostic and disease control strategy n this part of the country.

MATERIALS AND METHODS

Data was collected for three years (1999-2001) by regular survey of the 25 commercial broiler farms of Hyderabad district, *i.e.* Hyderabad, Hala, Tando Mohammad Khan, Tando Allahyer, and Tando Jam and official record of the Project Director Disease Diagnostic Laboratory of Directorate of Poultry Production Hyderabad, Sindh. Five farms were selected from each taluka to record the most commonly occurring bacterial disease. During this survey the number of birds affected with *Samonella* and fowl cholera diseases, and number of those which died due to those diseases were recorded. The sick birds were brought to the laboratory for postmortem examination. The disease was diagnosed on the basis of symptoms, and postmortem lesions for serological evidences. Rapid whole blood agglutination test, and haemagglutination test were performed. The data was statistically analyzed using test of least significant difference.

RESULTS AND DISCUSSION

In this study a total of 799,750 birds were examined in the five taluka of Hyderabad district *i.e.* Hyderabad, Hala, Tando Mohammad Khan, Tando Allahyer, and Tando Jam for two bacterial diseases *Salmonella pollorum* and fowl cholera. 398,150 birds were examined for *Salmonella pollorum* 1231 (0.31%) birds wee affected out of which 1049 (85%) died and 182, which is 15% of affected birds, survived in *Salmonella pollorum* (Table I). The 401,600 birds were examined for fowl cholera in five taluka of Hyderabad districts broiler farms; 1344 (0.33%) birds were affected, out of which 790 (59%) birds died and 554 (41%) survived (Table I). The highest mortality occurred due to *Salmonella pollorum* as compared to fowl cholera. During external examination, the affected birds were depressed and had decreased

TABLE I.- PERCENTAGE OF BIRDS AFFECTED, DIED, SURVIVED FROM THE BACTERIAL DISEASES REPORTED FROM COMMERCIAL BROILER FARMS OF 5-TALUKA OF HYDERABAD DISTRICT, DURING YEAR 1999-2001.

No. of disease	Total No. of birds examined For bacterial diseases	No. of birds affected (%)	No. of birds died (% of affected)	No. of birds survived (% of affected)
Salmonella	398,150	1231 (0.31%)	1049 (85%)	182 (15%)
Fowl cholera	401,600	1344 (0.33%)	790 (59%)	554 (41%
Total	799,750	2575 (0.32%)	1839 (71%)	(29%)

appetite, swollen wattles was the feature of chronic fowl cholera. Internal examination of bird showed gross lesions. In acute cases internal hemorrhages and congestion of liver, spleen and kidneys were found. In chronic fowl cholera cheesy exudates were found between the intestines and on liver and heart. In the case of *Salmonella pollorum*, the chicks had typical, white bacillary diarrhea with pasted cloacas and high mortality and during internal examination affected birds showed generalized infection with swollen livers, spleen, and kidneys and hemorrhages. Mortality was usually high (25 to 60%). This trend and pattern for two bacterial diseases may indicate that the contaminations for bacterial infection in broiler farms were probably admissible for carcasses and poultry processes. Timoney *et al*. (1970), Simmons and Byrnes (1972) and McGarr (1976) reported a high rate of *Salmonella* contamination of carcasses around poultry processing plants. Kenneth (1971) reported the *Salmonella* in fowl and infection was widespread in birds, mammals, and man, Duitschciever (1977) showed 34.8% increase in retail chicken products for *Salmonella* disease in Canada. Rhoades and Rimler (1985) reported fowl cholera caused by *Pasteurella multocida*. Mortality rate in Turkey reached as high as 100%. The control and treatment of disease cannot be successfully achieved until sufficient data is available on disease occurrence. Yadev *et al*. (1976) reported the outbreak of fowl cholera, but the disease outbreak in a commercial broiler farm may be due to neighboring farms with bacterial infection. It is suggested that standard commercial feed be provided in the poultry farm as the poultry feed may get some times contaminated with *Salmoella*. It is, further, recommended that the bacteriological examination of fish and bone meal sold in market be carried out regularly.

REFERENCES

DIUTSCHAEVER, C.L., 1977. Incidence of salmonella in raw cut-cup chicken. *J. Fd. Protect*., **400**: 191-192.

KENNETH, L. AND BUILS, 1971. The history of avian Medicine in the U.S. 111. *Salmonelosis. Avian Dis*., **21**: 430-435.

McGARR, C., 1976. *A longitudinal study of* Salmonella *in broiler chickens production*. M.Sc. thesis, University of Guelph.

RHODES, K.R. AND RIMILER, R.B., 1985. Avian pasturellosis. In: *Disease of poultry* (eds. M.S. Hofstad, H.J. Barnes, B.W. Calnek, W.M. Reid and H.W. Yoder Jr.), 8th ed. Pp. 114-156. Iowa State University Press, Ames, Iowa.

SIMMONS, G. AND BYMES, R., 1972. The origin of *Salmonella* in chicken carcasses. *Austral. Vet. J*., **48**: 186-189.

TIMONEY, J.E., KELLY, J.H. AND REEVES, D., 1970. A study of *Salmonella* contamination in some Dublin poultry processing plants. *Vet. Res*., **87**: 158-161.

YADEV, M.P., SHARMA, V.D. AND SETHI, M.S., 1976. An outbreak of fowl cholera due to *Pasteurella gallinarum* in Uttar Pardesh (India). *Avian Dis*., **21**: 323-325.

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MICROBIOLOGICAL ANALYSIS OF HONEY OF *APIS MELLIFERA*

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Abstract.- The honey samples of *Apis mellifera* from cider and sunflower were analysed and found to harbor aerobic bacteria such as *Bacillus circulans*, *B. coagulans, B. brevis, B.subtilis, B. alvei* and *Nocardia* sp., as well as anaerobic bacteria such as *Lactobacillus* sp., *Clostridium* sp. and *Bifidobacterium* sp. According to total count of colony forming units of bacteria, Chunian honey sample was found to be the most heavily contaminated followed by honey from Khokhrapar, Punjab University and NARC, Islamabad. There was no pathogenic bacterial strain isolated from honey samples. The bacterial contamination in honey is apparently contracted during the process of deposition of honey in the comb by honey bee, and or during extraction and packing procedure. It was concluded that none of the honey samples had health hazardous bacterial strains.

Key words: Honey and bacteria, microbial flora of honey, honey and bacterial contamination.

INTRODUCTION

Honey is condensed nectar from flowers. It contains 12-17% moisture by weight, while rest is the assortment of simple sugars. It takes about 12-13 pints of nectar to make one pint of honey (Crane and Walker, 1984). The bees put the nectar in open cells of comb and fan it with their wings to drive off the excess moisture. Then each cell is sealed with a wax cap. There is popular misconception that pollen is an ingredient of honey. There can be trace amounts of pollen mixed into honey but it is not actually part of honey (White *et al*., 1977). Honey has long preservation property. Besides that is a good preservative and contains less moisture and hence can be stored for long duration without spoiling its quality. It literally sucks the moisture out of bacteria killing it in the process. Some endospore forming bacteria are, however, able to survive in honey, and are implicated with contamination in honey. The primary sources of microbial contamination are likely to include pollen, the digestive tracts of honey bees, dust, air, earth and nectar sources

which are very difficult to handle (Baron *et al.,* 1994). The same secondary sources that influence any food product are also sources of contamination for honey. These include air, food handlers, cross contamination, equipment and buildings. Secondary sources of contamination are controlled by good and hygienic extraction practices. From different studies it has been observed that both aerobic and anaerobic bacteria are found to be present in honey (Snowdon and Cliver, 1996). The aerobic bacteria mainly include *Bacillus* species, while anaerobic strains include *Lactobacillus* sp., *Bifidobacterium* sp. and *Clostridium* sp. All these bacterial strains isolated from honey samples in aerobic and anaerobic conditions were found to be non pathogenic.

MATERIALS AND METHODS

Honey samples of *Apis mellifera* (European bee) taken from different localities of Punjab such as Khokhrapar, Chunian, Punjab University and NARC, Islamabad. These honey samples were diluted (1:1000ml for aerobes and 1:100ml for anaerobes) spread on LB agar (1g tryptone, 1g NaCl, and 0.5g yeast extract and 3g agar in 100ml of distilled water, autoclaved at 15lb for 20 minutes) and on cooked meat medium plates (100g cooked medium and 15g of agar in 1000ml of distilled water, flasks were cotton plugged, autoclaved at 15lb and 121°C for 20 minutes and 20ml medium poured in Petriplates) (Cheesbrough, 1993; Cappucino and Shermann,1996), in triplicate.The plates were incubated both aerobically and anaerobically at 37°C. The aerobic bacteria showed visible colonies after 12-15 hours, while the anaerobic bacteria showed visible colonies after 24-48 hours. These developed colonies were marked, counted and isolated to perform morphological and biochemical tests for purpose of identification. Following biochemical tests were performed to identify the aerobic and anaerobic bacterial isolates from honey samples: Catalase test, Urease test, Gelatin hydrolysis test, Starch hydrolysis test, Casein hydrolysis test, Carbohydrate fermentation test, Litmus milk test, Triple sugar iron test, MRVP test, Citrate test, Oxidase test, Indole test, Hydrogen sulphide production test, Nitrate reduction test, MacConkey agar test, Blood agar test (Brooks *et al.,* 1998; Benson, 2002). Antibiotic disc method was used to check the antibiotic resistance of bacterial isolates. The antibiotic discs used were Impenem (10µg), Ciprofloxacin (5 µg), Gentamicin (10 µg) and Amikacin (30µg). The zones of inhibition were measured and sensitivity recorded. Heavy metal resistance of bacteria was also studied by exposing these bacteria to different concentrations of heavy metals such as (Ag^{2+}, Pb^{2+}, Cr^{6+}, Cu^{2+}, Ni^{2+} and

Cd^{2+}). The stock solutions of salts of these metals were prepared and filter sterilized. The bacterial isolates were streaked on Lb plates containing 10-100μg of these metals. These plates were incubated at 37°C for 24 hours. The growth was observed the next day. The colonies were again streaked with increasing concentration of each metal. The process was continued until the minimum inhibitory concentration for each metal was determined (Cheesbrough, 1993).

RESULTS

Table I shows the physical and chemical characteristics of the bacterial isolates from honey of *Apis mellifera.* The bacterial species identified from honey samples in aerobic conditions were *Bacillus subtilis, B. alvei, B. circulans, B. coagulans, B. brevis* and *Nocardia* sp. The anaerobic strains isolated were *Lactobacillus* sp., *Bifidobacterium* sp. and *Clostridium* sp. Honey from chunian had the maximum bacterial contamination, which was followed by samples from NARC-2, and Khokrapar, Punjab University and NARC-1 (Table II). Antibiotics resistance test showed that the aerobic and anaerobic bacterial isolates were sensitive to Ciprofloxacin, and showed maximum resistance to Amikacin (Table III). Similarly, in heavy metal resistance test, the isolates showed maximum sensitivity to silver (Ag^{2+}) and were least sensitive to lead (Table IV). Among all bacteria, *Bacillus* spp. were frequently found in all honey samples. Total count of colony forming units (CFUs) showed that Chunian sample was the most contaminated followed by Khokhrapar, Punjab University and NARC samples (Table V).

DISCUSSION

Honey is not the basic medium for bacteria and hence most of the bacteria are unable to survive in honey. Honey has high sugar content and very low moisture level, due to which microbes are unable to survive in honey (Cox and Hinkle, 2002; Fenecia *et al.,* 1993). Honey has low level of microbes when it is being produced (Snowdon and Cliver, 1996). The microbial contamination of honey is done after its improper handling. In honey mostly endospore forming bacteria are present. Vegetative forms of bacteria are usually not found. The vegetative forms are non resistant and cannot survive in high levels of sugars in honey (Nevas *et al.,* 2002). The endospore forming bacteria are resistant to high temperature. On exposure to

TABLE I.- PHYSICO-CHEMICAL CHARACTERISTICS OF BACTERIAL ISOLATES FROM HONEY SAMPLES OF *APIS MELLIFERA*.

Physical / biochemical characteristics	*Nocardia* sp.	*B. circulans*	*B. subtilis*	*B. coagulans*	*Lactobacillus* sp.	*Clostridium* sp.	*B. brevis*	*B. alvei*	*Bifidobacterium* sp.
Motility test	+	+	+	+	+	+	+	+	-
Acid fast stain test	+	-	-	-	+	-	-	-	+
Endospore stain test	-	+	+	+	-	+	+	+	-
Gram stain test	+	+	+	+	+	+	+	+	+
Catalase test	+	+	+	+	+	+	+	+	+
Indole test	+	-	-	-					
Starch test	+	+	+	+					
Urease test	+	-	-						
Oxidase test	-	-	-						
Glucose test	+	+	+	+					
Lactose test	+	+	+	+					
Sucrose test	-	-	-	-					
Blood haemolysis test	-	+	+	+					
Casein test	+	-	-	-					
Nitrate test	-	-	+	-					
Citrate test	+	-	-	-					
MR test	-	-	-	-					
VP test	-	-	+	-					
Gelatin test	+	+	+	-					
Hydrogen sulphide test	-	-	-	-					
Growth on MacConkey agar	+	+	+	+					

+ positive; -, negative.

TABLE II.- BACTERIAL SPECIES ISOLATED FROM DIFFERENT LOCALITIES.

Strain	Khokhra par	Chunian	Punjab University	NARC 1	NARC 2
B. subtilis	+	+	+	+	+
B. alvei	-	+	-	-	+
B. circulans	+	+	+	-	-
B. coagulans	+	+	+	-	-
B. brevis	-	+	-	+	+
Nocardia sp.	+	+	-	-	-
Lactobacillus sp.	-	-	+	+	+
Bifidobacterium sp.	-	-	-	+	-
Clostridium sp.	-	+	-	-	+

TABLE III.- ANTIBIOTIC RESISTANCE OF BACTERIA ISOLATED FROM HONEY SAMPLES.

Strain	Ciprofloxacin (mm)	Impenem (mm)	Amikacin (mm)	Gentamicin (mm)
B. coagulans	(4) S	(6) S	(6) S	(4) S
B. circulans	(5) S	(8) S	(5) S	(3) S
B. brevis	(0) R	(6) S	(5) S	(3) S
B. alvei	(8) S	(9) S	(8) S	(7) S
B. subtilis	(7) S	(14) S	(9) S	(8) S
Nocardia. sp.	(12) S	(15) S	(9) S	(8) S
Clostridium sp.	(2) S	(2) S	(0) R	(0) R
Lactobacillus sp.	(13) S	(19) S	(7) S	(6) S
Bifidobacterium sp.	(13) S	(13) S	(4) S	(5) S

S, sensitive; R, resistant; Figures in bracket show zones of inhibition.

TABLE IV.- HEAVY METAL RESISTANCE OF BACTERIA ISOLATED FROM HONEY SAMPLES.

Strains	Cd^{2+} (µg)	Cu^{2+} (µg)	Pb^{2+} (µg)	Cr^{6+} (µg)	Ni^{2+} (µg)	Ag^{2+} (µg)
B. coagulans	350	450	1400	220	600	30
B. circulans	300	450	1600	210	600	30
B. brevis	350	450	1400	225	600	30
B. alvei	300	400	1400	220	700	20
B. subtilis	350	450	1400	220	650	30
Nocardia.	350	450	1600	215	700	30
Clostridium	380	400	1900	230	425	75
Lactobacillus	150	250	1950	220	620	35
Bifidobacterium	100	350	1850	230	325	30

TABLE V.- MEAN COLONY FORMING UNITS (CFUs) ISOLATED FROM HONEY SAMPLES FROM DIFFERENT LOCALITIES.

Sample sources	Mean CFUs ± SEM	
	Aerobic	Anaerobic
Khokhrapar	13.7±0.98	-
Chunian	30±0.47	0.67±0.27
Punjab University	8.3±0.27	0.33±0.27
NARC 1	5±0.47	1±0.47
NARC 2	3.67±0.27	1.33±0.27

high temperature the vegetative bacteria are destroyed but the endospore formers survive in this condition.

Honey contains both aerobic and anaerobic bacteria. The aerobic bacteria are mostly Gram positive rods and are catalase positive (Fleche *et al*., 1997). The Gram positive rods which are also endospore formers include different species of *Bacillus* and *Nocardia*. The *Bacillus* species are rods (short or long) and usually non-branching, while *Nocardia* species are long rods with branched structure (Murray *et al*., 2002). *Nocardia* sp. is non-endospore forming bacteria and shows positive acid fast stain. The anaerobes include *Clostridium* sp., *Lactobacillus* sp. and *Bifidobacterium* sp. From amongst the honey samples studied, Chunian sample was found to be the most contaminated one which may be due to improper handling during processing. The samples collected from NARC, Islamabad showed minimum bacterial contamination.

REFERENCES

BARON, E.J., PETERSON, L.R. AND FINEGOLD, S.M., 1994. *Bailey and Scott's Diagnostic microbiology* 9th ed. Mosby Limited.

BROOKS, G.F., BUTEL, S.J. AND MORSE, S.A., 1998. *Medical microbiology* 21st ed. Appleton and Lange, Stamford, Connecticut.

BENSON, H.J., 2002. *Microbiological applications* 8th ed. Mc-Graw Hill, New

CAPPUCINO, J.G. AND SHERMAN, N., 1996. *Microbiology: A laboratory manual* 4th ed. Rockland Community College, Suffern, New York.

CARR, C. 1981. Infant botulism. In: *Gleanings in bee culture*, pp. 254, 267.

CHEESEBROUGH, M., 1993. *Medical microbiology manual for tropical countries,* vol.II. *Microbiology* ELBs, University Press, Cambridge.

COX, N. AND HINKLER, R., 2002. Infant botulism. *Am. Fam. Physician Apr.,* **65**: 1388-1392.

CRANE, E. AND WALKER, P., 1984. Composition of honey from some important honey sources. *Bee Wld.*, **4**: 65.

FENECIA, L., FERRINI, A.M., AURELI, P. AND POCECCO, M., 1993. A case of infant botulism associated with honey feeding in Italy. *Eur. J. Epidemiol.*, **9**: 671-673.

FLECHE, C., CLEMENT, M.C., ZEGGANE, S. AND FAUCON, J.P., 1997. Contamination of bee products and risk for human health. *Rev. Sci. Tech.*, **16**: 609-619.

MURRAY, P.R., ROSENTHAL, K., KOBAYASHI, G. AND PFALLER, M.A., 2002. *Medical microbiology,* 4th ed. A Harcourt Health Science Company, London.

NEVAS, M., HIELM, S., LINDSTORM, M., HORN, H., KOIVALEHTO, K. AND KORKEALA, H., 2002. High prevalence of *Clostridium botulinum* types A and B in honey samples detected by polymerase chain reaction. *Fd. Microbiol.*, **72**: 45-52.

SNOWDON, J.A. AND CLIVER, D.O., 1996. Microorganisms in honey. *Fd. Microbiol,,* **31**: 1-26.

WHITE, J.R., REITHOF, M.L., SUBERS, M.H. AND KUSHNIR, I., 1977. Composition of honey. VI. The effect of storage on carbohydrates, acidity and diastase content. *J. Fd. Sci.*, **26**: 63.

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EFFECT OF SINGLE DOSE OF DIMETHAZINE ON REGENERATION OF PARTIALLY HEPATECTOMIZED RABBIT LIVER

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Abstract.- Effect of a single dose of dimethazine, an anabolic steroid, has been studied on the various biochemical components and histological structure of regenerating rabbit liver after partial hepatectomy (PH). Twenty adult, male rabbits were partially hepatectomized in which about 30-35% of the liver was removed. Their livers were taken out 12, 18, 60, 72 and 144 hours after PH. Another group of six rabbits was partially hepatectomized as above and dimethazine was administered intraperitoneally as a single dose of 5 mg/kg body weight immediately after PH. After PH the body growth rate was considerably hampered. The hepatic glutamate oxaloacetate transaminase (GOT) activity decreased significantly 60 and 72 hours after PH by 37.8 and 44.8%, but after 144 hours, it was normalized. LDH activity after initial decrease during first 60 hours after PH was normalized after 144 hours. The DNA and RNA content remained unaltered till 72 hours but increased 11% and 66%, respectively, 144 hours after PH. The soluble protein content decreased continuously till 144 hours after PH, but total protein content remained unaltered during post hepatectomy period. The glucose content decreased drastically after PH and remained significantly decreased till 144 hours after PH. The urea content decreased by 46%, 18 hours after PH, but then increased gradually non-significantly till 144 hours. The cholesterol content decreased by 43%, 12 hours after PH but increased significantly 144 hours after PH. During initial periods of PH, the size of cell, their nuclei and nucleoli decreased, but then increased gradually during later period. The number of nuclei/cell remained unaffected, while the number of nucleoli/nucleus decreased till 72 hours after PH, but then increased significantly 144 hours after PH. In the presence of single dose of dimethazine, the body wt./liver wt. ratio, and GOT (55%) and GPT (51%) activities and glucose contents were significantly decreased after 18 hours of administration, whereas other enzymes (AP, LDH), nucleic acids, total proteins, urea and cholesterol contents remained unaltered. The hepatic cells and the nuclei got hypertrophied after dimethazine treatment, while the number of nuclei/nucleus decreased. Dimethazine, in general, seems to hasten the recovery process.

Key words: Hepatic resection, liver function tests, hepatic regeneration.

INTRODUCTION

After partial hepatectomy (PH) a series of morphological and biochemical changes occur in the remaining liver that results in

reconstitution of the original tissue mass. Regeneration of liver does not involve regrowth of remnant stumps, instead it consists of hypertrophy and hyperplasia of all major cellular elements of the remaining unresected lobes of the liver (Bucher, 1967). Studies in rats have shown rapid cell division during the first four days after a 2/3rd partial PH, with the size of the liver restored within two to three weeks (Higgins and Anderson, 1931). The rate of regeneration in man is slower. Liver scanning, liver biopsy and repeat laparotomy have shown that though regeneration begins within three days of surgical resection, the process is not complete for about six months (Lin *et al*., 1979). PH is, therefore, a normal clinical/surgical need in a number of cases and is also very common practice for understanding mechanism of liver function.

The effects of numerous dietary components (Gurd *et al*., 1948), hormones (Canzanelli *et al*., 1949; Einhorn *et al*., 1954; Bucher, 1976; Leffert *et al*., 1979; Garuana and Gage, 1980; Strecker *et al*., 1980a,b) and vitamins (Gentile *et al*., 1970) and certain poisons and drugs like actinomycin D (Guidice and Novelli, 1963), hydroxyurea (Yarbro *et al*., 1965), puromycin (Gottlieb *et al*., 1964), vinyl chloride and As_2O_3 (Norpoth *et al*., 1980), phenobarbital (Ngala Kenda and Lambotte, 1981), aflatoxin (De Recondo *et al*., 1966), fluorouracil (Nagasue *et al*., 1978), certain carcinogens (Hsu, 1962; Craddock, 1975a,b; Date *et al*., 1976; Dixit and Rao, 1980) etc. have been studied on the various aspects of metabolism in partially hepatectomized livers of different animals. The sequence of biochemical changes during hepatic regeneration following PH and their relation to intracellular control has been reviewed extensively by Bucher and others (Bucher, 1963, 1967; Bucher *et al*., 1969; Gentile *et al*., 1970; Bucher and Malt, 1971; Levi and Zeppa, 1971; Murray *et al*., 1980, 1981). Oppenheimer and Flock (1947) have reported elevation of alkaline phosphatase levels in plasma and liver after 70% PH. Sekas and Cook (1979) have reported changes in γ-glutamyl transpeptidase, lactase dehydrogenase, glutamic oxaloacetate transaminase and alkaline phosphatase activities, which increase during very early hours of post-PH in rats. The peak of DNA synthesis in the liver of rat after 68% resection occurred 21 to 23 hours after resection, while in 85% resections, it was delayed ten to fifteen hours. In dog the maximum DNA synthesis appears to be three to four days after 70% resection (Sigel *et al*., 1965). In the early phases of regeneration the heptatocytes, their nuclei, and nucleoli were found to double in size and cytoplasm filled with lipid and other inclusions (Harkness, 1957; Weinbren, 1959).

Dimethazine (Fig. 1) is an anabolic steroid known for its anabolism of protein, weight gain and myotrophic-androgenic activity (Bianco *et al*., 1962; Lupo *et al*., 1962). Partially hepatectomized rabbits were treated with single dose of dimethazine to evaluate the recovery process.

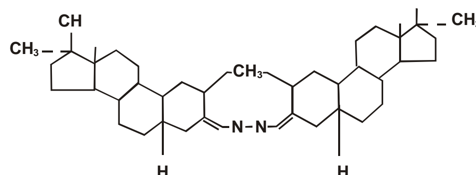

Fig. 1. Structure formula of dimethazine (2α, 17α-dimethyl-17β-hydroxy-5α andro-stan-3,3-azine)

MATERIALS AND METHODS

Animals

Fifty healthy male domesticated rabbits, *Oryctolagus cuniculus*, weighing 550-750 gms were purchased from the local market. They were kept in a group of 2-3 in each cage in the Animal House of the Department of Zoology, where semicontrolled temperature conditions were maintained. Fresh green fodder and tap water was supplied *ad libitum*. The total body weight of rabbits and the food consumed by them was recorded daily for 3 weeks. The regular gain in body weight and increase in the consumption of food was considered as an indicator of adjustment to the pre-experimental acclimatization period.

Dimethazine treatment

Dimethazine (2α, 17α-dimethyl-17β-hydroxy-5α andro-stan-3,3-azine), an anabolic steroid preparation of Ormonoterapia, Richter, Milan, Italy was administered intraperitoneally as a single dose of 5 mg/kg body weight.

Procedure adopted

Fifteen rabbits were partially hepatetomized and were designated as pH group, while another group of six was administered, intraperitoneally, with anabolic steroid after PH. The later group was designated PH + DM. Three animals were slaughtered 12, 18, 60, 70 and 144 hours after hepatic surgery,

whereas three animals were slaughtered after 12 hours, while another 3 were slaughtered after 18 hours from PH + DM group. The piece of liver that was removed after PH was used as control sample.

The livers were taken out, weighed and analyzed for various biochemical components. A piece of liver was also fixed in Bouin's fluid every time and processed for histological studies.

The food was withdrawn and the animals were weighed before hepatectomy. The total body weight was also recorded regularly during post-hepatectomy period.

Biochemical analysis of liver

Saline extract was prepared by homogenizing a piece of known weight of liver in 0.89% NaCl solution in a glass homogenizer. The homogenate was centrifuged at 5,000 rpm for 15 minutes to obtained clear supernatant. This supernatant was then used for the estimation of activities of various enzymes like alkaline phosphatase (AP) according to Bessey *et al*. (1949), glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transamianse (GOT) according to Reitman and Frankel (1957), lactate dehydrogenase (LDH) according to Cabaud and Wroblewski (1958), and various other biochemical contents like glucose (Hartel *et al.,* 1969), urea (Natelson *et al*., 1951) and soluble proteins (Lowry *et al*., 1951).

For cholesterol estimation a known weight of liver was crushed in ethanol which was then centrifuged. Supernatant was used for the determination of cholesterol, according to Lieberman and Bruchardt (Henry, 1964).

A portion of liver was weighed and processed for nucleic acids (DNA and RNA) and total protein contents. The nucleic acids were extracted according to Shakoori and Ahmad (1973), while DNA and RNA estimation were done according to Schmidt and Thennhauser procedure described by Schneider (1957).

Histological studies of liver

A small piece of liver fixed in Bouin's fixative was processed for embedding by the routine histological technique, 6-8 μ thick sections were

cut and then stained with hematoxylin and eosin. The stained sections were then studied both under low and high power to note various changes in hepatic structure. Morphological studies were conduced which included the following parameters: (1) Number of hepatic cells per microscopic field; (2) number of nuclei/cell; (3) number of nucleoli/nucleus; (4) size of hepatic cells; (5) size of nuclei; (6) size of nucleoli.

The counting for number of hepatic cells per microscopic field was done at a magnification of 500 x from at least three different parts of the section. The counting for number of nuclei/cell and number of nucleoli/nucleus was done at magnification of 1250 x (oil emersion lense). Different cells were studied from each slide for recording the number of nucleoli/nucleus. The values represent the mean of 130 readings.

The measurements for dimensions of hepatic cells, their nuclei and nucleoli were done with the help of ocular micrometer at a magnification of 1250x. For these measurements both the length and breath were recorded. The 2 dimensions were later multiplied with each other to represent the data in the from of area (μ^2). The number of observations recorded were the same as described for the number of nuclei and number of nucleoli.

RESULTS

Total body growth rate

Figure 2 shows the effect of PH on the total body growth rate. A control rabbit shows a daily increase of 1.38±0.13% (n=28) in the body weight. This growth rate is reduced to 9.18% per day 12 hours and 10% per day 18 hours post PH but it starts recovering during the subsequent period. Six days post pH the decrease in growth rate is 2.58% per day. This decrease, however, is in contrast to the constant gradual increase in the control body growth rate, which is 1.7% per day at the end of one week (Fig. 2). In PH + DM group the body weight was also drastically decreased after 12 and 18 hours, but this decrease was of lesser magnitude compared to that of PH group.

A control rabbit shows body weight/liver weight ratio 38.93±0.53 (n=15). Following PH this ratio decrease 9%, 16%, 19% and 21% after 12, 18, 60, 72 and 144 hours. After DM treatment following PH, the ratio is

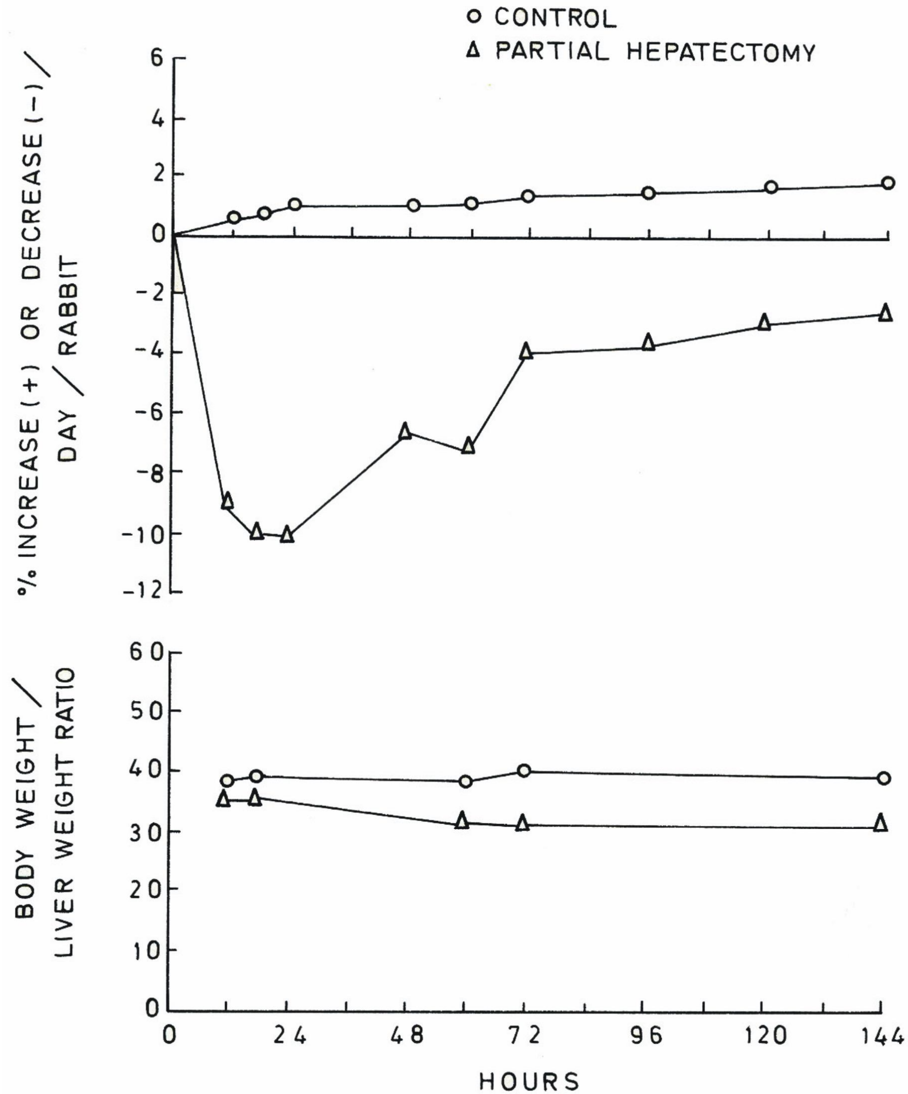

Fig. 2. Increase or decrease in the total body weights of male rabbits following partial hepatectomy. The figure also shows the change in body weight/liver weight ratios following partial hepatectomy.

normalized within 12 hours, though after 18 hours the ratio is it still show significant decrease of 8.2%. In the PH + DM group, however, the decrease

was significant (7%) after 12 hours and (10%) after 18 hours. The body wt./liver wt. ratio decreased 2% and 8.5%, respectively, 12 hours and 18 hours post PH. After DM treatment following PH, the ratio is normalized within 12 hours, though after 18 hours, the ratio still shows significant decrease of 8%.

Biochemical components of liver

PH led to extensive molecular readjustment in the liver. Table II show changes in various biochemical components of liver during post-hepatectomy period. After an initial decrease of all the biochemical parameters studied immediately following PH, some of them (LDH, AP) recovered 60 hours after PH, whereas others such as GOT, GPT, RNA, soluble proteins and glucose showed lower values throughout the experimental period of 144 hours post PH as compared with their respective controls. The DNA and total proteins remained unchanged throughout, whereas cholesterol and urea appeared to recover 72 hours post PH. Twelve hours after PH, AP, GPT, LDH, RNA, soluble protein, glucose, urea and cholesterol decreased 21%, 44%, 32%, 37%, 28%, 85%, 20% and 43%, respectively. GOT, DNA was not much affected, whereas total proteins increased 18%. After 60 hours of PH, the AP, GOT and GPT activities and soluble protein, glucose, urea and cholesterol content were still 20%, 38%, 17%, 27%, 28%, 17% and 12% below control values, respectively. The LDH however, increased 41% during this period. At the end of 144 hours after PH the GOT and GPT activities, and soluble protein and glucose content were 27%, 10%, 28% and 65%, lower as compared with control, respectively. The LDH activity RNA, urea and cholesterol content were 23%, 66%, 31% and 51% higher as compared with control liver.

The AP activity decreased 21% and 23%, respectively, 12 and 18 hours after PH. After dimethazine treatment this decrease was 10% after PH, whereas it showed 7% increase over the control value, 18 hours after PH. The GOT activity is decreased 55%, 18 hours after PH, but in the presence of dimethazine this decrease was only 7% compared to control. In GPT activity this decrease was 45% and 31%, respectively, 12 and 18 hours after PH, whereas after administration of single dose of dimethazine immediately following PH, this decrease was 7% and 28%, respectively, 12 and 18 hours post-PH.

TABLE I.- EFFECT OF DIAMETHAZINE, ADMINISTERED AS A SINGLE DOSE (5 mg/kg body) IMMEDIATELY AFTER SURGERY, ON THE VARIOUS ENZYMATIC ACTIVITIES AND BIOCHEMICAL COMPONENTS OF REGENERATING RABBIT LIVER FOLLOWING PARTIAL HEPATECTOMY.

Parameters	Hours of observations	Control (n=6)	Partial hepatectomy (n=3)	PH + Diamethazine (n=3)
AP (mU/g)	12	0.82±0.19a	0.65±0.10	0.74±0.49
	18	0.87±0.21	0.67±0.09	0.93±0.14
	60	0.72±0.09	0.57±0.07	
	72	0.84±0.12	1.03±0.18	
	144	0.67±0.05	0.73±0.05	
GOT (IU/g)	12	6.05±1.9	6.33±1.08	5.62±0.25
	18	14.28±3.24	10.28±1.4	6.31±0.22[b]
	60	7.89±0.80	4.91±0.29 d	
	72	11.52±2.08	6.35±1.00 b	
	144	9.41±0.56	6.81±0.510 c	
GPT (IU/g)	12	3.02±0.40	1.67±0.95	2.81±0.19
	18	4.58±0.92	3.17±0.07	2.27±0.83[b]
	60	3.90±1.38	3.22±0.62	
	72	5.01±0.61	3.90±1.09	
	144	5.25±1.01	4.69±1.30	
LDH ($X10^3$ IU/g)	12	28.96±5.29	19.71±1.10	44.6±9.94
	18	32.13±5.49	24.20±2.71	35.5±4.54
	60	65.49±4.16	92.39±50.9d	
	72	48.09±8.29	78.43±12.17	
	144	73.46±10.38	92.14±6.85	
DNA (mg/g)	12	1.62±0.25	1.67±0.08	0.96±0.07[b]
	18	1.78±0.23	1.67±0.05	1.29±0.14
	60	1.91±0.15	1.64±0.02	
	72	1.91±0.19	2.14±0.29	
	144	1.82±0.04	2.02±0.02d	
RNA (mg/g)	12	7.12±0.45	4.49±0.86b	6.55±0.26
	18	6.71±0.79	5.71±0.26	7.81±0.66
	60	6.82±0.95	7.86±0.13	
	72	7.09±0.65	7.93±1.13	
	144	4.26±0.28	7.07±0.89b	

Continued

Parameters	Hours of observations	Control (n=6)	Partial hepatectomy (n=3)	PH + Diamethazine (n=3)
Soluble protein (mg/g)	12	237.23±18.85	171.68±1.18c	275.39±47.87
	18	255.02±34.77	156.34±4.33b	315.31±26.30
	60	252.19±9.20	182.78±14.26c	
	72	236.90±22.11	173.07±26.32b	
	144	237.69±10.62	185.11±6.39c	
Total protein (mg/g)	12	296.47±39.20	348.34±63.99	333.92±18.27
	18	340.0±16.21	329.79±12.0	355.79±14.74
	60	363.54±15.52	390.24±30.91	
	72	320.69±27.95	286.57±4.26	
	144	377.26±21.34	389.34±36.01	
Glucose (mg/g)	12	12.98±1.26	1.93±0.13d	8.73±2.50[b]
	18	13.20±2.08	6.88±1.85b	8.05±0.58[b]
	60	15.53±1.05	11.17±0.95c	
	72	15.71±0.92	6.36±1.35d	
	144	12.92±1.28	7.76±0.45c	
Urea (mg/g)	12	15.80±1.78	12.55±2.33	20.26±1.54
	18	15.17±1.95	8.24±1.61b	17.77±2.94
	60	17.21±2.31	14.24±1.82	
	72	14.85±1.22	11.41±1.14	
	144	10.86±1.83	14.31±4.01	
Cholesterol (mg/g)	12	3.71±0.35	2.13±0.22c	3.64±0.4
	18	3.54±0.17	3.90±0.38	3.26±0.23
	60	5.25±0.40	4.60±0.56	
	72	6.40±0.50	5.72±1.41	
	144	3.40±0.28	5.19±0.55b	

[a]Mean±SEM; Student's t test, [b]P<0.05; [c]P<0.01, [d]P<0.001.

LDH activity decreased 32% and 25%, 12 and 18 hours after PH, but in the presence of dimethazine the activity increased 54% and 11%, respectively, after 12 and 18 hours post-PH. This increase was statistically non-significant.

The DNA content remained unaltered after PH, but after dimethazine treatment, DNA decreased 41% after 12 hours and 28% after 18 hours of PH. The RNA content likewise were not affected after PH. After dimethazine treatment the RNA content showed 8% decrease 12 hours, and 16% increase 18 hours after PH. The soluble protein decreased 28% and 39%, 12 and 18

TABLE II.- EFFECT OF DIAMETHAZINE ON THE VARIOUS HISTOLOGICAL PARAMETERS OF REGENERATING RABBIT LIVER FOLLOWING PARTIAL HEPATECTOMY.

Parameters	Hours of observations	Control (n=120)	Partial hepatectomy (n=120)	PH + Dimethazine (n=120)
Size of cell (μm^2)	12	346.52±10.68	288.41±8.42[d]	406±10.62[d]
	18	339.00±15.43	336.34±9.03	323.71±8.53
	60	380.06±81.71	362.66±1.28	
	72	348.10±11.37	349.27±9.47	
	144	342.76±13.05	294.54±11.41[c]	
Size of nucleus (μm^2)	12	56.015±2.34	45.93±0.91[d]	52.54±0.94
	18	59.33±3.57	52.29±1.09[d]	51.99±0.76[b]
	60	51.86±2.00	60.44±1.59[d]	
	72	56.15±1.80	52.61±1.55	
	144	56.01±2.34	52.72±0.88	
Size of nucleolus (μm^2)	12	3.37±0.20	2.39±0.15[d]	2.89±0.13[b]
	18	2.99±0.17	3.33±0.18	4.15±2.22[d]
	60	2.84±0.19	3.67±0.24c	
	72	2.84±0.19	6.16±0.27d	
	144	3.18±0.18	3.43±0.24	
No. of nuclei/cell	12	1.16±0.05	1.19±0.04	1.37±0.05[c]
	18	1.24±0.04	1.25±0.04	1.23±0.04
	60	1.31±0.05	1.29±0.05	
	72	1.15±0.09	1.27±0.04	
	144	1.2±0.03	1.25±0.04	
No. of nucleoli/ nucleus	12	1.64±0.07	1.42±0.06b	1.61±0.06
	18	1.67±0.06	1.48±0.05b	1.39±0.06[a]
	60	1.83±0.08	1.66±0.07	
	72	1.64±0.07	1.38±0.07b	
	144	1.65±0.06	1.90±0.09b	
No. of cells/mf.	12	220.38±4.28	219.52±11.85	208.5±5.39
	18	220.38±4.28	218.07±10.50	227.57±5.95
	60	218.63±4.44	204.0±5.31b	
	72	218.63±4.44	197.71±6.50b	
	144	219.28±4.35	284.0±12.12d	

[a]Mean±SEM; Student's t test, [b]P<0.05; [c]P<0.01, [d]P<0.001.

hours after PH. In the presence of dimethazine, the soluble protein increased 16% and 24%, respectively, compared to control. Dimethazine caused 10.2%

increase in soluble protein when compared to PH group. The total proteins do not make any significant change after PH. The content remained unchanged even treatment after dimethazine.

Within 12 hours of PH, the sugar, urea and cholesterol contents decreased 85%, 20% and 43%, respectively. The DM treatment tends to maintain these values as close to control values as possible. After DM treatment the sugar is decreased 33%, whereas urea showed 28% increase.

Hepatic histological structure

Control

Figures 3-5 show the structure of liver of a control rabbit. A typical lobule with a central vein, cords of hepatic cells are visible (Figs. 3, 4). A hepatic cell has a well defined vesicular nucleus surrounded by uniformly dense cytoplasm is obvious (Fig. 5). It occupies an area of 351.29±26.45 μm^2 (n=600), while that of nucleus and nucleolus are, respectively, 55.87±2.41 μm^2 (n=600) and 3.04±0.19 μm^2 (n=600) at a magnification of X 1250 (Table II). The number of hepatic cells per micrscopical field (at a magnification of X500) were 219.46±4.36 (n=107). In this area the number of nuclei/cell was 1.21±0.05 (n=600), while number of nucleoli/nucleus were 1.69±0.07 (n=600) (Table II) at magnification of X 1250. The area occupied by the cell is 6.29 times that of the nuclear area.

After 12 post-hepatectomy hours: Figures 6-9 show the structure of liver after 12 hours of PH. Although the general pattern of the structure of a typical lobule was the same as in the control liver, the surgical and or toxic insult has obviously led to the general shrinkage of hepatic cells (Figs. 7, 8). The hepatic cells with vacuolated cytoplasm and those with uniform cytoplasm (Fig. 9), large sinusoidal spaces and vacuolation is towards periphery are visible (Figs. 7, 9).

The total number of cells/microscopical field at magnification of X500 and number of nuclei/cell remain unchanged 12 hours following partial hepatectomy. The number of nucleoli/nucleus decreased from 1.69±0.07 (n=600) to 1.42±0.06 (n=132) ($t=2.44$; $P<0.02$). The size of cell, nucleus and nucleoli showed a significant decrease 12 hours after PH. The size of cell decreased to 288.41±8.42 μm^2 ($t=4.275$, $P<0.001$) as against 351.29±26.45 μ^2 (n=600) of the control liver. The size of nuclei and nucleoli decreased

Figs. 3-9. Histological structure of normal rabbit liver (3-5) and of liver 12 hours post-hepatectomy. The typical hepatolobular architecture, consisting of central vein (3, 6), cords of hepatic cells (4, 7) and hepatic cells (5,8). In Figure 9 hepatic cells are more vacuolated than in control.
Magnification: 3, 6, 9, 50x; 4, 7, 200x; 5, 8, 500x.

significantly by 18 and 29%, respectively ($t = 4.034$, $P<0.001$) and ($t=3.967$; $P<0.001$) (Table II).

After 18 post hepatectomy hours: Figures 10-15 show the structure of liver on hour 18 of post-hepatectomy period. The cells from the periphery region showed degeneration of hepatic lobule (Fig. 13). Figure 14 shows cell close to the region of resection where hepatocellular architecture is totally disturbed. The hepatic cells were highly vacuolated and had hypertrophied nuclei (Fig. 12) as compared with control (Fig. 5). Except for the number of nucleoli/nucleus which decreased significantly 1.477 ± 0.052 ($t=2.431$; $P<0.02$) as against 1.686 ± 0.069 ($n=600$) of the control liver after hepatectomy on hour 18, all other histological parameters remained unaltered (Table II).

After 60 post hepatectomy hours: Figures 16-19 show the general pattern of liver after 60 hour post hepatectomy period. The hepatolobular architecture, consisting of central vein portal area, hepatic cell (Figs. 16-18) and hepatic cords with Kupffer cells (Fig. 19). The various histological parameters like size of cells, number of nuclei/cell and number of nucleoli/nucleus were not significantly deviated from the control liver parameters. Number of cells/microscopic field, decreased significantly to 204 ± 5.31 ($n=14$) as against 219.46 ± 4.36 ($n=107$) of control liver. But size of nuclei and size of nucleoli increased significantly about 17% and 29%, respectively 60 hours following PH (Table II).

After 72 post hepatectomy hours: Figures 20-32 show the structure of liver 72 hours after PH. A portion of liver was completely devoid of hepatolobular architecture (Fig. 20). Most of hepatic cells did not show any typical structure (Figs. 20-21). The entire area appeared to be infested with muscle fibers (Fig. 22). Some portions were found to be completely free from hepatic cells and infested heavily with regenerated muscle fibers and connective tissue (Figs. 23-32). The number of cells/microscopic field decreased to 197.71 ± 6.50, 72 hours after PH as against control value of 219.46 ± 4.356 ($n=500$). The number of cells/microscopic field and number of nuclei/cell remained unaltered. The size of nuclei decreased non-significantly. The number of nucleoli decreased to 1.38 ± 0.07 ($t=2.566$, $P<0.02$). This decrease also reflected the size of nucleoli which increased significantly from 3.04 ± 0.19 of control liver to 6.16 ± 0.27 ($n=5$) (Table II).

Figs. 10-15. Histological structure of rabbit liver 18 hours after partial hepatectomy. General hepatolobular architecture, consisting of central vein, portal area (10), cords of hepatic cell (11) and hepatic cells (12). Degenerated hepatic lobules (13), disturbed hepatolobular architecture at the region of resection (14), cords of hepatic cells and central vein (15) visible.
Magnification: 10, 13, 14, 50x; 11, 15, 200x; 12, 500x. Stain: Haematoxylin and eosin.

Figs. 16-19. Histological structure of rabbit liver 60 hours after partial hepatectomy. The general hepatolobular architecture (16, 17), cords of vacuolated hepatic cells (18, 19) and Kupffer cells (19).
Magnification: 16, 20x; 17, 50x; 18-19, 200x. Stain: Haematoxylin and eosin.

Figs. 20-25. Histological structure of rabbit liver 72 hours after partial hepatectomy. Degenerated hepatic lobule (20, 21), extensive packing of cells just beneath. The regenerated muscular area (22, 23) and connective tissues (23). Extensive packing of cells just beneath the regenerating muscular area and interolobular region flooded with was connected tissue (25).
Magnification: 20, 20x; 21, 23, 24, 50x; 19, 22, 200x. Stain: Haematoxylin and eosin.

Figs. 26-32. Histological structure of rabbit liver 72 hours after partial hepatectomy. Degenerated hepatic lobule with central vein (26), completely degenerated hepatic cells infested with muscle fibers and connective tissues (27, 28) and invaded by blood vessels (28). Figures 30 and 31 show cords of hepatocytes with blood vessels, whereas Figure 32 shows cords of hepatocytes in an area away from resected region (32).
Magnification: 26, 30, 32, 50x; 27, 28, 30, 32, 200x. Stain: Haematoxylin and eosin.

After 144 post hepatectomy hours: Figures 33-36 show the structure of liver 144 hours after PH. Although general pattern of the structure of typical hepatic lobule, is the same as in control liver, but in few sections of liver hepatic lobule is completely degenerated (Figs. 34-35). The various histological parameters like number of nuclei/cell, size of nuclei and size of nucleoli remained unaltered, 144 hours after PH. The number of cells/microscopic field at 500X magnification increased significantly to 284.0±12.12 as against 219.46±4.36 (n=21) in control liver. The size of hepatic cell decreased to 294.54±11.41 μm^2 ($t=2.78$, $P<0.01$), from 351.29±26.45 μm^2 of control liver. The number of nucleoli also increased significantly by 15% (Table II).

Dimethazine treatment

Figures 37-39 show the effect of single dose of dimethazine administered immediately after PH on the histological structure of regenerating rabbit liver. The hepatic cells appeared to be hypertrophied though the typical signs of degeneration and vacuolated cytoplasm which was obvious in the PH rabbits after 12 hours (Table II) were not seen.

After PH, the size of cell, nucleus and nucleolus decreased, respectively, 17, 18 and 29% within 12 hours whereas after dimethazine treatment these values respectively increased 4.5% and decreased 6 and 14% after DM administration following PH during the same period. After 18 hours of PH + DM administration, the cell and nucleus size got smaller by 5% and 12% compared to PH group. The nucleolar size was however 39% higher, as against 1 and 12% decrease in the size of cell and nucleus, respectively in the PH group. The nucleolar size is however 11% higher than the control.

The number of nuclei/cell increased by 15% after DM administration following PH, whereas in PH group alone it showed less than 3% increase during 12 hours after PH. The number of nucleoli/nucleus and number of cells/field however decreased. In the PH group this decrease was 13% and less than 0.4%, whereas in PH + DM group, this decrease was, respectively, 2% and 5%. The number of cells/microscopic field showed 3% increase after PH + DM treatment, 18 hours after the PH.

Figs. 33-36. Histological structure of rabbit liver 144 hours after partial hepatectomy. Note general hepatolobular structure (33,34), cords of hepatocytes with increase in sinusoidal area (35). Note hepatic lobule, degenerated area and complete distinguishable hepatic lobule (36).
Magnification: 33, 36, 20x; 34, 50x; 35, 200x. Stain: Haematoxylin and eosin.

Figs. 37-39. Histological structure of rabbit liver treated with a single dose of dimethazine (5 mg/kg body wt. Administered immediately after PH), 12 hours after partial hepatectomy. Note hepatolobular architectures showing blood vessels, central vein, portal area {37), central vein and portal area (38), hepatic cells not arranged in definite cords (39).
Magnification: 37, 20x; 38, 50x; 39, 200x. Stain: Haematoxylin and eosin.

Figs. 40-41. Histological structure of rabbit liver treated with a single dose of dimethazine (5 mg/kg body wt. Administered immediately after PH), 18 hours after partial hepatectomy. Note hepatic lobule (40), hepatic cells (41). Magnification: 40, 50x; 41, 200x. Stain: Haematoxylin and eosin.

DISCUSSION

Partial hepatectomy is a process in which a part of a liver is surgically removed to observe the molecular readjustment in the remaining organ and to understand the mechanism of hepatic regeneration. The extent of damage and the time required for molecular readjustment depends upon the amount of liver taken out; 10-25% removal may not cause any appreciable disturbance in the molecular architecture and hence the function of liver, whereas 90% removal of liver will be more stressful for the animal. Sekas and Cook (1979) suggested that most of the molecular readjustment sets in and is completed to a large extent, within a period of one week after surgical removal of liver in

rats. Shakoori *et al*. (1984) also concluded that PH brings about molecular readjustment in the liver of rabbits which is actively accomplished during the first 10 days.

Body weight

The PH causes significant decrease in the total body weight and body weight / liver weight ratio. Within 12 hours after PH the total body weight of rabbits decreased at a rate of 9.18%/day. But weight is gained during the subsequent period. Six days after PH the decrease in total body weight is 2.58%/day. Significant decrease immediately after PH is because of the pre-hepatectomy starvation. Loss of body weight in the absence of food is a well established factor (Peters, 1967; Porta and Hartoft, 1970; Cascarano *et al*., 1978; Goodman and Ruderman, 1980). Loss in body weight is also due to PH (Shakoori *et al*., 1984). Feeding leads to recovery of body weights in subsequent recovery period.

Biochemical components of PH liver

PH had no significant effect on AP and GOT activities. The GOT activity decreased 38% and 45% respectively 60, 72 hours after PH. But this activity normalized after 6 days. LDH activity increased significantly 60 hours after PH, but is normalized 144 hours after PH. Sulkin and Gardener (1948) and Bucher and Malt (1971) have described changes in the liver structure and some enzymatic components of liver after PH in rats. The present studies deal with rabbits.

The enzymatic activities may be decreased (1) as a result of enzyme release from the cells due to hepatic tissue necrosis or (2) may be due to leeching of enzymes into blood with increased cell membrane permeability without cell necrosis (Bartsokas, 1974; Schmidt *et al*., 1974; Cerdan *et al*., 1978). Cytoplasmic enzymes are released into circulation within a few hours of injury, which is reflection of cell membrane injury or permeability changes before the onset of frank necrosis. Possibly enzyme activities are increased as a result of its absorption of enzymes release by necrosis of the small amount of hepatic tissue remaining distal to the site of ligation, or it could also be attributed to the increased synthesis of the enzymes after PH.

Nucleic acids contents remained unaltered upto 72 hours after PH, but increased on day 6 of post hepatectomy period. The increase in DNA

following PH, and corollary of hepatic regeneration has been reported from different laboratories (Cater *et al.*, 1956; Grisham, 1960; Bucher, 1963). The glucose content decreased during the post hepatectomy period consistently. Caruana *et al.* (1981) also suggested that glucose utilization after hepatectomy is a unique process. Shakoori *et al.* (1985) also described that glucose content decreased during the first fortnight of post hepatectomy period. Soluble protein content decreased just like glucose during the post hepatectomy period. This is due to greater mobilization of protein into amino acid during post hepatectomy. Cholesterol content decreased 12 hours after PH, but increased significantly 144 hours after hepatectomy. The liver fat, neutral fat concentration and glycogen contents sharply increased 48 hours after operation (Gurd *et al.*, 1948).

Histological structure of PH liver

The regeneration does not involve regrowth of the remnant stumps, instead it consists of hypertrophy and hyperplasia of all major cellular elements of the remaining unresected lobes of liver. Although a comparable regenerative response can be produced by extirpation, irradiation or toxic destruction, the two latter methods have excessive amounts of necrotic hepatic tissues and an inflammatory resection which complicate the analysis of an already complex problem (Hays, 1974).

The histological structure of liver is drastically affected 12 hours after PH. PH leads to reduction in the size of cell, nucleus and its nucleoli. The number of nucleoli/nucleus also decreased but number of nuclei/cell and number of cells per microscopic field remain unaltered. Vacuolation of cytoplasm is also prominent. This decrease in size of histological parameter causes changes in the constituents of the cytoplasm of the cell and not in the cell membrane. Various histological parameters 18 hours after PH remained unaffected except for the number of nucleoli/nucleus.

The size of nuclei and nucleoli increased significantly 60 hours after hepatectomy, whereas number of cells decreased 60 hours after PH. Sinusoidal spaces were enlarged. The increase in size of nucleoli accounted for increased synthesis of RNA, observed in the biochemical analysis of the liver. This also accounted for greater synthesis of protein. The size of nucleoli increased 72 hours after PH significantly, but the number of nucleoli/nucleus and number of cell/microscopical field decreased significantly. Degeneration of some portion of hepatic tissue was prominent.

At some places hepatolobules got disintegrated, were gradually resorbed, and ultimately were replaced by muscular tissue.

Six days after PH the number of cell/mf and number of nucleoli/nucleus increased significantly. Increase in the number of cell was reflected in the size of cell which decreased significantly. Degeneration of hepatic lobule was also observed. The increase in number of cells and decrease in the size of cell was an indication of cell division. This increase in cell number also correlated with the increase in DNA content and increase in size of nucleoli with the increase of RNA synthesis. Dixit and Rao (1980) had also been reported the increase in DNA, RNA and protein of rat and mouse liver following PH.

Effect of dimethazine on PH rabbit liver

Dimethazine, anabolic steroid, was administered to PH rabbits to assess its possible growth promoting role during hepatic regeneration. At a dose of dimethazine 5 mg/kg body weight, administered immediately after the PH, the followings changes were recorded as against those without dimethazine administration; (1) the body weight showed increase after PH as against decrease without the drug, (2) the SGOT and SGPT activities decreased significantly, (3) the DNA content decreased within 12 hours after PH, but the amount was recovered later on, (4) soluble protein contents were kept normal, as against drastic decrease in the absence of DM, (5) the glucose content were kept low but decrease was less against drastic decrease in the absence of DM, (6) the urea content were also maintained at the normal level as against significant decrease without DM treatment, (7) the cholesterol level also appeared to be corrected. In the absence of DM, the cholesterol content decreased considerably, (8) the size of cell and number of nuclei/cell increased, during earlier period of PH. The nucleolar size later on also increased 18 hours after PH. All these observations point towards the rapid recovery process of DM during hepatic regeneration.

Dimethazine has been known to promote growth rate, cause increase in RNA synthesis (Lone and Matty, 1989) and protein synthesis (Matty and Cheema, 1978), besides its role in mobilization of macromolecules and concentration of enzyme. Dimethazine, therefore, appears to accelerate the process for normalization in which biochemical and structural adjustment are involved, after partial hepatectomy. Inspite of the fact that three different types of doses of dimethazine were administered to the rabbits, all point

towards normalization of enzymatic activities and other biochemical components initially disturbed by partial hepatectomy in rabbit. The previous studies from this lab. showed that in contrast to steroid, thioacetamide would further delay the process of readjustment because of its known hepatotoxic and hepatocarcinogenic effect.

REFERENCES

BARTSOKAS, S.K., 1974. Protection of liver function in thioacetamide poisoned rats by heparinoids. *Res. exp. Med.*, **162**: 105-108.

BESSEY, O.A., LOWRY, O.H. AND BROCK, M.I., 1946. A method for the rapid determination of alkaline phosphatase with 5 cc of serum. *J. biol. Chem.,* **164**: 321-329.

BIANCO, L., LAZARINI, E., LUPO, C. AND PIETRA, E., 1962. Confronts dell attivita anabolica della dimethazine e del metiltestosterone con metodo radiochimico. *Farmco Ed. Sci*., **17**: 621-626.

BUCHER, N.L.R., 1963. Regeneration of mammalian liver. *Int. Rev. Cytol*., **15**: 245-300.

BUCHER, N.L.R., 1967. Experimental aspects of hepatic regeneration. *New Engl. J. Med*., **277**: 686-696, 738-746.

BUCHER, N.L.R., 1976. Insulin, glucagons and the liver. *Adv. Enzyme Regul*., **15**: 221.

BUCHER, N.L.R. AND MALT, R.A., 1971. Regeneration of liver and kidney. In: *New England Journal of Medicine*, Medical Progress series, pp. 1-176. Little and Brown, Boston.

BUCHER, N.L.R., SHROCK, T.R. AND MOOLLEN, F.E., 1969. An experimental view of hepatic regeneration. *Johns Hopkins Med. J*., **125**: 250.

CABAUD, P.G. AND WROBLEWSKI, F., 1958. Colorimetric measurement of lactate dehydrogenase activity of body fluids. *Am. J. Clin. Pathol*., **30**: 234.

CANZANELLI, A., RAPPORT, D. AND GUILD, R., 1949. Control of liver regeneration and nucleic acid content by the thyroid: with observation on the effects of pyrimidines. *Am. J. Physiol*., **157**: 225.

CASCARANO, J., MIGLER, R.A. AND WILSON, M.A., 1978. Starvation and refeeding in rats: Effect on organismic respiration, cytoplasmic constituents of liver, and succinic dehydrogenase activity in liver, kidney and heart. *J. Nutr.*, **108**: 1606-1615.

CATER, D.B., HOLMES, B.E. AND MEE, L.K., 1956. Cell division and nucleic acids synthesis in the regenerating liver of the rat. *Acta Radiol*., **46**: 655.

CERDAN, S., CASCALES, M., CHACON, P., CASCALES, F. AND SANTOS-RUIZ, A., 1978. Hepatotoxic effect of thioacetamide (TAM) on glutamate dehydrogenase. *Arch. Toxicol. Suppl*., **1**: 221-224.

CRADDOCK, V.M., 1975a. Effect of single treatment with the alkylating carcinogens dimethyl-nitrosamine, diethylnitro-samine, and methyl methanesulphonate on liver regeneration after partial hepatectomy. 1. Test for induction of liver carcinomas. *Chem. Biol. Interact*., **10**: 313-321.

CRADDOCK, V.M., 1975b. Effect of single treatment with the alkylating carcinogens dimethylnitrosamine and methyl methanesulphonate on liver regeneration after partial hepatectomy. III. Effect on DNA synthesis *in vivo* and on DNA polymerase activity assayed *in vitro*. *Chem. Biol. Interact*., **15**: 247-256.

DATE, P.A., GOTHOSKAR, S.V. AND BHIDE, S.V., 1976. Effect of partial hepatectomy on tumor incidence and metabolism of mice fed thioacetamide. *J. Natl. Cancer Inst*., **56**: 493-497.

DE RECONDO, A.M., FRAYSSINET, C., LAFARGE, C. AND LEBRETON, E., 1966. Action de laflatoxine sur le metabolism due DNA au course de l'hypertrophic compensatrice du foil après hepatectomie partielle. *Biochim. biophys. Acta*, **119**: 322.

DIXIT, A. AND RAO, A.R., 1980. Influence of ascorbic acid on action of DMBA on DNA, RNA and protein levels in mouse liver following partial hepatectomy. *Indian J. exp. Biol*., **18**: 1494-1496.

EINHORN, S.L., HIRSCHBERG, E. AND GELLHORN, A., 1954. Effect of cortisone on regenerating rat liver. *J. Gen. Physiol.*, **37**: 559.

GARUANA, J.A. AND GAGE, A.A., 1980. Increased uptake of insulin and glucagons by the liver as a signal for regeneration. *Surg. Gynecol. Obstet*., **150**: 390-394.

GARUANA, J.A., Jr., GOLDMAN, J.K., CAMARA, D.S. AND GAGE, A.A., 1981. Insulin, glucagons and glucose in regeneration of liver surgery. *Gynecol. Obstet.*, **153**: 726-730.

GENTILE, J.M., AVILA, L. AND GRACE, Jr., J.T., 1970. Liver regeneration-old and new concepts. *Am. J. Surg*., **120**: 2-6.

GOTTLIEB, L.I., FAUSTO, N. AND VAN LANCKER, J.L., 1964. Molecular mechanism of liver regeneration: Effect of puromycin on deoxyribonucleic acid synthesis. *J. biol. Chem*., **239**: 555-559.

GOODMAN, M.N. AND RUDERMAN, N.B., 1980. Starvation in the rat. I. Effect of age an obesity on organ weights, RNA, DNA and protein. *Am. J. Physiol*., **239**: E269-E276.

GRISHAM, J.W., 1960. Deoxyribose nucleic acid synthesis and cell renewal in regenerating rat liver. *J. Histochem. Cytochem*., **8**: 330.

GUIDICE, G. AND NOVELLI, G.D., 1963. Effect of actinomycin D in synthesis of DNA polymerase in hepatectomized rats. *Biochem. Biophys. Res. Commun*., **12**: 383-387.

GURD, F.N., VARS, H.M. AND RAVDIN, I.S., 1948. Composition of the regenerating liver after partial hepatectomy in normal and protein depleted rats. *Am. J. Physiol*., **152**: 1148.

HARKNESS, R.D., 1957. Regeneration of liver. *Br. Med. Bull*., **13**: 87.

HARTEL, A., HELGER, R. AND LANG, H., 1969. A method for determination of glucose. *Z. Klin. Chem. Klin. Biochem*., **7**: 183.

HAYS, D.M., 1974. Surgical research aspects of hepatic regeneration. *Surg. Gynecol. Obstet*., **139**: 609-619.

HENRY, R.J., 1964. *Clinical chemistry: Principles and techniques*. Harper and Row, New York.

HIGGINS, G.M. AND ANDERSON, R.M., 1931. Experimental pathology of liver: restoration of liver of white rats following partial surgical removal. *Arch. Pathol*., **12**: 186-202.

HSU, K.H., 1962. The effect of a carcinogenic substance (O-amino axotoluene) on the reactivity of liver cells following partial hepatectomy. *Bull. Eksp-Biol. Med*., **53**: 116-118.

LONE, K.P. AND MATTY, A.J., 1984. Oral administration of an anabolic androgenic steroid Dimethazine increases the growth and food conservation efficiency and brings changes in molecular growth responses of carp (*Cyprinus carpio*) tissues. *Nutr. Rep. Int.,* **29**: 621-638.

LEFFERT, H.L., KOCH, K.S., MORAN, T. AND RUBALCAVA, B., 1979. Hormonal control of rat liver regeneration. *Gastroenterology*, **76**: 1470-1482.

LEVI, J.U. AND ZEPPA, R., 1971. Source of humoral factor that initiates hepatic regeneration. *Ann. Surg.,* **174***:* 364-370.

LIN, T.Y., LEE, C.S., CHEN, C.C., LIAU, K.K. AND LIN, W.S.J., 1979. Regeneration of human liver after hepatic lobectomy studies by repeated liver scanning and repeated needle biopsy. *Ann. Surg*., **190**: 48-53.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A. AND RANDALL, R.J., 1951. Protein measurement with the Folin phenol reagent. *J. biol. Chem*., **193**: 265-275.

LUPO, C.R., MATSCHER, R. AND DeRUGGIERI, P., 1962. Determination biologica delle attivita ormonalli secondary della dimetazine. *Boll. Soc. Ital. Biol. Sper*., **38**: 990-995.

MATTY, A.J. AND CHEEMA, I.R., 1978. The effect of some steroid hormones on the growth and protein metabolism of rainbow trout. *Aquaculture*, **14**: 163-178.

MURRAY, A.B., STRECKER, W. AND SILZ, S., 1980. Changes in hepatocyte volume and liver weight in the early phase after partial hepatectomy. *Hepato-gastroenterol*., **27**: 4-8.

MURRAY, A.B., STRECKER, W. AND SILZ, S., 1981. Ultrastructural changes in rat hepatocyte after partial hepatectomy and comparison with biochemical results. *J. Cell Sci*., **50**: 433-448.

NAGASUE, N., KOBAYASHI, M., IWAKI, A., YUKAYA, H., KANASHIMA, R. AND INOKUGHI, K., 1978. Effect of 5-flurouracil on liver regeneration and metabolism after partial hepatectomy in the rat. *Cancer*, **41**: 435-443.

NATLESON, S., SCOTT, M.L. AND BEEFA, C., 1951. A rapid method for determination of urea in biological fluids by means of the reaction between diacetyl and urea. *Am. J. Chem. Pathol*., **21**: 275.

NGALA-KENDA, J.F. AND LAMBOTTE, L., 1981. Oxidative phosphorylation, enzyme induction and rat liver regeneration: Effect of phenobarbital. *Eur. Surg. Res*., **13**: 169-177.

NORPOTH, K., GOTTSCHALK, D., GOTTSCHALK, I., WITTING, U., THOMAS, H., EICHNER, D. AND SCHMIDT, E.H., 1980. Influence of vinyl chloride monomer (VCM) and As_2O_3 on rat liver cell proliferation after partial hepatectomy. *J. Cancer Res. Clin. Oncol*., **97**: 41-50.

OPPENHEIMER, M.J. AND FLOCK, E.V., 1947. Alkaline phosphatase levels in plasma and liver following partial hepatectomy. *Am. J. Physiol*., **149**: 418-421.

PETERS, J.M., 1967. Effect of absolute starvation and refeeding on organ weights and water content of albino rats. *Growth*, **31**: 191-203.

PORTA, E.A. AND HARTROFT, W.S., 1970. Protein deficiency and liver injury. *Am. J. Clin. Nutr*., **23**: 447.

REITMAN, S. AND FRANKEL, S., 1957. A colorimetric method for the determination of serum glutamate oxaloacetates, and glutamate pyruvate transaminase. *Am. J. Clin. Pathol*., **28**: 56.

SCHMIDT, E., SCHMIDT, F.W., MOHR, J., OTTO, P., VIDO, I., WROGEMANN, K. AND HERFARTH, C., 1974. Liver morphology and enzyme release: Further studies in the isolated perfused rat liver. In: *Pathogenesis and mechanisms of liver cell necrosis* (ed. D. Keppler), University Park Press, Baltimore.

SCHNEIDER, W.C., 1957. In: *Methods in enzymology* (eds. S.P. Colowick and N.O. Kaplan), vol. 3. Academic Press, New York.

SEKAS, G. AND COOK, R.T., 1979. The evaluation of liver function after partial hepatectomy in the rat: Serum changes. *Br. J. exp. Path*., **60**: 447-452.

SHAKOORI, A.R. AND AHMAD, M.S., 1973. Studies on the liver of chicken, *Gallus domesticus*. 1. Liver growth and nucleic acids content. *Pakistan J. Zool*., **5**: 111-117.

SHAKOORI, A.R., CHEEMA, A.I.A., RANI, A. AND ALI, S.S., 1984. Evaluation of liver function after thioacetamide treatment of partially hepatectomized rabbits. *Acta Physiol.*

Latinam., **34**: 304-312.

SHAKOORI, A.R., RANI, A., CHEEMA, A.I.A. AND ALI, S.S., 1985. Some biochemical and histological changes in regenerating liver after thioacetamide treatment of partially hepatectomized rabbits. *Pakistan J. Zool*., **17**: 271-288.

SIGEL, B., PECHET, G., QUE, M.Y. AND MacDONALD, R.A., 1965. Tritiated thymidine autoradiography in the regenerating liver of the dog. *J. Surg. Res*., **5**: 72.

STRECKER, W., SILZ, S., RUHENSTROTH-BAUER, G. AND BOTTGER, I., 1980a. Insulin and Glukagon in Blutplasma teilhepatektomierter Ratten. *Z. Naturf.*, **35c**: 65-71.

STRECKER, W., SILZ, S., SALEM, A.Y., RUHENSTROTH-BAUER, G., 1980b. Metabolic changes in the serum of partially hepatectomized rats. *Horm. Metabol. Res*., **12**: 563-642.

SULKIN, N.M. AND GARDNER, J.H., 1948. The acid alkaline phosphatase activity in the normal and recovering liver in the rat. *Anat. Rec*., **100**: 143.

WEINBREN, K., 1959. Regeneration of the liver. *Gastroenterology*, **37**: 657.

YARBRO, J.W., NIEHAUS, W.G. AND BARNUM, C.P., 1965. Effect of hydroxyurea on regenerating rat liver. *Biochem. Biophys. Res. Commun*., **19**: 592-597.