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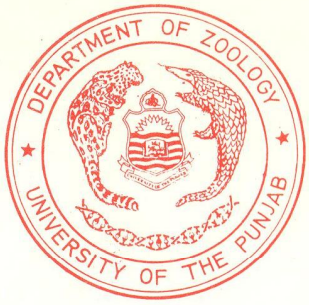


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# EFFECT OF DIFFERING CALCIUM CONTENTS IN RATIONS ON SERUM CALCIUM AND INORGANIC PHOSPHORUS IN CYCLE AND EARLY PREGNANCY OF DWARF GOAT

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**Abstract**: Total serum calcium, inorganic phosphorus and their ratio have been studied in dwarf goat provided with varying amount of dietary calcium in their dry rations i.e. deficient, normal and supplemented in addition to routine grazing on vegetation with inadequate supply of calcium to the grazers. The significant increase in circulating calcium in calcium supplemented goats only, significant enhancement in inorganic phosphorus and decrease in calcium : phosphorus ratio in all the three groups have been observed within two week of the experiment. In prostaglandin F2$\alpha$ synchronized cycle calcium: phosphorus ratio increased markedly in normal ration goats at estrus although it was greater in all the groups compared to acclimation phase. The ratio elevated in calcium deficient at diestrus and it was maximum in all the three groups at early pregnancy compare to other phases.

**Key words:** Dwarf goat, dietary calcium, estrus cycle, serum calcium, serum phosphorus, pregnancy.

## INTRODUCTION

Dietary factors affect various phases of reproduction (Rattray, 1977). The unavailability of balanced ration prior to a fertile cycle and then progressively during the cycle affects ovulation and fertilization; and both the anabolic and catabolic phases of the pregnancy (Vernon *et al.*, 1981). Calcium is one such factors, which plays vital role in the chemical balance, hormonal homeostasis and many other metabolic processes such as skeletal integrity (Adeloye and Akinsoyinu, 1984; Navch-Many and Justin, 1990), muscular contraction and relaxation, blood pressure regulation and many reproductive processes. Calcium in circulation is index of the inter-relationship of hormones like parathyroid hormone (PTH) and calcitonin (CT) (Furlanetto *et al.*, 1990).

The $Ca^{2+}$ intake is important for preventing milk fever and there is an increased incidence of dystocia and retained placenta in cows with milk fever which reduced the first service and conception rate (Morrow *et al.*, 1980). Phosphorus concentration may not be of great significance directly, however, indirectly affect calcium regulatory mechanism. Phosphate withdrawal does not evoke an immediate response, but over several days serum phosphate concentration fall, leading to a rise in production of 1,25-dihydroxy-cholecalciferol and consequently increases in intestinal absorption of $Ca^{2+}$, raises serum calcium, suppresses PTH, decreases the renal clearance of phosphate and increases renal clearance of calcium (Rasmussen *et al.*, 1974).

Interdependence of $Ca^{2+}$ and P is also well demonstrated in several works. Infusion of calcium into normal subjects have been reported to lower phosphate excretion (Chambers *et al.*, 1956; Nordin and Fraser, 1954; Chen and Neuman, 1955; Wallach

and Carter, 1961) whereas others observed enhancement in its excretion (Hiatt and Thompson, 1957; Pak, 1971; Spencer *et al.*, 1978; Greger *et al.*, 1981; Zemel and Linkswiler, 1981). A number of physiological interactions can occur when dietary levels and forms of protein, phosphorus, electrolytes and calcium are altered. Ward *et al.* (1972) concluded that supplementation of $Ca^{2+}$ has better effect on reproductive performance of dairy cows. Supplemented minerals are most critical during the wet season when cattle are gaining weight rapidly and energy and protein supplies are adequate and the economic return on mineral supplementation is high (McDowell *et al.*, 1986). An extra calcium in diet is excreted through urine (Bailey, 1991), thus circulatory level remain within a range. $Ca^{2+}$ and P retention was not increased by feeding these elements in excess of their estimated requirements and were reduced when $Ca^{2+}$ and P or P alone are reduced proportionately to about 0.75 of the requirement (Zahari *et al.*, 1990).

In conventional farming, the goats form marginal lands receive reduced amount of calcium in grazing. Thus the present study was undertaken to investigate effects of dietary calcium contents on serum calcium and phosphorus in prostaglandin synchronized cycling and at early pregnant goats.

## MATERIALS AND METHODS

Eighteen adult nanny goats which were not in any noticeable stage of pregnancy were selected from the herd maintained at the Bio-Saline Research Substation of Nuclear Institute for Agriculture and Biology at Lahore. These were divided into three categories of 6 goats in each group, and acclimatized for 15 days on their respective rations i.e., normal feed with 1% dicalcium phosphate (DPC), calcium deficient feed without DCP and calcium supplemented with 1% marble powder. The composition of calcium deficient base diet was cotton seed cakes 29%, wheat bran 20%, molasses 25%, rice polishing 20%, wheat straw cuttings 04%, urea 01% and common salt 01%.

The goats were given dry ration early in the morning and then allowed to graze in the fields for rest of the day and kept isolated from the males of the herd. The goats were synchronized for cycle with the injection of prostaglandin F2 (Estrumate, ICI, England). Two intramuscular injections at a concentration of 62.5$\mu$g Estrumate per goat at the spacing of 10 days were given. Estrus cycle was counted from second injection. Twenty five day after another Estrumate injection was given for the synchronization of fertile estrus and the pregnancy. Goats of all the three experimental groups were sampled for blood at a time during acclimation, synchronization and in cycle and early pregnancy.

Serum calcium level was determined with methylthymol blue method (Cheesbrough, 1981), where as serum inorganic phosphorus was estimated according to Zilversmit and Davis (1950) and Bogatzki (1938).

## RESULTS

*Serum calcium*

The average serum $Ca^{2+}$ level was $1.543 \pm 0.165$, $1.445 \pm 0.181$ and

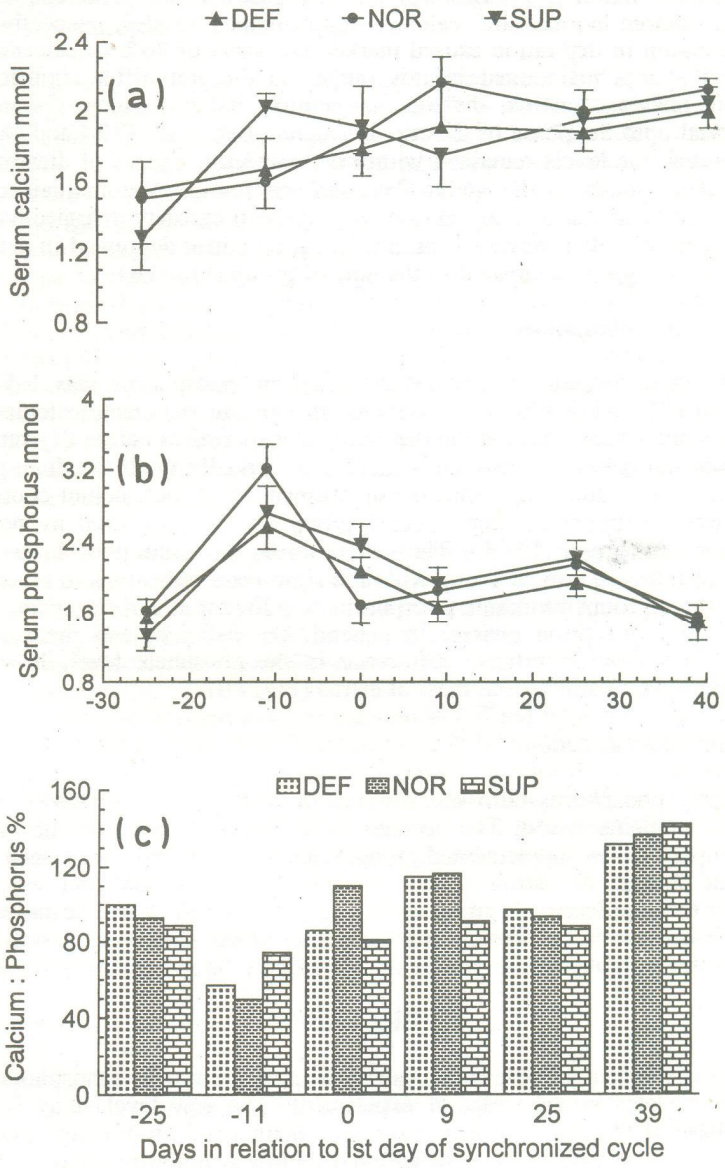


**Fig. 1**. Average serum calcium (a), phosphorus (b), and their ratio (c) in calcium deficient (DEF), normal (NOR) and supplemented (SUP) group during cycle and early pregnancy. Days 0, 9, 25 and 39 indicates estrus, diestrus, conception and early pregnancy respectively

1.281 ± 0.183 mmol at the onset of the experiment in the goats chosen for calcium deficient, calcium normal and calcium supplemented groups, respectively. Calcium supplementation in dry ration caused marked elevation of 76% in calcemia within two weeks and it was maintained in this range, as did not differ significantly, at the subsequent phases of estrus, diestrus, conception and pregnancy. Calcemia increases were gradual upto the phase of diestrus in calcium deficient (33%) and calcium normal (65%) groups, the levels remained within the respective ranges of diestrus calcemia at the subsequent phases of the study. Calcemia was found to be maintained in a limited range of levels at estrus, conception and early pregnancy irrespective of calcium availability in their diet, however, at diestrus, it remained depressed in deficient as well as supplemented group compared to the normal group (Fig. 1A).

*Serum inorganic phosphorus*

The average concentration of serum inorganic phosphorus was 1.546 ± 0.244, 1.623 ± 0.131 and 1.328 ± 0.17 mmol in calcium deficient, calcium normal and calcium supplemented group at the beginning of experiment before dry ration provision to the goats. Inorganic phosphotemia increased markedly in all the three groups within two weeks of dry ration supplementation irrespective of the calcium content in it. The increase was maximum in supplemented group (277%), followed by normal (237%) and the deficient group (191%). Thereafter, serum inorganic phosphorus was lowered markedly at estrus in normal group with non significant reductions in other groups also. In all the three groups inorganic phosphorus was lowest at early pregnancy compare to cycle and the conception phases. In general, the deficient and the supplementation group did not show significant difference in the phosphate level, however, normal group pattern was distinct from these at estrus (Fig. 1B).

*Calcium phosphorus ratio*

Calcium : phosphorus ratio was lowered in all the groups following two weeks of dry ration supplementation. The decrease was greater in the deficient and the normal group compare to the supplemented group. Thereafter, the ratio remained higher at the subsequent phases. At estrus it was maximum in the normal and was minimum at diestrus in the supplemented group among all the three groups. The ratio did not vary among the groups at conception and early pregnancy, however, it was maximum at early pregnancy among all the phases studied (Fig. 1C).

## DISCUSSION

The goats have shown low calcemia and inorganic phosphotemia in non reproductive states at the onset of experiment. The low levels may be due to non cycling phase of the goats or these have been maintained on grazing only on the plant materials of salinity affected area which provide low availability calcium to the animals. The elevation of both circulating calcium as well as inorganic phosphate level during the acclimation phase on the dry rations with varying degree of calcium contents in general, reflect that supplementation may have been the cause of the minerals elevation. This response is certainly evident in calcium supplemented goats. In deficient and

normal groups, however, it is found that onset of cycling activity, perhaps, brings gradual increase in calcemia.

The significant lowering of calcemia in supplemented group is due to the consequent effects on parathyroid hormone (PTH) with the result of elevated serum calcium level. Aurbach *et al.*, (1985) have analyzed that rise in ionized calcium leads to suppression of PTH secretion with the consequent increased renal clearance of calcium and eventually brings low calcemia; also the suppression of PTH causes decreased clearance of phosphate thus raising the serum phosphate. To this view, the increase in inorganic phosphate in serum along the elevation in calcemia is well understsood. The response of phosphate increase is marked in normal compare to other groups. Again higher calcium concentration in normal group at estrous indicate the calcium supplementation more or less resemble to calcium deficient state in the maintenance of these minerals. Thus restoration of calcium to a normal level is more important for reproductive activity of the dwarf goat and excess dietary calcium is of no advantage in these mechanisms. Reddy and Reddy (1988) have observed that nitrogen, calcium and phosphorus balances were best with normal feeds. Because hormonal interaction of calcitonin (CT), parathyroid hormone (PTH) and vitamin D3 are most conclusive with normal proportion of calcium in diet.

The pattern of response in different cycling condition and early pregnancy resemble in all the three experimental groups of the study. It is found low calcium intake for short time apparently does not affect its production and may elevate resistance to calcium stress (Belyea *et al.*, 1976). Thus in reproductive activity in calcium deficient goats calcemia has been elevated through such mechanism.

In normal conditions elevated level of calcium mobilized by PTH reduces inorganic phosphate in circulation (Hiatt and Thompson, 1957). In hypoparathyroid subjects calcium infusion is accompained by an acute rise in serum phosphate (Eisenberg, 1965). The optimal elevation of serum calcium in cycling and early pregnancy and consistent lower phosphate, particularly at onset of pregnancy, evident from calcium : phosphorus ratio, reflect that increased PTH secretion is important at these phases.

In conclusion, restoration of normal calcium level in the diet of ruminants are necessary and extra supplementation may have adverse effects. Even in deficient state animal resist calcium deficiency through PTH mobilization.

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# PATHOGENICITY OF *ASPERGILLUS NIGER* AGAINST VARIOUS SPECIES OF TERMITES

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**Abstract**: The workers of *Microtermes unicolor*, *Microcerotermes championi* and *Heterotermes indicola* were infected with a surface culture of *Aspergillus niger* for a period of 10, 20, and 30 minutes and the mortality pattern was described. Their 100% death occured quickly when the insects were crawled over a conidial culture of *A. niger* for a longer period of time. Out of 3 termite species *M. championi* was found to be more susceptible to *A. niger* than *M. unicolor* and *H. indicola*.

**Key words:** *Aspergillus niger*, pathogenicity, termites.

## INTRODUCTION

Several ectoparasitic genera, *Ectomyces*, *Termiteria* (Tate, 1927, 1928) and *Antennopsis* (Buchli, 1951) are recorded to cause a mycosis in termites. Krejzova (1976) isolated a strain of *Paecilomyces fumoso-roseus* from *Zootermes formosanus* and *Reticulitermes lucifugus*. A few parasitic fungi on termites were also reported and described by several investigators (Bao and Yenndol, 1971; Kimbrough *et al.*, 1972; Yendol and Rosario, 1972; Rossi, 1974; Rossi and Rossi, 1977 a,b; Blackwell and Kimbrough, 1976, 1978.)

The mucoralian fungus, *Absidia coerulea* (Bainer) has also been found to cause a mycosis in *R. virginicus* (Lund and Engelhard, 1962). Comparative pathogenicity studies of *Metarrhizium anisopliae* (Metch) and *Beauveria bassiana* (Balsamo) against workers and soldiers of the formosan termite *Coptotermes formosanus* (Shiraki) have been made by Leong (1966). These two entomogenous fungi showed similar symptoms with *B. bassiana* being more pathogenic.

In laboratory test Beal and Kias (1962) exerimentally infected two subterranean species *Reticulitermes virginicus* (Banks) and *R. flavipes* (Kollar) with *Aspergillus flavus*. Sannasi (1968) observed dead queen and king of *Odontotermes obesus* and found *A. flavus* as the causative agent. *Aspergillus flavus* was also isolated from *Bifiditermes beesoni* collected from stem of *Pyrus communis* and it was fairly pathogenic to *M. championi*, *Heterotermes indicola* and *B. beesoni* (Khan, 1981).

According to Lenz (1968) a strain of *Aspergillus niger* was known to produce at least two toxins and one of them was found to be toxic to the termite *H. indicola*. But infection by *A. niger* on termites was not thoroughly described. Therefore, the present work was undertaken to test the pathogenicity of *A. niger* against the various species of termites.

## MATERIALS AND METHODS

In order to test pathogenicity of *A. niger* against various species of termites in Pakistan, a pure culture of *A. niger* was obtained from the laboratory of Professor G. Becker (Bundesanstalt fur Malerialprufung, Berlin, West Germany). Its pathogenicity was tested against *Microtermes unicolor* Snyder, *Microtermes championi* Snyder and *H. indicola* (Wassman).

The inoculum was prepared by growing *A. niger* on Sabouraud's dextrose agar medium with yeast extract (SDA+Y) for 7 days as described by Yendol and Rosario (1972). The culture was homogenized in 100ml sterile distilled water. One ml aliquot of this homogenized suspension was then added to each petri dish (100x15mm) containing SDA+Y medium. These cultures were incubated at $25 \pm 1$ $^{O}$C for 72 hours.

*Microtermes unicolor*, *Microcerotermes championi* and *H. indicola* workers were divided into four groups. Each had 25 termites. Group No. 1 of each species of termite was kept as control. While workers of group Nos. 2,3 and 4 of each species of termite were directly placed on 72 hours sporulating culture of *A. niger* and allowed to crawl over the fugal surface for 10, 20 and 30 minutes respectively. The control groups were treated in the same fashion, except that the fungal inoculation was omitted. When the inoculating period terminated, termites of each group were transferred to other petri dishes containing a double layered filter paper as a bed, slightly dampened with sterile water and their mortality pattern was noted after every 24 hours and the dead termites were carefully examined.

## RESULTS AND DISCUSSION

In order to demonstrate the etiological agents, 72 hours infected dead specimens were removed from the holding Petri dishes, surface sterilized with 5.25% sodium hypochlorite and then rinsed several time in sterile water. The specimens were then placed on SDA+Y medium and incubated for 3 days at $25 \pm 1$ °C. Within this period, mycelium and a few spores of *A. niger* grew out of the infected termite onto the medium. The fungus was reisolated in pure culture, identified and Koch's postulates were fulfilled as described by Bao and Yendol (1971).

Observations after every 24 hour indicated that termites became gradually inactive and tend to appear in groups leaving behind the dead individuals. Their percent mortality was calculated daily and presented in Table 1-3. However, the data of $LT_{50}$ and $LT_{100}$ is shown in Table-4 for comparison. The termite workers (25 individuals) were divided into four groups as mentioned earlier. Group 1 of each species of termite was kept as control while group 2,3 and 4 were allowed to crawl over 72 hours conidial culture of *A. niger* for 10, 20 and 30 minites respectively.

At 48 hours following infection of *Microtermes unicolor* by *A. niger*, there was 52%, 60% and 48% mortality in group Nos. 2,3 and 4 respectively. There was 100% motality at 144 hours, 144 hours and 120 hours, in group Nos. 2,3 and 4 respectively (Table-1).

**Table I. Percentage mortality of workers of *Microtermes unicolor* infected by 72 hour old culture of *Aspergillus niger***

| Hours after infection | Group-1 (control) | Group-2 (crawling) 10 min. | Group-3 (crawling) 20 min. | Group-4 (crawling) 30 min |
|---|---|---|---|---|
| 24 | 4 | 24 | 28 | 32 |
| 48 | 4 | 52 | 60 | 48 |
| 72 | 4 | 68 | 80 | 72 |
| 96 | 4 | 80 | 88 | 84 |
| 120 | 4 | 88 | 92 | 100 |
| 144 | 4 | 100 | 100 | - |

The $LT_{50}$ was calculated as 46.8 hours hours, 41.8 hours and 50.4 hours, in group Nos. 2,3 and 4, respectively (Table-IV)

While at 48 hours following infection of *Microcerotermes championi* by *A. niger*, there was 76%, 50% and 80% mortality in group Nos. 2,3 and 4 respectively. However, their 100% mortality occurred at 96 hours, 96 hours and 72 hours following infection respectively (Table-II)

**Table II. Percentage mortality of *Microcerototermes championi* infected by 72 hours old culture of *Aspergillus niger***

| Hours after infection | Group-1 (control) | Group-2 (crawling) 10 min. | Group-3 (crawling) 20 min. | Group-4 (crawling) 30 min. |
|---|---|---|---|---|
| 24 | 4 | 40 | 28 | 32 |
| 48 | 4 | 76 | 50 | 80 |
| 72 | 4 | 92 | 76 | 100 |
| 96 | 4 | 100 | 100 | - |

$LT_{50}$ was calculated as 30 hours, 48 hours and 32.4 hours in group Nos. 2,3 and 4 respectively.

At 48 hours following infection of *H. indicola* by *Aspergillus niger*, there was 28%, 32% and 24% mortality in group Nos. 2,3 and 4, respectively; and their 100% mortality occurred at 264 hours, 216 hours respectively (Table-III).

**Table III. Percentage mortality of *Heterotermes indicola* infected by 72 hours old culture of *Aspergillus niger***

| Hours after infection | Group-1 (control) | Group-2 (crawling) 10 min. | Group-3 (crawling) 20 min. | Group-4 (crawling) 30 min. |
|---|---|---|---|---|
| 24 | 0 | 8 | 16 | 16 |
| 48 | 0 | 28 | 32 | 24 |
| 72 | 4 | 32 | 44 | 44 |
| 96 | 4 | 44 | 64 | 60 |
| 120 | 4 | 52 | 72 | 76 |
| 144 | 4 | 72 | 80 | 84 |
| 168 | 4 | 76 | 88 | 92 |
| 192 | 4 | 80 | 96 | 96 |
| 216 | 4 | 88 | 100 | 100 |
| 240 | 4 | 92 | - | - |
| 264 | 4 | 100 | - | - |

$LT_{50}$ was calculated as 115.2 hours, 79.2 hours and 81.4 hours in group Nos. 2,3 and 4, respectively.

An analysis of the results showed that all the species of termites were susceptible to *A. niger* infection. The comparative data on 100% mortality pattern shows that when the termites were allowed to crawl for a longer duration of time *i.e.*, up to 30 minutes, their mortality occured more quickly. Similarly, in case of *H. indicola*, the data of $LT_{50}$ also showed that less time was required to kill 50% of the termites when these were allowed to crawl over a fungal growth for longer period *i.e.* 20 or 30 minutes. However, in case of *Microterms. unicolor* and *Microcerotermes. championi*, the mortality pattern based on $LT_{50}$ did not show any significant difference in causing 50% death of termites when crawled for 10 or 30 minutes.

The comparative data on the mortality pattern of all three species of termites shows that *Microcerotermes championi* died in a shorter time than the other species of termites (Table-IV)

**Table IV. $LT_{50}/LT_{100}$ (in hour) of *Microtermes unicolor* infected by 72 hours old culture of *Aspergillus niger***

| Termite species | $LT_{50}/LT_{100}$ (crawling) 10min. | $LT_{50}/LT_{100}$ (crawling) 20min. | $LT_{50}/LT_{100}$ (crawling) 30min. |
|---|---|---|---|
| *Microtermes* unicolor | 46.8/144 | 41.8/144 | 50.4/120 |
| *Microcerotermes championi* | 30/96 | 48/96 | 32.4/72 |
| *Heterotermes indicola* | 115.2/264 | 79.2/216 | 81.4/216 |

These findings indicated that the workers of *Microcerotermes championi* were more susceptible to *Aspergillus niger* infection as compared to *Microtermes unicolor* and *Heterotermes indicola* workers.

These findings support the view of Becker (1965) that differences exist in the action of a strain of fungus to various species of termites. The time interval from fungal invasion to death of the insect host varies considerably among different species of fungi and even between host as found in the present studies and others (Becker and Kerner-Gang, 1964; Leong, 1966; Bao and Yendol, 1971).

*Aspergillus niger* appears to be a potential pathogen for the control of termites in laboratory conditiions. Further studies are needed for testing the pathogenicity of this fungus against termites in the field conditions.

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# CASTE POLYMORPHISM IN FIELD COLONY OF *ODONTOTERMES REDEMANNI* (WASMANN)

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**Abstract**: Total population of the nest opened on 20th December, 1986 consisted of 99033 individuals. The soldier to worker ratio in the nest population was 1:8 and workers constituted 6% and 48% of the total population, respectively.

Two types of workers minor and major, were found in the nest. The major worker passes through five instars, first three larval and the last two worker instars. Minor worker also develops from 3rd instar larva and after another moult changes into minor worker.

Origin of the pre-soldier takes place from 3rd larval instar and this pre-soldier after another six moults develops into the soldier caste. Neither alates nor developmental stages leading to alates were found in the nest opened in December *i.e.*, after the swarming season was over.

**Key words**: Caste polymorphism, field colony, termite.

## INTRODUCTION

*Odontotermes redemanni* whose developmental pathways are discussed in the present report was first of all desccribed by Wasmann in 1893 from Ceylon (now Sri Lanka). It was thought to be confined to Ceylon only. Later on it has been recorded from many localities of India by Bose (1984) who has further pointed out that *O. redemanni* and *O. obesus* cannot be easily separeted and preferred to conider them two species in a group, till their taxonomic problem is solved. The present material collected from Lahore has been compared with *O. redemanni* collected by Escherich on 17th Feb. 1900 from Ceylon and was determined by Holmgren. It has also been compared with *O. redemanni* collected from Ceylon on Oct. 15, 1928 which was identified by Ahmad. The specimen whose developmental pathways are described here come more close to *O. redemanni* than to *O. obesus*.

## MATERIALS AND METHODS

The material used in the present study consisted of a population of a nest of subterranean termites *O. redemanni*. The nest was opened in the month of Dec. 1986 and was dug out for an area of four $m^2$.

For caste composition soldiers were counted individually whereas number of undifferentiated population of nymph was counted by volume method as explained by Thorne (1985).

The population of the nest was preserved in 80% alcohol. The specimens were measured under Leitz stereoscopic microscope with built in magnification changer. Measurements of different instars of worker and soldier lines were taken with the aid of

calibrated ocular micrometer. Illustrations were prepared with the help of Camera Lucida wherever necessary photographs of the various developmental stages were taken under microscope.

Taxonomic terms and measurements used in the present study are as explained by Emerson (1945), Ahmad (1950) and Noirot (1985).

To trace the origin of the worker and soldier lines following characters were measured:

1. Total body length.
2. Length of head to side base of mandible.
3. Maximum width of head.
4. Length of hind tibia.

Numerical data for various characters was analyzed for mean, standard deviation, coefficient of variation, according to Sokal and Rohlf (1973).

In the tables mean is represented as X, standard deviation as S.D. and coefficient of variation is represented by C.V.

## RESULTS

Caste composition of *Odontotermes redemanni*

The subterranean nest of *O. redemanni* was opened on Dec. 12, 1986, and a total of 4.0 $M^2$ area was dug out. Caste composition of the nest is given in Table I.

Soldier worker ratio in the nest population was 1:8.

**Table I. Cast composition of a nest of *Odontotermes redemanni***

| Caste | Number | Percentage of total |
|---|---|---|
| Soldier | 6583(1:8)* | 6% |
| Worker | 48189 | 48% |
| Nynph | 44261 | 44% |
| Total Population | 99033 | |

*Soldier to worker ratio

*(1) Description of developmental pathways of workers line*

*a) First instar larva-12 antennal segments*

Head and body whitish, unpigmented; head round with brain area much enlarged, occupying nearly the whole of head capsule. Mandibles unpigmented, left mandible with minute indication of apical and first marginal tooth; right mandible with minute

indication of apical and first marginal tooth. Antennae 12 segmented with second segment as long as third and fourth combined. Abdomen with two styli, tarsi 4-jointed (Table II).

**Table II. Biometric analysis of different characters of first instar larva (measurements in mm)**

| Characters | Range (N=20) | Mean±S.D | C.V. |
|---|---|---|---|
| Total body length | 1.15-1.40 | 1.27±0.089 | 7.00 |
| Length of head to side base of mandible | 0.26-0.32 | 0.29±0.013 | 4.48 |
| Maximum width of head | 0.39-0.45 | 0.43±0.023 | 5.34 |
| Length of hind tibia | 0.18-0.20 | 0.19±0.012 | 6.31 |

*b) Second instar larva - 13 antennal segments*

Head and body whitish, unpigmented, head round, brain area visible through cuticle, much enlarged, occupying nearly the whole of head capsule. Mandible whitish, unpigmented, right mandible with apical and first marginal tooth more developed than first instar nymph. Notch between first marginal and second marginal tooth slightly indicated. Left mandible with apical first and second marginal tooth well indicated, notch between first and second marginal tooth slightly indicated. Mandibular differentiation in *O. redemanni* starts much earlier than *O. gurdaspurensis*. Antennae 13 segmented, abdomen with a pair of styli, tarsi 4-jointed (Table 3).

**Table III. Biometric analysis of different characters of second instar larva (measurements in mm)**

| Characters | Range (N=7) | Mean±S.D. | C.V. |
|---|---|---|---|
| Total body length | 1.85-2.11 | 1.93±0.099 | 5.12 |
| Head length to side base of mandible | 0.36-0.46 | 0.39±0.030 | 7.69 |
| Maximum width of head | 0.56-0.64 | 0.60±0.027 | 4.5 |
| Length of hind tibia | 0.36-0.41 | 0.38±0.016 | 4.21 |

*c) Third instar larva - 14 antennal segments*

Head and body whitish, unpigmented, head round, with brain area much enlarged occupying nearly the whole of the head capsule. Mendible unpigmented and whitish. Right mandible with apical and first marginal tooth, more developed than second instar

worker. Notch between first marginal and second marginal tooth slightly indicated. Left mandible with apical and first marginal tooth well developed, yet unpigmented. Antennae 14 - segmented, abdomen with a pair of styli, tarsi 4-jointed (Table 4).

**Table IV. Biometric analysis of different characters of third instar larva (measurements in mm)**

| Characters | Range (N=20) | Mean±S.D. | C.V. |
|---|---|---|---|
| Total body length | 1.99-2.44 | 2.19±0.188 | 5.38 |
| Head length to side base of mandible | 0.41-0.54 | 0.48±0.033 | 6.87 |
| Maximum width of head | 0.67-0.77 | 0.70±0.045 | 6.42 |
| Length of hind tibia | 0.41-0.48 | 0.43±0.023 | 5.34 |

*d) Fourth instar worker - 15 antennal segments*

Head and abdomen whitish, unpigmented. Head round, brain area visible through the cuticle much reduced. Mandible whitish, and more differentiated than third instar. Right mandible with a distinct notch between first and second marginal and third marginal tooth. Second notch between posterior margin of third marginal and molar plate slightly indicated, well differentiated teeth; tip only weakly pigmented, posterior margin of third marginal tooth in this instar separated from the molar plate, molar plate not well differentiated. Antennae 15 segmented, abdomen with a pair of styli (Table V).

**Table V. Biometric analysis of different characters of fourth instar larva (measurements in mm)**

| Characters | Range (N=20) | Mean±S.D. | C.V. |
|---|---|---|---|
| Total body length | 2.47-2.73 | 2.58±0.084 | 3.25 |
| Head length to side base of mandible | 0.45-0.61 | 0.54±0.043 | 7.96 |
| Maximum width of head | 0.74-0.80 | 0.76±0.019 | 2.50 |
| Length of hind tibia | 0.48-0.61 | 0.53±0.047 | 8.86 |

*e) Fifth instar worker - 16 antennal segments*

Head and thorax slightly darker than abdomen, weakly sclerotized, head oval narrowing posteriorly, brain not clearly visible. Mandible more darkly pigmented than fourth instar; teeth well developed. Antennae 16 segmented. Abdomen with a pair of styli (Table VI)

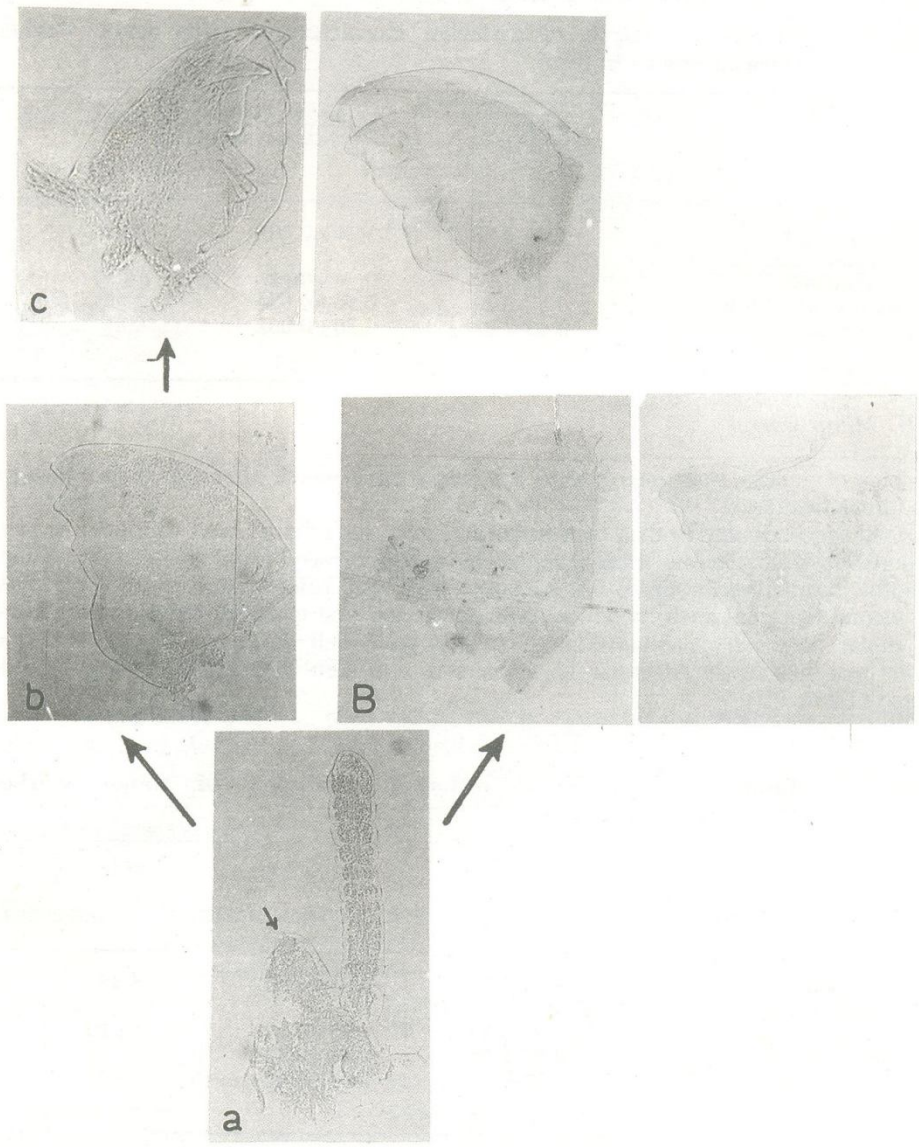


**Fig. 1**. Stages of mandibular development of worker of *O. redemanni* (Wassman). a, first larval stage; b. second stage larva of minor worker; c, third stage larva of minor worker; d, third stage larva of major worker.

**Table VI. Biometric analysis of different characters of fifth instar worker (measurements in mm)**

| Characters | Range (N=20) | Mean±S.D. | C.V. |
|---|---|---|---|
| Total body length | 2.82-3.52 | 3.20±0.203 | 6.34 |
| Head length to side base of mandible | 0.49-0.94 | 0.76±0.126 | 16.57 |
| Maximum width of head | 0.77-1.23 | 0.96±0.024 | 2.50 |
| Length of hind tibia | 0.65-0.82 | 0.70±0.062 | 8.85 |

*f) Minor worker*

(This originates from one type of 3rd instar larva which after one moult changes into minor worker)

Head and body darker than fifth instar and more sclerotized; head yellowish brown, abdomen brownish yellow. Head oval narrowing posteriorly; brain area clearly visible. Mandible strongly sclerotized; left mandible with well differentiated apical tooth, first and second marginal teeth; notch between apical and first marginal tooth, second tooth and molar plate very distinct; right mandible with well developed apical, first and second marginal teeth. Antennae 17 segmented, abdomen with a pair of styli, tarsi 4-jointed (Table VII).

**Table VII. Biometric analysis of different characters of minor worker (measurements in mm)**

| Characters | Range (N=20) | Mean±S.D. | C.V. |
|---|---|---|---|
| Total body length | 3.84-4.35 | 4.04±0.22 | 5.45 |
| Head length to side base of mandible | 0.81-1.22 | 1.05±0.17 | 16.19 |
| Maximum width of head | 0.97-1.58 | 1.24±0.23 | 18.54 |
| Length of hind tibia | 0.92-1.22 | 1.07±0.12 | 11.21 |

*g) Major worker*

(This originates from a second type of 3rd instar larva which after two successive moults changes into the major worker).

Head much darker than rest of the body. Head oval, brain visible but much reduced.

Mandibles strongly sclerotized with well differentiated notches and fully developed teeth. Antennae with 17 segments, first article as long as second and third combined. Abdomen with a pair of styli, tarsi 4-jointed (Table VIII).

**Table VIII. Biometric analysis of different characters of major worker (measurements in mm)**

| Characters | Range (N=20) | Mean±S.D. | C.V. |
|---|---|---|---|
| Total body length | 4.86-5.52 | 5.11±0.24 | 4.69 |
| Head length to side base of mandible | 1.12-1.33 | 1.23±0.09 | 7.31 |
| Maximum width of head | 1.48-1.58 | 1.56±0.05 | 3.20 |
| Length of hind tibia | 1.12-1.28 | 1.16±0.06 | 5.17 |

*2. Soldier line*

In spite of best efforts, first and second instar pre-soldiers could not be found in the field nest population. The most primitive pre-soldier instar found in nest was third instar which is described.

*a) Third instar pre-soldiers*

Head and body whitish, head nearly round. Mandibles differentiating within the intact cuticle of older second instar pre-soldier; older mandible with first marginal tooth present, aterior margins wavy; new mandible developing within older mandible provided with minute tooth much below the first marginal tooth of older mandible. Antennae 16 segmented (Fig. 2, Table IX).

**Table IX. Biometric analysis of different characters of third instar pre-soldier (measurements in mm)**

| Characters | Range (N=5) | Mean±S.D. | C.V. |
|---|---|---|---|
| Total body length | 3.48-4.04 | 3.76±0.21 | 5.58 |
| Head length to side base of mandible | 0.87-1.02 | 0.92±0.06 | 6.52 |
| Maximum width of head | 0.92-1.12 | 0.99±0.07 | 7.07 |
| Length of hind tibia | 0.76-0.87 | 0.83±0.04 | 4.81 |

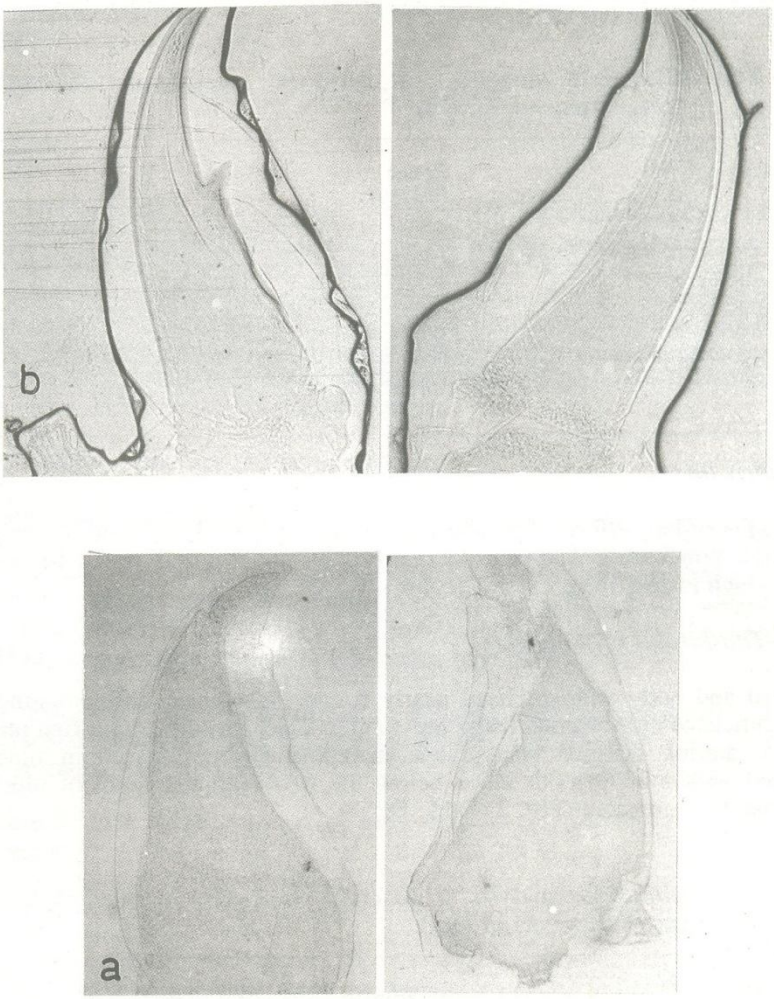


**Fig. 2.** Stages of mandibular development during soldier differentiation. a. third instar pre-soldier; b. fifth instar pre-soldier.

*b) Fourth instar pre-soldier*

Like third instar pre-soldier, but with left mandible tooth much more developed.

**Table X. Biometric analysis of different characters of fourth instar pre-soldier (measurements in mm)**

| Characters | Range (N=5) | Mean±S.D. | C.V. |
|---|---|---|---|
| Total body length | 3.43-4.35 | 3.84±0.38 | 9.97 |
| Head length to side base of mandible | 0.71-0.92 | 0.80±0.08 | 10.00 |
| Maximum width of head | 0.87-1.12 | 0.98±0.08 | 8.16 |
| Length of hind tibia | 0.87-0.97 | 0.92±0.03 | 3.26 |

*c) Fifth instar pre-soldier*

Head and body whitish like fourth instar. Head still round, left mandible with well differentiated tooth and right mandible with a distinct denticle, tips of mandible slightly more sclerotized (Table XI).

**Table XI. Biometric analysis of different characters of fifth pre-soldier instar (measurements in mm)**

| Characters | Range (N=5) | Mean±S.D. | C.V. |
|---|---|---|---|
| Total body length | 3.78-4.35 | 3.98±0.23 | 5.77 |
| Head length to side base of mandible | 0.76-0.92 | 0.82±0.07 | 8.56 |
| Maximum width of head | 0.97-1.07 | 1.00±4.04 | 4.47 |
| Length of hind tibia | 0.81-1.02 | 0.84±0.10 | 11.40 |

*d) Sixth instar pre-soldier*

Head elongately oval, mandible with reddish brown in upper two third, bases not sclerotized. Antennae slightly more sclerotized than 5th instar, distal articles not distinctly darker than basal one (Table XII).

**Table XII. Biometric analysis of different characters of sixth instar pre-soldier (measurements in mm)**

| Characters | Range (N=5) | Mean±S.D. | C.V. |
|---|---|---|---|
| Total body length | 4.04-4.60 | 4.28±0.22 | 5.14 |
| Head length to side base of mandible | 0.92-1.12 | 1.03±0.07 | 6.69 |
| Maximum width of head | 1.02-1.17 | 1.12±0.06 | 5.35 |
| Length of hind tibia | 0.97-1.02 | 1.00±0.02 | 2.00 |

*e) Soldier*

Final instar with mandibles strongly sclerotized. Antennae with distal articles darker, proximal lighter in colour (Table XIII).

**Table XIII. Biometric analysis of different characters of soldier (measurements in mm)**

| Characters | Range (N=20) | X±S.D. | C.V. |
|---|---|---|---|
| Total body length | 4.64-5.77 | 5.38±0.28 | 5.20 |
| Head length to side base of mandible | 1.41-1.49 | 1.43±0.02 | 1.39 |
| Maximum width of head | 1.18-1.20 | 1.22±0.03 | 2.45 |
| Length of hind tibia | 1.13-1.29 | 1.17±0.04 | 3.41 |

## DISCUSSION

Developmental pathways of only few species of fungus growing termites are known. Noirot (1985a) reported that development stages through which the worker passes after differentiation from third instar larva may vary in *Macrotermitinae*. Okot Kotber (1985) reported that in case of *Macrotermes* michaelsoni the third stage larva moults into adult workers both major and minor. Akhtar and Rana (1988) reported that in a colony of *Odontotermes gurdaspurensis* worker develops after five successive moults. Present studies with a field colony of *O. redemanni* revealed that the egg hatches into larva and this larva after two moults may differentiate into worker line or the soldier line (Fig. 3).

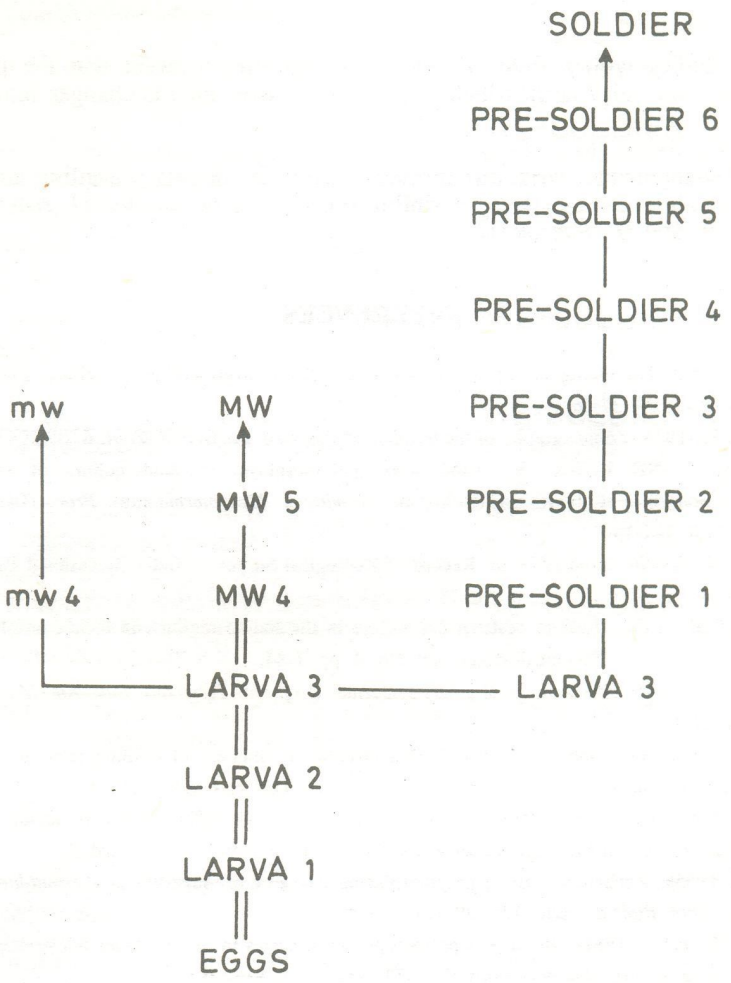


**Fig. 3**. A scheme of post embryonic development in a mature colony of *O. redemanni*. $L_1$-$L_3$, larval instars; $Mw_4$-$Mw_5$, minor worker instar; Mw, minor worker; 1-6, presoldiers.

The third instar larvae are of two types. One type leads to minor worker after one moult; and the second type of larvae after two successive moults change into the major worker.

The development of minor pre-soldiers from third instar is a common phenomenon in *Macrotermitinae* (Noirot, 1955, 1969) and the results obtained from causal observations of field material and from laboratory incipient colonies where only minor soldiers are produced show that *M. michaelsoni* is no exception (Okot Kotber,

1981a,b).

Present studies with a field colony of *O. redemanni* reveal that the pre-soldier differentiates from third larva which after six successive moults changes into a mature soldier.

These developmental variations further confirm the doubts prevailing amongst the termitologists about the existence of sibling species like *O. obesus*, *O. redemanni*, *O. gurdaspurensis* and *O. assamensis*.

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# EFFECT OF STARVATION ON MORTALITY AND TOTAL BODY WEIGHT OF LARVAE AND ADULTS OF *TRIBOLIUM CASTANEUM**

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**Abstract**: The sixth instar larvae of *Tribolium castaneum* showed 100% mortality after 16 days and adult beetles after 18 days of total starvation under laboratory conditions at 35 °C and 75% relative humidity. Rate of wet weight loss was greater in sixth instar larvae than adult beetles. Larvae reduced their wet weight sharply during initial 48 hours of starvation and thereafter maintained steady reduction. In contrast adults reduced their wet weight gradually throughout the experimetation. However, both stages of *T. castaneum* decreased their dry wieght abruptly during the initial 48 hours followed by steady reduction.

**Key words**: Starvation, *Tribolium castaneum*, red flour beetle.

## INTRODUCTION

The ability to withstand starvation could have an important evolutionary cosequrnces, if stored grain insects are forced to undergo some periods of starvation (Sverdlov and Wool, 1975). Although the response of higher animals to such stress is fairly understood (Scharrer and Scharrer, 1963), very little informatioin is available on the effect of total starvation on physiological and bio-chemical systems of insects (Dahlman, 1973). In the absence of sufficient bio-chemical data on starved *Tribolium castaneum* (Sverdlov and Wool, 1975), and since insecticides were planned to be administered to these insects in this laboratory under starved conditions, it was felt necessary to generate basic data for *T. castaneum* under total starvation conditions.

Some of the previous studies in other laboratories describe the effects of starvation on the length of the interlarval period in the tsetse fly, *Glossina morsitans orientalis* (Saunders, 1972); on survivorship curves, weight loss and percent dry weight of larvae of tobacco hornworm, *Manduca sexta* (Dahlman, 1973); on growth development and survival in the red trunip beetle, *Entomoscelis americana* (Gerber, 1984) and on survival and maintenance of soldier proportion in laboratory groups of the *Formosan subterranean* termite, *Coptotermes formosanus* (Su and La-Fage, 1986).

Review of literature revealed that very little published information was available on these aspects of stored grain insects particularly *T. castaeum*. Sverdlov and Wool (1975) reported some aspects of survival of starved adult *T. castaneum*, whereas Malik and Galley (1976) studied combined effects of starvation and radiation on feeding activity of this beetle. Likewise Rosinski *et al.* (1979) studied the effect of starvation on trehalase activity of closely related stored grain insect, *Tenebrio molitor* and

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*Part of the thesis submitted to the University of the Punjab by the first author for award of Ph.D. degree in Zoology

Buscarlet *et al.* (1986) demonstrated the effect of fasting and irradiation on free amino acids of *T. castaneum*. From this laboratory we have already reported effect of total starvation on mortality, total body weight, some enzyme activities and some biochemical components in 6th instar larvae of *T. castaneum* (Saleem and Shakoori, 1993) at 30±1 °C and 75% relative humidity.

## MATERIALS AND METHODS

*Rearing of beetles*

The methods have already been described elsewhere (Saleem and Shakoori, 1986, 1993). The master culture of the red flour beetle, *T. castaneum* was obtained from the Food Storage Division of the Pakistan Agricultural Research Council (PARC), Karachi and was maintained in a temperature controlled laboratory at 30±1 °C with 60% relative humidity (p.h). The insects were reared in empty jam jars covered with muslin cloth and whole meal wheat four was used as the culture medium. Sixth instar larvae collected 28±1 days after egg laying and adult beetles collected after 10±1 days after emergence from pupae were used in the present study.

*Determination of mortality and loss of body weight*

Seventeen glass vials (diameter 2cm., height 4cm.) each containing 10 final instar larvae and 10 glass vials (diameter 2cm., height 4cm) each containing 10 adult beetles were weighed with and without insects and then placed in desiccators maintained at 75% r.h. with the help of saturated sodium chloride solution according to the procedure described by O'Brien (1948), Ernst (1957) and Minnick *et al.* (1973). The desiccators were in turn kept in a temperature controlled lab. maintained at 35±1 °C.

These larvae and adult beetles were forced to undergo total starvation till 100% mortality was achieved. Mortality, dry and wet weight data was recorded after 24 and 48 hours for larvae and beetles, respectively.

First of all insects were weighed to obtain total body wet weight. Thereafter, one vial from each category was kept in an oven at 110 °C for 24 hours for estimation of thier total dry weight as described by Dahlman (1973). Hence total body water content was the difference of live wet weight and dry weight obtained by keeping the insects overnight at 110 °C (Woodring, 1984). The experiments lasted 16 and 18 days for larvae and beetles, respectively. That way 68 glass vials each containing 10 final instar larvae and 40 vials each containing 10 adult beetles were used in the present study. Each experiment had 4 replicates.

## RESULTS

*Mortality*

All sixth instar larvae died after 16 days of total starvation. About 23% mortality occured within 24 hours, while 50% insects died between 3rd and 4th days of starvation. The daily mortality recorded is shown in Table I. Hence total starvation

caused the highest mortality during the early period of 48 hours of forced starvation, and then it showed gradual increase in mortality with the lapse of time period (Table I).

**Table I. Effects of starvation on total body wet weight, total body dry weight, total body water content and mortality of 6th instar larvae of *Tribolium castaneum* at 35 °C and 75% r.h. Each value is a mean of 10 beetles**

| Days of starvation | Wet weight in mg (n=4) | % loss of wet weight | dry weight in mg (n=4) | % loss of dry weight | Total body water content in mg | % loss of total body water content | Dead larvae (n=4) | Mortality (%) |
|---|---|---|---|---|---|---|---|---|
| 0 | 24.10±0.42 | 0 | 11.25±0.16 | 0 | 12.85 | 53.38 | 0 | 0 |
| 1 | 17.33±1.15 | 28.09 | 9.8±0.64 | 12.89 | 7.53 | 43.45 | 9 | 22.5 |
| 2 | 12.78±0.50 | 46.97 | 6.90±0.88 | 38.67 | 5.88 | 46.01 | 18 | 15.0 |
| 3 | 12.70±0.60 | 47.30 | 6.63±0.41 | 41.07 | 6.07 | 47.80 | 19 | 47.5 |
| 4 | 12.18±1.6 | 49.46 | 6.53±0.19 | 41.95 | 5.65 | 46.39 | 23 | 57.5 |
| 5 | 10.83±1.29 | 55.06 | 5.73±0.48 | 49.07 | 5.10 | 47.09 | 21 | 52.5 |
| 6 | 10.43±0.73 | 56.72 | 6.37±0.84 | 43.38 | 4.06 | 38.93 | 26 | 65.0 |
| 7 | 10.38±1.51 | 56.93 | 6.20±0.93 | 44.89 | 4.18 | 40.27 | 29 | 72.5 |
| 8 | 10.43±2.23 | 56.72 | 5.73±1.14 | 49.07 | 4.70 | 45.06 | 28 | 70.0 |
| 9 | 8.63±0.73 | 64.19 | 5.23±0.57 | 53.51 | 3.40 | 39.40 | 29 | 72.5 |
| 10 | 8.23±0.46 | 65.64 | 5.60±0.67 | 50.22 | 2.68 | 32.37 | 32 | 80.0 |
| 11 | 8.03±0.39 | 66.68 | 5.40±0.19 | 52.00 | 2.63 | 32.75 | 33 | 82.5 |
| 12 | 7.68±0.39 | 68.13 | 5.00±0.64 | 55.55 | 2.68 | 34.89 | 34 | 85.0 |
| 13 | 6.80±0.51 | 71.78 | 4.90±0.54 | 56.44 | 1.90 | 27.94 | 33 | 82.5 |
| 14 | 6.23±0.68 | 74.15 | 4.40±0.80 | 60.88 | 1.83 | 29.37 | 35 | 87.5 |
| 15 | 6.25±0.59 | 74.07 | 4.38±0.16 | 61.07 | 1.87 | 29.92 | 36 | 90.0 |
| 16 | 6.00±0.93 | 75.10 | 4.35±0.93 | 61.33 | 1.65 | 27.50 | 40 | 100.0 |

Table II shows the average number of beetles which died due to the forced starvation and thir corresponding percent mortality. No mortality was recorded during the first 144 hours of starvation. The 18% mortality occured first after 8 days of starvation, while 100% mortality was recorded after 18 days of deprivation of food. The percent mortality on day 10, 12, 14 and 16 was 42.5, 75.0, 95.0%, respectively.

*Total body weight*

Table I shows the effect of total starvation on total body wet weight of 6th instar larvae of *T. castaneum* kept at 35±1 °C and 75 r.h. The total body wet weight of 10 larvae recorded on day 0 was 24.10±0.42mg (n=4) which reduced 47% during the first 48 hours of total starvation. Thereafter, the reduction in body wight was gradual

till it reached its maximum on day 16. The 10 larvae weighed after 16 days of starvation as 6.00±0.93mg (n=4), showing thereby a 75% decrease in the total body wet weight.

In contrast to the 6th instar larvae, ten adult beetles manifested gradual decrease in their total body wet weight from day 0 through day 18 from 20.25±0.42mg to 6.15±0.25mg showing about 70% loss in weight (Table II).

**Table II. Effect of total starvation on total body wet weight, total body dry weight, total body water content and mortality of adult beetles of *Tribolium castaneum* at 35 °C and 75% r.h. Each value is a mean of 10 beetles**

| Days of Starvation | Wet weight in mg (n=4) | % loss of wet weight | dry weight in mg (n=4) | % loss of dry weight | Total body water content in mg | % loss of total body water content | Dead beetles (n=4) | % Mortality |
|---|---|---|---|---|---|---|---|---|
| 0 | 20.25±0.42 | 0 | 9.73±0.27 | 0 | 10.52 | 51.95 | 0 | 0 |
| 2 | 18.63±0.50 | 8.00 | 3.70±0.40 | 10.58 | 9.63 | 51.69 | 0 | 0 |
| 4 | 16.20±0.19 | 20.00 | 7.70±0.19 | 20.86 | 8.50 | 54.47 | 0 | 0 |
| 6 | 16.28±0.54 | 19.60 | 8.15±0.39 | 16.24 | 8.13 | 49.94 | 0 | 0 |
| 8 | 14.20±0.65 | 29.88 | 7.33±0.30 | 24.66 | 6.87 | 48.38 | 7 | 17.5 |
| 10 | 11.45±0.78 | 43.46 | 6.43±0.16 | 33.91 | 5.02 | 43.84 | 17 | 42.5 |
| 12 | 8.93±0.28 | 55.90 | 5.73±0.43 | 41.11 | 3.20 | 35.83 | 30 | 75.0 |
| 14 | 6.88±0.18 | 66.02 | 5.23±0.26 | 46.25 | 1.65 | 23.98 | 38 | 95.0 |
| 16 | 6.30±0.15 | 68.89 | 5.25±0.35 | 46.04 | 1.05 | 16.67 | 38 | 95.0 |
| 18 | 6.15±0.25 | 69.63 | 5.20±0.44 | 46.56 | 0.95 | 15.45 | 40 | 100.0 |

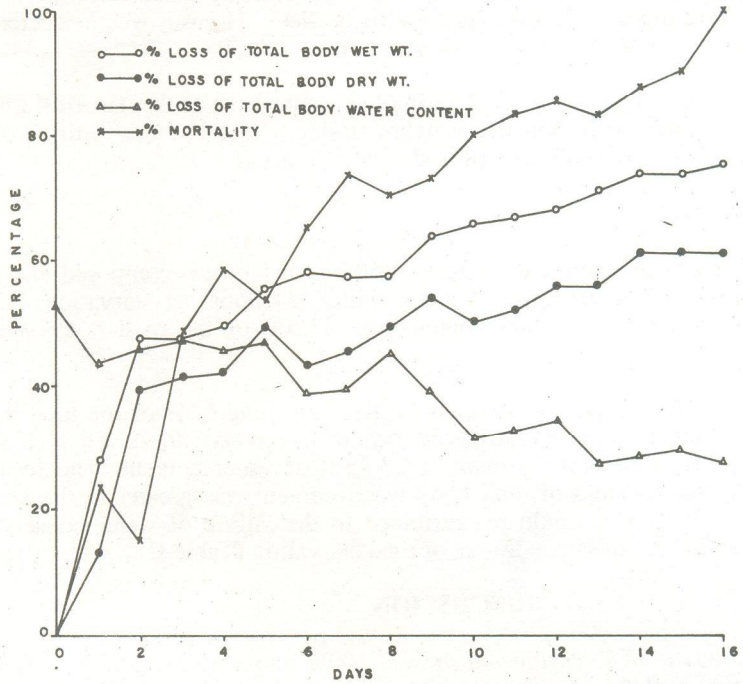
*Total body dry weight*

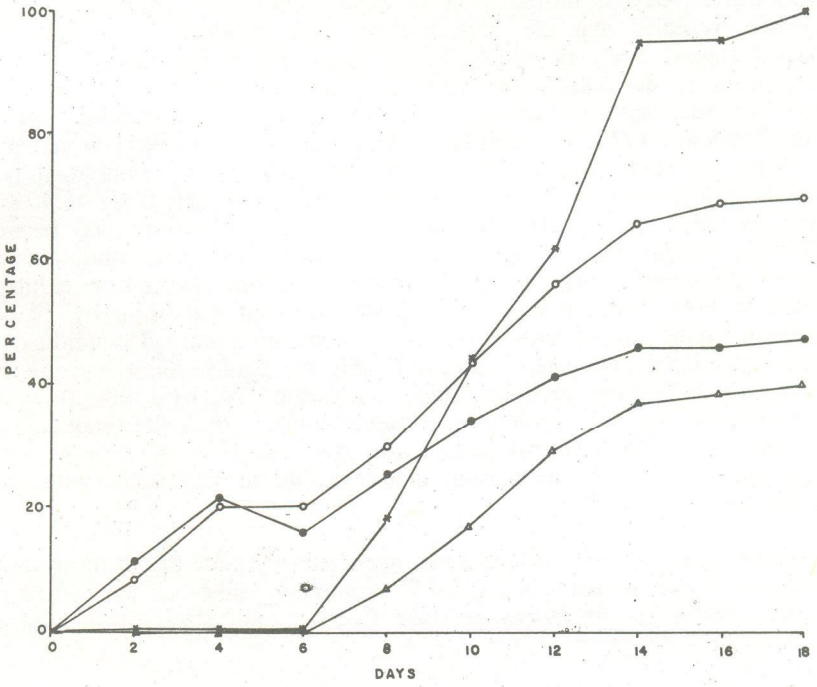
Table I shows the effect of starvation on the total body dry weight of sixth instar larvae of *T. Castaneum*. Fifty three percent of the body weight was lost after drying of day 0 larvae at 110 °C (from 24.10±0.42 to 11.25±0.16 mg for 10 larvae). The loss of dry body weight is reduced after starvation. The wet weight is lost by 75% and dry weight by 61% after 16 days of starvation. Under total starvation conditions the maximum reduction of 38.67% was noted during the first 48 hours of starvation (*i.e.* from 11.25±0.16 to 6.90±0.88mg per 10 larvae at day 0 and day 2, respectively). On day 16 the dry weight was 4.35±0.93mg for 10 beetles, which was 61.33% less than that of day 0 larvae.

**Fig. 1**. Effects of total starvation on mortality and body weight loss at 75% relative humidity and 35 °C of 6th instar larvae of *Tribolium castaneum*

**Fig. 2.** Effects of total starvation on mortality and body weight loss at 75% relative humidity and 35 °C of adult beetles of *Tribolium castaneum*

EFFECT OF STARVATION ON *TRIBOLIUM CASTANEUM*



The total body wet weight decreased 52% after drying of day 0 adult beetles at 110 °C (from 20.25±0.42 mg to 9.73±0.27mg for 10 beetles). The wet weight decreased 70% and dry weight by 47% after 18 days of starvation (Table II).

The reduction in dry weight was gradual until it reached the maximum of 42.45% on day 16 when compared with their dry weights on day 0. Percent loss in dry weight of adult beetles from day 2 through day 18 is shown in Table II.

*Total body water content*

Fifty three percent of the total body weight of 6th instar larvae is composed of water at day 0. The water content decreased 43.45% within 24 hours of starvation, while after 16 days of starvation, the water content was 27.5% of the total body weight (Table I).

The total body water content of the adult beetles constitute 52% of the total body weight. The water content decreased during 28 days of forced starvation. After 18 days of total starvation, the adult beetle contained 15.45% of water content. The results, therefore, indicated that the loss of total body water content was greater in the larvae than in the adult beetles. This could be attributed to the failure of water conserving mechanism in larvae under stress conditions of total starvation (Table II).

## DISCUSSION

The sixth instar larvae of *T. castaneum* showed 100% mortality in 16 days, whereas the adult beetles achieved this mortality in 18 days at 35±1 °C and 75% r.h. The results, therefore, revealed that the adult beetles survived longer than their fully developed larval stages. Such an ability is not surprising as the adult beetles are relatively less prone to desiccation by certain devices such as waxy epicuticle, the tracheal system and the impressive ability of the excretory system to produce vey dry products (Nicolson et al., 1974). The ability to restrict water loss varies. This statement could be further confirmed from the aforementioned results that percent total body water loss in the 6th instar larvae during starvation period ranged from 41.40% to 98.68% while its range in the adult beetles under similar conditions was between 3.61% to 90.97%. Further, loss of body water in the adult beetles was continuous and slow throughout the experimental period. In contrast the 6th istar larvae exhibited abrupt decrease in body water during the first 48 hours of starvation by 54.24% followed by steady reduction of body water in the remaining starved conditions till death occured. Presumably the beetles are well able to regulate their haemolymph composition during desiccation produced under starvation. We have not, however, followed changes in the organic constituents of haemolymph so that we cannot rule out the possibility that such changes contribute to death or that other events are responsible. The results of the present experimentation, therefore, are in accordance with those described by Nicolson *et al.* (1974).

The results of the present study revealed that mean survival time of 6th instar larvae was smaller than those of the adult beetles of *T. castaneum*. Likewise the rate of wet weight loss was greater in the immature stage than in adult stage throughout the

experimental period. Moreover the larvae reduced their wet weight during the experiment. The more rapid larval wet weight loss can be correlated with the higher initial larval mortality than.

The percent dry matter of larvae and adults, however, manifested almost similar pattern of reduction. Both stages decreased their dry weight abruptly during the initial 48 hours of starvation followed by steady reductions. This could be due to the utilization of energy reserves such as trehalose, glycogen, lipids and proteins for sustenance of life during starvation.

The findings of these experiments, therefore, revealed that the relative loss of solids and water (as indicated through the reduction of wet weight and total body water loss) occured at approximately equal rates in the 6th instar larvae and adult beetles during the first 48 hours of starvation, and thereafter all these parameters revealed gradual reduction till death. The increase with time of percent dry matter and percent weight loss prior to death presumably suggests the failure of water conserving mechanism in larvae than adults under stress conditions induced by total starvation. Dahlman (1973) also reported similar results in one of his studies in connection with the effects of starvation on survival, weight loss and percent dry weight loss of larvae of tobacco hornworm, *Manduca sexta*.

The results of similar experiment reported earlier from this lab (Saleem and Shakoori, 1993) revealed that 10 day old adult beetles, when kept under starved conditions at 30±1 °C and 60% r.h., survived longer *i.e.* up to 34 days. In contrast 30 day old beetles survived up to 18 days when kept at 35 °C and r.h. of 75%. This could be either attributed to the age of beetles (as younger beetles survived longer than elders) or to the temperature requirements (as the beetles sustained longer at the optimum temperature of 30 °C than 35 °C). Requirements of optimum temperature of 30 °C was confirmed from another experiment also reported from this laboratory. (Saleem and Shakoori, 1986), where the 6th instar larvae survived longer at this temperature than at 35 °C. Likewise age of the beetles may also play an important role in their survival as the 10 days old beetles may possess more energy reserves than those of elder ones for the sustenance of life. In a similar study Gray (1948) described that several factors affect the rapidity of growth of flour beetles, the more important of which are temperataure, relative humidity and the food medium. He further reported that temperature is probably the most important factor, while relative humidity has little effects than temperature (Gray, 1948). Holsapple and Florentine (1972) reported about the thermal perception of red flour beetle, *T. castaneum*. Likewise Edwards (1976) carried out experiments on age of *T. castaneum* and correlated it with its susceptibility against synthetic juvenile hormone and Heller-Haupt and Varma (1982) studied the effect of age on susceptibility of two species of African ticks (*Ixodidae*) to synthetic pyrithroid insecticides.

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# EFFECT OF ACCLIMATION TEMPERATURE ON PROXIMATE BIOCHEMICAL ANALYSIS OF SOME GASTROPOD SNAILS*

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**Abstract**: The present paper describes the relation of proximate biochemical changes as a function of acclimation temperature in four gastropod snails. The results showed that variation in the moisture, crude protein, fat, glycogen and ash contents of all the snail species studied were found associated with difference in acclimation temperature ($P < 0.001$).

**Key words**: Gastropod snails, acclimation temperature, proximate biochemical analysis.

## INRODUCTION

To ensure survival, all organisms show some degree of adjustment to the fluctuating environmental temperature, that influences the rate of almost all biological processes. Effect of temperature on various physiological and metabolic processes of pulmonate snails has been admirably reviewed by Aldridge (1983) and McMahon (1983). However, proximate biochemical changes in snails following acclimation to different temperatures seems to have received little attention. The only work of significance regarding effects of various acclimation temperatures on the survival and proximate biochemical anslysis of snail's tissues as well as some other related species have been reported by Nagabhushanum and Azmatunnisa (1976), Loomis and Deborah (1987), Wolmarans (1987), Barber *et al.* (1988) and Tanveer (1989b). Present study examines the proximate biochemical constituents of four gastropod snail species acclimated for 14 days at different temperatures ranging from 10-40 °C with an interval of 5 °C. The temperature acclimation relation of these snails is of considerable importance in facilitating further studies on their various physiological aspects.

## MATERIALS AND METHODS

Gastropod snails used in the present study were *Lymnaea acuminata* (*Lymnaeidae*), *Indoplanorbis exustus* (*Planorbidae*), *Physa acuta* (*Physidae*) and *Bellamaya bengalensis* (*Viviparidae*). Excluding *B. bengalensis* all these snail species are intermediate hosts for various helminth parasites of medical importance. The snails collected and maintained following the methods described by Tanveer (1989a) and Tanveer *et al.* (1989). In the present study unless otherwise stated laboratory bred snails were used. The required number of each snail species were removed from the

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holding tank and acclimatized separately at different temperatures ranging from 10-40 °C with an interval of 5 °C for 14 days. *B. bengalensis* utilized in this study was 70 week old, while the rest three snail species were 24 weeks old. For each determination of proximate biochemical analysis, homogenized soft tissue material of 20 samples were treated individually for each snail species. Total water content of body was estimated by completely drying it at 100 $^{O}$C untill a constant weight was reached. Glycogen was estimated from the dried samples by Kemp *et al.* (1954) method. The fat was extracted from the powdered tissue by using soxhlet apparatus. Protein level was measured by Kjeldahl micromethod (Hawk *et al.*, 1954) using the value of 6.25 as a conversion factor between nitrogen content and protein. Ash contents were determined by ashing the tissue in a muffle furnace.

The results correspond to the mean of percentage $\pm 5.0$ of all the determinations calculated both on wet and dry soft tissue basis. All the weighing were made to the nearest of 0.01 mg. Significance level was tested according to Student's `t' test (Sokal and Rohlf, 1969).

## RESULTS

The results presented as mean percentage on wet and dry weight basis were found statistically significant ($p < 0.001$) when analyzed by Student's `t' test (Figs. 1-4). The results clearly revealed the variation in the proximate chemical constituents of snails have been associated with difference in the ambient temperature. It was observed during present studies that increase in the temperature increased the body water content in all the snails under observation. This increase was more pronounced in *I. exustus* and *P. acuta* than *L. acuminata* and *B. bengalensis*. The results on dry weights were in the opposite direction. These changes in water content and dry weights due to temperature for the species under observation were highly significant ($p < 0.001$) when compared with the lowest temperature acclimated snails.

In general, there seemed to be a reciprocal relationship between the water content and the crude proteins of the species studied. As the water content increased, the protein content decreased. Snails kept at highest temperature had highest water contents but the lowest protein value.

Apart from the water, and crude protein content, crude fats, glycogen and ash content were also observed. Total fat and glycogen content of all the species showed an opposite response to water contents when described on wet weight basis. When observed on the dry weight basis, the fat content of *L. acuminata* remained constant when the temperature increased from 10 to 20 °C. After this, the fat content decreased. In the other three species, the fat content first increased with the inccrease in temperature (10-25 °C) and then decreased.

The glycogen contents showed a gradual decrease with increse in temperature. With the increase in temperature from 10 °C to the optimum temperature of each snail, the ash contents when expressed on wet weight basis increased and further raising the temperature caused release of the minerals from the body. Similar trend was observed

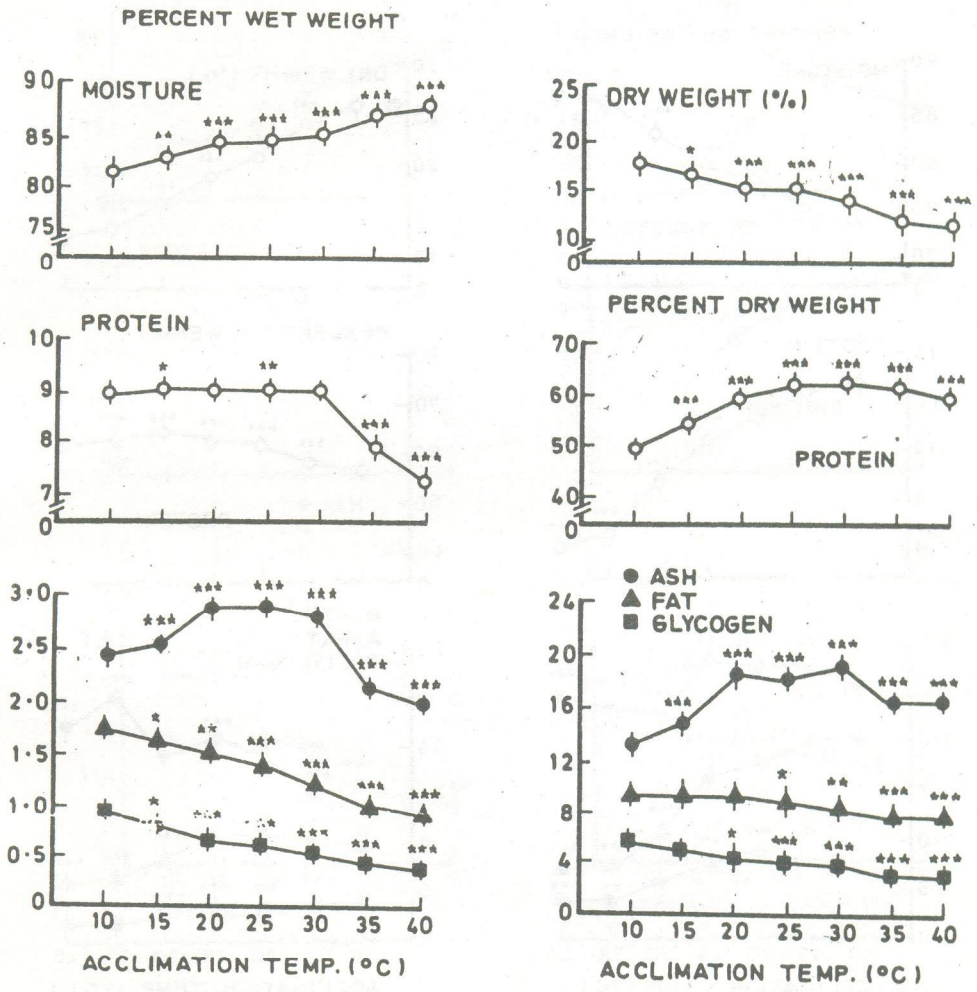


**Figure 1.** Effect of acclimation temperature (2 weeks) on the proximate composition of the total soft parts of *Lymnaea acuminata*. Values given are mean ± S.D. of 20 samples treated individually. Values with asterisks are significantly different from the snails kept at 10 °C according to `t' test. * = $P<0.05$, ** = $p<0.01$, *** = $p<0.001$.

on the dry weight basis also. The response of *P. acuta* was different from rest of the three species, and therefore, warrants further comments. In this snail the ash, when described on the dry weight basis, showed an inverse relationship with the dry weight and a positive correlation with the moisture contents. This was not seen in other snail species.

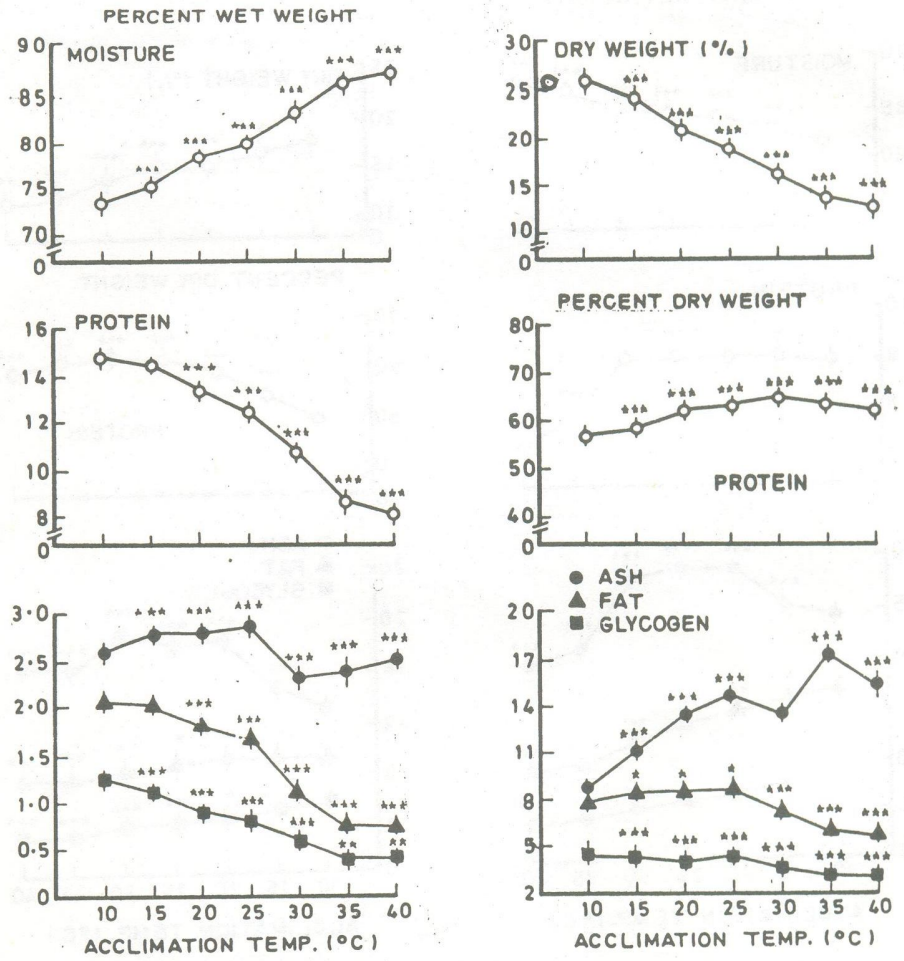


**Figure 2.** Effect of acclimation temperature (2 weeks) on the proximate composition of the total soft parts of *Indoplanorbis exustus*. Values given are mean ± S.D. of 20 samples treated individually. Values with asterisks are significantly different from the snails kept at 10 °C according to `t' test. * = $P<0.05$, ** = $p<0.01$, *** = $p<0.001$.

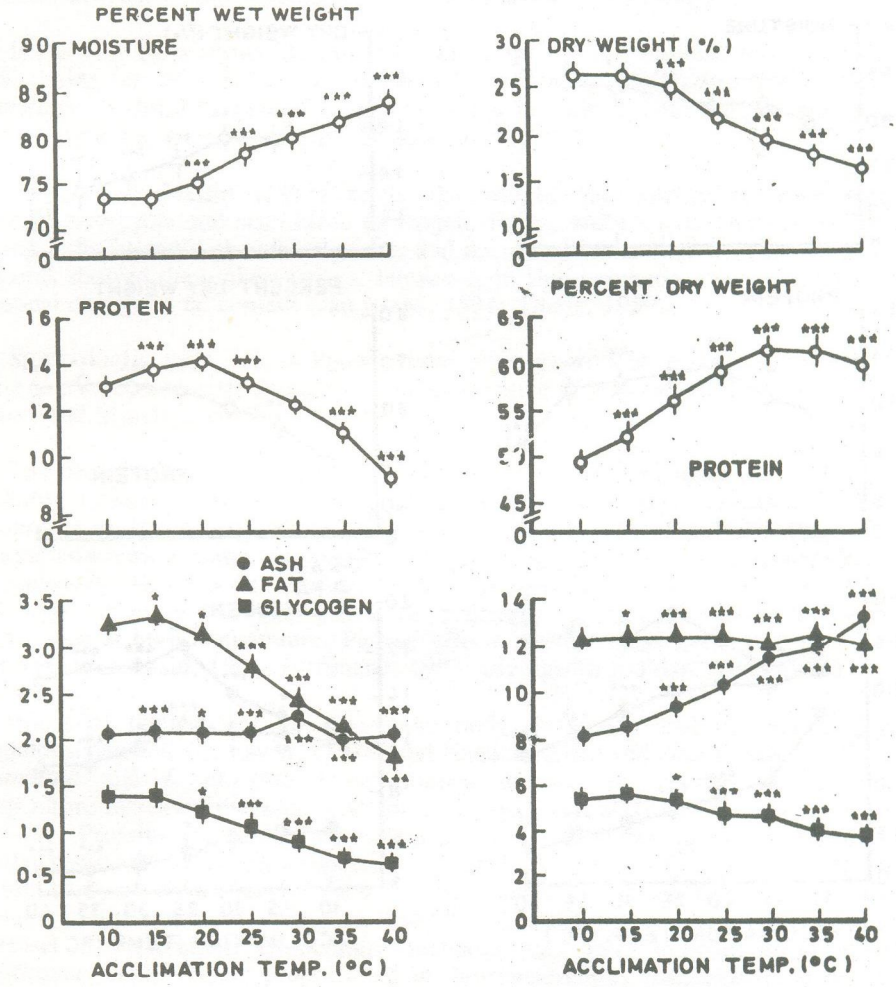


**Figure 3**. Effect of acclimation temperature (2 weeks) on the proximate composition of the total soft parts of *Physa acuta*. Values given are mean ± S.D. of 20 samples treated individually. Values with asterisks are significantly different from the snails kept at 10 °C according to `t' test. * = $P<0.05$, ** = $p<0.01$, *** = $p<0.001$.

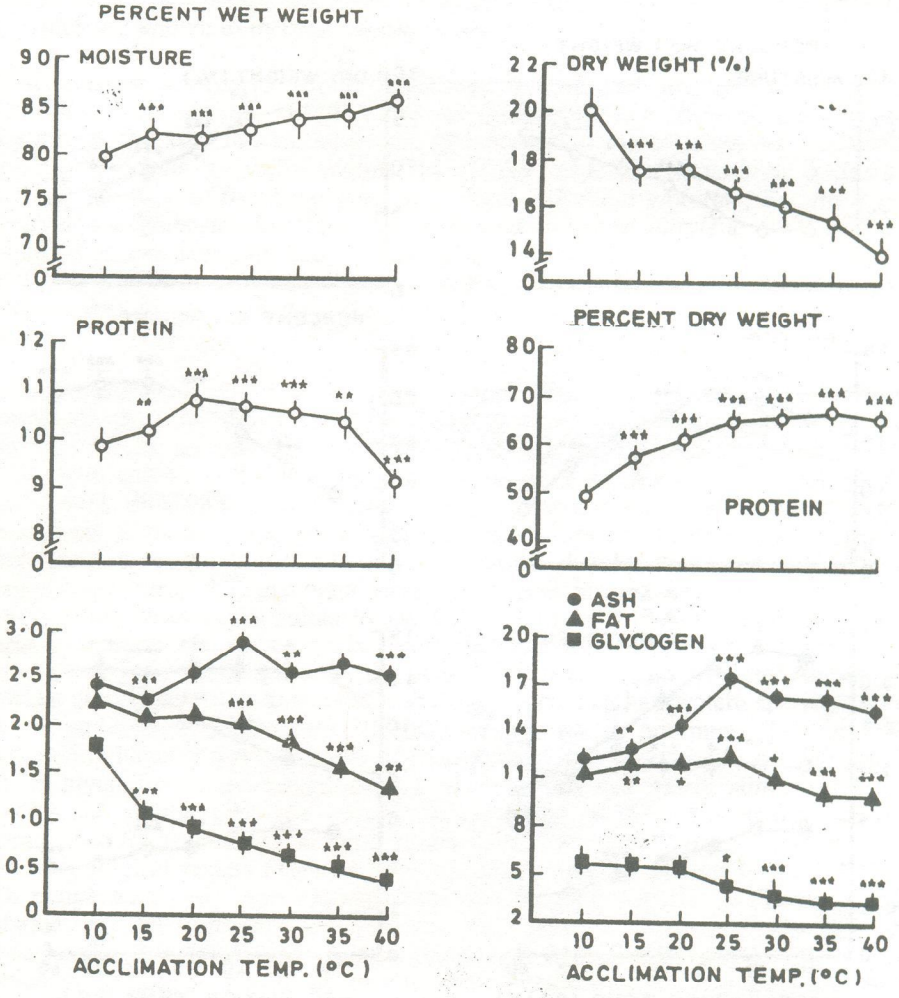


**Figure 4**. Effect of acclimation temperature (2 weeks) on the proximate composition of the total soft parts of *Bellamaya bengalensis*. Values given are mean ± S.D. of 20 samples treated individually. Values with asterisks are significantly different from the snails kept at 10 °C according to 't' test. * = $P<0.05$, ** = $p<0.01$, *** = $p<0.001$.

## DISCUSSION

Glycogen has been found in the body of pulmonates and other molluscs (Goddard and Martin, 1966) and has been reported to the most readily catabolized substrate to meet the energetic burden posed by infection (Barber *et al.*, 1988).

It has also been shown that in molluscs, glycogen is mobilized more quickly than fats (Baker *et al.*, 1942). There is aslo indication, though indirect, that high temperataure (July) decreased the glycogen and fat contents but the proteins increased to a maximum and then decreased (Masumoto *et al.*, 1934).

Castro and Mattio (1987) relate the weight and energy reserves with the reproductive cycle and starvation. Glycogen, lipids, and protein contents increased in spring with the rapid growth of gonads and decreased in summer with spawning, while proteins showed the highest values. However, in Helix pomatia, there was no apparent seasonal change in fat content van Brand, 1931; Thiele, 1959).

Surprisingly, very little is known about other gastropods regarding this aspect and there is also controversy about the dominant source of energy whether glycogen or fat (Barry and Munday, 1959).

The decrease in the glycogen content of the body with increase in the temperature probably reflects an increase in metabolic rate at moderately increased temperature (around optimal temperature) and at extreme temperature, it is possible that the snails entered anaerobic metabolism thereby stripping their body reserves of glycogen. It will be interesting to study in these snails whether they exhibit the pasture effect or not. This type of study will also prove the contention made above regarding anaerobic metabolism at high temperature. Pasture effects in snails have been studied by various workers (Meenakshi, 1964; Hoffmann, 1983; Livingston and de Zwaan, 1983).

Effect of temperature on the whole body proximate analysis was studied by Nagabhushnan and Chintawar, (1976) and Nagabhushnan and Azmatunnisa (1976) in *L. acuminata* and *I. exustus*. These studies showed that increase in acclimation temperature increased the water content of the body but decreased the glycogen and fat contents. Proteins increased first but then showed a decreasing trend. Surprisingly, similar results have also been reported in the fish (Mearow and Houston, 1980). In the present study we have also estimated the water content, proteins, fats, glycogen and ash contents of the snails acclimated at 10-40 $^{o}$C. The results of the analysis showed that increase in acclimation temperature increases the water content while all other constituents are inversely proportional to the temperature. Although these results are similar to the results of Nagabhushnan and Chintawar (1976) and Nagabhushnan and Azmatunnisa (1976) but there are some points that warrant further comments.

Increasing the acclimation temperature increased the water contents of *L. acuminata* and *B. bengalensis* to the tune of 7.50% while *I. exustus* and *P. acuta* had their body water increased by 18.37 and 14.29 %. Increase in water contents of the body upon acclimation to high temperature has also been reported in other snails (van Brand, 1955

and Nagabhushnan and Chintawar (1976) and Nagabhushnan and Azmatunnisa (1976). Reasons for this increase are not known, but at high temperature it is possible that the snails switch to anaerobic metabolism (van Brand, 1955). This anaerobic metabolism will also explain the acute decrease in the glycogen and fat contents of the body. Another factor in increase in the water content of the body is increased breakdown of fats which are known to increase metabolic water.

Acclimation to high temperature decreased the proteins, glycogen and fat contents of the body of all the snails studied. Increase or decrease in acclimation temperature not only effects the ash, fat, carbohydrate, protein and water contents but various other excretory products as well. Wolmarans (1986) made a comparative study in the excretory products of five fresh water snail species (*Bulinus globosus*, *Bulinus tropicus*, *Biomphalaria glabrata*, *Lymnaea natalensis* and *Helisoma duryi*) at 4 $^{O}$C and 25 $^{O}$C and reported that concentration of their acids varied not only from species to species, but also in the same species at different experimental temperatures. It was found that concentration of most of the acids was higher at 4 $^{O}$C than at 25 $^{O}$C except *L. natalensis*.

In the present study increase in temperature from 10 to 40 $^{O}$C had the following changes on the three major energy yielding cellular constituents. Total body protein (nitrogen x 6.25) decreased 20.67, 46.09, 26.83 and 6.85%, total fat decreased 46.02, 63.20, 5.29 and 41.17% while glycogen decreased to the tune of 59.18, 64.62, 57.04 and 74.44% for *L. acuminata*, *I. exustus*, *P. acuta* and *B. bengalensis*, respectively. An analysis of these figures revealed that *L. acuminata* and *P. acuta* had more or less similar responses, but *I. exustus* and *B. bengalensis* differed from them. It is also clear from these values, that glycogen values decreased maximally, followed by fat and protein. This observation points to the fact that these snails had response similar to mammals, where aslo, glycogen is a preferred fuel for energy. When glycogen stores are nearly exhausted, fat provides the energy followed by the proteins. Another thing which is discernible from these data is that, glycogen also has protein sparing property. For example in *B. bengalensis*, decrease in protein is minimum, but comparable decrease in glycogen was maximum when compared with other snails. Similar examples can be given for *L. acuminata* and *P. acuta* which had nearly similar decrease in proteins and similar glycogen and fat values. Based on these biochemical studiees it appears that *B. bengalensis* is more resistant to temperature change, a response also seen in ecological results (Tanveer, 1989b). Further that *I. exustus* is least tolerant of high temperature. All these changes exhibited by these specific snails are mean, to countract the environmental changes and help survival of the species. The detail physiological and biochemical changes underlying these gross changes will have to be studied in order to put them in the right perspective to analyze their biology thoroughly.

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# MANGANESE RESISTANT BACTERIUM FROM POLLUTED WATER: SOME ENVIRONMENTAL FACTORS INFLUENCING CONJUGAL TRANSFER OF $Mn^{2+}$-RESISTANT PLASMID

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**Abstract:** A manganese resistant strain AnMn-1 was examined, which could prevent the toxic action of $Mn^{2+}$ up to 2000 μg/ml in the medium. It was Gram-negative, pleomorphic, motile with high convex, undulate and circular colonies. It favoured neutral to alkaline media and was sensitive to $Co^{2+}$, $Hg^{2+}$, Cm and Sm but could tolerate $Ni^{2+}$, $Zn^{2+}$, $Ba^{2+}$, $Sn^{2+}$, $Fe^{3+}$, $Cu^{2+}$, Ap, Tc and Km in the medium. It was facultative anaerobic, spore-former and had catalase, oxidase and urease enzymes. It had the ability to produce acid from arabinose, rhamnose and glucose. It could hydrolyse urea, decarboxylase lysine and ornithine. Single plasmid was present in it. Conjugation experiments under different set of environmental conditions like time (0-24hrs), pH (6.5-8.5), temperature (25°, 28°, 32°, 37°) and donor to recipient ratio (1 to 10) revealed that maximum transfer frequency was found at 37°C when pH of the medium was 8 and also at donor to recipient ratio 10. It shared maximum characters with Gram-negative *Bacillus*.

**Key words:** $Mn^{2+}$ resistant bacteria, plasmid, conjugal transfer

## INTRODUCTION

Heavy metal burdened industrial effluents pose serious problems to biological life since many of them are toxic with long decay time. Majority of these metals exist in insoluble forms in the industrial wastes (Vallee and Ulmer, 1972; Moore and Ramammoorthey, 1984). One of these metals is manganese, traces of which are essential for plant and animal life. It activates certain enzymes such as decarboxylase, dehydrogenase and oxidase (Bidwell, 1979); cotrols nitrogen metabolism and assimilation reaction in plants (Bidwell, 1979). It also has a structural role in chloroplast membrane system and in the photosynthetic split of $H_2O$ (Salisbary and Ross, 1986). Nevertheless, excess of manganese is harmful to living organisms. Under low redox potential manganese is reduced which is more soluble in acidic pH. Reduced manganese absorbs at the root surface and cause adverse effect on plant growth (Jeffrey and Helm, 1987). Increased amount of $Mn^{2+}$ (0.1-1.0mM) in combination with reduced pH effect more significantly the content of chlorophyll a, carotene and ultimately the biomass production (Kummerova and Buresova, 1989a,b). Excess of manganese also causes iron deficiencies (Brady, 1984) and destruction of IAA (Devlin and Witham, 1986)

In animals surplus amount of manganese is pneumotoxic (Richard and Morris, 1989), and in rat kidney it blocks the induction of hemo-oxygenase (Drummond and Kappas, 1989). Excess amount of manganese stimulates lysozyme production in *Micrococcus lysodektious* and cause lysis of cells (Sack, 1981). It also effects the transport system of microbes (Perry and Silver, 1982). Furthermore $Cd^{2+}$ and $Mn^{2+}$ are the competitive ihibitors of each other (Perry and Silver, 1982; Nies and Silver, 1989). Metal resistant bacteria can detoxify the adverse effects of metals (Wood and

Wang, 1985). For the said reasons the present work deals with the isolation and characterization of metal resistant bacteria from industrial wastes. Effects of some environmental factors on conjugal transfer of Mn-resistant plasmid, present in this strain, have also been discussed.

## MATERIALS AND METHODS

From the effluents of Shan Ghee which was odourless, colourless, with oily gradients and pH 7, a manganese-resistant bacterial strain was isolated. Fifty $\mu$l of sample water was plated onto nutrient-agar plates containing 25$\mu$g/ml of $MnSO_4$ (having 9.09$\mu$g/ml of $Mn^{2+}$). Bacterial growth was observed within 24 hours at 37$^{o}$C. Purified strain, which was designated as AnMn-1 was taken progressively to higher levels of $MnSO_4$ in the medium. AnMn-1 was characterized morphologically, physiologically as well as biochemically (Gerhardt *et al.*, 1981). Twenty biochemical and the cytochrome oxidase tests were performed by using QTS-20 (20 Quick Test Strips for Bacterial Characterization) and CO-strips (Cytochrome oxidase strips), respectively (DESTO Laboratories, Karachi). Spore forming ability was authenticated by the method of Moir (1981). AnMn-1 was also checked for the resistance against antibiotics Ap (ampicillin), Km (kanamycin), Sm (streptomycin), Cm (chloramphenicol), and Tc (tetracycline); (300, 50, 500, 5 and 25$\mu$g/ml, respectively) and other metallic salts *i.e.* $ZnSO_4$ (250$\mu$g/ml), $NiCl_2$, $CoCl_2$ and $HgCl_2$ (25$\mu$g/ml), $FeCl_3$ (50$\mu$g/ml), $CuSO_4$ (200$\mu$g/ml), $SnCl_2$ (600$\mu$g/ml), $BaCl_2$ (200$\mu$g/ml) and $CdCl_2$ (50$\mu$g/ml). For the detection of plasmid total cell lysate method (Thomas, 1984) was used. For characterization of plasmid, broth mating technique of Willetts (1984) was followed and conjugation experiments were performed using *E. coli* K12 strain CSR603 (*recA1 phr1* derivative of AB1886 (*thr-1 leu-6 lacY1 galK2 ara-14 xyl-5 mtl-1 proA2 his-4 str-31 tsx33 sup37 uvxA6*) as recipient. Transconjugants were selected on media containing 2000$\mu$g/ml $MnSO_4$ and 500$\mu$g/ml streptomycin. For determining the effect of environmental factors on the transfer frequency, time (0-24 hrs), pH (6.5-8.5), temperature (25, 28, 32, 37$^{o}$C) and donor to recipient ratio (1-10) were taken into consideration. For studying transfer frequency, AnMn-1 (donor) and CSR603 (recipient) were grown overnight in 5ml of L. broth with continuous agitation (150 rpm) at 37$^{o}$C. Initial recipient density was determined by plating dilutions ($10^{3}$, $10^{4}$, $10^{5}$) on nutrient-agar containing 500$\mu$g/ml of Sm. Afterwards the mating mixture was diluted and spread on double inhibitor-supplemented plates. Colonies obtained on double inhibitor-supplemented (Mn+Sm) agar at 37$^{o}$C were scored as transconjugants. Frequency of transfer was calculated as the number of presumptive transconjugants per initial amount or number of recipients.

## RESULTS AND DISCUSSION

Strains purified at conceentration of 2mg/ml $MnSO_4$, was characterized morphologically, physiologically as well as biochemically. Single colonies obtained after 24 hours of incubation were used for study. They were opaque, creamy-yellow, circular, undulate with high convex elevation, ranging in size from 3-3.5mm. The cells were motile, pleomorphic, and Gram-negative. When isolate was taken to elevated levels of $MnSO_4$the growth rate declined which clearly indicates that cellular growth was inhibited by extremes of environmental metallic toxicity. In addition to metallic

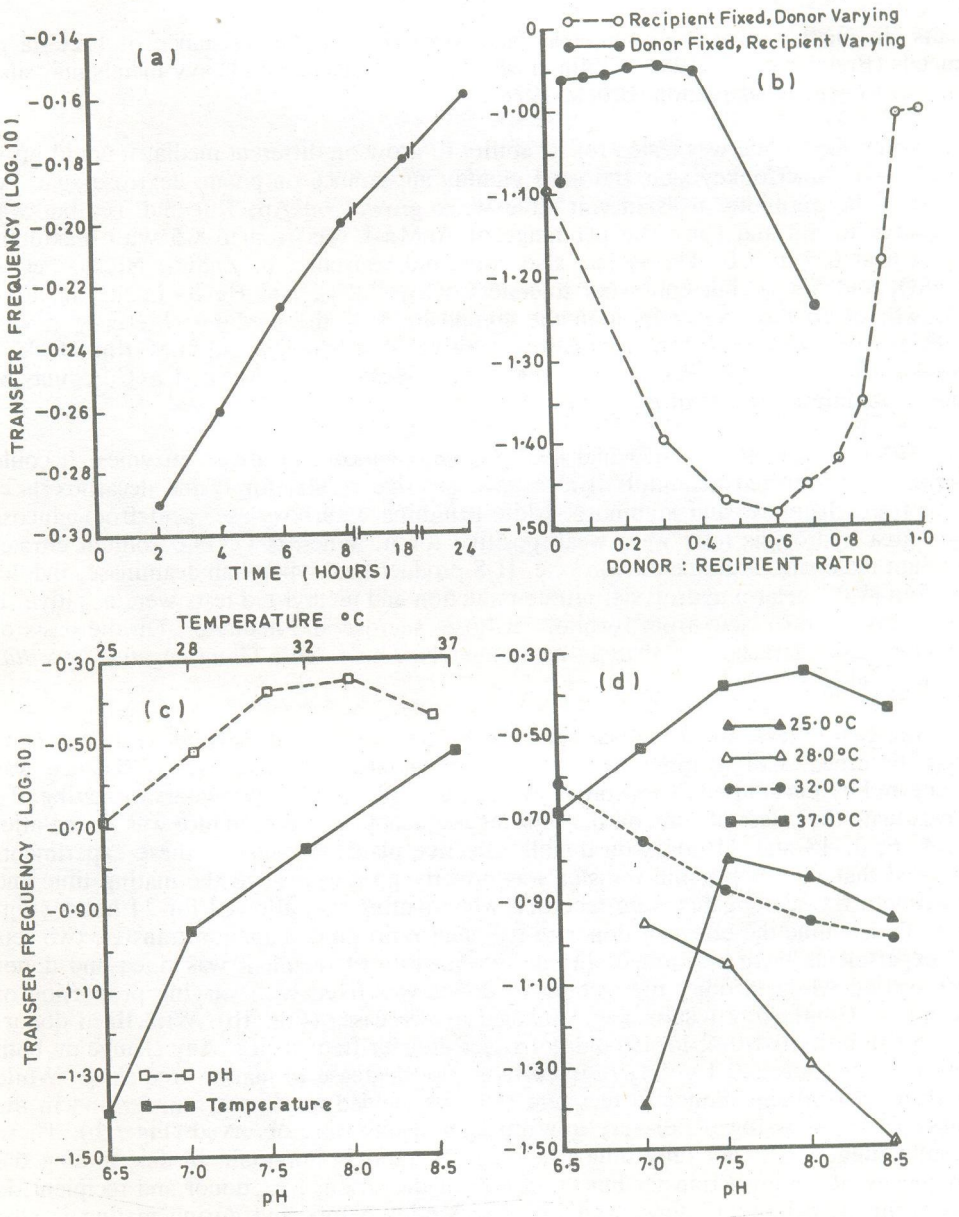


**Fig. 1.** Factors influencing conjugal transfer of Mn-resistant plasmid from AnMn-1 to *E. coli* strain CsR603. (a) Time, (b) Donor to recipient ratio, (c) Temperature and pH, (d) Combined effect of varying temperature and pH.

salts (in media) other environmental factors also effect the resistance of bacteria to metals (Breteler *et al.*, 1981; Giblin *et al.*, 1983). Resistance to heavy metals may also be due to genetic adaptation (Brock. 1978).

When the isolate was tested for its ability to grow on different media, it could grow on L-agar, MacConkey agar and gave gummy appearance on potato dextrose agar. As regards the antibiotic resistance it gave weak growth on Ap, Km and Tc, but was sensitive to Sm and Cm. The pH range of AnMn-1 was from 6-8.5 with maximum growth at 6.5 to 7.0. The isolate also conferred resistance to $ZnSO_4$, $NiCl_2$, $FeCl_3$, $CuSO_4$ and $SnCl_2$, but could not tolerate $CoCl_2$, $CdCl_2$, and $HgCl_2$ in the medium. Growth of bacteria depends upon the composition of the medium (Mergeay *et al.*, 1985). $Cd^{2+}$ and $Mn^{2+}$ are competitive co-rival of each other. 0.2$\mu$M Km of $Cd^{2+}$ hinder $Mn^{2+}$ transport (Perry and Silver, 1982). Sensitivity of AnMn-1 to $Cd^{2+}$ may be due to inhibitory effects of manganese.

AnMn-1 was spore former and had oxidase, catalase and urease enzymes. It could ferment glucose and mannitol. It also gave positive results for lysine decarboxylase, acid from rhamnose and arabinose, while ornithine decarboxylase, acid from glucose and urea hydrolysis tests were weak positive for it. Whereas ONPG, sodium citrate, sodium malonate, arginine dihydrolase, $H_2S$ production, tryptophan deaminase, indole, acetoin (VP), gelatin hydrolysis, nitrate reduction and methyl red tests were negative. It could not produce acid from sorbitol, maltose, sucrose and mannitol. On the basis of biochemical characters it shared maximum characters with Gram-negative *Bacillus* (Krieg and Holt, 1984).

Manganese resistant strain was screened for the presence of plasmid. Only one band was discerned. Conjugation experiments demonstrate that manganese resistance was conferred by plasmid and transconjugants could be obtain after two hours of mating. To investigate the effect of time on the transfer frequency, mating mixture was plated after 2, 4, 6, 8, 18 and 24 hours on double selective plates. Results of these experiments showed that rate of plasmid transfer was directly proportional to the mating time and maximum transconjugants were recorded when mating was allowed for 24 hours (Fig. 1a). To examine the effect of donor to recipient ratio on conjugation transfer, two sets of experiments were performed. In one the quantity of recipient was fixed and donor was varied while in other the amount of donor was fixed with varying proportion of recipient. Contrasting results were obtained in two cases (Fig. 1b). With fixed donor, change in ratio (0.1-0.4, log10) did not affect transfer frequencies. Any change on both sides of this range (0.1 - 0.4) resulted in abrupt decrease in mating frequency. While with fixed recipient, donor to recipient ratio 10 yielded maximum transfer and in the range of 0.3-0.8 (log scale) very low transconjugants were observed (Fig. 1b). These results suggest that not only donor to recipient ratio is important in determining the frequency of conjugal transfer but the nature of the strain, *i.e.*, donor and recipient, is also critical. pH of the donor and recipient strains before and during mating is also important factor in scoring transconjugants.

Different studies on the transfer of drug resistance and metallic compounds have shown optimum pH 6.0 - 7.5 for conjugal mating (Shenderov, 1971; Harada and

Mitsuhashi, 1977; Singleton and Anson, 1983). In the results presented here maximum transconjugants were obtained at pH 8.0 (Fig. 1c). These results demonstrate that plasmid could be transferred between pH 6.5 and 8.5. Beyond both limits of pH no transconjugants were recorded whereas the bacteria could grow at pH 6. Temperature being an important factor of environment also effects the conjugation. Many antibiotic resistant plasmids have been reported to transfer maximally at temperature between 20 - 30°C (Gauthier *et al.*, 1985; Altherr and Kasweck, 1982; Kelly and Reanney, 1984). An increase in the temperature from 27 to 37°C led to a large increase in the transfer frequency of Mn resistant plasmid from AnMn-1 strain (Fig. 1c). Higher optimum temperature for maximum transfer frequency may be attributed to the hot climate from which this strain was isolated. Influence of low temperature on the mating frequency have also been reported by other workers (Harada and Mitsuhashi, 1977; Kelly and Reanney, 1984). Since temperature and pH have coordinated action on plasmid transfer (Singleton and Anson, 1983; Rochelle *et al.*, 1989), the effects of varying pH (6.5 to 8.5) in combination with different temperatures (25, 28, 32, 37°C) on the plasmid transfer were also determined (Fig. 1d). Generally 37°C yielded maximum transconjugants at all pH values (Fig. 1d) when compared with other temperatures at respective pH. At this temperature, up to pH 8.0 progressive augmentation in transfer frequency with the increase in pH value was recorded after which a decrease in conjugal transfer was observed. With low temperature (25, 28°C) no plasmid transfer was observed at pH 6.5. With increasing pH value, 32°C and 25°C caused gradual dismount in transfer frequency of this plasmid. With 28°C maximum conjugal transfer was observed at pH 7.5 but even it was significantly less than that of 37°C at respective pH value. Thus the Mn-resistant plasmid described here favours pH 8 at 37°C for its maximal transfer and acidic pH with low temperature (25, 28°C) hinder its conjugation.

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# STUDY OF SEASONAL VARIATIONS IN PHYSICO-CHEMICAL PARAMETERS OF A FISH FARM

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**Abstract**: Various types of physico-chemical parameters were studied in a fish farm near Multan, (Pakistan). The air temperature ranged from 23 $^{o}$C to 44.2 $^{o}$C and the water temperature was found to be 18.8 $^{o}$C and 34.1 $^{o}$C. Secchi's disc reading varied from 14.6 cm to 31.6 cm. The pH fluctuated within a narrow limit 7.51-8.57 and did not show seasonal pattern. However, dissolved oxygen showed an inverse relationship with water temperature. Total solids ranged from 1.0 to 2.2 mg/g.

**Key words**: Seasonal variation, physico-chemical, fish farm.

## INRODUCTION

In recent years, aquaculture is being projected as a possible solution to the food problems faced by the masses. It gives higher productivity/unit as compared to agriculture and animal husbandry. Water quality studies are important and have been taken up because these play a key role in aquaculture (Sinha and Srivastava, 1991). The water quality ultimately determines the survival and growth of the cultured animals and plants (Dehadri, 1992). The productivity depends on the physico-chemical characteristics of the pond water. The maximum production is obtained when the physical and chemical factors are at the optimum level (Huet, 1986). The fresh water fisheries resources of Pakistan are not being adequately exploited or developed due to lack of active research. Research on the limnological aspects is of paramount significance in developing freshwater fisheries. The present study describes the seasonal variation in physico-chemical factors in a commercial fish farm.

## MATERIALS AND METHODS

This study was carried out on Zaidi Aqua farm located on the left side of Muzaffargarh road, at a distance of 16km from Multan. This is a commercial aquatic complex covering an area of 6 hectares. The pond selected for sampling in the present study covers a water area of 0.5 hectare with a maximum depth of 7 feet. The pond had a stocking combination of major carps *i.e. Labeo rohita, Catla catla* and *Cirhinus mrigala* alongwith two Chinese carps *i.e. Hypophathalmicthys molitrix* and *Ctenopharyngodon idella*. The study was carried out for a growth period of eight months *i.e.* from March to October, 1993.

At the time of sampling, air and water at 6" depth temperature was recorded using a mercury thermometer. The light penetration was recorded with the help of Secchi's disc. The dissolved oxygen was determined using an oxygen meter (Model 9070) immediately after samplig. The pH was determined using a digital pH meter (Model CD. 640) in the laboratory. For the determination of total solids, water samples were

taken at 6" depth in preweighed glass bottles and then reweighed. The samples were evaporated to dryness at 80 °C in a drying oven (Memmert). After evaporation, the glass bottles were reweighed and the amount of total solids in the sample was determined and expressed as mg/g of water. The data for humidity was collected from Meteriological department, Multan.

## RESULTS

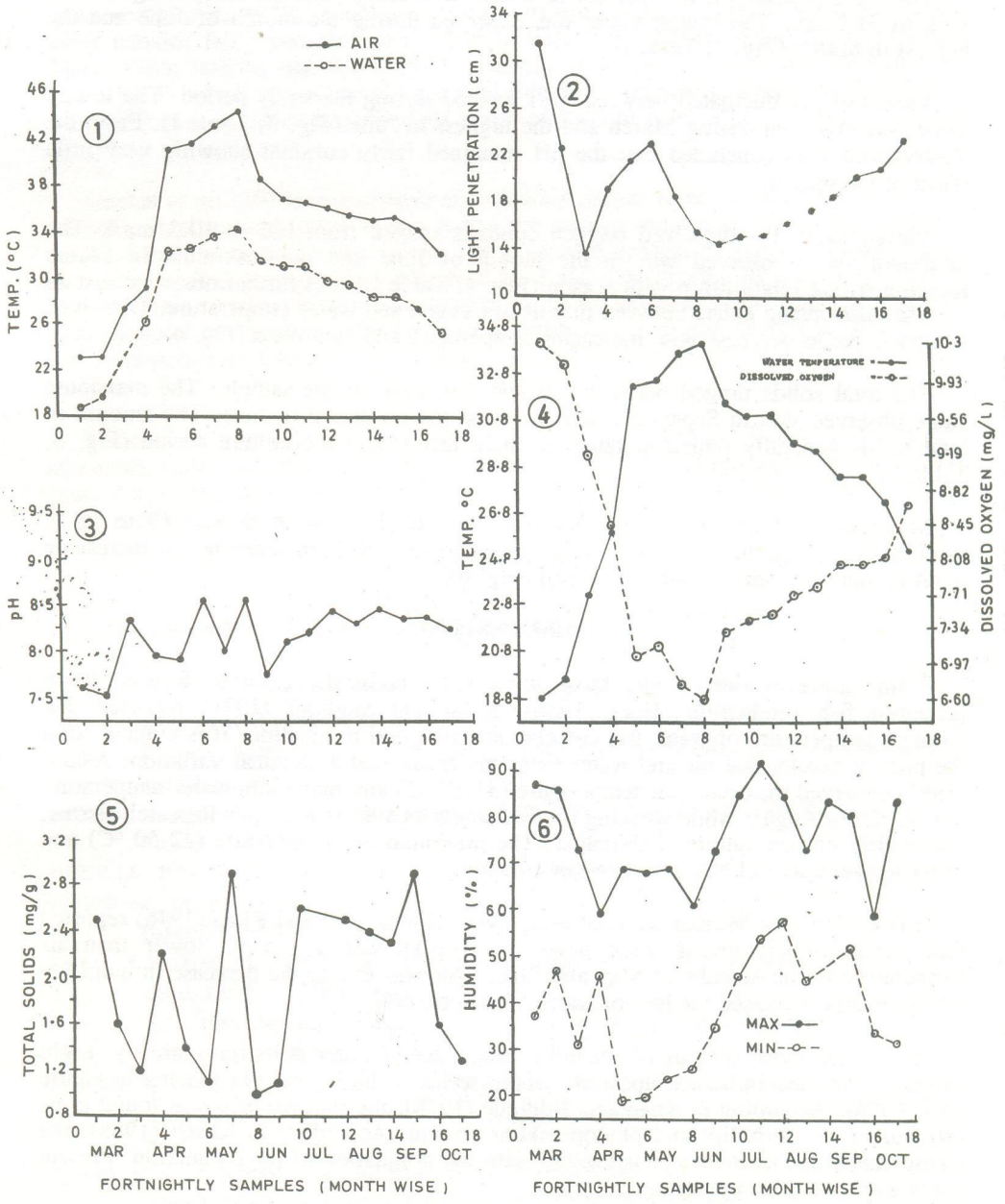
The overall range in atmospheric temperature observed was 23 - 44 °C while the water temperature fluctuated between 18.8 and 34.1 °C. The maximum and minimum temperatures were recorded during months of March and June, respectively (Fig. I, Table I). There is a direct relationship in the seasonal fluctuations between atmospheric and water temperature (Fig. 1).

**Table I. Seasonal changes in physico-chemical parameters of a fish farm**

| Date | Temperature | | Light | pH | Dissolved | Total solids | Humidity | |
|---|---|---|---|---|---|---|---|---|
| | Air (°C) | Water (°C) | penetra-tion | | Oxygen (mg/l) | (mg/g) | max.(%) | min.(%) |
| 1.3.93 | 23.0 | 18.8 | 31.6 | 7.61 | 10.3 | 2.5±.0054 | 87 | 37 |
| 15.3.93 | 23.0 | 19.6 | 22.6 | 7.51 | 10.1 | 1.6±.0015 | 86 | 47 |
| 30.3.93 | 27.3 | 23.2 | 13.7 | 8.35 | 9.15 | 1.2±.0003 | 73 | 31 |
| 13.3.93 | 29.1 | 26.0 | 19.2 | 7.96 | 8.45 | 2.2±.0004 | 59 | 46 |
| 28.4.93 | 41.0 | 32.3 | 21.7 | 7.91 | 7.05 | 1.4±.0016 | 69 | 19 |
| 13.5.93 | 41.4 | 32.5 | 23.2 | 8.55 | 7.16 | 1.1±.0003 | 68 | 20 |
| 28.5.93 | 43.0 | 33.7 | 18.6 | 8.07 | 6.72 | 2.9±.0045 | 69 | 24 |
| 12.6.93 | 44.2 | 34.1 | 15.6 | 8.57 | 6.60 | 1.0±.0003 | 61 | 26 |
| 27.6.93 | 38.5 | 31.5 | 14.6 | 7.85 | 7.28 | 1.1±.0029 | 73 | 35 |
| 12.7.93 | 36.7 | 31.0 | 15.3 | 8.11 | 7.40 | 2.6±.0033 | 85 | 46 |
| 27.7.93 | 36.6 | 31.0 | 15.4 | 8.30 | 7.49 | 2.3±.0023 | 92 | 54 |
| 11.8.93 | 36.1 | 29.8 | 16.4 | 8.45 | 7.69 | 2.5±.0014 | 85 | 58 |
| 26.8.93 | 35.5 | 29.5 | 17.6 | 8.30 | 7.77 | 2.4±.0021 | 73 | 74 |
| 10.9.93 | 35.1 | 28.4 | 18.7 | 8.45 | 8.01 | 2.3±.0011 | 84 | 49 |
| 25.9.93 | 35.3 | 28.4 | 20.5 | 8.34 | 8.01 | 2.9±.0030 | 81 | 52 |
| 10.10.93 | 34.3 | 27.3 | 21.1 | 8.36 | 8.06 | 1.6±.0010 | 59 | 34 |
| 25.10.93 | 32.1 | 25.2 | 23.5 | 8.22 | 8.64 | 1.3±.0021 | 84 | 32 |

**Fig. 1-6**. Some physico-chemical parameters of a fish farm: temperature (1), light penetration (2), pH (3), dissolved oxygen and relationship between dissolved oxygen and temperature (4), total solids (5) and humidity (6).

# SEASONAL VARIATIONS IN PARAMETERS OF A FISH FARM



The light penetration interpretted in terms of Secchi's disc visibility ranged from 14.6 to 31.6 cm. The lowest value was observed during the month of June and the highest in March (Fig. 2, Table I).

Values of pH fluctuated between 7.61 to 8.57 during the study period. The lowest value was observed during March and the highest in June (Fig. 3, Table I). From the observations it is concluded that the pH remained fairly constant showing very little effect of the season.

Variations in the dissolved oxygen contents ranged from 6.6 to 10.3 mg/l. The minimum value observed was in the month of June and the maximum in March showing strong relationship with season (Fig. 4, Table I). It is further observed that an inverse relationship exists between dissolved oxygen and water temperature. Dissolved oxygen contents decrease with increasing temperature and vice versa (Fig. 4).

The total solids ranged between 1.0 and 2.9 mg/g of the sample. The maximum value observed was in September and May and the minimum in June. The amount of total solids generally remained fairly constant throughout the culture period (Fig. 5, Table I).

Maximum and minimum humidity ranged between 59 to 92% and 19 to 58%, respectively, being the highest in August and lowest in April showing an increasing trend in humidity with season after April (Fig. 6).

## DISCUSSION

Temperature is one of the most important among the external factors which influence fish production (Huet, 1986). Khan and Siddiqui (1978) reported that constant temperature of water has correlation with pond production. It is evident from the present results that air and water temperature showed a seasonal variation. Ashraf (1987) observed maximum air temperature (35.57 °C) and maximum water temperature (33.50 °C) in August while working on fish ponds located at Fisheries Research Farms, Universitly of Agriculture, Faisalabad. The minimum air temperature (12.50 °C) and water temperature (11 °C) was noted in January.

It is evident that Multan has relatively severe climate. Ali and Khan (1976) reported that water temperature of some ponds in Aligarh was 2 - 5 °C lower than air temperature in the Months of May and June. This was due to the decrease in humidity which greatly increased the loss of water by evaporation.

One of the most obvious and familiar properties of water is its transparency. Light exerts a profound influence upon the whole series of biological phenomena in nature (Ali, 1976). According to Khan and Siddique (1978), the transparency was found to be effected by the turbidity and phytoplankton growth. According to Khatri (1985), the transparency has an inverse relationship with the population of phytoplankton. Present study confirms this observation.

In ponds, seasonal and diurnal variations in pH may be noticeable and are usually

related with photosynthesis and respiratory processes of the various organisms concerned (Boyd, 1979). Biological conditions are considered better when pH of the water remains fairly constant rather than in water undergoing considerable variations (Huet, 1986; Jeffries and Mills, 1992). The pH of the pond in this study ranged from 7.51 to 8.57 which was relatively constant showing little effect of season. Mahboob *et al.* (1988) also observed a similar pH range of 7.45 to 8.50 while working on Ajmla Fish Farm, Faisalabad.

Singhal *et al.* (1986) investigated significantly higher values of dissolved oxygen in the colder months and lower values during the warmer months of the season similar to the observations made in this work. The dissolved oxygen of the pond under study showed a gradual decrease from March to June and then gradual increase from June to October. This trend in seasonal fluctuation of the dissolved oxygen is inversely related to pond water temperature. The decrease in oxygen content was correlated with rise in water temperature. Quadri *et al.* (1981) reported similar pattern in dissolved oxygen contents.

The concentration of total solids in our study varied between 1.0 and 2.9 mg/g. The maximum value was observed in May and September while minimum was observed in June. Ali, (1976) also observed maximum concentration of total solids in the month of September and minimum in June.

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# LIGHT AND SCANNING ELECTRON MICROSCOPIC STUDIES ON THE GILL RAKERS AND TASTE BUDS OF TWO INDIAN HILL-STREAM FISHES

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**Abstract**: Light and scanning electron microscopic studies of the gill-arch and gill-rakers in two Indian hill-stream fishes, *Garra lamta* (Hamilton) and *Noemacheilus rupicola* (McClelland) show many striking features. In the former fish, due to its herbivorous nature and for leading an active life even in the midst of high current of water with the help of its adhesive apparatus, gill-rakers are reduced without showing taste-buds, whereas in the later one, due to is carnivorous nature and a burrowing and a sluggish life at the bottom in sand/mud, the system is elaborate, with stumpy gill-rakers, provided with girdle like lamellae at the surface, many taste-buds with transverse like openings, provided with thick rim. Origin of the gill rakers has been suggested from the gill-arch tissues, by invagination.

**Key words**: Gill rakers, taste buds, hill-stream fish.

## INTRODUCTION

Although in recent years considerable advancement has been made in the study of animal adaptation to different types of environment, little attention seems to have been paid to the wonderful modifications exhibited by the fauna of mountain torrents (Hora, 1992). Hill-stream fishes constitute an interesting group of small sized fishes, belonging to different genera like *Lepidocephalichthys, Noemacheilus, Botia, Garra, Glyptothorax, Glyptosternum* etc., which due to their peculiar habitat have undergone a variety of modifications specially in their external features, scales, paired fins, girdles, caudal fins, mouth and associated structures, eyes, skin, gill-openings etc. The chief factors which mainly influence for modifications are the strength of the current, food, shallow rocky water bed, water with constant motion and plenty of oxygen.

Light and electron microscopic studies on the gill-rakers and taste buds of Indian teleosts have attracted the attention of different workers very recently (Rooj, 1984; Munshi *et al.*, 1984; Ray *et al.*, 1987; Ghosh *et al.*, 1988; and Munshi *et al.*, 1989). The importance of gill-rakers of various types of morphology in connection with different types of feeding habits in fishes are well known. These structures are well represented in large number of teleosts in their buccopharyngeal cavity attached with the gill-arches, either in one or two rows on each arch. They serve mainly to prevent the food from escaping out along with the respiratory water current (Khanna, 1970). However, the taste buds or taste receptors have been seen to be generally situated on the tip of gill-rakers (Ghosh *et al.*, 1989) and buccopharynx of a number of species (Ray *et al.*, 1987). The presence of the taste buds at the base of the gill-rakers and on the gill-arch epithelium have also been observed earlier (Rooj, 1984; Ojha and Singh, 1986 and Ojha, 1987).

In the present study the presence of taste buds at the base of the gill-rakers and

beneath the gill-arch epithelium of two Indian hill-stream fishes *Noemacheilus rupicola* (McClelland) and *Garra lamta* (Hamilton) have been shown with the help of light and scanning electron microscopes.

## MATERIALS AND METHODS

Live specimens were collected from Jonha Fall (Gautamdhara) near Ranchi (India) during the month of March when the rush of water in the stream remains low.

*Light microscopy*

Live specimens were anaesthetized by MS-222, 40mg/liter, opercula were removed, gills were taken out carefully, washed in Ringer's solution and small pieces were fixed in Bouin's fixative. After washing, the gill-pieces were decalcified, re-washed and dehydrated in graded ethanol, embedded in paraffin and horizontal sections (6-7$\mu$m) were obtained by a Weewox (India) microtome, the slides were stained in haematoxylin, counter stained with eosin and microphotographs were taken.

*Scanning electron microscopy*

Gills were carefully taken out from freshly anaesthetized (MS-222, 40mg/liter) specimens, and small pieces were fixed in 5% glutaraldehyde solution for about two hours inside a thermos flask filled with ice to maintain a cold temperature possible at the site. The required dilution of glutaraldehyde was obtained by cacodylate buffer (pH 7.4). The materials were transferred to 12% cacodylate buffered glutaraldehyde and stored inside ice-filled thermos flask for about 48 hours. After washing with buffer to remove fixative, the materials were dehydrated through a graded ethanol series and kept in acetone, before critical point drying. Gill pieces were glued on stubs with silver paint and coated with a thin layer of gold and observed under a Philips scanning electron microscope (PSEM/500), at regional Sophisticated Instrumentation Centre, Bose Institute, Calcutta and photographs were taken.

## RESULTS

*Light microscopy*

In horizontal sections the gill-arch in *N. rupicola* shows short and stumpy projections of gill-rakers with shallow, concave tips (Fig. 10), whereas in *G. lamta* these are without any concavity (Fig. 11) and are lined with large cuboidal epithelium.

Both the figures indicate their origin as evaginations of gill-arch tissues. The gill arch of *N. rupicola* shows distinct epithelium. Thick layer of striped musculature is also distinct in the gill-arch above which many mucous gland cells, supporting cuboidal epithelium cells and groups of large taste buds are visible (Figs. 7-9). Each taste buds is more or less circular in shape, provided with many spindle shaped gustatory cells.

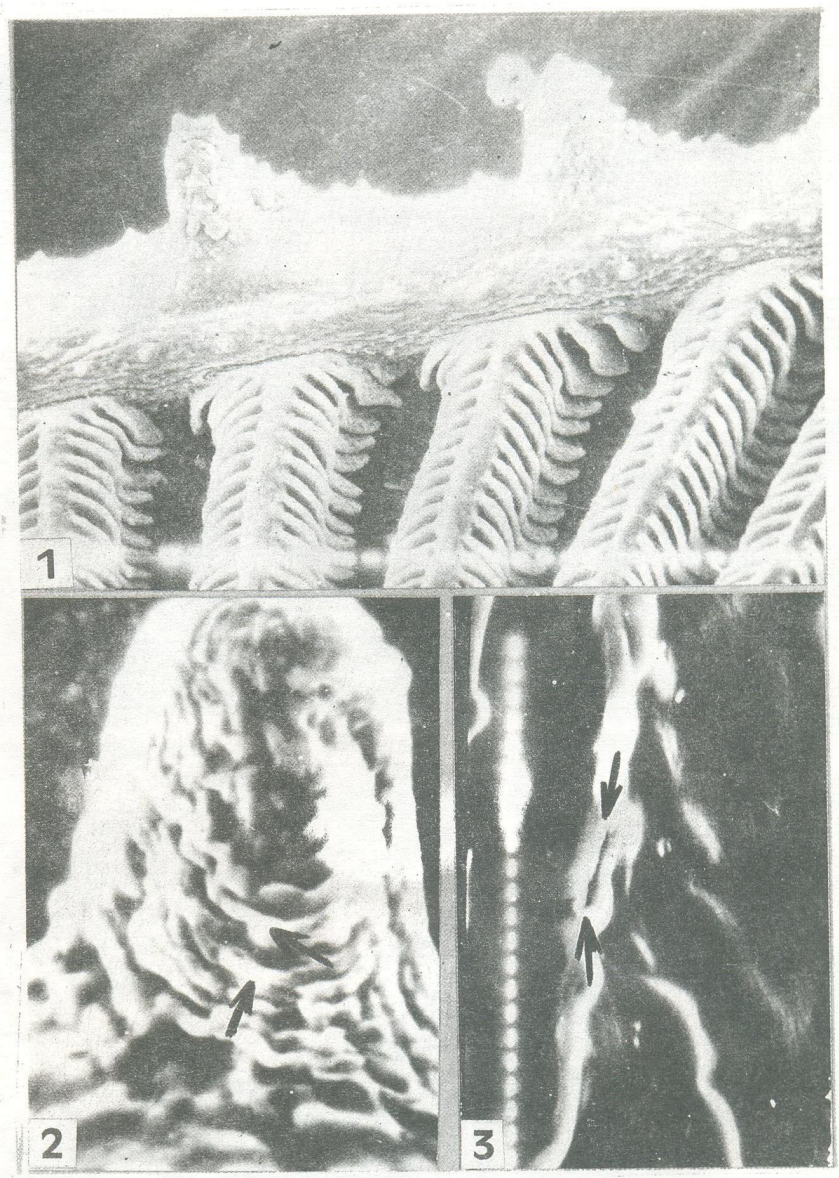


**Fig. 1**. Low power SEM of the part of the gill of *N. rupicola*, showing gill-arch, gill-rakers, primary and secondary gill lamellae (x157).

**Fig. 2**. High power SEM of a single gill-raker of *N. rupicola*, showing girdle like parts (x700 arrows)

**Fig. 3**. High power SEM of the base of a gill-raker of *N. rupicola* showing opening of a taste bud (arrow, x1600)

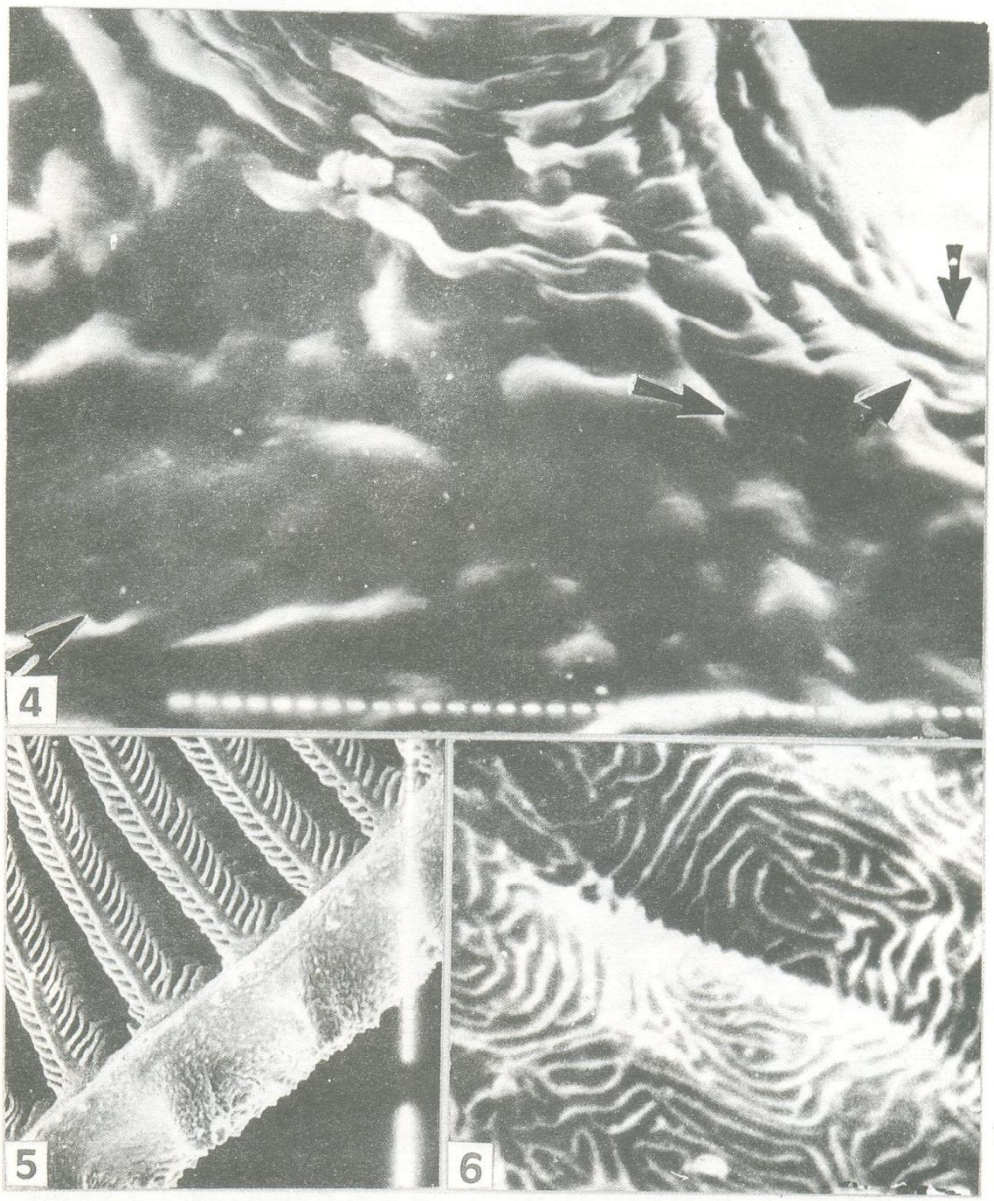


**Fig. 4**. High power SEM of the base of a gill-raker of *N. rupicola*, showing girdle like parts and openings of taste buds (arrow x5750).
**Fig. 5.** Low power SEM of a part of the gill of *G. lamta* (x80).
**Fig. 6**. High power SEM of the surface structure of the gill-rakers of *G. lamta* (x5750).

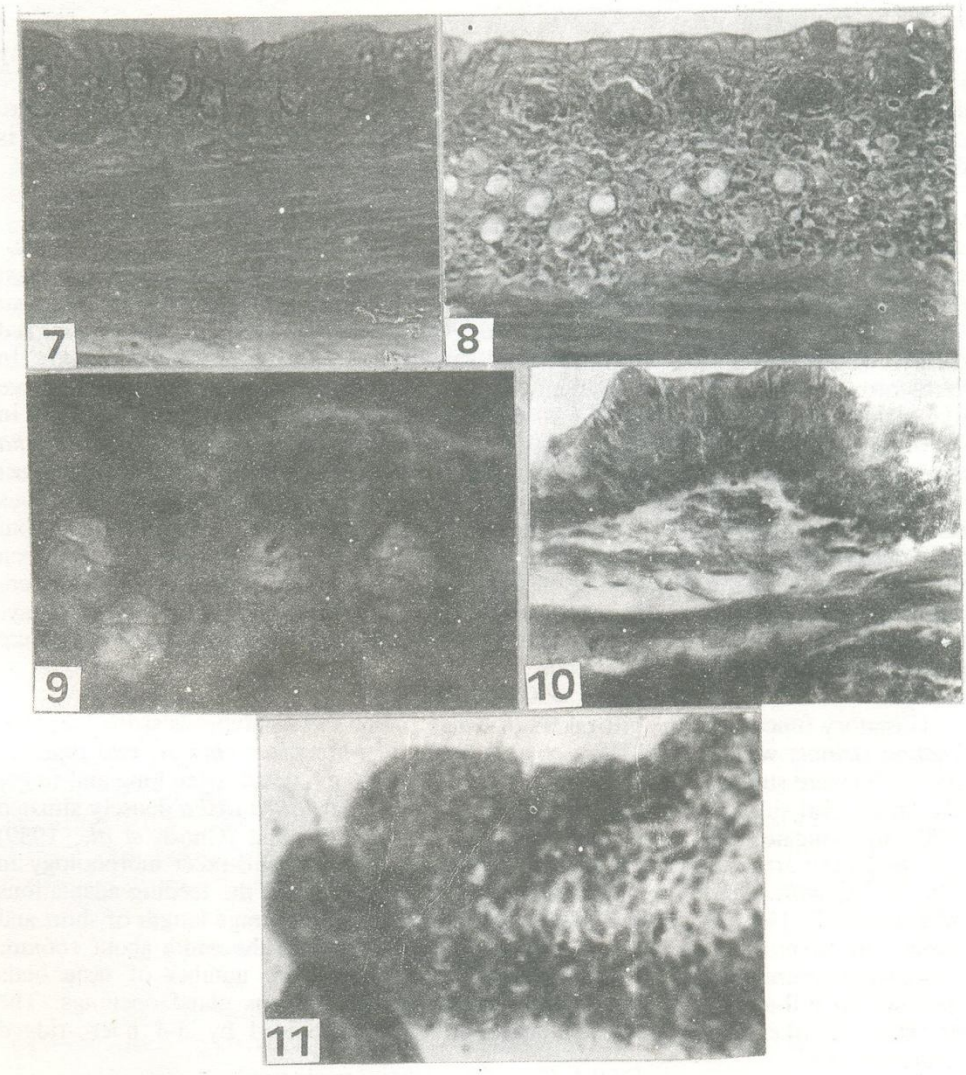


**Fig. 7**. H.S. paraffin section of the gill-arch of *N. rupicola* showing taste buds, mucous glands, and muscles (x100).

**Fig. 8**. Fig. 7 magnified, showing the structure of the gill-arch more distinctly (x400).

**Fig. 9**. Fig. 8 magnified, showing the mucous glands (x100).

**Fig. 10**. H.S. paraffin section of the gill-arch of *N. rupicola*, showing the origin of a gill-raker (x400)

**Fig. 11**. H.S. paraffin section of the gill-arch of *G. lamta*, showing the histology of a gill-raker (x400).

*Electron microscopy*

Each gill-raker in *G. lamta* and *N. rupicola* is short and stumpy structure (Figs. 1 and 5). Surface of the gill-rakers in *N. rupicola* is not smooth but provided with many transverse girdle like structures, openings of taste buds, and of mucous glands (Figs. 2 and 4). Each taste bud opening forms an elongated transverse slit like structure encircled by wide rim like portion (Fig. 3). Surface of the gill-raker in *G. lamta* is differently organized and shows micro-ridged sculpturing (Fig. 6).

## DISCUSSION

Tooth like gill-rakers have also been reported in large number of carnivorous and predatory Indian teleosts, like *Wallago attu*, *Mystus seenghala*, *M. aor*, *Channa marulius*, *C. punctatus*, *C. gachua* and *Notopterus chitala*. In the generalized omnivorous species like *Tor tor, Puctius sarana, P. ticto*, they are short and stumpy. In herbivorous forms like *Labeo rohita, Cirrhina mrigala*, gill-rakers form broad sieve like structures across the gill-slits for filtering the water in order to retain the food in the bucco-pharynx. This function is best developed in plankton feeders like *Hilsa ilisha* and *Gadusia chapra*, in which the gill-rakers are long and thin, and form a perfect sieve so as to retain zooplankton and phytoplankton in the buccal cavity. Numerous taste buds have been observed on the lips and buccopharynx of a number species, but their number is smaller in some forms and absent in others. Taste buds have also been found in the epithelium covering the gill-arch (Khanna, 1970). Taste buds were reported also in the barbels, tongue and gill rakers of Clarias batrachus (Linn) (Ray, 1987), and the gustatory pores of the taste buds were found to be provided with minute projections of the neuro-epithelial cells.

Gustatory function of the gill-rakers in *Labeo rohita* and carnivorous striped murrel, *Channa striatus* was doubted (Ray *et al.*, 1987). In *Notopterus chitala*, two types of gill-rakers were studied (Ghosh *et al.*, 1988) on its gill-arch which were long and finger like on the oral side, while knob like on the aboral side. In *Hilsa ilisha* densely situated (120/cm) elongated gill-rakers with flattened tips were studied (Ghosh *et al.*, 1989) with biserially arranged taste receptors (about 380-400/cm). Gill-raker morphology in *Cirrhina mrigala*, *Danio dangilla* and *Channa striatus* showed the feeding adaptations (Munshi *et al.*, 1989). Ojha and Singh (1986) measured the average length of short and stumpy gill-rakers of *Danio dangilla*, to be about 222$\mu$m and the width about 166$\mu$m. The two adjacent gill-rakers were about 185$\mu$m apart. The number of taste buds openings in gill-arch were found to be more than the mucous gland openings. The elevated rim like opening of each taste bud was surrounded by 3-4 micro-ridged epithelial cells.

Gill-rakers and taste buds are concerned mainly with feeding. Many carnivorous and predacious fishes, like *Harpodan nehereus* and *Murasnesox telabon* feed by sight and taste buds are rare or absent. Some species like *Tor tor*, *Catla catla Cirrhina mrigala* depend more on the gustatory faculty for feeding and possesses a large number of taste buds. The loach *Noemacheilus rupicola* as most of the times remains buried in the sand and mud and leads to a rather sluggish and sedimentary life (Rooj, 1984), presence of gill-rakers and taste buds in good number in gills is a part and parcel of its adaptation

for survival, while *G. lamta* as is an active fish and lives even in the midst of high current of water, with its adhesive disc (Bose *et al.*, 1971; Ojha *et al.*, 1982 and Ojha and Singh, 1992) is a herbivore, hence the system is not well developed in the gill-arch rather is simple.

## SUMMARY

Fish gills are provided with gill-lamellae towards the outside, and in many cases, gill-rakers internally. Gill-rakers are pointed structures, projecting into the pharyngeal cavity and are arranged either in one or two rows on each arch. They serve to prevent the food from escaping out through the respiratory current of water. Taste buds are also present in fishes in the epithelium covering the gill-arch and gill-rakers, in addition to bucco-pharynx, lips, barbels and even on the surface of the body. Species which do not feed by sight (as many catfishes and loaches, who keep themselves embedded in mud and under the stones) have a large number of taste buds on the lips and barbels. Each taste bud is oval or circular in shape. Fishes with their taste buds can sense salty, sweet, bitter and acid stimuli, and many fishes are seen to reject the unsuitable food after taking it in the buccal cavity.

Presence of large number of taste buds in Noemacheilus rupicola a cobitid of hill-streams is a special adaptation for survival which help the species to detect the chemical nature of water and also of food material, which are of immense importance to the fish. Gill-rakers are evaginations of gill-arch epithelium, a fact which is corroborated also by histological studies. Taste buds are smaller in gill-arch epithelium.

Scanning electron microscopy reveals the morphological details of gill-rakers, their mucous glands and taste bud openings etc, in *N. rupicola*. In *G. lamta* it is simple.

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# METAMORPHOSIS OF THE THORACIC NERVOUS CENTRE OF *PIERIS BRASSICAE* (LINN) (*LEPIDOPTERA, PIERIDAE*)

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**Abstract**: The histological changes occurring during the metamorphosis of the thoracic nervous centre of *Pieris brassicae* are described. The meso- and metathoracic ganglia fuse with the first and the second abdominal ganglia to form one centre. These and the prothoracic ganglion make up the adult thoracic nervous centre. The different histological changes taking place in the cortex and neuropile are relatively more complex in thorax as compared to the abdominal region. These are correlated with the functional responses.

**Key words**: *Pieris brassicae*, thoracic ganglia, prothoracic ganglia.

## INTRODUCTION

Insects have different types of locomotion but walking and flying are the commonest. For these activities centrally generated programs produced by pattern generators are thought to control the basic pattern of acitvity in motor nerves supplying the muscles of the legs and wings, while input from peripheral sensory receptor systems provide a feedback system for precise control of locomotary movements. The nervous system plays the central role in these activities, which possesses electrical and chemical mechanisms for information reception, transmission and processing. The neurons, which are specialized for nervous function, produce axons and dendrites. These are especially adapted for neural transmission (Heitler and Burrowa, 1977; Pearson, 1982; Steaves and Pearson, 1982; Eaton, 1985). Synaptic junctions between the neurones lie in the ganglionic neuropile, especially in the various glomerular bodies where associations occur between axon branches or dendrites (Richards and Davies, 1977). Thus, extremely complicated networks are formed which are integrated to produce functional integrity in different activities. These enable the insects to receive, process and effectively use enormous amounts of information (Shankland and Frazier, 1985). The degree of complexity of a parhway depends upon the type of activity. As locomotion is a complicated activity in which different sets of sensory and effector organs are involved, the thoracic nervous centre, which is the locomotary centre, has complicated sets of nerve tracts. Fusion of the ganglia occur to increase the efficiency of the system. In *Pieris brassicae* during metamorphosis, the meso- and meta-thoracic ganglis beecome fused with the first and second abdominal ganglia to form the thoracic locomotary centre. There is such a complexity of network that in the present study attention has been paid only to the major nerve tracts and their neurons.

## MATERIALS AND METHODS

The various larval, pupal and adult stages needed for this work were reared at 20-22°C from the first instar of *Pieris brassicae*. The various larvae were killed for

dissection and histological treatment in the middle of the instar. Material was fixed in Bouin's solution or Zenker's or Gilson's fixative for staining in Mallory's Triple Stain or Heidenhain's Iron Haemotoxilin. The material was embedded in paraffin wax and serial sections were cut at 5-8$\mu$. To study the different nerve pathways and for additional information on cellular constituents, Wigglesworth's (1957, 1959) method of osmium tetroxide fixation followed by ethyl gallate treatment was used.

## RESULTS AND DISCUSSION

In the present study metamorphic changes concerning meso- and metathoracic ganglia and those occuring in the first and second abdominal ganglis are described. Prothoracic ganglia which is also part of the thoracic motor centre has been described earlier in detail (Ali, 1993).

*Peripheral nerves*

Six pairs of nerves are given off from this part of the nervous system. Four of them are large but the other two are very delicate. The mesothoracic ganglion gives off one strong and one feeble pair; the other feeble nerve comes from the abdominal part of this centre.

*Cortex*

The cellular components of these ganglia are similar to those which have already been described (Ali, 1973; 1993). The structure of the first and second abdominal ganglia is essentially similar to the other more posterior abdominal ganglia in the larval stages but during metamorphosis they decrease in volume and finally lose their separate identity completely. Some of their cells undergo pyknosis and histolysis but most of their neurones apparently migrate to the thoracic nervous centre. This is indicated by the fact that the posterior region of the compound thoracic centre in the later pupal atages and adult contains cell groups and fibre tracts which correspond in position and arrangement to those previously present in the first and second abdominal ganglia of the larva.

*Neuropile and axonal tracts*

The pattern of the neuropile is generally the same in all the three thoracic ganglia of the 5th instar larva, while in the first and second larval abdominal ganglia it is like that the third abdominal ganglion (Ali, 1991). Considering this the fibre tracts of the adult meso- and meta-thoracic ganglia have only been described here in detail, but comparison has been made between the two stages of development among the different ganglia.

The following are the paired tracts and other features of the adult mesothoracic ganglion:

Tract at 1: From dorsolateral groups of motor neurones. Runs around neuropile ventrally.

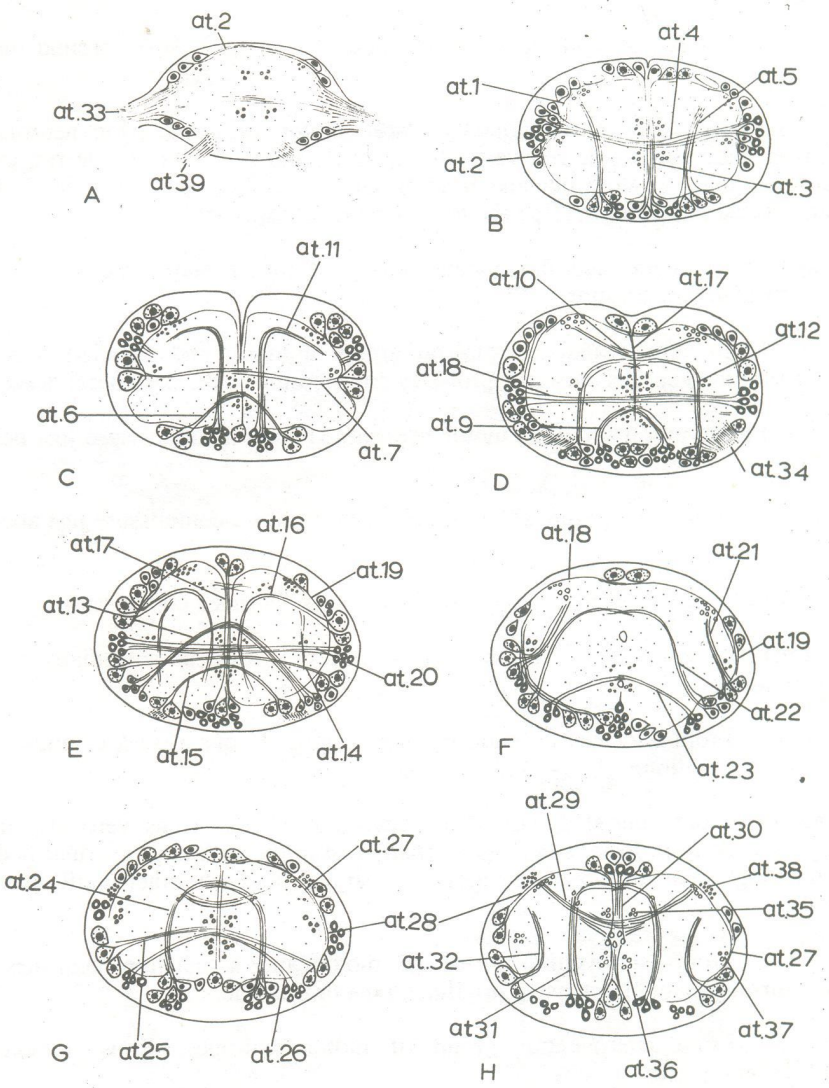


**Fig. 1.** Diagrammatic serial drawings of transverse sections of adult mesothoracic ganglion showing the major fibre tracts with neurones. Cells with dark nuclei and dotted cytoplasm represent the large motor neurones; cells with dark nuclei and white cytoplasm represent medium-sized motor neurones; cells with dark cytoplasm represent association neurones; small clusters of circles represent axons cut transversely; heavily shaded areas represent glomerular bodies.
at1, at2.... adult mesothoracic fibre tracts.

Tract at 2: From lateral and dorsolateral motor neurones. Runs around neuropile dorsally.

Tract at 3: From ventrally and dorsally placed motor and association neurones. The fibres from the ventral side run vertically upwards and from dorsal side run vertically downwards, some curving out and running along periphery of neuropile. It forms a median vertical partition dividing the ganglion into a right and left half.

Tract at 4: From lateral and dorsolateral group of motor and association neurones. Forms a median commissure.

Tract at 5: From ventral and ventrolateral group of motor and association neurones. Runs upward then curves outwards probably contributing to the peripheral nerve.

Tract at 6: From ventral group of motor neurones. Forms a commissure just below the centre.

Tract at 7: From internal group of motor neurones. Forms a commissure just above tract at 6.

Tract at 8: From lateral group of association neurones. Forms a commissurre.

Tract at 9: From ventral group of association neurones. Runs dorsomedially forming a weak commissure.

Tract at 10: From dorsolateral boundary of neuropile. Forms a weak commissure near dorsal end of ganglion.

Tract at 11: From ventral group of association neurones. Runs vertically upwards curving outward near dorsal side, and is finally lost in the ventral glomerular body. The corresponding tracts of opposite sides run parallel to each other until they curve outwards.

Tract at 12: From lateroventral group of motor and association neurones. Runs upwards turning medially crossing midline on the dorsal side.

Tract at 13: From lateroventral group of motor neurones. Runs dorsomedially forming a commissure.

Tract at 14: From laterally placed motor neurones. Forms a commissure.

Tract at 15: From ventrally placed motor neurones. Runs dorsomedially forming a commissure just below tract at 14.

Tract at 16: From dorsolateral and lateral motor neurones. Runs dorsomedially. Curves downwards and run down, where it is lost in ventral glomerular body.

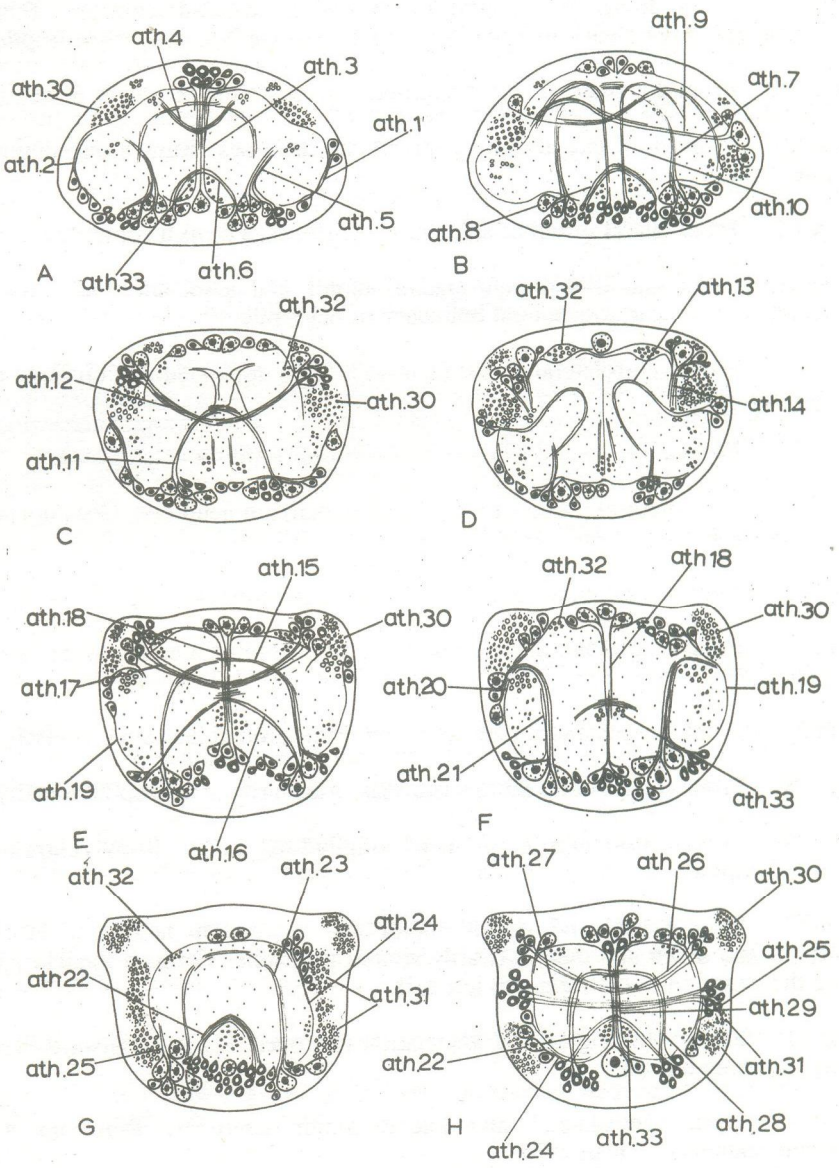


**Fig. 2.** Diagrammatic serial drawings of adult metathoracic ganglion cut transversely. (expalnations as Fig. 1).
ath1, ath2.... adult metathoracic fibre tracts.

Tract at 17: From dorsal and ventral motor and association neurones. Fibres run vertically up and down then curve outwards running along boundary of neuropiles.

Tract at 18: From dorsolateral motor neurones. Runs around boundary of neuropile.

Tract at 19: From dorsolateral and lateral motor neurones. Runs along boundary of neuropile ventrally.

Tract at 20: From lateral group of association neurones. Forms a commissure.

Tract at 21: From lateral and ventrolateral motor and association neurones. Goes upwards and is lost near dorsolateral boundary of neuropile.

Tract at 22: From ventrolateral motor neurones. Runs dorsomedially crossing midline on dorsal side.

Tract at 23: From ventrolateral association neurones. Forms a commissure.

Tract at 24: From ventral group of motor and association neurones. Goes dorsalwards curving medially crossing midline.

Tract at 25: From lateral motor neurones. Forms a commissure.

Tract at 26: From ventrolateral association neurones. Forms a commissure just below tract at 25.

Tract at 27: From dorsolateral motor neurones. Runs around neuropile ventrally.

Tract at 28: From ventrolateral motor neurones. Runs around neuropile dorsally.

Tract at 29: From a dorsolateral group of longitudinal axons. Runs ventromedially and crosses midline.

Tract at 30: From dorsal and ventral motor and association neurones. Fibres run ventrally up and down and then outwards around neuropile. Forms a median partition dividing the ganglion into a right and left half.

Tract at 31: From ventral group of association neurones. Runs dorsalwards and then medially, crossing midline.

Tract at 32: From ventrolateral and lateral motor neurones. Runs up towards dorsolateral boundary of neuropile.

Tract at 33: From neuropile and some motor neurones. Forms the first peripheral nerve of this ganglion. It leaves laterally.

Tract at 34: From neuropile. Forms the second peripheral nerve, leaving the ganglion ventrolaterally.

Tract at 35: From intraganglionic connectives at the anterior end of ganglion. A thick bundle of axons, some of them are lost in the neuropile while the others pass through the whole length of ganglion and join the connectives at the posterior end.

Tract at 36: The interganglionic connectives at the anterior end of ganglion. Runs longitudinally at the posterior end of gnglion.

Tract at 37: From connectives at the anterior end of ganglion. Runs through the ganglion longitudinally and joins the connectives at the posterior end of the ganglion.

Tract at 38: From connectives at the anterior end of ganglion. It traverses the whole ganglionic length and continues into the meta-thoracic ganglion.

The last four tracts are longitudinal while the remainder are transverse ones. Comparable tracts can be found among those of the adult prothoracic ganglion but there is no corresponding tract to at.35 (Ali, 1993) in the mesothoracic ganglion. It 4, 5, 15, and 29 are the only larval tracts not represented in the adult while at 10, 13, 14, 15, 20, 21, 24, 25, and 29 are adult tracts not represented in the larva (Ali, 1993).

Paired tracts and other features of adult metathoracic ganglion are as following:

Tract ath.1: From dorsal and lateral motor neurones. Runs around neuropile ventrally.

Tract ath.2: From lateral motor neurones. Runs around neuropile dorsally.

Tract ath.3: From dorsal and ventral motor and association neurones. Fibres run vertically up and down then curve outwards to run around neuropile. It forms a median vertical partition dividing the ganglion into a right and left half.

Tract ath.4: From group of axons cut transversely on dorsal boundary of neuropile in the sections. Runs medioventrally crossing the midline.

Tract ath.5: From ventral and ventrolateral motor and association neurones. Runs upwards and then outwards towards dorsolateral boundary of neuropile, probably contributes to the peripheral nerve.

Tract ath.6: From ventral group of motor neurones. Forms weak commissure.

Tract ath.7: From ventrolateral motor and association neurones. Runs dorsalwards, then curves medially crossing midline on dorsal side.

Tract ath.8: From ventral group of association neurones. Froms a commissure.

Tract ath.9: From dorsolateral and lateral motor neurones. Runs medially and the corresponding opposite tracts cross each other at mid-dorsal line, making a chiasma. After crossing their fibres continue along peiphery of neuropile.

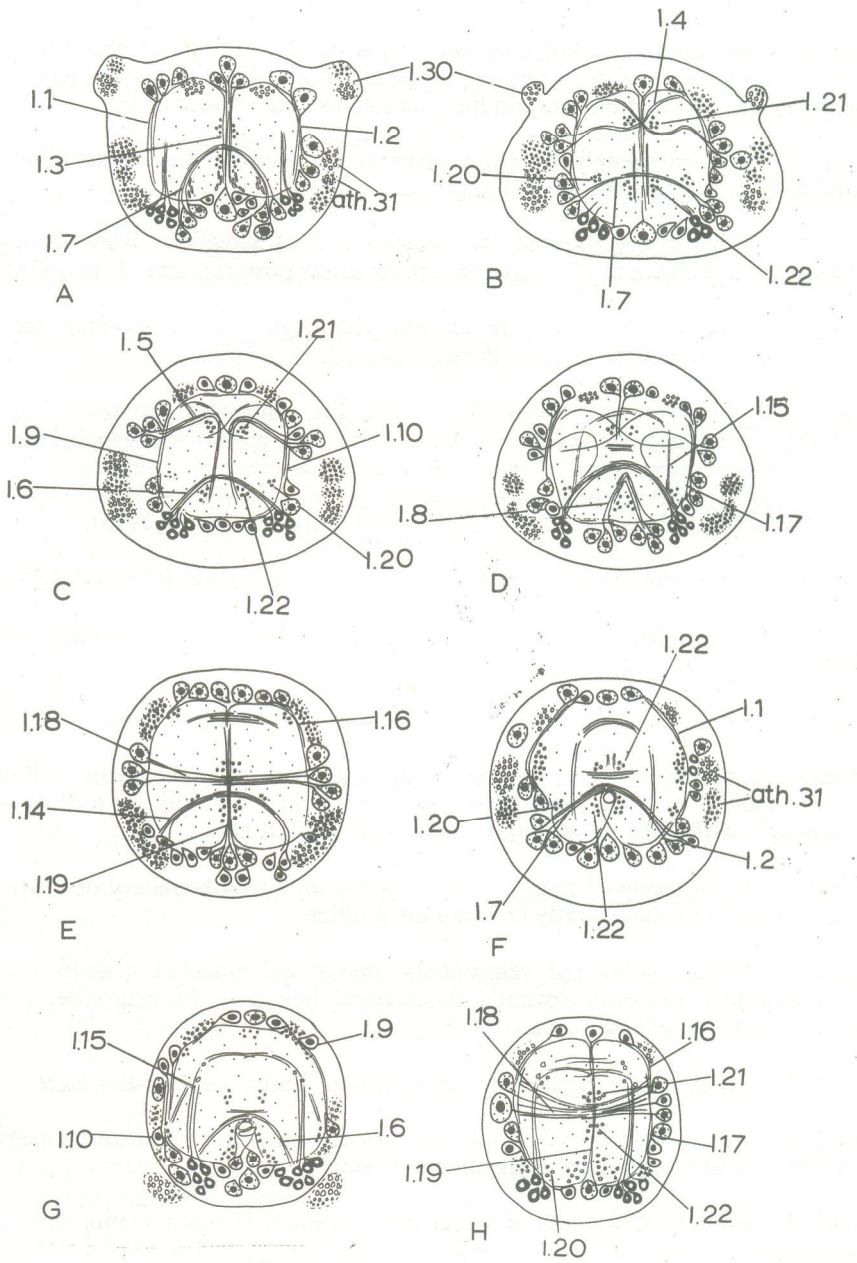


**Fig. 3.** Diagrammatic serial drawings of adult first and second abdominal ganglia cut transversely and showing the same features and with the same explanations as Fig. 1. A-E, first abdominal ganglion (explanations as Fig. 1)
1.1, 1.2 Adult abdominal fibre tract.

Tract ath.10: From venral group of association neurones. Runs dorsalwards and then curves outwards, finally lost in lateral glomerular body.

Tract ath.11: From ventrolateral motor neurones. Runs dorsomedially and crosses midline.

Tract ath.12: From dorsolateral motor and association neurones. Runs dorsomedially and crosses midline.

Tract ath.13: From lateral motor neurones. Runs dorsomedially, curving outwards near midline, then runs outwards and is finally lost in ventral glomerular body.

Tract ath.14: From dorsolateral motor and association neurones. Runs downwards and is then lost in neuropile.

Tract ath.15: From ventrolateral motor and association neurones. Runs downwards and crosses midline on dorsal side.

Tract ath.16: From ventrolateral motor neurones. Runs dorsoventrally and crosses midline.

Tract ath.17: From dorsolateral motor and association neurones. Runs ventrolaterally and crosses midline near tract arh.16.

Tract ath.18: From dorsal and ventral motor and association neurones. Fibres run vertically up and down and then outwards along periphery of neuropile forming a median vertical partition.

Tract ath.19: From dorsolateral and lateral motor neurones. Runs around neuropile ventrally.

Tract ath.20: From lateral motor neurones. Runs around neuropile dorsally.

Tract ath.21: From ventrolateral motor and association neurones. Runs upwards and then turns outwards. It seems to contribute to motor axons of peripheral nerve.

Tract ath.22: From ventral association neurones. Forms a commissure.

Tract ath.23: From ventrolateral motor and association neurones. Runs dorsalward and then turns medially to cross midline.

Tract ath.24: From dorsolateral motor neurones. Runs around neuropile ventrally.

Tract ath.25: From ventrolateral motor neurones. Runs around boundary of neuropile dorsally.

Tract ath.26: From dorsolateral motor neurones. Runs ventromedially forming a

*Glomerular bodies*

In the larval stages two pairs of glumerular bodies are found in each thoracic ganglion. The first pair appears in the middle of the ganglion on the ventral side. It ends towards the posterior end of each ganglion. The second pair is lateral, also in the middle of the ganglia and ending towards the posterior end. The fibres of tracts It. 9, 11 and 15 break up in both lateral and ventral bodies (Ali, 1991; 1993).

In the adult only traces of the larval ventral glomerular body can be seen, but the lateral pair is quite prominent. Apart from these there is also a dorsal glomerular body in the adult ganglia. It is near the posterior end of each ganglion and appears after 72 hours of pupal life when the ventral glomerular body loses its prominences, till in the 120 hours pupa they assume the adult condition.

*First and second abdominal ganglia*

In general appearance the first and second abdominal ganglia of the larva are exactly the same as any other simple abdominal ganglia, though they are in some ways more akin to the sixth abdominal ganglion which fuses with the seveth and eighth abdominal ganglia during metamorphosis. In the same way the first and second abdominal ganglia fuse with the mtathoracic ganglion and thus lose their external identity forming a part of the thoracic nervous centre of the adult.

In the stages when these ganglis are intact, all the normal abdominal fivre tracts can be seen but later they comprise only an undifferentiated mass of vacuolated perineurial cells surrounding the connectives. Peripheral nerves are still seen to run through the perineurial tissue and leave the ganglis. Even in the late pupal stages and the adult some of the abdominal tracts can be seen. For example, the first abdominal ganglion has the following tracts in the adult: 1. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 14, 15, 16, 17, 18, 19, 20, 21, and 22. The second abdominal ganglion has the following tracts in the late pupal stages and the adult: 1. 1, 2, 6, 7, 9, 10, 15, 16, 17, 18, 19, 20, 21, and 22 (Ali, 1991). The remaining 5th instar larval tracts of these ganglia, as described by Ali (1993), evidently disappear at metamorphosis and no specifically adult tracts arise in theie place.

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# DDT-INDUCED HAEMOTOXICITY IN SPRAGUE DAWLEY RATS*

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**Abstract**: The various haematological parameters in albino rats were prominently altered after oral administration of DDT (100mg, 20mg and 10mg/kg body wt/day) alongwith the feed for a total period of 48 hours, 15 days and 18 months respectively. Total erythrocytic count (TEC) and haemoglobin (Hb) content was reduced upto 16 and 11% (48 hours), 17 and 9% (15 days) and 14 and 8% in case of 18 month treatment, respectively. The decrease in PCV was 7 and 8% after 15 days and 18 months of DDT feeding. Among the slight but various indices values, the meam capuscular haemoglobin concentration (MCHC) exhibited significant decline at 48 hours (10%) and 15 days (5%). Mean carpuscular volume (MCV) and mean carpuscular haemoglobin (MCH) increased up to 19 and 12% during 48 hours, 26 and 9% during 15 days and 13 and 11% in 18 months experiments respectively. Significant increase (48%, 64%, and 29% was also observed in total leukocytic count (TLC) in all three treatments, respectively. The study suggests that DDT is extremely toxic compound which involves variety of pathology in the blood components of non-targent systems.

**Key words**: DDT toxicity, blood morphology haematology, rats.

## INTRODUTION

Haematological characteristics have been widely used in the diagnosis of variety of diseases and pathologies, induced by industrial components, drugs, dyes, heavy metals, pesticides and several others (Morgan and Stockdale, 1980; Hermanowicz *et al.*, 1982; Szubartowska, 1983; Ali *et al.*, 1988; Ali and Shakoori, 1988; Pain and Rattner, 1988; Haniffa and Vijayarani, 1989).

The measurement of haemoglobin, erythrocytic and leukocytic counts, erythrocyte sedimentation rate, haematocrit estimation and various haematological indices values are considered sensitive and valuable indicators of mild and sublethal exposure of animals and humans to variety of toxic pollutants (Ali and Shakoori, 1981, 1990, 1993; Nishihara and Utsumi, 1983; Shakoori *et al.*, 1988, 1990, 1992; Jabbar *et al.*, 1991). Organochlorine pesticides constitute a very significant proportion of these environmental pollutants which have been used quite extensively and indiscriminately in the past several decades for control of agricultural and public health insect pests (Artemev *et al.*, 1992; Douthwaite, 1992). Although the use and manufacture of these pesticides has been banned or restricted in many parts of the world, they are still being used in several third world countries including India and Pakistan (Metcalf, 1973; Suzuki *et al.*, 1976; Parveen and Masud, 1983; Jabbar *et al.*, 1991; Lodha and Saxena, 1991; Bhatnagar *et al.*, 1992; Calero *et al.*, 1992; Chandra *et al.*, 1992).

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Highly stable and persistent nature of these compounds and their metabolites, which may be equally toxic as the parent compound, in plants, animals, soil, water and air, is the real cause of concern for toxicologists and public health scientists ans engineers (Suzuki *et al.*, 1976; Mugambi *et al.*, 1989; Radulescu *et al.*, 1990; Bhatnagar *et al.*, 1992; Chandra *et al.*, 1992; Hitch and Day, 1992; Sahu *et al.*, 1992). Because of this property these organochlorine pesticides enter and move in the food chains (Atuma, 1985; Sitarska *et al.*, 1991; Hargrave *et al.*, 1992; Hovinga *et al.*, 1993), and inflict variety of toxic effects in the animal systems (Mukharjee *et al.*, 1980; Saigal *et al.*, 1982; Ali *et al.*, 1988; Ali and Shakoori, 1981, 1988; Shakoori *et al.*, 1988, 1992).

This study was undertaken to investigate the short and long term effects of strong and weak doses of DDT on some haematological parameters of laboratory rats.

## MATERIALS AND METHODS

A colony of Sprague Dawley albino rats, raised in the animal house of the Department of Zoology, University of the Punjab was used in the present study. For short term experiments two groups of female rats, about 6 months of age were used. One group with 180 g average weight was used for feeding insecticide for 48 hours, while the second with average weight 196 g was used for feeding insecticide for 15 days. For long term experiments, the rats weighing about 90-105 g and four months of age were used. They were provided with feed (see Shakoori *et al.*, 1988) and water *ad libitum*.

*Insecticide used and its administration*

A chlorinated insecticide DDT; 1,1,1,- trichloro-2,2-bis (4-chlorophenyl) ethane, was obtained from the Plant Protection Division of Punjab Agriculture Department, in the form of 75% powder which was administered to animals alongwith feed. DDT was administered to rats as strong and weak doses. For short term (ST) experiments, two levels of strong doses of DDT were administered. In one group of rats a strong dose of 100 mg/kg body weight/day (0.4 $LD_{50}$) was administered for a total period of 48 hours (ST-I). In the second group 20 mg/kg body weight/day (0.08 $LD_{50}$) was administered for a total period of 15 days (ST-II).

A weak dose at a rate of 10mg/kg body weight/day (0.04 $LD_{50}$) was administered to another group of rats for 18 months. For ST-I experiment, DDT mixed diet was prepared by mixing 800 mg of 75% DDT in 1 kg of dry feed. Since each experimental rat, on the average, consumed 30 g of feed daily, it will take 100 mg/kg body wight/day. For ST-II, the insecticide mixed diet was prepared by mixing 525 mg of 75% DDT pewder in about one liter molasses-mixed water and added to 3 kg of feed mixture. Each rat of 196 g average weight consumed about 30 g of this feed daily. In this way rats got a DDT dose of 20 mg/kg body weight/day.

For long term experiment, 87.5 mg of 75% DDT powder was mixed/kg of ingredient-mixed feed. That way the rats consumed 10mg DDT/kg body weight/day.

*Procedure adopted*

For short (ST-I and ST-II) and long term experiments three groups of rats, with 8, 20 and 9 animals in each, respectively were fed on three different doses of insecticide mixed diet for various durations as mentioned above. A group of four rats was anaesthetized and slaughtered after 24 and 48 hours in ST-I experiment. Similarly 4 animals each were slaughtered at 3, 6, 9, 12, and 15 day intervals in case of ST-II experiment. In long term experiment a group of three DDT fed animals was slaughtered at 6, 12, and 18 month durations. After stipulated periods the blood samples were collected from inferior vena cava with 10 ml disposable syringe and used for various types of analyses. About 1 ml of blood was quickly collected in tubes containing EDTA as an anticoagulant and mixed gently. This sample was used for study of various haematological parameters which involved the estimation of haemoglobin (Hb) content according to Van-Kampan and Zijlstra (1961), packed cell volume (PCV) according to microhaematocrit method of Strumia *et al.*, (1954) and total erythtocytic cout (TEC) and total leukocytic count (TLC) according to routine clinical methods. The data obtained was then utilized for calculating different haematological indices *i.e.* mean carpuscular haemoglobin (MHC) mean carpuscular volume (MCV) and mean carpuscular haemoglobin concentration (MCHC) according to Dacie and Lewis (1977).

## RESULTS

Administration of DDT alongwith feed as three different concentrations and durations induced significant changes in rat blood. Tables I, II, and III show the effects of DDT feeding at 100, 20 and 10 mg/kg body weight/day for total durations of 48 hours, 15 days and 18 months, respectively on various haematological parameters.

The Hb content, TEC and PCV exhibited significant reduction. The Hb content decreased upto 11% in 48 hours (Table I), 9% in 15 days (Table II) and 8% in case of 18 months (Table III) DDT feeding experiment. The decrease in TEC was 16% (48 hours), upto 17% (15 days) and 15, 13 and 14% in case of 6, 12 and 18 months DDT intoxication, respectively. The PCV content did not show any significant change in 48 hour experiment at 100 mg/kg dose level but reduced upto 7% in 15 days and upto 8% in 18 months toxicant feeding.

Corresponding changes were also observed in various haematological indices. Statistically significant but slight decrease was also found in MCHC which was 10% in 48 hours and upto 5% in 15 days DDT treatment and remained unchanged in long term experiment (Table III). DDT at all doses produced significant increase in MCV and MCH. The rise in MCV in ST-I experiment was 19%, in ST-II experiment it was upto 26%, while 13% in case of 18 months of un-interrupted DDT feeding.

Another important parameter which showed maximum and consistent increase was TLC. The rise in this case was upto 48% in 48 hours treatment, from 13-64% in 15 days treatment and upto 19% in case of 18 months experiment (Table I-III).

**Table I. Effect of feeding DDT mixed diet (100mg/kg body weight/day) for 48 hours on the various haematological parameters of albino rats**

| Parameters | Control | DDT - fed animals | |
|---|---|---|---|
| | (n=7) | 24 hours (n=4) | 48 hours (n=4) |
| Hb (g/dl) | 13.27±0.14[a] | 12.47±0.25* | 11.87±0.22*** |
| TEC ($X10^6/\mu l$) | 6.84±0.25 | 5.76±0.17** | 5.74±0.13** |
| TLC ($X10^3/\mu l$) | 6.36±0.20 | 9.00±0.44*** | 9.44±0.20*** |
| PCV (%) | 42.09±0.34 | 41.94±0.29 | 41.81±0.20 |
| MCV (fl) | 61.56±0.17 | 72.95±1.66*** | 72.93±1.34*** |
| MCH (pg) | 19.38±0.08 | 21.67±0.53** | 20.69±0.42** |
| MCHC (g/dl) | 31.52±0.09 | 29.72±0.39** | 28.39±0.39*** |

[a]Mean±SEM, student's `t' test; *P<0.05; **P<0.01; ***P,0.001
[b]Abreviations used; TEC, total erythrocytic count; TLC, total leukocytic count; Hb, haemoglobin; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; PCV, packed cell volume; dl, deci=100ml; fl, femtolitre=$10^{-15}$ litres; pg, picogram=$10^{-12}$g.

**Table II. Effect of feeding DDT mixed diet (20mg/kg body weight/day) for 15 days on the haematological parameters of albino rats.**

| Parameters | Control | DDT - fed animals | | | | |
|---|---|---|---|---|---|---|
| | (n=6) | 3days (n=4) | 6days (n=4) | 9 days (n=4) | 12 days (n=4) | 15days (n=4) |
| Hb (g/dl) | 13.04±0.16[a] | 12.37±0.39 | 12.28±0.36 | 11.98±0.37* | 11.84±0.41* | 11.85±0.36* |
| TEC ($X10^6$ cells/$\mu l$) | 6.91±0.12 | 6.36±0.12* | 6.29±0.11** | 5.9±0.09*** | 5.88±0.14*** | 5.76±0.14*** |
| TLC ($X10^3$ cells/$\mu l$) | 6.55±0.52 | 7.70±0.56 | 8.67±0.51* | 9.50±0.72** | 9.01±0.66* | 9.57±0.23* |
| PCV (%) | 43.28±0.40 | 42.17±0.93 | 41.76±1.09 | 40.85±0.82* | 40.50±0.97* | 40.48±0.06*** |
| MCV (fl) | 62.64±0.46 | 66.26±0.36*** | 66.34±0.67** | 79.21±0.89*** | 68.82±0.25*** | 70.41±0.68*** |
| MCH (pg) | 18.88±0.28 | 19.42±0.27 | 19.50±0.26 | 20.29±0.17** | 19.75±0.16* | 20.56±0.24** |
| MCHC (g/dl) | 30.15±0.36 | 29.31±0.32 | 29.39±0.14 | 29.31±0.05* | 28.70±0.23** | 29.22±0.19* |

[a]Mean ± SEM, Student's `t' test; *P<0.05; **P<0.01; ***P<0.001
For other details, see Table I.

**Table III. Effect of feeding DDT mixed diet (10mg/kg body weight/day) for 6-18 months on the various haematological parameters of albino rats.**

| Parameters | DDT feeding experiment (months) | | | | | |
|---|---|---|---|---|---|---|
| | 6 | | 12 | | 18 | |
| | Control (n=6) | DDT fed (n=3) | Control (n=4) | DDT fed (n=3) | Control (n=6) | DDT fed (n=3) |
| Hb (g/dl) | 13.79±0.32[a] | 12.79±0.51 | 13.14±0.23 | 12.64±0.26 | 13.04±0.16 | 12.02±0.19** |
| TEC ($X10^6$ cells/$\mu$l) | 7.09±0.12 | 6.08±0.11*** | 7.05±0.13 | 6.14±0.15** | 6.91±0.12 | 5.99±0.13** |
| TLC ($X10^3$ cells/$\mu$l) | 6.55±0.40 | 7.20±0.11 | 6.24±0.13 | 7.43±0.32* | 6.55±0.52 | 8.43±0.28* |
| PCV (%) | 45.92±0.44 | 42.45±0.22*** | 42.90±0.20 | 40.37±0.39*** | 43.28±0.40 | 40.75±0.63* |
| MCV (fl) | 64.38±0.65 | 69.81±0.81** | 60.90±0.86 | 69.06±1.10*** | 62.64±0.46 | 68.01±0.92** |
| MCH (pg) | 19.45±0.32 | 21.01±0.55* | 18.64±0.06 | 20.60±0.22*** | 18.88±0.28 | 20.05±0.21* |
| MCHC (g/dl) | 30.24±0.65 | 30.12±1.05 | 30.62±0.41 | 29.84±0.33 | 30.15±0.36 | 29.48±0.09 |

[a]Mean ± SEM, Student's `t' test; *P<0.05; **P<0.01; ***P<0.001
For other details, see Table I.

## DISCUSSION

The Hb, TEC, PCV and MCHC decreased in almost all treatments after DDT treatment, while TLC, MCV and MCH increased at the same time. The TEC decreased 16%, 17% and 14% after DDT treatment for 48 hours, 15 days and 18 months administration at their respective doses. On analysing the data, it is clear that strong doses of DDT induced immediate reduction in Hb and TEC while in other two experiments the effect was delayed and induced gradually, which was indicated by absence of any change at 3 and 6 day treatments in 15 days experiment and at 6 and 12 months in 18 month DDT feeding experiments. No change was recorded in PCV in 48 hours experiment and during early part (up to 6 days) of 15 days experiment. Similarly MCH and MCHC remained resistant to DDT toxicity up to day 6 in 15 day experiment.

The parameters, most sensitive to DDT intoxication were TEC and TLC which showed alterations in all treatments. The study indicates that DDT administration at all above doses and durations is responsible for the induction of anemia and leukocytosis in rats. Effects of DDT and various other pesticides on blood and its main cellular constituents were reported by different laboratories (Vrochinskii *et al.*, 1976; Traczyk *et al.*, 1978; Raalte and Jansen, 1981; Nisihara and Utsumi, 1983).

The decrease in Hb, PCV and TEC was also observed with another organochlorine insecticide, aldrin (Ali and Shakoori, 1990). Similar changes in haematological parameters were reported in birds with other pesticides (Ali and Shakoori, 1988; Szubartowska, 1983; Mandal and Lahiti, 1985; Qadri *et al.*, 1987; Ali *et al.*, 1988, 1992). The decrease in TEC and Hb content could be attributed to breakdown of erythrocytes due to DDT treatment (O'Brien and Hamilton, 1979) or it may be ascribed to changes in haemopoietic tissue which according to Nohara (1986) may be caused by the disturbances in the metabolisms of nucleic acids which persist in this tissue. These chlorinated insecticides including DDT have also been shown to be responsible for the appearance of aplastic anemia and bone marrow atrophy in human body (Vrochinski *et al.*, 1976; Sternberg, 1979). Kamarova (1976) showed that prolonged contact with pesticides produce leukemia and hypoplastic anemia in pesticide workers. He further reported that amount of DDT and its main metabolite DDE was greater in the haemopoietic organs of persons who died of hypoplastic anemia than in subcutaneous tissue.

The TLC showed drastic increase after DDT feeding at different durations and doses. In 48 hours feeding experiment, the TLC increased 48%, while in 15 day experiment it was increased 64% at the end of experimental period. In long term experiment the TLC showed gradual increase which was maximum (29%) at 18 months DDT intoxication. The increase in TLC in the present study indicated the induction of body's defence against the foreign chemicals. The TLC may also increased in response to abnormal or subnormal leukocytic functioning due to toxic effects of DDT (Hermanowicz, 1982). DDT also stimulate the lymphocyte mitogenesis under certain condition (McCable and Nowak, 1986). The MCV and MCH also showed significant increase after DDT treatment, while MCHC either exhibited decrease as in both short term experiments or remained unchanged as in long term DDT treatment. The increased values of MCV and MCH and decreased MCHC revealed that anemia produced was of macrocytic type.

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# ISOLATION OF BACTERIA OF *PSEUDOMONAS* AND *ENTEROBACTER* Spp. FROM SOIL AND STUDY OF THEIR ABILITY TO DEGRADE ORGANIC POLLUTANTS

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**Abstract**: Fifteen isolates of *Enterobacter* and *Pseudomonas* spp. were checked for their ability to degrade naphthalene, salicylate and benzoate. Seven of the isolated strains were able to degrade all three hydrocarbons, three could degrade two hydrocarbons while six of the strains degraded only one of the hydrocarbons. The hydrocarbons used in the study are usual pollutants from industrial and urban waste. The possibility of the use of these bacteria in degrading environmental pollutants is discussed.

**Key words**: *Pseudomonas*, *Enterobacter*, hydrocarbon degradation, hydrocarbon pollutants, aromatic hydrocarbons.

## INTRODUCTION

Aromatic compounds are abundant in the biosphere as products of plant metabolism, wastes from industry, and as pollutants. Chlorinated aromatic compounds are usually toxic and are source of health hazards for animals and human beings due to their mutagenic and carcinogenic effects. A number of bacterial strains have been isolated from soil and waste-waters which play an important role in degrading organic compounds. These bacteria are capable of using aromatic compounds as sole source of their energy requirements and thus break down aromatic compounds. Many of these microorganisms degrade chlorinated benzoic acid, chlorinated phenols, chlorinated phenoxyacetic acid, and chlorinated benzene through a common pathway to ß-ketoadepate (Reincke and Knackmuss, 1988; Eaton and Chapman, 1992).

Study of the metabolic pathways involving breakdown of chlorinated hydrocarbons is usually undertaken with the objective that the elaboration of these pathways would lead to use of bacteria and the degradative enzymes in breaking down more and more toxic chlorinated hydrocarbons which are constantly appearing in the environment due to agricultural industry or other industries and due to dumping of wastes. In this way much information has collected about the enzymology and molecular regulation of aerobic pathways of aromatic compound degradation (Dagley, 1986; Harayama and Neidle, 1992; Ornston *et al.*, 1990; van der Meer *et al.*, 1992; Harwood *et al.*, 1994). It is important that many of the catabolic enzymes involved in these pathways are similar and the gene clusters that encode them are organized in similar fashions (Daubaras and Chakrabarty, 1992).

The objective of the present study was to isolate bacterial strains from local soil samples capable of degrading organic contaminants, like naphthalene, salicylic acid and benzoate. It was also aimed at checking the possibility of exploiting locally isolated strains for their hydrocarbon degrading potential.

## MATERIALS AND METHODS

*Sample collection*

Soil samples were collected from petrol pumps (service stations), in the city of Lahore, Pakistan using sterile vials and brought to the laboratory. The isolates were identified by staining and biochemical techniques.

*Hydrocarbon degradation*

Isolated strains of *Pseudomonas* and *Enterobacter* spp. were tested for their degradative capabilities by growing the cells on M9 salt medium having benzoate, salicylic acid or naphthalene as the carbon source for the bacteria. Agar plates were used for checking the colony formation.

*Media*

For selection of bacteria for their ability to metabolize aromatic hydrocarbon M9 medium was used but glucose was substituted by the respective hydrocarbon. The medium was prepared by dissolving 0.6g $Na_2HPO_4$, 3g $KH_2PO_4$, and 1g $NH_4Cl$ in distilled water so that the ultimate volume of the medium was one liter. The solution was autoclaved. One ml of 1M $MgSO_4.7H_2O$ and 10ml of 0.01M $CaCl_2$ were autoclaved separately and mixed. When the temperature lowered to about 60°C, benzoate and salicylate were added instead of glucose. The pH of all the solutions was adjusted to 7.0. Naphthalene was sprinkled on agar plates so that its vapors were available to the bacteria for their growth. Agar plates were incubated at 30°C and colonies were observed after 24 hours.

## RESULTS AND DISCUSSION

Various strains isolated from petrol pumps and different hydrocarbon contaminated soils were collected and grown on LB plates. After the appearance of growth these strains were checked for their naphthalene, salicylate or benzoate degrading ability. The culture of the respective strains was spread on plates containing M9 medium in which glucose was substituted with one of the hydrocarbons. Fifteen strains were screened in this way. The growth and appearance of colonies showed the ability of the strain to metabolize hydrocarbon present in the medium. The degradative capability of various strains is given in Table I.

*Pseudomonas* and *Enterobacter* strains isolated from soil samples exposed to petroleum spillage for a long duration harbored hydrocarbon degrading bacteria. Local strains are important in various kinds of operations which are especially beneficial for the local environment, like detoxification of chlorinated aromatic compounds present in the ecosystem. As these bacteria have been exposed to these chemicals for a long time hence they have developed metabolic pathways for degradation of the chemicals. This capability might have been acquired through the process of mutation and natural selection. *Pseudomonas* have some special genes related to catabolism of hydrocarbons.

which are not found in other bacteria. That has provided these bacteria the opportunity to survive in environment contaminated with hydrocarbons. Parallel to that, the scientists have got the opportunity to use these bacteria for the breakdown of certain toxic compounds.

All the fifteen strains of *Pseudomonas* and *Enterobacter* spp. were able to degrade and grow on benzoate, seven of the strains were capable of degrading all three hydrocarbons used in this study, three of the strains were able to catabolize two hydrocarbons and six were capable of degrading only one (Table I). The results show a widespread occurrence of hydrocarbon degradative pathways in many of the bacterial strains, particularly in those which are exposed to contaminated soils or waste-water. Naphthalene constitutes about 10% of the contaminants of our environment. The degradative capacity of a number of strains isolated in this study opens the opportunity for the environmentalists to exploit these bacteria for environmental cleanup. Chlorinated aromatic hydrocarbons are highly toxic, mutagenic and carcinogenic. Microbiological processing of these compounds would lead to cleaner environment with less secondary pollution resultant of any chemical operation.

**Table I. Growth of various isolated strains of *Pseudomonas* and *Enterobacter* spp. on different hydrocarbon sources**

| Strain | Growth on benzoate | Growth on salicylate | Growth on naphthalene |
|---|---|---|---|
| ATCC 50208 | +++ | +++ | + |
| *Pseudomonas* | | | |
| PS69 | +++ | +++ | ++ |
| PS75 | +++ | +++ | ++ |
| P2520 | +++ | +++ | ++ |
| P2556 | +++ | +++ | ++ |
| P2587 | +++ | +++ | ++ |
| P2588 | +++ | +++ | ++ |
| J49 | + | + | ++ |
| *Enterobacter* | | | |
| A3 | ++ | - | ND |
| A5 | + | - | ND |
| B12 | ++ | - | ND |
| B13 | ++ | - | ND |
| E28 | ++ | - | ND |
| E38 | + | - | ND |
| D20 | ++ | - | ND |
| D36 | + | - | ND |

Abbreviation / signs used: -, no growth; +, growth after 3 days; ++, growth after 2 days, +++, growth after 24 hours; ND, not determined.

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# GEOGRAPHICAL DISTRIBUTION OF FRESHWATER FISHES IN PAKISTAN: A REVIEW*

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**Abstract**: There are about 160 native species of freshwater fishes belonging to 69 genera, 23 families, 11 orders, 4 superorders and 3 cohorts of the teleostean fishes found in Pakistan and Kashmir. Of these, about 60% species are South Asian, about 8% species are High Asian, about 4% species are West Asian and about 24% species are endemic to Pakistan and Kashmir. The remaining native species are widely distributed. In addition, there are several species of exotic fishes which are not discussed in this paper. On the basis of the distribution of the native freshwater fishes, Pakistan and Kashmir can be divided into 5 ichthyogeographical provinces: I. Hindukush-Karakoram-Himalayan Province including the Northern Areas and the adjoining parts of NWFP and Kashmir; II. Abasinh-Kashmir Province including the northern montane area of North West Frontier Province and Kashmir south of the Great Himalayan Range; III. Yaghistan Province comprising the north eastern Baluchistan and upper parts of the Gomal and Kurram rivers and the middle part of the Kabul river in Pakistan and Afghanistan; IV. Mehran Province comprising the Indus Plain southeastern Baluchistan east of the Central Brahui and Hala ranges, and the submontane areas of Pakistan and Kashmir; and V. Gedrosian Province including the western Baluchistan and the adjoining parts of Afghanistan and Iran (Hingol river, Lora-Pishin river, Mashkhel river, Dasht river and minor streams along the Makran coast). Of these, the Hindukush-Karakoram-Himalayan Province is a part of the High Asian Region; Abasinh-Kashmir, Yaghistan and Mehran Provinces belong to the South Asian Subregion of the Oriental Region; while the Gedrosian Province may be included in the Southwest Asian or West Asian Transitional Region.

Key words: Freshwater fishes, ichthyogeographical provinces, fish fauna of Pakistan

## INTRODUCTION

Pakistan and Kashmir lie roughly between 24° to 37°N and 60° to 80°E. Excepting the Indus Plain, most of the area is composed of fold mountains, and valleys and plateaus between them. In the north at the border of Pakistan, Afghanistan, Tajikistan and Sinkiang lies the Pamir knot. From here various mountain ranges radiate out in all directions. The Hindukush Range runs in the southwest along the Pak-Afghan border and then passes into Afghanistan. The Tienshan runs towards the northeast into China. The Kunlun Range passes towards the east into China. The Karakoram Range passes towards the east slightly curving towards the south. The Great Himalayas run towards the southeast. In addition, there are many minor ranges such as the Sufaid Koh, the Sulaiman, the Toba Kakar, the Kirthars, the Central Brahui, the Pub, the Hala and the central and coastal Makran ranges.

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*This paper is dedicated to the memory of the late Professor Dr. Javed Ahmad Butt, Chairman, Department of Zoology, University of Peshawar, Peshawar, Pakistan.

Pakistan and Kashmir are drained mainly by the river Indus and its tributaries. The river Indus originates from the West Tibet and flows towards the west behind the Himalayan mountains. After receiving various tributaries in the Northern Areas and Kashmir, it takes a turn towards the southwest. It receives many small rivers such as the Kabul, the Kurram and the Gomal from the North West Frontier Province, and the Haro, the Soan, the Jhelum, the Chenab, the Ravi and the Sutlej from the Punjab and Kashmir. It ultimately falls into the Arabian Sea near Karachi. In addition, there are several small rivers in Baluchistan which are either endorheic (the Lora-Pishin and the Mashkhel) or fall into the Arabian Sea (the Hub, the Purali, the Hingol and the Dasht).

There are about 160 species of native freshwater fishes, belonging to 69 genera, 23 families, 11 orders, 4 superorders and three cohorts of the teleostean fishes. No higher taxon is endemic to Pakistan and Kashmir. Among the species about 60% are South Asian, which are found throughout except the trans-Himalayan parts of the Northern Areas; 8% are High Asian, 4% are West Asian, while about 24% are endemic. The remaining species are widely distributed. At the generic level, there are 45 South Asian, 9 High Asian and 2 West Asian genera, while the remaining 13 genera are widely distributed. No genus is endemic. There are about 65% South Asian, 13% High Asian, 3% West Asian and 13% widely distributed genera in the Palaeotropical regions, East Asia and Southwest Asia. It is remarkable that the genera *Aspidoparia*, *Crossocheilus*, *Schistura* and *Channa* are very widely distributed reaching the limits of Afghanistan in the north and Iran in the west. They are, however, absent in the trans-Himalayan areas.

*Zoogeographical provinces of Pakistan and Kashmir*

From the distribution of freshwater fishes in Pakistan, it appears that this country lies in the peripheral zone of the South Asian Subregion of the Oriental Region. Excepting the trans-Himalayan areas, which contain no representatives of the South Asian fishes, the South Asian genera are represented in almost all parts of this area. There is no genus among the freshwater fishes which could be regarded as endemic to Pakistan. Of 69 genera of freshwater fishes only 9 genera are High Asian and 2 West Asian, while most of the remaining genera are South Asian. A few genera of marine origin are widely distributed. On the basis of freshwater fishes, the following ichthyogeographical provinces of Pakistan may be recognized. In the delimitation of these provinces, the physiography also has been taken into consideration. The mountain ranges can serve as the effective barriers to the dispersal of fishes only if they serve as a watershed for the drainage systems on the two opposite sides and are not intersected by the water channels. In the absence of such a barrier, the environmental factors like the water current, temperature of water, nature of substratum, and the amount of dissolved oxygen and other materials, determine the dispersal of fishes. Thus most of the snow-carps are restricted to the trans-Himalayan parts of the Indus system and only a few come down to the submontane areas.

The limits of these ichthyogeographical provinces are more or less arbitrary as the faunas of the neighbouring ichthyogeographical provinces merge into oneanother often imperceptibly.

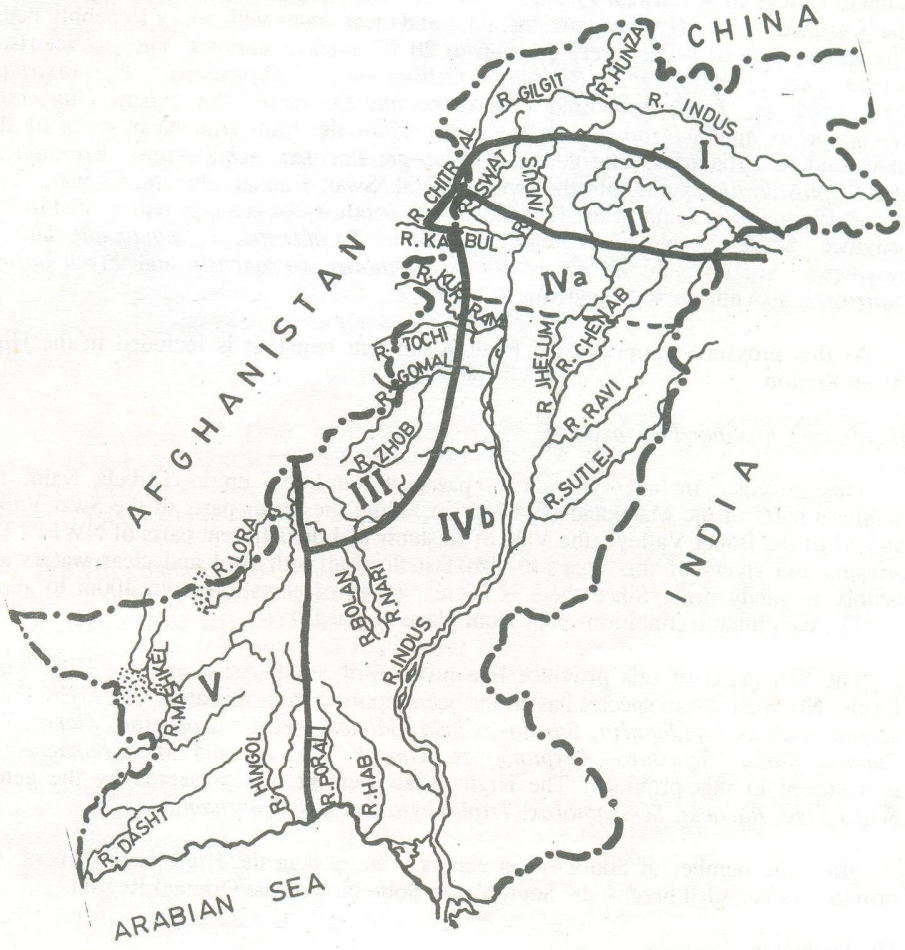


**Fig. 1.** Hydrography of Pakistan and Kashmir with Ichthyogeographical Provinces: I. Hindukush-Karakoram-Himalayan Province; II. Abasinh-Kashmir Province; III. Yaghistan Province; IV. Mehran Province; V. Gedrosian Province

*I. Hindukush-Karakoram-Himalayan Province*

This province comprises the northern montane areas of Pakistan and Kashmir above 1500m including the Northern Areas and the upper parts of the Chitral, Swat and Kaghan valleys in Northwest Frontier Province. The streams and rivers of this area are characterized by strong water currents, cool and clear water with rocky to pebbly beds. The temperature of water rarely goes up to 20 °C even in summer. The characteristic genera are *Schizopyge*, *Racoma*, *Schizothorax*, *Diptychus*, *Ptychobarbus*, *Schizopygopsis*, *Triplophysa* and *Glyptosternum*. Of these, the genera *Diptychus*, *Ptychobarbus* and *Schizopygopsis* are restricted to the trans-Himalayan parts of the Indus and its tributaries. The genera *Schizopyge*, *Racoma*, *Schizothorax*, *Triplophysa* and *Glyptosternum* extend into the rivers Chitral, Swat, Kunhar, Jhelum, Chenab, Ravi etc. and Indus even south of the Himalayas. No South Asian genus is represented in this province. Some species of *Triplophysa* such as *T. microps*, *T. tenuicauda* and *T. trewavasae* are endemic or nearly so. *Schizothorax skarduensis* and *Ptychobarbus conirostris* also appear to be endemic.

As this province comprises the High Asian fish fauna, it is included in the High Asian Region.

*II. Abasinh-Kashmir Province*

This province includes Himalayan parts of the Indus up to Tarbela Dam, the southern parts of the Malakand Division (including the lower parts of the Swat Valley and all of the Buner Valley), the Vale of Kashmir and the adjacent parts of NWFP. The streams and rivers of this area are also fast-flowing with cool and clear waters and pebbly to sandy beds. Since there is a great range of elevation from 400m to above 1,000., the climatic conditions vary from place to place.

The fish fauna of this province is a mixture of South Asian and the High Asian forms. No West Asian species has so far been reported from this area. The South Asian genera, such as *Aspidoparia*, *Barilius*, *Chela*, *Salmostoma*, *Crossocheilus*, *Garra*, *Tor*, *Puntius*, *Botia*, *Schistura*, *Glyptothorax*, *Ompok*, *Channa* and *Mastacembelus* are represneted in this province. The High Asian element is represented by the genera *Schizopyge*, *Racoma*, *Schizothorax*, *Triplophysa* and *Glyptosternum*.

Since the number of South Asian genera is more than the High Asian genera, this province is included here in the South Asian Subregion of the Oriental Region.

*III. Yaghistan Province*

This province includes the northeastern parts of Baluchistan comprising the Zhob river and the upper parts of the Nari river system, the northwestern mountains along the Pak-Afghan border (upper parts of the rivers Gomal, Kurram and the middle part of the river Kabul in Afghanistan). It is demarcated from rest of Pakistan by the Sulaiman Range in the east, Marri-Bugti hills in the south and the Central Brahui Range in the southwest. There is no sharp boundary in the north and northwest where this province

extends into Afghanistan.

The fish fauna of this province is a mixture of South Asian, High Asian and West Asian forms. There are 6 species which appear to be endemic to this province. These are *Schizocypris brucei*, *Schistura arifi*, *S. harnaiensis*, *S. anambarensis* and *S. machensis* from the northeastern Baluchistan, and *Garra wanae* from South Waziristan. It is interesting that the genus *Schizocypris* in Pakistan is restricted to this province. It is also noteworthy that *Garra wanae* is endemic to the Wana Toi and has not been collected from the adjoining streams. The Wana Toi is a small tributary of the river Gomal in South Waziristan.

The South Asian genera are *Labeo*, *Tor*, *Naziritor*, *Garra*, *Crossocheilus*, *Chela*, *Salmostoma*, *Amblypharyngodon*, *Aspidoparia*, *Barilius*, *Puntius*, *Botia*, *Noemacheilus*, *Schistura*, *Ompok*, *Glyptothorax*, *Channa* and *Mastacembelus* etc. The High Asian genera are *Racoma*, *Schizothorax*, *Schizopyge*, *Schizocypris*. The genus *Schizopyge* is found in the river Kabul but not in other rivers of this province. The West Asian element is represented by the genus *Cyprinion* in the rivers Zhob, Gomal and Kurram (but not in the Kabul).

So this province also is included in the South Asian Subregion of the Oriental Region.

*IV. Mehran Province*

This province comprises about 50% area of Pakistan and Kashmir. It includes the Indus Plain and adjoining hills and the vale of Peshawar. The climatic conditions vary from marine type in the southwest with moderate temperature to subtropical type with cold winters and hot summers in other parts of this province.

The fish fauna as a whole is predominantly South Asian. In the hilly areas surrounding the Indus Plain in the north and west some West Asian forms, i.e., *Cyprinion watsoni* and *Aphanuns dispar* are met with. *Aphanius dispar*, however, is restricted to the coastal areas in the southwestern parts of this province, while *Cyprinion watsoni* is widely distributed in the Baluchistan - Sindh hills, northeastern montane areas, Potowar plateau, Salt range, and in the southern parts of Azad Kashmir (Mirpur and Kotli districts). In the vale of Peshawar, northern Punjab and submontane areas of NWFP and Azad Kashmir three genera of the snow carps, *i.e.*, *Racoma*, *Schizopyge* and *Schizothorax* are represented. High Asian genus *Triplophysa* is also represented in the Indus above Attock Khurd. The remaining genera are widely distributed in South Asia: *Notopterus*, *Chitala*, *Barilius*, *Salmostoma*, *Securicula*, *Amblypharyngodon*, *Catla* ( = *Gibelion*), *Acanthocobitis*, *Ailia*, *Sisor*, *Rita*, *Aplocheilus*, *Xenentodon*, *Sicamugil* etc., South and South East Asia: *Chela*, *Brachydanio*, *Danio*, *Barbodes*, *Osteobrama*, *Esomus*, *Puntius*, *Cirrhinus*, *Botia*, *Lepidocephalus*, *Noemacheilus*, *Ompok*, *Wallago*, *Batasio*, *Amblyceps*, *Bagarius*, *Clupisoma*, *Eutropiichthys*, *Pseudeutropius*, *Heteropneustes*, *Colisa*, *Nandus*, *Badis*, *Macrognathus*, etc.

Still other genera are widely distributed in South Asia, South East Asia, East Asia,

Soutwest Asia and even Africa: *Rasbora*, *Aspidoparia*, *Crossocheilus*, *Garra*, *Labeo*, *Tor*, *Schistura*, *Glyptothorax*, *Channa* and *Mastacembelus*. This is the only province where our major carps, viz., *Gibelion catla*, *Labeo rohita* and *Cirrhinus mrigala* are naturally found. In the Indus these fishes generally are found up to Kalabagh except *Cirrhinus mrigala* which has been recorded up to Khushhalgarh. This species can tolerate cold water to some extent. The limits of this Province in the east extend into India up to Aravalli range, which is the waterdivide between the Indus and the Ganges river systems. The fish fauna of the Indus and the Ganges river systems is quite similar but there are some families and many genera and species of fishes found in the Ganges river system, which do not extend into the Indus system. Only a few West Asian genera found in the Indus system are not represented in the Ganges system (Mirza, 1989).

Within this province two divisions can be recognized:

*IV a. Submontane Indus Division*, in which the major carps are absent and the snow carps and mahasheer are common. This division comprises the Vale of Peshawar, Kohat Valley, Potowar Plateau and the Salt Range and adjoining hilly areas.

*IV b. Indus Plain Division,* in which the major carps are common and the snow carps are absent. The mahasheer is rarely found. The palla (*Tenualosa ilisha*) is found in the lower part of the Indus. Several other such species are also found.

This province also is a part of the South Asian Subregion of the Oriental Region.

*V. Gedrosian Province*

This province comprises the western Baluchistan west of the Central Brahui and Hala ranges. Topographically, it varies from the high mountain areas in the east and the arid basins with a few marshy lakes in the inner Baluchistan. The temperature also varies greatly. In the northern part of this province, drained by the Lora-Pishin river and its tributaries, only a few species belonging to the South Asian genus *Crossocheilus*, West Asian *Discognathus* (subgenus of *Garra*), *Cyprinion* and *Schistura* and High Asian *Triplophysa* are found. It is interesting to note that none of the *Schizothoracine* genera distributed in the river Zhob in the east and the river Helmand in the north of the Lora-Pishin river is found in this river. In the river Mashkhel, the South Asian genera *Aspidoparia*, *Labeo*, and *Channa*, and the West Asian genus *Aphanius* are added but the High Asian genus *Triplophysa* is not represented. In the coastal areas, the fish fauna is represented by the South Asian genera *Aspidoparia*, *Crossocheilus*, *Puntius*, *Tor* and *Channa* alongwith the West Asian genera *Cyprinion* and *Aphanius*. In addition, there are several fishes of marine origin that frequently enter the streams and rivers in the coastal areas.

It is noteworthy that the South Asian genera such as *Notopterus*, *Danio*, *Salmostoma*, *Mystus*, *Gudusia* and *Mastacembelus*, which are found in the river *Porali* in the east of the Hala range, have not so far been collected from the rivers west of this range. There are only three endemic species: *Labeo gedrosicus* is endemic to the river Rakhshan, while *Labeo macmohoni* is endemic to the river Dasht, *Cyprinion milesi* is also endemic to this province.

The fish fauna is mainly South Asian and West Asian with one species of High Asian genus *Triplophysa* (restricted to the river Lora-Pishin). So the transitional aspect is apparent and hence this province may be included in the Southwest Asian Transitional Region.

## DISCUSSION AND CONCLUSION

The freshwater fish fauna of Pakistan is predominantly South Asian. Some High Asian genera, *Schizopyge*, *Racoma*, *Schizothorax*, *Diptychus*, *Ptychobarbus*, *Schizopygopsis*, *Triplophysa* and *Glyptosternum*, however, have penetrated into the northern montane areas. Of these, the genera *Racoma* and *Schizothorax* extend into the nothwestern mountains up to the northeastern Baluchistan. Two snow-carp species, viz., *Racoma labiata* and *Schizothorax plagiostomus*, descend into the upper parts of the rivers of the Punjab sporadically. The genus *Schizocypris* is not found in the Northern Areas but is restricted to the Yaghistan Province. This is in contrast to the other *Schizothoracine* genera. One West Asian genus (*Cyprinion*) has been able to disperse into Baluchistan and the submontane areas surrounding the Indus Plain up to the Sufaid Koh and Kala Chitta ranges in the north, and into submontane areas of Azad Kashmir. The West Asian subgenus *Discognathus* of the genus *Garra* is restricted to the northwestern Baluchistan (in Lora-Pishin and Mashkhel rivers).

Thus, the fish fauna of Pakistan is composed of three elements: the South Asian, the High Asian and the West Asian. Among these, the South Asian element is predominant. Excepting the trans-Himalayan parts, which contain exclusively the High Asian genera, the South Asian genera are represented in all the areas of Pakistan. At the species level there are about 60% South Asian, 8%, High Asian, 4% West Asian and 24% endemic forms among the freshwater fishes; while the remaining forms are widely distributed in the Oriental Region.

The fish faunas of the South Asia and the High Asia originated from the same ancestral South-East Asian stock. The present dissimilarity between them is "probably due to their differentiation in different geological ages, long isolation and resulting segregation" (Hora, 1937). The fish fauna of High Asia belongs to three basic groups: *Schizothoracinae*, *Noemacheilidae* and *Glyptosternum*. Among these, the *Schizothoracinae* are specially modified "Oriental Barbels", the *Noemacheiline* loaches have evolved from the South-East Asian ancestral stock, and *Glyptosternum* is closely related to (and probably derived from) the Oriental *Glyptothorax*. The ancestors of these fishes migrated to the High Asian areas probably in the Pliocene and differentiated into the present forms after their isolation due to the Himalayan orogeny. Their dispersal from east to west had been possible through a system of interconnected water bodies forming a westward flowing river along the northern face of Himalayas. Thus the High Asian fauna has been able to penetrate as far as northeastern Iran up to Tehran. Regan (1922) included the Central Asia in the Palaearctic Region. Similarly, Berg (1940) recognized this area as the High Asian Subregion in the Holarctic Region. But according to Banarescu (1975), the High Asia should be included as a subregion in the Oriental Region. Mirza (1989, 90), however, recognized it as a separate region.

There is no controversy about the zoogeographical position of the South Asia. It has

been included in the Oriental Region by almost all the zoogeographers (Beaufort, 1951; Darlington, 1957).

There exists some controversy about the zoogeographical position of the West Asia. It was included in the Palaearctic Region by Regan (1922) and in the Mediterranean Subregion of the Holarctic Region by Berg (1940). According to Banarescu (1960), the freshwater fish fauna of West Asia has an indisputable South Asian (Indo-Malayan) character. He proposed the inclusion of this area as a subregion in the Oriental Region. Subsequently, however, he changed his view and was of the opinion that the fish fauna of the West Asia was closer to the Holarctic than to the Oriental Region (Banarescu, 1973). But the fish fauna of the West Asia shows a transition between the Oriental, the Palaearctic and perhaps the Ethiopean Regions. Darlington's (1957) conclusion that "the southwestern Asia is a region of double transition of fish faunas, the transition being from African to Oriental forms in one direction, and from tropical to northern forms in another" seems quite valid even today. The West Asia, therefore, should be treated as a transitional region (Mirza, 1975). Banarescu (1992) has now came to the same conclusion.

From the above discussion it follows that the freshwater fish fauna of Pakistan is predominantly Oriental and that the Hindukush - Karakoram - Himalayan Provinces should be included in the High Asian Region; the Abasinh-Kashmir Province, the Yaghistan Province and the Mehran Province belong to the South Asian Subregion of the Oriental Region; while the Gedrosian Province may be included in the West Asian Transitional Region as defined by Mirza (1990).

**Acknowledgement**

The author is indebted to Professor Mrs. Habibah Makhdum, Head of the Department of Zoology, Government College for Girls, Gulberg, Lahore, for providing recent literature.

**Table I. Distribution of higher taxa of freshwater fishes in the Ichthyogeographical Provinces of Pakistan**

| Name of Taxa | Ichthyogeographical Provinces | | | | | Status |
|---|---|---|---|---|---|---|
| | I | II | III | IV | V | |
| CLASS ACTINOPTERYGII | | | | | | |
| SUBCLASS NEOPTERYGII | | | | | | |
| INFRACLASS TELEOSTEI | | | | | | |
| COHORT ARCHAEOPHYLACES | - | - | - | + | - | Tropicopolitan |

| Name of Taxa | Ichthyogeographical Provinces | | | | | Status |
|---|---|---|---|---|---|---|
| | I | II | III | IV | V | |
| SUPERORDER OSTEOGLOSSOMORPHA | - | - | - | + | - | Tropicopolitan |
| I. ORDER OSTEOGLOSSIFORMES | - | - | - | + | - | Tropicopolitan |
| FAMILY (1) NOTOPTERIDAE | - | - | - | + | - | Palaeotropical |
| 1. Genus *Chitala* | - | - | - | + | - | Oriental |
| 2. *Notopterus* | - | - | - | + | - | Oriental |
| COHORT CLUPEOCEPHALA | - | - | - | + | + | World-wide |
| SUPERORDER CLUPEOMORPHA | - | - | - | + | + | World-wide |
| II. ORDER CLUPEIFORMES | - | - | - | + | + | World-wide |
| FAMILY (2) CLUPEIDAE | - | - | - | + | + | World-wide |
| 3. *Gudusia* | - | - | - | + | + | Oriental |
| 4. *Tenualosa* | - | - | - | + | + | Oriental |
| COHORT EUTELEOSTEI | + | + | + | + | + | World-wide |
| SUPERORDER OSTARIOPHYSI | + | + | + | + | + | World-wide |
| III. ORDER CYPRINIFORMES | + | + | + | + | + | Megagaeic |
| FAMILY (3) CYPRINIDAE | + | + | + | + | + | Megagaeic |
| SUBFAMILY CULTRINAE | - | + | + | + | - | Eurasian |
| 5. *Chela* | - | + | + | + | - | Oriental |
| 6. *Salmostoma* | - | + | + | + | - | Oriental |
| 7. *Securicula* | - | - | - | + | - | Oriental |

| Name of Taxa | Ichthyogeographical Provinces | | | | | Status |
|---|---|---|---|---|---|---|
| | I | II | III | IV | V | |
| SUBFAMILY RASBORINAE | - | + | + | + | + | Palaeotropical |
| 8. *Amblypharyngodon* | - | - | + | + | - | Oriental |
| 9. *Aspidoparia* | - | + | + | + | + | Oriental |
| 10. *Barilius* | - | + | + | + | - | Oriental |
| 11. *Brachydanio* | - | - | - | + | - | Oriental |
| 12. *Danio* | - | - | - | + | - | Oriental |
| 13. *Esomus* | - | - | - | + | - | Oriental |
| 14. *Rasbora* | - | - | - | + | - | Oriental |
| SUBFAMILY GARRINAE | - | + | + | + | + | Palaeotropical |
| 15. *Crossocheilus* | - | + | + | + | + | Oriental |
| 16. *Garra* | - | + | + | + | + | Palaeotropical |
| SUBFAMILY BARBINAE | - | + | + | + | + | Palaeotropical |
| 17. *Cirrhinus* | - | - | - | + | - | Oriental |
| 18. *Cyprinion* | - | - | + | + | + | West Asian |
| 19. *Gibelion* (=*Catla*) | - | - | - | + | - | Oriental |
| 20. *Labeo* | - | + | + | + | + | Palaeotropical |
| 21. *Naziritor* | - | - | + | + | - | Oriental |
| 22. *Osteobrama* | - | - | - | + | - | Oriental |
| 23. *Puntius* | - | + | + | + | + | Oriental |

| Name of Taxa | Ichthyogeographical Provinces | | | | | Status |
|---|---|---|---|---|---|---|
| | I | II | III | IV | V | |
| 24. *Tor* | - | + | + | + | + | Oriental |
| SUBFAMILY SCHIZOTHORACINAE | + | + | + | + | - | High Asian |
| 25. *Diptychus* | + | - | - | - | - | High Asian |
| 26. *Ptychobarbus* | + | - | - | - | - | High Asian |
| 27. *Racoma* | + | + | + | + | - | High Asian |
| 28. *Schizocypris* | - | - | + | - | - | West Asian |
| 29. *Schizopyge* | + | + | + | + | - | High Asian |
| 30. *Schizopygopsis* | + | - | - | - | - | High Asian |
| 31. *Schizothorax* | + | + | + | + | - | High Asian |
| FAMILY (4) COBITIDAE | - | + | + | + | - | Oriental & Palaearctic |
| 32. *Botia* | - | + | + | + | - | Oriental |
| 33.*Lepidocephalus* | - | - | - | + | - | Oriental |
| FAMILY (5) NOEMACHEILIDAE | + | + | + | + | + | Oriental, East Asian, High Asian, West Asian & Palaearctic |
| 34. *Acanthocobitis* | - | - | + | + | - | Oriental |
| 35. *Noemacheilus* | - | - | + | + | - | Oriental |
| 36. *Schistura* | - | + | + | + | + | Oriental |
| 37. *Triplophysa* | + | + | - | + | + | High Asian |

| Name of Taxa | Ichthyogeographical Provinces | | | | | Status |
|---|---|---|---|---|---|---|
| | I | II | III | IV | V | |
| IV. ORDER SILURIFORMES | + | + | + | + | + | World-wide |
| FAMILY (6) BAGRIDAE | - | - | - | + | - | Palaeotropical |
| 38. *Aorichthys* | - | - | - | + | - | Oriental |
| 39. *Batasio* | - | - | - | + | - | Oriental |
| 40. *Mystus* | - | - | - | + | - | Oriental |
| 41. *Rita* | - | - | - | + | - | Oriental |
| FAMILY (7) SISORIDAE | + | + | + | + | - | Eurasian |
| 42. *Bagarius* | - | - | - | + | - | Oriental |
| 43. *Gagata* | - | - | - | + | - | Oriental |
| 44. *Glyptosternum* | + | + | - | - | - | High Asian |
| 45. *Glyptothorax* | - | + | + | + | - | Eurasia |
| 46. *Nangra* | - | - | - | + | - | Oriental |
| 47. *Sisor* | - | - | - | + | - | Oriental |
| FAMILY (8) SILURIDAE | - | + | + | + | - | Oriental & Palaearctic |
| 48. *Ompok* | - | + | + | + | - | Oriental |
| 49. *Wallago* | - | - | - | + | - | Oriental |
| FAMILY (9) SCHILBEIDAE | - | - | - | + | - | Palaeotropical |
| 50. *Alia* | - | - | - | + | - | Oriental |
| 51. *Clupisoma* | - | - | - | + | - | Oriental |

| Name of Taxa | Ichthyogeographical Provinces | | | | | Status |
|---|---|---|---|---|---|---|
| | I | II | III | IV | V | |
| 52. *Eutropiichthys* | - | - | - | + | - | Oriental |
| 53. *Pseudeutropius* | - | - | - | + | - | Oriental |
| FAMILY (10) HETEROPNEUSTIDAE | - | - | - | + | - | Oriental |
| 54. *Heteropneustes* | - | - | - | + | - | Oriental |
| FAMILY (11) AMBLYCIPITIDAE | - | - | - | + | - | Oriental |
| 55. *Amblyceps* | - | - | - | + | - | Oriental |
| SUPERORDER ACANTHOPTERYGII | - | - | - | + | + | World-wide |
| SERIES MUGILOMORPHA | - | - | - | + | + | World-wide |
| V. ORDER MUGILIFORMES | - | - | - | + | + | World-wide |
| FAMILY (12) MUGILIDAE | - | - | - | + | + | World-wide |
| 56. *Sicamugil* | - | - | - | + | + | Oriental |
| SERIES ATHERINOMORPHA | - | - | - | + | + | World-wide |
| VI. ORDER BELONIFORMES | | - | - | + | + | World-wide |
| FAMILY (13) BELONIDAE | - | - | - | + | + | World-wide |
| 57. *Xenentodon* | - | - | - | + | - | Oriental |
| VII. ORDER CYPRINODONTIFORMES | - | - | - | + | + | Tropicopolitan |
| FAMILY (14) APLOCHEILIDAE | - | - | - | + | - | Tropicopolitan |
| 58. *Aplocheilus* | - | - | - | + | - | Oriental |
| FAMILY (15) CYPRINODONTIDAE | - | - | - | + | + | Tropicopolitan |
| 59. *Aphanius* | - | - | - | + | + | West Asian & Mediterranean |

| Name of Taxa | Ichthyogeographical Provinces | | | | | Status |
|---|---|---|---|---|---|---|
| | I | II | III | IV | V | |
| SERIES PERCOMORPHA | - | - | - | + | + | World-wide |
| VIII. ORDER CHANNIFORMES | - | - | - | + | + | Palaeotropical |
| FAMILY (16) CHANNIDAE | - | - | - | + | + | Palaeotropical |
| 60. *Channa* | - | - | - | + | + | Palaeotropical |
| IX. ORDER SYNBRANCHIFORMES | - | - | - | + | - | World-wide |
| FAMILY (17) SYNBRANCHIDAE | - | - | - | + | - | World-wide |
| 61. *Monopterus* | - | - | - | + | - | Oriental |
| X. ORDER MASTACEMBELIFORMES | - | + | + | + | - | Palaeotropical |
| FAMILY (18) MASTACEMBELIDAE | - | + | + | + | - | Palaeotropical |
| 62. *Macrognathus* | - | - | - | + | - | Oriental |
| 63. *Mastacembelus* | - | + | + | + | - | Oriental & West Asian |
| XI. ORDER PERCIFORMES | - | - | - | + | + | World-wide |
| SUBORDER PERCOIDEI | - | - | - | + | + | World-wide |
| FAMILY (19) CHANDIDAE | - | - | - | + | - | World-wide |
| 64. *Chanda* | - | - | - | + | - | Oriental |
| 65. *Parambassis* | - | - | - | + | - | Oriental |
| FAMILY (20) NANDIDAE | - | - | - | + | - | Tropicopolitan |
| 66. *Nandus* | - | - | - | + | - | Oriental |
| FAMILY (21) BADIDAE | - | - | - | + | - | Oriental |

| Name of Taxa | Ichthyogeographical Provinces | | | | | Status |
|---|---|---|---|---|---|---|
| | I | II | III | IV | V | |
| 67. *Badis* | - | - | - | + | - | Oriental |
| SUBORDER GOBIOIDEI | - | - | - | + | + | World-wide |
| FAMILY (22) GOBIIDAE | - | - | - | + | + | World-wide |
| 68. *Glossogobius* | - | - | - | + | + | Tropicopolitan |
| SUBORDER ANABANTOIDEI | - | - | - | + | - | Palaeotropical |
| FAMILY (23) BELONTIIDAE | - | - | - | + | - | Oriental |
| 69. *Colisa* | - | - | - | + | - | Oriental |

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