MECHANISM OF SALINITY ADAPTATION IN FISH, *OREOCHROMIS MOSSAMBICUS* (TILAPIA) SPECIES

THESIS SUBMITTED TO GOA UNIVERSITY, FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY

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CERTIFICATE

This is to certify that, the thesis entitled "**Mechanism of salinity adaptation in fish, *Oreochromis mossambicus* (Tilapia) species**", submitted by Miss. Kalpana V. Kamat for the award of the Degree of Doctor of Philosophy in Zoology, is based on her original and independent work carried out by her during the period of study and that it has not previously formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or similar other titles in any University or Institute.

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STATEMENT

I state that the thesis entitled, **"Mechanism of salinity adaptation in fish, *Oreochromis mossambicus* (Tilapia) species"**, is my original contribution and that the same has not been submitted on any previous occasion for any other Degree or Diploma of this University or any other University / Institute. To the best of my knowledge, the present study is the first comprehensive study of its kind from the area mentioned.

The literature conceiving the problem investigated has been cited. Due acknowledgements have been made wherever facilities and suggestions have been availed of.

Kalpanakamat

KALPANA V. KAMAT.

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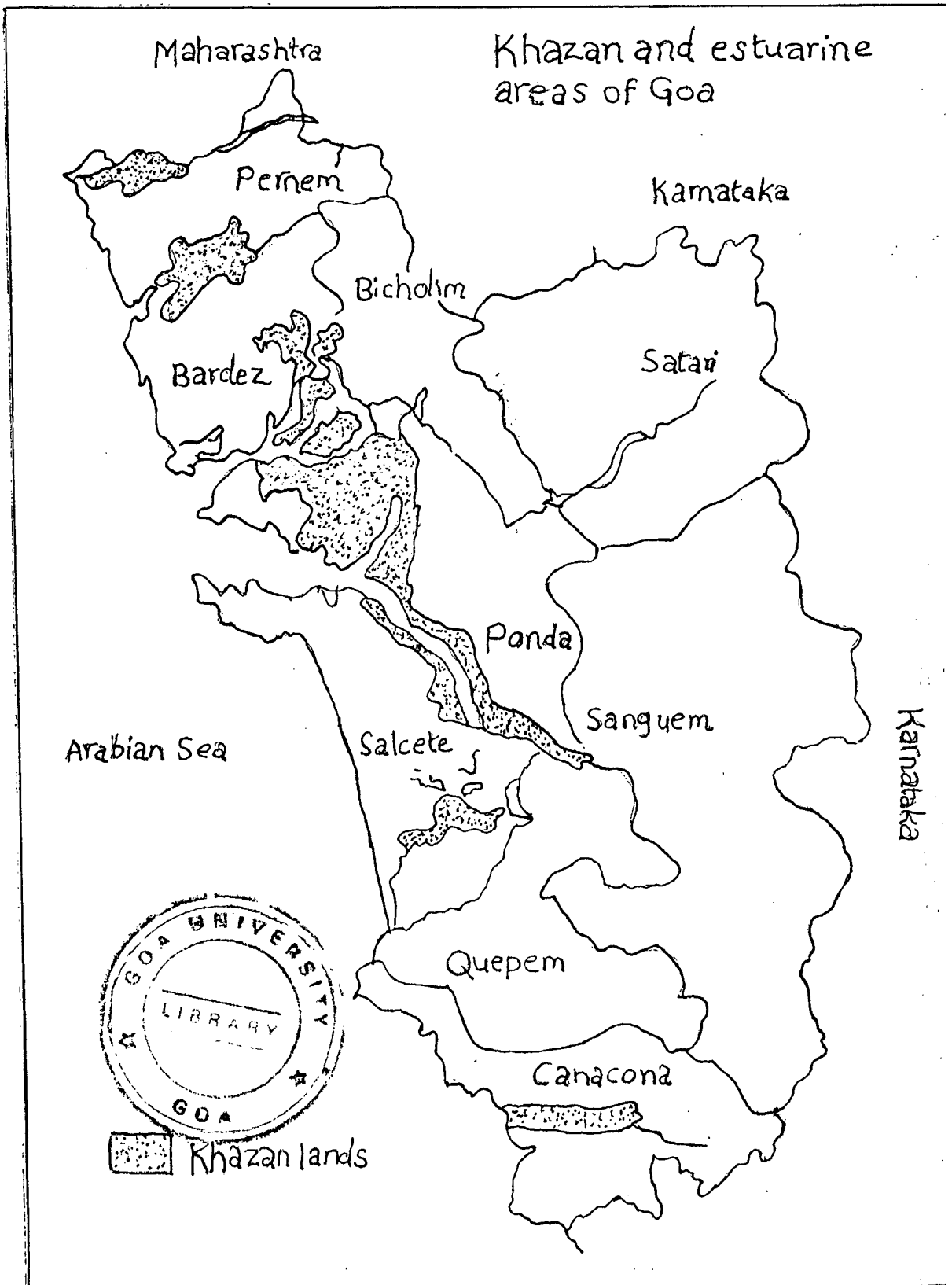
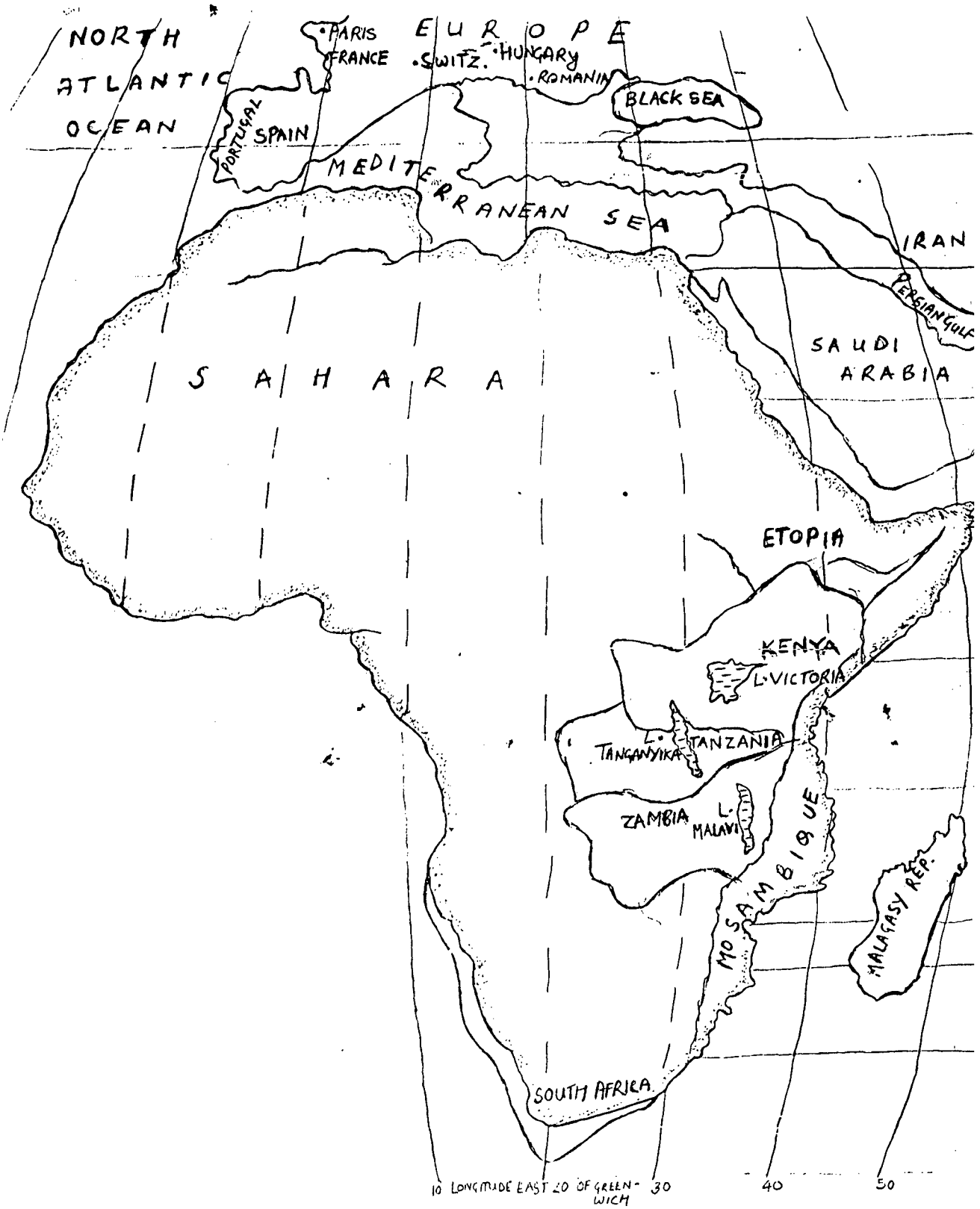
CONTENTS



(SOURCE: ANONYMOUS, 1994) FIG. 1.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EUROPE
PARIS FRANCE
SWITZ.
HUNGARY
ROMANIA
BLACK SEA
PORTUGAL
SPAIN
MEDITERRANEAN SEA
IRAN
PERSIAN GULF
SAUDI ARABIA
SAHARA
ETOPIA
KENYA
L. VICTORIA
L. TANGANYIKA
TANZANIA
ZAMBIA
L. MALAWI
MOSAMBIQUE
MALAGASY REP.
SOUTH AFRICA
10
LONGITUDE EAST OF GREENWICH
20
30
40
50

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ii) LIST OF FIGURES

iii) LIST OF TABLES

1. GENERAL INTRODUCTION

Utilisation of aquatic renewable resources through fishing practices has long been closely associated with the ecology of man, and has been a part of his civilization. The term "fisheries" include not only fin-fishes, but all those aquatic species of plants and animals, that are exploitable interest to man. Most of these yield protein-rich meat for direct human consumption, while others are used for important, economic industries viz. pearl from pearl oyster, resins from seaweeds. More recently, the term "aquaculture" has come to be used, denoting international farming and husbandry, of economically important aquatic animals and plants, under controlled conditions. The ultimate objective of aquaculture, is to create a significant aquatic food and manure producing industry, analogous to agriculture, with reasonable, predictable and controlled yield.

India has made dramatic advances in marine fisheries during the last 50 years, and occupies the seventh position in global fish production. The consistent growth of this sector till now with the 1997-98 production touching 2.71 million tonnes out of a potential of 3.9 million tonnes, from the EEZ (Extended Economic Zone) of 2.02 million sq. km. is the result of development measures taken during successive plan periods. In the Indian marine fisheries there are nearly 1,570 species of finfish and about 1,000 species of shellfish. Capture fisheries employs various types of crafts and gears for the exploitation of the commercial stocks. Development activities helped increase the harvest from about six lakh tonnes in 1950 to 2.71 million tonnes in 1977, showing an average annual growth of 6.4%. However the annual growth rate declined from 6.5% during 1950-60 to 2.3% during 1960-70, increased to

4.3% during 1970-80 and 4.8% during 1980-90, and declined to 4% during 1990-96. This fall in growth is reflected in the annual catch attaining the optimum levels in the inshore fishing grounds extending up to the 50m depth of about 180,000 sq. km. (Devaraj, 1999).

Currently around 1.91-lakh non-mechanised craft (including 32,000 motorised craft), 47,000 small mechanised craft and 180 large fishing vessels operate in the Indian EEZ. The contribution from the mechanised sector to the total catch is 67%, motorised 20% and non-mechanised 13%. The deep-sea fishing in India is a potential beyond the 50m depth in the EEZ is estimated to be 1.7 million tonnes consisting of 0.74 million tonnes pelagic, 0.65 million tonnes demersal and 2.09 million tonnes oceanic stocks. There is also a potential of about 100 million tonnes mesopelagic in the outer areas of Arabian Sea. Nearly 55 categories of marine products are exported to countries in South-East Asia, Europe and the U.S. The total quantity exported increased from 97,000 tonnes in 1987-88 to 3.85 lakh tonnes during 1997-98. In terms of value, it increased from Rs. 530 crores to Rs. 4,697.5 crores.

India leads in Asia, next only to China, in freshwater aquaculture production with a modest beginning in coastle shrimp aquaculture which began in the current decade. It is expected that India may achive a production of about two million tonnes through coastle mariculture by 2025, that is 3.9% of the global aquaculture production of 51.8 million tonnes projected for 2025. Coastle mariculture and seafarming of finfish (seabass, pompano, groupers, red snappers and breams) are suitable for farming along the South-West and South-East coasts; sea-cucumber along the coast of Tamil Nadu (Mandapam and Tuticorin), Andhra and Gujarat coasts; edible oysters in Andhra, Tamil

Nadu, Kerela, Karnataka, Goa and northern Maharashtra; windowpane oysters and blood clam in the Kakinada Bay and nearby areas. Commercial shrimp hatcheries have come up all along the Indian coast, along with the Coastle Regulation Zone (CRZ) rules and regulations which have helped to evolve eco-friendly and diverse aquaculture practices (Devaraj, 1990).

Indian freshwater aquaculture have evolved from the stage of domestic activity in West Bengal and Orissa to that of an industry in recent years with states like Andhra Pradesh, Punjab, Haryana and Maharashtra taking up fish culture as a trade. With technological inputs, entrepreneurial initiatives and financial investments, pond productivity has gone up from 600-800 kg/ha/yr to 8-10 tonnes/ha/yr in several parts of the country with the national average being around 2000 kg/ha/yr. A range of technologies such as pen culture, cage culture, running water fish culture, sewage fed fish culture, rice cum fish culture and integrated farming systems have made freshwater aquaculture increasingly popular across the country. It is significant that the freshwater aquaculture sector contributes a third to the total fish production of 4.95 million tonnes in the country with an annual growth rate of 6% and production potential of 4.5 million tonnes (Ayyappan, 1999).

Aquaculture development in the country has been phenomenal with the quantity increasing from 7.88 lakh tonnes in 1987 to 1.77 million tonnes during 1996. the values rose from 827 million to 1980 million. The share of the inland sector which was 29% in 1950-51 exceeded 45% by 1995-96 and the percentage share of aquaculture in the total inland fish production increased from 46% to 72% during the same period. Two specific aquaproduce, carps in

freshwater aquaculture and shrimps in brackish water aquaculture, have contributed to the growth of the sector.

The freshwater aquaculture resources in the country comprise 2.25 million hectares of ponds and tanks, 1.3 million hectares of bheels and derelict waters, 2.09 million hectares of lakes and reservoirs and 1.2 lakh km of irrigation channels and 2.3 million hectares of paddy fields, a portion of which is amenable to fish culture. India being basically a carp country, indigenous and exotic carps (catla, rohu, mrigal, kalbasu, silver carp, grass carp, common carp) accounts for 82% of the total production. The freshwater prawns, *Macrobrachium rosenbergii* and *Macrobrachium malcolmsonii* receive attention with regard to the establishment of hatcheries and grow-out systems. The aquaculture systems not only utilise the wastes as nutrient inputs but process and treat a large number of wastes no other farming system can. This aspect is assuming importance in the context of environmental management (Ayyappan, 1999).

It is quite understandable that for constant increase in production of aquatic products, there should be congenial environment apart from appropriate genetic mileu. In this regard, physico-chemical parameters such as turbidity, colour, smell, pH, chloride, alkalinity, hardness, calcium, magnesium, phosphate, sulphate and iron exert profound impact on the productivity, survival, reproduction including evolution at large. Thus, study of these parameters are of prime importance.

1.1 PHYSICO CHEMICAL PARAMETERS

Nearly three fourth of the earth's surface is covered by water, which is the most abundant single substance in the biosphere being, 1,500,000,000 cubic

kilometres in volume. Although the vast water body is inhabited everywhere, the abundance of flora and fauna varies from place to place, depending primarily on the physico-chemical character of water. The environmental factors that are of major significance are salinity, temperature, pressure, solar illumination and water movements. The interactions of these factors, has made the fresh water body a unique habitat, very favourable for living organisms (Pillai, 1986). Water quality affects the abundance, species composition, stability, productivity and physiological conditions of, indigenous populations of aquatic animals. Therefore the nature and health of the aquatic communities, is an expression of the quality of water.

Water temperature exerts a major control over the distribution and activities of aquatic fauna. Temperature regulates many physiological processes. Temperature tolerance for embryo and larvae stages is less than that of adults. Further, each organism has its own temperature range suitable for growth. Within this range, the growth increases with increase in temperature. But beyond this range, the higher or lower temperature will decrease growth rate or even kill the animal. Differences in the optimum temperature in the range are clearly related to the ecological habit of the animal (Zeng Zhong *et al.*, 1989).

Salinity in the open ocean, is usually in the range of 32-38ppt. Highest salinities occur in the area of excess evaporation, either in the subtropical central regions of the ocean basins, or in the landlocked seas of arid regions. The lowest salinity occurs, where precipitation exceeds evaporation, primarily at coastal or equatorial regions. Changes in the salinity, alters the specific

gravity of water, and this in turn influences the pelagic organisms indirectly through its effect on buoyancy (Pillai, 1986).

Measurement of pH is one of the most important and frequently used tests in water chemistry. Practically every phase of water supply and waste water treatment eg. acid-base neutralization, water softening and corrosion control is pH dependent. pH of water body is largely determined by the bicarbonate concentration, and usually ranges from 7.5 to 8.5. Changes in pH may be due to photosynthesis and respiration of organisms. Increase in temperature or pressure causes a slight decrease in pH (Pillai, 1986). At a given temperature the intensity of the acidic or basic character of a solution is indicated by pH or hydrogen ion activity. Alkalinity and acidity are the acid and base neutralizing capacities of water and usually are expressed as milligrams $CaCO_3$ per litre.

Chloride in the form of chloride (Cl^-) ion, is one of the major inorganic anions in water and waste water. In potable water the salty taste produced by chloride concentrations is variable and dependent on chemical composition of water. Some water containing 250 mg Cl^-/L may have a detectable salty taste if the cation is sodium. On the other hand, the typical salty taste may be absent in waters containing as much as 1000 mg/L when the predominant cations are calcium and magnesium. The chloride concentration is higher in wastewater than in raw waters because sodium chloride of diet passes unchanged through the digestive system. Along the sea coast, chloride may be present in high concentrations because of leakage of salt water into the sewage system or industrial processes. A high chloride content may harm metallic pipes and structures, as well as growing plants (APHA, 1985).

Alkalinity of water is its acid-neutralizing capacity. The measured values, varies significantly with the endpoint pH used. Alkalinity is significant in treatments of natural waters and waste waters. Alkalinity of surface water is primarily a function of carbonate, bicarbonate and hydroxide content. Raw domestic wastewater has an alkalinity less than, or only slightly greater than, that of the water supply. Properly operating anaerobic digesters typically have supernatant alkalinities in the range of 2000-4000 mg $CaCO_3$ ($CaCO_3$)/L (APHA, 1985).

Hardness is a measure of the capacity of water to precipitate soap. Soap is precipitated chiefly by Ca and Mg ions present. Hardness is defined as the sum of the calcium and magnesium concentrations, both expressed as calcium carbonate in milligrams per litre. When hardness is numerically greater than the sum of carbonate and bicarbonate alkalinity, the amount of hardness equivalent to the total alkalinity is called "carbonate hardness", the amount of hardness in excess of this is called "non carbonate hardness". The hardness may range from zero to hundred milligrams per lite, depending on the source and treatment to which the water has been subjected (APHA, 1985).

Inorganic constituents like calcium, magnesium and sulphates, present in seawater, contributes to the alkalinity and total hardness of the water, which has a bearing on the biological composition of the aquatic system. In some cases these may become limiting factors for plankton growth, due to their availability and not due to their absolute concentration (Wickstead, 1976). Hence, the studies of these properties of water apart from the quantitative analysis, is important to elucidate their inter-relationship (APHA, 1985).

The presence of calcium, which is fifth among the elements in order of abundance in water supplies results from passage through or over deposits of limestone, dolomite, gypsum and gypsiferous shale. The calcium content may range from zero to several hundred milligrams per litre, depending on the source and treatment of the water. Small concentrations of calcium carbonate combat corrosion of metal pipes by laying down a protective coating (APHA, 1985).

Magnesium ranks eighth among the elements in order of abundance and is a common constituent of natural waters. Important contributors to the hardness of water, magnesium salt breakdown when heated, forming scale in boilers. Concentration greater than 125mg/L also can have a cathartic and diuretic effect. Chemical softening, reverse osmosis, electrodialysis or iron exchange reduces the magnesium and associated hardness to acceptable levels. The magnesium concentration may vary from zero to several hundred milligrams per litre, depending on the source and treatment of water (APHA, 1985).

Phosphorus occurs in natural waters solely as phosphates. They occur in solution, in particles or detritus or in the bodies of aquatic organisms. Small amounts of certain condensed phosphates are added to some water supplies during treatment. Large quantities of the same compound is added in laundries for cleaning and also in the treatment of boiler waters. Orthophosphates applied to agricultural or residential cultivated land as fertilizers are carried into surface water. Phosphorus is essential to the growth of organisms and can be the nutrient that limits the primary productivity of a

body of water. Phosphate rich waters may stimulate growth of photosynthetic aquatic micro and macro organisms in nuisance quantities (APHA, 1985).

Phosphate and nitrates are minor constituents, but essential nutrients. They are extracted from surface waters, by the photosynthesising planktons to make organic tissue. They are totally depleted in surface waters, where biological production is higher and hence, called as 'Biolimiting Constituents' (APHA, 1985).

In filtered samples of oxygenated surface waters, iron concentration, seldom reach 1mg/L. Some ground waters and acid surface drainage may contain considerably more iron. Iron in water causes staining of laundry and porcelain. A bittersweet astringent taste is detectable by some persons at levels above 1mg/L. In water samples iron may occur either in ferrous or ferric, suspended or dissolved state (APHA, 1985).

1.2 LC_{50} AND BEHAVIOUR

"La fixite 'du milieu intre' riecur est condition de la vie libre", i.e. "Constancy of the internal environment is essential for an independent life". Most species in nature choose their habitat based on several physiological, behavioural and ecological factors. Homeostasis or internal regulation is a mechanism employed in maintaining body fluid concentration is called osmoregulation and is achieved by specialized regulatory device. No doubt life first originated in sea, and the marine organisms body fluid somewhat resemble sea water in the proportion of major ions like Na, K, Ca, Cl etc. K tends to be more abundant and Mg less in their body fluids. Thus for smooth functioning of enzymatic and protoplasmic systems, a closely regulated ionic environment is essential. A Poikilothermic animal is an osmoconformer and responds to

environmental osmatic changes, by drifting with it. Animals that can withstand aquatic media of wider range of concentration are called euryhaline species. This class includes anadromous (fishes that migrate from sea to freshwater eg. Salmons) and catadromous (fishes that migrate from freshwater to sea eg. Eel). As the fish migrates from freshwater to sea they increase the tonicity of their blood by raising the accumulation of urea /TMAO (Nagabhushanam *et al.*, 1989).

Osmosis is the process by which water flows from a low medium solute concentration to one across high concentration across a membrane permeable to water but not to dissolved substrates. Animals, which have a permeable body surface and which maintain their blood concentration at level differing from that of the medium in which they live, tend to gain or lose water, accordingly. Life probably began in the sea and reflecting the basic biochemical mechanism in the environment, the functioning of most modern cells is dependent upon their being bathed by a medium which, like sea water, contains a high proportion of sodium chloride and lesser amounts of K, Ca, Mg and SO_4. Both, the total concentration and the concentration of individual chemical constituents of the blood have to be maintained within certain limits. The degree to which salinity change in the medium can be tolerated varies widely (Hoar, 1984).

Since the osmotic pressure (a function of concentration) of the blood, is the same as that of the media, these animals require no special means to restrict water flow across their body surface and in general the permeability is very high. If such forms are transferred to more dilute medium, water enters the body by osmosis, salts are lost in the urine and across the body surface and

the blood concentration falls. Few sub-littoral marine species tolerate a prolonged drop in blood concentration of more than about 30%. A greater degree of tolerance of dilute media is shown by some inshore and estuarine species such as the common mussel, *Mytilus edulus*, and the lugworm *Arenicola marina.*

Cellular adaptations requires that the internal osmotic pressure of the cells be matched to that of the blood to avoid gross water shifts across the cell surface. Adaptations involves two components, first, regulation of ionic composition and secondly, control of the amount of small organic molecules in the cells. Replacement of the ionic lost from the body is the function of active transport. Ions such as Na and Cl can be taken up across the body surface of most aquatic animals in the direction opposite to that in, which they are tending to move passively. Parts of the surface specialized, as respiratory organs are usually also the sites of active transport, the sodium uptake across the gills in freshwater fishes. The rate of active transport can be regulated by the individual to meet its ion requirements. If the blood concentrations falls below normal, the rate of transport is increased (Hoar, 1984).

The well known modes of progression / locomotion such as walking, running, hopping, flying, swimming and crawling are special modes of vertebrates to meet special requirements. Among these are the walking fish or climbing fish (Anabas) of southern Asia and Africa. When the impulse moves, these strange creatures climb up the bank out of water, take a stroll on the ground and even climb into shrubs and trees. They really walk on the tip of their modified fins. Individual fish being known to have travelled unhurriedly more than 300 feet in 30 minutes. Flying fish (*Exocoetus*) do not actually fly.

They merely gain speed in the water, break through the surface into the air and glide with the aid of their large, wing like pectoral fins, which serve as plane (Prasad, 1991).

Orientation is the assumption of definite direction in response to stimulus. Orientation within the environment is one of the most important aspects of animal behaviour, dependent upon the sense organs, which allows the creature to position itself or move in its habitat. Electric fish rely upon electric orientation. The way in which the animals are able to orientate themselves is often considered in connection with migration, however, many animals which do not migrate for eg, ants, bees, frogs, sedentary birds, small animals and fishes have well developed and highly complex methods of orientation. Orientation thus helps these non-migratory animals to move quickly and efficiently from place to place within their home range (Prasad, 1991).

Aquatic animals are particularly sensitive to chemical stimuli, either attracted, or avoids it. The most dramatic response is seen in the scallops, which literally leaps away from the starfish by opening and snapping its shells together, jetting away in leaps and bounds, salmons on both coasts on North America are hatches in freshwater stream. After a time they migrate into the ocean to develop to maturity. Eventually they migrate back into freshwater to spawn. Each adult returns to the stream in which it was hatched. They appear to accomplish this task by chemical orientation. Experiments have shown that blocking of the nostrils disorients the fish so that the home stream is not chosen. Also, study has shown that a sample of water from the home stream stimulates the electrical activity of the brain while water from other streams does not. The explanation for this is each stream has its own particular

chemical composition and composite odour. Most probably the chemical constituency is imprinted in the memory of the fish, years later when the salmons returns to freshwater to spawn, it follows the odour pails of the stream, leading it back home (Prasad, 1991).

In general, freshwater animals are more tolerant of raised salinities than marine species are of dilute medium. Most die when exposed to salinities more concentrated than their normal blood concentration. Various physiological mechanisms have been adapted by different animals to assist in the maintenance of low blood concentration in the face of the tendency for water to be withdrawn from the body by osmosis, and for the ions to penetrate down the gradient from seawater. NaCl is one of the most common substance that can occur in any water body and aquatic animals exhibits a wide range of responses to sodium chloride depending on the concentration of the compound, exposure time, water condition and species (Eisler, 1977).

The ways, in which the animals cope with life's many problems to survive and reproduce, have fascinated mankind since ancient times. Initially, animals were supposed to be guided by readymade, inborn, unalterable instincts. Nevertheless, the problem of how the adaptive behaviour develops, how an animal's behaviour comes to 'fit' in its natural environment, remains as intriguing as ever and is a topic of much current research. To some extent the terms 'innate behaviour' and 'instinct' overlap, if an instinct is taken to imply a complex, species predictable behaviour, developed largely as a consequence of adaptation. However, the word 'instinct' is not often used as a technical term nowadays, it lacks precision and means too many different things to different people (Weidmann, 1971).

Innate behaviour dealing in turn with motor performance, responds to stimuli, rhythms and orientation. Any one looking for the movements of an animal soon discovers elements of behaviour, which appears again and again in more or less the same form. Such relatively stereotyped motor components of behaviour, which are typical for the members of the species have been called 'fixed (action) pattems' or 'instinctive movements' (Lorenz, 1965).

Some of the best documented, examples of innate behaviour, concern are, activity rhythms and mechanisms of orientation. Most activities occur in definite cycles, with periodicity ranging from fractions of a second to one year. Many of these cycles reflect environmental rhythms such as the changes of the seasons, the 24 hour cycle of day and night, the phases of the moon, and the changing tides. But others, the so-called 'private rhythms', are endogenous and self sustained. How animals find their way poses problems of baffling complexity. For example many fish cover thousands of miles under water and return to the same area. The features of an organism, including its behaviour, may be visualized as the outcome of a web of interrelated developmental programs, determined by the genes and the cytoplasm, acting in conjunction with the changing environmental factors throughout ontogeny. (Weidmann, 1971).

Correspondence between innate behaviour and the natural situation in which it first occurs can sometimes be ascribed to chance. In all such instances, where chance fits and direct experience of the relevant situation can be ruled out, evolutionary adaptations must be implicated. Studies have shown that behaviours such as orientation, feeding defence, schooling, spawning and migration are largely dependent on olfactory cues, in such a

way, that changes in the olfactory function induced by a toxicant may affect the normal adaptive response of fish (Hara, 1986). With the exceptions of endothermic fishes, all living members of the vertebrate classes Agnatha, Chondrichthyes and Osteichthyes are ectothermic, with their body temperatures being determined by the thermal characteristics of the environment. Mean excess tissue temperatures for both muscle and brain were approximately $0.45^{0}C$ in Tilapia weighing between 40 and 200gm, but averaged less than 0.15 in smaller fish.

Although changes in body and ambient temperature are obligatory linked, fish manage and compensate for thermal stress by a variety of mechanisms Crawshaw *et al.*, (1990), including, behavioural adjustments, physiological or autonomic responses to acute temperature change and acclimatory (within the lifetime of an individual) or adaptational (over evolutionary time) adjustments to persistently altered temperatures. The first two of these mechanisms are complementary and activated within seconds to minutes, where as the third requires some period of exposure to altered body temperatures before becoming effective. Although most fish are obligate poikilotherms, they do not regulate their body temperature if given the opportunity to do so (Crawshaw and Hammel, 1974). Fish, like all other vertebrates, initiate thermoregulatory behaviour by responding to sensory input from both peripheral and central thermoreceptors (Crawshaw *et al.*, 1990). Processing and integration of this information is accomplished in the preoptic anterior hypothalamus (Crawshaw 1980).

Fish displays a variety of alternate mechanisms for coping with the extremes of environmental temperature. Some fish simply respond to

seasonal extremes of temperature, (frequently correlated with other changes in the environment such as water or food availability), by entering a state of dormancy, thereby circumventing environmental extremes. For example American eels *Anguilla rostrate* swim actively and feed regularly at summer temperatures 13-17^{0}C, but at temperatures below 8^{0}C, cease feeding and burrow in the mud (Walsh *et al.*, 1983).

Though there is much circumstantial evidence supporting the idea that natural selection influences behaviour, such influences have rarely been tested directly in the field, partly because the expected evolutionary changes would, as a rule, be too slow for detection during a researcher's life span. However, laboratory experiments on artificial selection provide evidence that is fully compatible with the inference drawn. Genes exert their influence on the development of behaviour. In spite of much current research this is not yet well understood. Genes could act directly on processes of growth affecting relevant neuronal networks. This might be called as adaptations in the strict sense. However, genes may sometimes influence behaviour also indirectly, in a variety of ways: the development of the effector organs might in turn channel the development of behaviour, for instance, an animal could program some of its behaviour by discovering what it can do with the 'tools' – fins, mouth and so on. The study of these intimate developmental interactions is a new field in which students of behaviour work hand in hand (Weidmann, 1971).

When exposed to chemical stimuli associated with food or chemical, fish initiate behavioural patterns, which enhance their chances of locating and consuming the food source and maintaining homeostasis (Bateson, 1890).

The contents of these food search patterns vary from species to species, but in many cases, they are easily discerned even by the untrained observer. Indeed in some instances, as during feeding frenzies, food search behaviour can be quiet spectacular (Greene, 1925; Tester, 1963). Changes in locomotion are not limited to activity. Chemical stimulation appears to override those neural mechanisms controlling all aspects of locomotory behaviour (Kleerekoper, 1967). Some complex behaviour may be overridden as well.

The most studied behavioural response of fish to metals in preference or avoidance behaviour, a sensitive indicator of sublethal exposure that can be objectively and rapidly quantified (Cherry and Cairns, 1982; Beitinger and Freeman, 1983; Giattina and Garton, 1983; Atchison *et al.*, 1987). Avoidance or preference responses vary greatly with fish species and contaminate. Black and Bridge (1980), noted that bluegills, *Lepomis macrochirus*, and largemouth bass, *Micropterus salmoides*, had lower response threshold concentrations than trout, for cadmium copper and zinc.

Fish swim for a wide and complex set of adaptive movements to perform numerous activities necessary to survive in diverse habitats and to bring about homeostasis. Three muscle fibres are important for locomotion are the red or slow oxidative fibres, white or fast glycolyte fibres and pink of fast oxidative fibres. Separate muscle system of fin blade of median, caudal, anal, Dorsal, paired pectoral and pelvic fins controls, used in homeostasis (Webb, 1993).

Generally fish lives in a constant habitat and restrict their movement within particular territorial limits, but there are many others which migrate from one type of habitat to another and travel long distances moving from freshwater to

sea water or vice versa, for the purpose of feeding, spawning or shelter called migration. During this process, population mortality of numerous individuals occur due to different ecological hazards such as temperature fluctuation, scarcity of food, predation etc. Migration provides better survival, and important migratory fishes include Pacific salmon (*Onchorhyrrchus*), Atlantic salmon (*Salmo salar*), European eel (*Anguilla rostrate*), American eel (*Anguilla vulgaris*), Herring (*Clupea harangus*), Swordfish (*Xiphias gladus*), Hilsa (*Hilsa ilisha*), Codfish (*Gadus morhua*), Plaice (*Pleuronectis platessa*), Barracudas (*Sphyraena zygaena*), Mackerals (*Scomber microlepidotus*), Tunnas (*Thunnus thynnus*), milkfish (*Chanos*) (Gundevia and Singh, 1996).

The relative close confinement of the fish causes stress in the establishment of 'territories', in which one fish reserves part of the tank for itself and vigorously repels intruders. This behaviour is especially noticeable at the spawning season (Brawn, 1961b). Other aggressive behaviour is also shown, which fairly leads to a social caste system, or 'peckorder', among fish, similar to that seen in domestic animals (and of course man also) when they are kept together. Small fishes repeatedly chased by the others eventually refuse food and die (Brawn, 1961a).

The temperature tolerance zone, represents the thermal arena within which, individuals of a species can operate. Critical Thermal Methodology (CTM), a laboratory method to quantify temperature tolerance, a random sample of fish is subjected to a constant, linear change in temperature until a predefined sublethal, but near lethal endpoint is reached. The endpoint either the CTmaximum or CTminimum, is the pre-death thermal point at which locomotory movements become disorganised and a fish loses its ability to

escape from conditions that may lead to its death. This end point of the CTM test, i.e., ecological death is more obvious, dramatic and easier to interpret than other types of bioassays in which a behavioural or physiological process are used as a biomarker. This explains as unambiguous, biological defensible and important attributes of an individual (Beitinger *et al.,* 2000).

For over a hundred years following the publication of Darwin's theory, biologist consistently misunderstood an important element of his reasoning, and the 'theory of natural selection' they promulgated, which was accepted by both professional biologists and the lay public, was actually a misinterpretation of Darwin's theory of natural selection. Biologist came to substitute for Darwin's precise statements about natural selection their own imprecise version, which may now be called 'the good of the species' concept. It became implicit in virtually all discussions of natural selection that evolution works for the good of the species. Natural selection favours what is good for the species, came to an abrupt end in the mid 1960s. We now recognise that natural selection can, in principle, act at any level of biological organization, although it acts at the level of the individual.

Like all branches of science, ethology began with qualitative descriptions of the patterns of behaviour of different species of animals in different situations. With the accumulation of a large amount of excellent qualitative information, the transition from qualitative to quantitative ethology has began to take place. While it is logical for any field of enquiry to begin in a qualitative way and mature into a quantitative science, emphasis is laid that the qualitative phase is no way inferior and certainly cannot be dispensed with. Quantitative studies that are not based on adequate qualitative studies are

likely to be flawed. It is therefore essential that careful qualitative descriptions should always precede quantitative studies. It is true however, that, quantitative ethology, has its own unique methodological problems, which are often ignored. Typically, quantitative ethology involves 'measuring' animal behaviour. The first problem in quantitative ethology, perhaps more than any branch of biology, has the potential of generating completely useless information, if certain precautions are not taken. Animal behaviour is often transient and extremely context dependent. In addition it may also very dependent on the internal state of the animal. Thus unless these variables are carefully controlled or adequately recorded, information gathered for measuring behaviour cannot be interpreted, or worse, can lead to misinterpretation (Gadagkar, 2001).

An even more serious problem has to do with potential biases that often exist in measuring behaviour. Quantitative ethology may attempt to measure the rate of a particular behaviour of different individuals and relate these rates to the age of the animal. Many investigators do not appear to recognise that very often, we are only sampling the behaviour and not enumerating it. Because of the mistaken notion that we are enumerating rather than sampling inadequate attention is usually, paid to precautions that need to be taken to ensure a reasonably random sample. The possibility of obtaining a biased sample is especially high when we are observing not one but a whole group of animals and we are recording not one but a whole range of behaviours. Swimming behaviour is the most sensitive indicator of exposure to any perturbant. Behaviour represents a sensitive tertiary stress response of ecological relevance. The four very common sources of bias and simple ways

of avoiding them are, some animals are more conspicuous than others, some behaviours are more conspicuous than others, behaviours are non uniformally distributed over space and time, and the human mind is more sensitive to habituation and fatigue than most of us suspect (Gadagkar, 2001).

Every behaviour can be thought of as an event or a state. For example, one can think of feeding as an event and compute the number of times that an animal feeds per day. Behaviours which are of long duration are more appropriately thought of as states rather than events (time spend in sleeping rather than number of times the animal sleeps), and short duration behaviours are more appropriate thought of as events (number of times an animal fights per day rather than proportion of time spent in the state of fighting). Similarly information on differences in the behaviours performed by different animals can be computed either as time spent in each behaviour or the rate at which each behaviour is performed. Scan sampling is a very convenient and widely used method for treating behaviours as states and computing time budgets and focal animal and focal behaviour sampling are convenient and widely used methods for treating behaviours as events and computing rates. During focal animal sampling it is relatively easy to keep a continuous record of all behaviours shown by the focal animal during the specified duration. This is not possible in scan sampling and is difficult in focal behaviour sampling. Focal animal sampling therefore is also a convenient method for understanding associations between different behaviour, by computing and analysing different sequences of behaviour. For example, one might want to know if wasps always groom themselves after feeding or if they rest after a foraging trip. Scan sampling is a convenient method for understanding

associations between different animals. For example one might want to know if the workers in a wasp colony work and rest in co-ordination with active and inactive states respectively of the queen (Gadakar, 2001).

Since the time of Charles Darwin it has become increasingly possible to explain the role of behaviour in the survival and reproduction of the animal. Just as an animals, eyes, ears, legs or wings can be viewed as mechanisms designed to enable the animal to cope with its particular mode of life, so too can the mechanisms controlling behaviour (McFarland, 1985).

Behaviour patterns result from the complex interactions of external stimuli and internal conditions. However, any behaviour pattern is constrained by the ways in which information is processed by the animals. The internal information processing systems are established during the course of development from the fertilized egg, to the embryo, to the adult animal, a process called ontogeny. In *Aplysia*, the action of the genes is of direct importance in guiding the production of a stereotyped behaviour pattern. While genetic influences underlie all behaviours at some level (for instance, the embryogenesis through which the body structure is laid down), there are also varying degrees of environmental influences that can modify the behavioural results of development. The same genes may have different phenotypic effects when the animal is subjected to different environmental influences during development. Many movement patterns appear to develop in the absence of practise or example (Hinde, 1970).

The term 'innate' has different meanings. Lorenz, (1939) view of behaviour as being hereditary, individually fixed and thus, open to evolutionary analysis. Tinbergen, (1942), refers similarly to instinctive acts as being highly

stereotyped coordinated movements, the neuromotor apparatus of which belongs, in its complete form, to the hereditary constitution of the animals. According to Lehrman, (1953) behaviour is influenced both by genetic factors and the experience of the individual. Thus for the study of behaviour it is convenient to use the term 'innate behaviour' as short hand for 'behaviour that develops without obvious environmental influence'.

Animals are considered as machines. We should focus our attention on the individual animal and ask how it adjusts to changes in the environment. The type of explanation we are seeking is a mechanistic one. How are we to explain, in terms of immediate causes, changes in the animal behaviour. Ideally we might wish to describe behavioural mechanism in terms of the physiological hardware responsible – the events in the brain and other parts of the nervous system. However our knowledge of physiology is not yet sufficiently developed to enable us to do this. Thus alternative ways of describing behavioural mechanisms are with respect to nervous, sensory and muscular activity (McFarland, 1985).

Animal behaviour is controlled by the nervous system made of specialized cells called neurons, which operate on the same principles throughout the animal kingdom. The nervous system receives information from the environment through sensory receptors. There are nerve cells specialized as energy transducers. They may provide information about the animal's external environment and about its internal state. Further, behaviour is affected through the action of muscles and glands. The muscles are responsible for animal movement, and the gland produce substances, which may play a variety of behavioural roles such as communication with other animals,

protection against predators and adherence to the substrate. Muscular activity is controlled and co-ordinated by means of the somasthetic part of the nervous system. This provides feedback to the central nervous system about the position, tension, and length of joints, tendons and muscles. The nervous commands to the muscles are modulated by this feedback of information .The most distinctive feature of the nervous system in vertebrates is highly developed brain and associated sense organs, chemoreception (exteroreceptors, interoreceptors), thermoreception, which responds to the changes in the external environment, pheromones and other endocrine produced hormones play important behavioural role. Fish has thermoreceptors in the skin, lateral line and brain making them sensitive to temperature changes. Catfish shows response to changes in temperature less than $0.1^{0}C$ (McFarland, 1985).

1.3 OSMOSIS

The fishes have evolved to occupy all but a few types of natural waters ranging from low ionic strength ('near-distilled') freshwater to those with salinities of 80-142‰ (Kinne, 1964; Parry, 1966; Griffiths, 1974). Some are restricted to narrow ranges of salinity (stenohaline) while others tolerate broad ranges (euryhaline). Some spend a major part of their pre-adult lives in freshwater and migrate to spawn in the sea (catadromous), others begin life in freshwater, go to sea as juveniles, grow and mature, and return to spawn in estuaries or coastal habitats of reduced salinities, others spend their entire life in oceanic salinities, or in freshwater. There also is much variation within species. The various developmental stages, the fertilized egg, larva, juvenile, may have particular salinity optima, including those catadromous and

anadromous forms where relatively rapid changes occur in salinity tolerance with juvenile metamorphosis or pre spawning migration.

Some fishes are osmoconformers (poikilosmotic), their body fluids tend to follow and confirm with changes in osmotic properties of the external medium. Teleost fishes generally are osmoregulators (homiosmotic), their body fluids remain relatively constant with alterations of external medium. Many teleost regulate in the central portion of their ranges of tolerance and confirm at the extremities. Hyperosmotic regulators (most freshwater teleost) maintain body fluid concentration that of their external surroundings. Conversely, hypoosmotic regulators (most marine teleosts) maintain body fluid concentration below that of the external medium. Osmoconformers tolerate wider range of external concentration (Prosser, 1973).

It is recognised that water molecules may cross plasma membranes in two ways, through the matrix of the membrane by solubility diffusion process, or by passage through transmembrane channels. The primary basis for regulative capacity can be considered as the semi permeable membrane of the individual cell and its tolerance and response to external and internal osmotic and ionic alteration. A second level of regulative capacity is provided in the development of particular tissues and effector organs, whose cells have specific regulatory functions. A third level, is the intact animal, involves neurosecretory activity, which tends to modulate cell, tissue or organ functions governing regulations. Osmotic and ionic regulation during early development of teleosts then poses certain questions:

- What are the mechanisms that may provide regulative capacity in the egg, embryo and larva?

- What patterns can be identified in particular species?
- When or what developmental stages do these regulatory mechanisms becomes functional?

The first question has received much attention often from the studies of oocytes, fertilized eggs, juveniles or adults of non-teleost. With respect to the second question, (Evans, 1979) suggests that there are three general patterns of regulation viz. regulation among the fishes, electrolyte iso-osmolarity with sea water and renal ion regulation.

Teleosts normally maintain their plasma osmolarity within a narrow range (290-340 mOsmol/L) irrespective of the salinity of the external medium, and failure to do so for a prolonged period results in death. The transition from freshwater to seawater requires a reversal from net ion influx to net ion efflux which is regulated primarily by the gills but also involves the kidney, gut and urinary bladder. For most teleost Na^+K^+ATPase activity increases after transfer from freshwater to sea water (McCormick and Saunders, 1987). Ion exchange which may occur in the integumental slime, Evans, (1984) offers intriguing evidence that parallel Na^+/H^+ and Cl^-/HCO_3^- exchange systems, generally associated with sodium chloride regulation in fresh water vertebrates. In the elasmobranchs, the blood level of NaCl is below that of the external medium, but fluid levels are maintained isosmotic or somewhat hypertonic by the retention of urea and trimethylamine oxide (TMO). The third pattern found in the teleosts, the principle effector organs and the branchial epithelium, the renal complex, the gut, and Marshall and Nishioka, (1980), the integument. Similarities with the teleost patterns are found in the

Chondrosteans (sturgeons), Holostens (gars, bowfin), Dipnoans (lungfish) and Petromyzonid (lamprey) Agnathids.

Keeping *Tilapia mossambica*, a freshwater fish, for even a short time in an aquarium has been found to affect seriously the secretion of acid in the stomach because of a shortage of salt, this species depends on the ingested salt from suitable food to maintain its digestive secretion (Fish, 1960).

The ion regulatory needs of teleost fishes living in freshwater and seawater are diametrically opposed so animals moving between these environments are presented with a physiologically challenging situation. These fishes must realign their transepithelial ion transport mechanisms to successfully adapt (ion uptake in freshwater and ion elimination in sea water). A number of salmonid species make these migrations and have thus been a popular group to study from both academic and commercial perspectives. Juvenile coho salmon, *Oncorhynchus kisutch* raised in freshwater and transferred in seawater showed NHE 2-like protein which is associated with NaCl elimination.

Juvenile salmonids inhabit a hyposmotic environment and continuously loose salts by diffusion and gain water by osmosis. Achievement of hydromineral balance is by absorption of inorganic ions from the medium, possibly via mitochondrion rich cells on the body surface while excess water is excreted via the kidney. Although drinking has no obvious physiological function, it represents a route for water uptake, as well as for the uptake of substances from the environment. Toxic substances in water may directly enter via the body surface if they are sufficiently lipid soluble. Under natural conditions the salmon, *Salmo salar m.* displays a life pattern similar to those

of their anadromous conspecefics. Physiological changes associated with smolting and desmolting when examined in 1-2 years hatchery showed early developmentof the fish accelerated by increasing temperature from 4 to 8^0C. Sodium uptake across the freshwater fish gill, involving electroneutral Na^+/H^+ exchange Krogh, (1937), was not tenable. Instead, it was postulated Avella *et al.*, (1989), that Na^+ across the apical membrane of gill epithelial cells was linked energetically to active H^+ extrusion via the H^+V-ATPase. (vacuolar H^+ATPase).

Love, (1970) stated that an increase in the proportion of either, water, lipid or protein leads to the decrease of the other, so that the sum remains approximately constant. Rao, (1967) and Jafri and Khawaja, (1968), have shown an inverse relationship between water and fat in freshwater fishes.

1.4 RESPIRATION

Few events in the history of vertebrates throw light on how the transition of life first from water to land took place. Such transition was possible due to direct utilization of atmospheric oxygen. Hence, fishes occupy a crucial phylogenetic position, to show how fundamental progress of breathing with gills is modified or assisted by air breathing organs for aerial respiration. Therefore aerial respiration in fishes merits considerable attention, because it throws light on physiological mechanism of adaptation and profound effect on the direction of vertebrate evolution. Thus the coexistence of water and air breathing mechanism in one animal, promises information of general physiological interest.

In order the organism may survive, there are three courses open for it.

- Change in distribution.
- Tolerance.
- Evolution.

If none of these responses is possible, the organism must perish and cease to exist as an independent unit by extinction. All these responses have been utilised by fishes in course of evolution. Among the vertebrates, fishes are most plastic poikilotherms inhabiting diverse habitats; sea, freshwater, estuaries, ponds, streams, rivers etc which demonstrates numerous and extremely varied examples of how the fundamental process of breathing with gills in water and /or air breathing organs in air (Raveendran, 2000).

The amount of oxygen in the atmosphere is relatively constant at 200 cc/l, the partial pressure of oxygen is therefore fixed, with slight variation. Hence the amount of oxygen that can go into solution in seawater also varies

according to salinity and temperature. Oxygen is slightly soluble in water, the saturation value for oxygen at 0^0C is 8cc/l in sea water salinity 35ppt, 8.6cc/l at 27ppt. Sea water receives oxygen from the diffusion of oxygen from the atmosphere and also through photosynthetic activity of plants, but it depends on salinity, temperature, solar illumination, nature of ocean surface etc. the rate of diffusion is 100 times greater, if sea surface is agitated. Generally more oxygen enters cold, less saline waters of higher latitudes and there is less oxygen in warm, highly saline waters of lower latitudes (Terdalkar, 2000).

Air breathing fishes perhaps represent the zenith in bimodal breathing among the vertebrates. Air breathing has evolved independently among many species of fishes, which as a result, possess a variety of anatomical, physiological, biochemical and behavioural specialization for aerial gas exchange (Johanson, 1970; Graham, 1976).

The literature on numerous air breathing fishes on the capacities of accessory respiratory structures, skin, air bladder and lungs to obtain oxygen and eliminate carbon dioxide are regulated, are presumably adaptive. The adaptive structure of the gill and aquatic respiration of fishes to drastic perturbed environment in the recent decade have become a subject of great interest, partly because of population explosion which is causing environmental deterioration, and partly because of many convergence to the ancestral vertebrates, which made the transition from, water to land (Raveendran, 2000).

Morphologist, anatomist, physiologist and biochemists have long been interested in understanding the phenomena of respiration in fishes. Biologists hope to find the biological solutions to the environmental problems associated

with altered aquatic respiration. In the past decade, we are witnessing ever-increasing research effort in environmental biology of aquatic and air breathing fishes. A number of workers including Carter, (1931, 1957); Carter and Beadle, (1931); Hora, (1935) and Das, (1940) have compiled the earlier literature relating to the bionomics structure and physiology of respiration in fishes. The functional morphology of the air breathing apparatus and the ecophysiological adaptations are reviewed by (Dutta *and* Munshi, 1985). The common trend in this wealth of anatomical, physiological and biochemical papers on respiration in fish is a search for how evolution has optimised respiration for the fitness of the organisms, moulded by natural selection posed by pre-existing hostile environment.

Obligate and facultative air breathers can be differentiated on the basis of whether or not water oxygen content controls air breathing (Johansen *et al.*, 1970). Obligate air breathers respond to hypoxia, in the air breathing organs (ABO) by increasing the frequency of air breathing without altering branchial breathing. They do not appear to be affected by low water oxygen content. Facultative air breathers, however, increase air breathing frequency and branchial breathing in response to low water oxygen content (Gee and Graham, 1978). In recent years the category of facultative air breathers study with various species *Umbra*, Gee, (1980); *Corydoras*, (Kramer and McClure, 1980) and (Kramer, 1983) that not obligate air breathers but breath air in normoxic water and mearly increase air breathing frequency in order to endure hypoxia. Often species with well developed air breathing organs have reduced gills, hence they are obligate air breathers, unable to meet their oxygen demands by exclusive water breathing even in normoxic water

(Johansen, 1970; Singh, 1976). Other bimodal species have well developed gills and can meet their oxygen demands by water breathing down to quiet low levels of dissolved oxygen (DO). Such facultative air breathers may have well developed or very simple ABO. Certain species generally referred to as "Continuous air breathers" use ABO over the whole range of DO while others, which may be called "Threshold air breathers" use only water except in hypoxia. Perhaps unexpectedly, the continuous air breathers include both obligate and facultative species (Kramer, 1983). Air breathing fishes are also classified into continuous air breathers Gee and Graham, (1978), continuous facultative air breathers Bevan and Karma, (1987), and obligatory air breathers (Gee and Graham 1978). Johanson, (1970), classified these fishes into obligate and facultative. This is mainly based on the basis of whether or not water oxygen content controls air breathing. Obligate air breathers respond to hypoxia in the accessory respiratory organs by increasing the frequency of air breathing without water.

Origin of air breathing habitat among the fishes has been an important event in the evolution of terrestrial life and several workers have attempted to explain this phase of evolution from different aspects. ABO in the form of paired lungs evolved in the early bony fishes and placoderms. Bothriolepis, one of the placoderms of the upper Devonian, indicate the possibility of the presence of well-developed functional lungs (Colbert, 1955; Romer, 1966). Whether the osteichthyes inherited air-breathing organs from their placoderm ancestors or whether they acquired them independently, these organs have been of great advantage to piscine fauna of the Devonian. It is now generally

agreed that in most cases the lungs were converted into air bladder, which serves the purpose of hydrostatic organ.

Bimodal respiration (BR), first appeared in Palaeozoic fish stock, perhaps because of limiting oxygen tension (Liem, 1989). Among the teleost air breathing fish there are more than 140 species found in 16 families Bertin, (1958). However recent taxonomic survey reveals, 370 known air breathing fish species occurring among 125 genera in 49 families (Graham, 1994). The fresh water air breathing AB, fishes of India is generally found in tanks, ponds, rivers, pools, marshes and sometimes in rice fields during floods (Day, 1877). But very little is known about exotic, hardy and eurythermal fish like *Tilapia* in Indian waters. In India, the pools, ponds, creeks and streams often dry up during summer or their water becomes muddy, highly hypoxic and hypercarpic (Munshi, 1980). Under such ecological adverse conditions, there exist a very interesting group of fish, which has a bimodal gas exchange mechanism where the ABO exchanges oxygen with air, while the gills and /or skin exchanges gases with water. The air breathing (AB) respiratory chambers have developed an extension of the pharyngeal, brachial or opercular (extrabranchial) chamber (Hughes, 1980). Based on the phylogeny it can be classified into

- Derivative of digestive tube including lungs, gas bladder, pneumatic duct, stomach and intestine.
- Formed in the head region including the buccal, pharyngeal, branchial and opercular surfaces as well as the pouches formed adjacent to the pharynx.
- Existing organs such as the gills or skin.

The respiratory surfaces may be used either in air or water or some fishes make simultaneous use of two or more of them. All AB fishes irrespective of either obligate or facultative mode of breathing has, universally retained gills. Evolutionary, lung was a primitive characteristic and it was gradually transferred into respiratory gas bladder then to a non-respiratory physostomous gas bladder and finally to a physoclistous gas bladder (Graham, 1994).

In most primitive air breathers, air breathing occurred at irregular intervals. Thus in its earliest stages AB had little resemblance to rhythmic ventilation or the rhythmic AB pattern of higher vertebrates (Smatresk, 1994). The coexistence of regular gill ventilation with irregular air breathing was a new motor pattern that did not simply evolve from modest modification to the branchial rhythm generator. Establishment of air breathing function by accessory respiratory organs, may be due to variety of indirect evidences as given by Das, (1935), is summarized below.

- "Asphyxiation of the fish by preventing access to air."
- "Recovery after drowning by exposure to aerated water."
- "Effect of injury to special air breathing organs."
- "Survival out of water."

According to Das, (1927) and Hora, (1935), the ABO are meant exclusively for aerial respiration and only air is retained in them and not water in any stage of the existence of the fish. According to Das, (1927), the ABO in fishes living in tropical swamps, pools and ditches deficient in oxygen is so strongly developed that they must come to the surface to obtain atmospheric air in order to maintain life, no matter how well the water may be oxygenated.

Indian teleost fishes living in a wide range of habitat which are habitual air breathers are included in the families Notopteridae, Cyprinidae, Cobitiidae, Clariidae, Heteropneustidae, Siluridae, Bagridae, Anguillidae, Channidae, Symbranchidae, Amphipnoidae, Anabantidae, Muraenidae, Blennidae, Mastacembelidae and Gogiidae (Dubale, 1961).

Air breathing respiratory chambers has developed as an extension of pharyngeal, branchial or opercular (extra branchial) chamber (Hughes, 1980). These respiratory surfaces may be used in air or water and some fishes make simultaneous use of both of them. All air breathing fishes irrespective of either obligate or facultative mode of breathing have universally retained gills. In the evolutionary point of view, lung was seen in most primitive fish and it gradually transferred into a respiratory gas bladder then to a non-respiratory physostomous gas bladder (Graham, 1994). *Lepisosteus osseus* and *Lepisosteus oculatus* "living fossils" are aquatic and arguably facultative air breathers that use swim bladder "lungs", are a good example of early stages of transition from water to AB (Lauder and Liem, 1983).

The functional respiratory surface area of gills, and ABO can be correlated with other distribution of the gill elements and their development and the mode of respiration (Hughes and Al. Kadhomiy, 1986). According to Das, (1927), fishes living in tropical swamps, pools and ditches, deficient in oxygen has to come to the surface to obtain atmospheric air to maintain life. According to Willmer, (1934) tropical freshwater AB fish has to breathe air due to poor aerated oxygen in water, which shows a better correlation with environmental conditions than with fish activity habits. Das, (1927) has worked out the ecology, bionomics, structure, physiology and in majority of

cases a whole group of typical AB fishes of India, numbering at least a dozen included in six different families. Because of the limited solubility of oxygen in water, gill breathers must irrigate their respiratory surfaces with a 40 times larger volume than air breathers in order to extract an equivalent amount of oxygen (Block, 1991). In addition, the high heat capacity of water (3000 fold greater than air) combined with higher rates of thermal than molecular (ie. oxygen) diffusion, ensure that by the time blood in the gills is saturated with oxygen, it has also equilibrated with the water temperature (Carey, 1973). Thus, in atypical fish, the heat generated by metabolism is carried via the blood to the gills, where it is lost to the environment, Carey and Lawson, (1973), consequently, tissue temperatures are not, within, $1^{0}C$ of the ambient water temperature (Carey *et al.*, 1971; Reynolds *et al.*, 1976).

Johansen, (1966) pointed out, in South American AB fish, *Synbranchus marmoratus* gills are used as an organ of aerial respiration. Similar evidence has been reported for *Anguilla vulgaris* by (Berg and Steen, 1965). Fishes, which employ aquatic breathing in well-aerated water, are acutely sensitive to deoxygenated water and greatly augments their respiratory effects (Johansen and Lenfant, 1968). The use of atmospheric air as oxygen source has now been reported in at least 34 families of fishes and has been extensively studied in recent years (Gans, 1970; Hughes, 1976). The capacity of gills to extract oxygen from the water decreases as the temperature is progressively increased. At lower temperatures, the contribution of aquatic gas exchange machinery for the survival mechanism is greater. At very high temperatures, the air breathing apparatus probably loses its function and death ensures due to severe collapse of the blood and enzymatic systems. Temperature is one of

the most fundamental environmental stressor, altering almost all biological processes through its action on basic chemical reactions, supporting physiological processes.

Oxygen consumption is often considered as a reliable index of physiological rhythmicity in teleosts (Huang, 1990). Fish living in sub tropical habitat where the aquatic environment is frequently oxygen limited, Ross and McKinney, (1988), should demonstrate an adaptive significance in oxygen consumption. Studies on oxygen consumption of tropical and sub-tropical are rare and those in relation to changing environmental parameters are even rarer (Desilva *et al.*, 1986). Often species with well developed potentialities for AB have reduced gills and hence, obligate air breathers are to meet their oxygen demands by exclusive water breathing even in normoxic water. Other air breathing species have well developed gills and can meet their oxygen demands by water breathing down to quiet low levels of dissolved oxygen. Such facultative air breathers may have well developed or very simple ABO (Graham, 1976).

Rhythms in animals are related to rhythms in the environment, Pengelly, (1974). Palmer, (1976), provided many examples of these relations and presented definitions for the terminology of chronobiology. Circannual rhythms last about 12 ± 2months and circadian rhythms about 24 ± 2hours. Both circannual and circadian rhythms are endogenous or free running under constant conditions. The normal physiological conditions of an animal are entrained to diel cycles, animals do respond to stimulus equally throughout the 24 hours period of a day. The differential responses have been related to both endogenous cycles and to natural environmental changes including diel

changes in light, temperature, dissolved oxygen and pH. The ability of teleosts, to regulate their metabolic activities in order to meet environmental oscillations is quiet advantageous. Air breathing teleosts display various types of respiratory changes with fluctuations in environmental conditions with circadian rhythm representing one of these peculiar adaptations (Reddy and Natarajan, 1970). Circadian rhythm in AB fishes is affected by interactions between both the exogenous and endogenous rhythms and is species specific characteristic.

It is generally assumed that the ability of fish to resist hypoxia decreases with increasing temperature (EIFAC, 1973; Alabaster *and* Lloyd, 1980; Chapman, 1986). Davis, (1975) found that incipient sublethal response thresholds of adult fish were insensitive to temperature when expressed as a concentration (ie. mg l^{-1}). Ultsch *et al.*, (1978), on the other hand, reported that asphyxiation levels were actually lower at 20^0C than at 10^0C, even when expressed as partial pressures, in adults of six species of darters.

1.5 EXCRETION

During metabolic reactions a number of 'useless' and injurious products are formed. The removal of these from the body is excretion. Some of the main excretory products are carbon dioxide, undigested food materials, excess of water, salts and nitrogenous products of protein catabolism. Out of these, carbon dioxide along with a number of volatile substances (alcohol, ketone bodies, aromatic oils, water vapours etc.) are excreted directly by gills /lungs. Undigested food materials along with heavy metal ions are removed through the digestive system. Skin is one more excretory route through which

salts, water and fat derivatives are removed. The last category ie. nitrogenous wastes create the main problem of elimination (Nagabhushanam *et al.*, 1989).

Any organism that ingests and metabolises protein, generates bicarbonates. When the protein is hydrolysed and the constituent amino acids are oxidised, one bicarbonate ion is produced for each peptide bond in the protein plus one for each carboxylate or carboxyamide group in the side chains of glutamate, glutamine, aspartate and aspergine. Approximately the same amount of ammonia is produced. Normal aerobic metabolism of proteins and amino acids produce approximately equimolar amounts of ammonia and bicarbonate on the net basis (Raveendran, 2000).

Ammonia is the principle nitrogenous end product excreted by aquatic gilled animals (Smith, 1929). It is metabolically inexpensive, being formed during the catabolism of proteins and nucleic acids and requires absolutely no modifications prior to its elimination. In aqueous solutions, ammonia exists in both ionised and unionised forms. Ammonia is the small lipophilic molecule, which can diffuse easily across lipid membranes, whilst the diffusion permeability of ammonia is also substantial, although it is thought probable the diffusive loss occurs mostly in the unionised form. Evan, (1977), showed that ammonia is exchanged for sodium, although the importance of this pathway may differ among species. As well as this active exchange mechanism and the passive clearance of ammonia, it is now thought that a significant fraction of excreted ammonia in teleost fish arises from the deamination in the gill tissue.

Urea although secondary in importance to ammonia as an excretory product, can provide a significant contribution to the nitrogenous output,

values between 13 and 46% of the total output having been recorded for aquatic teleosts (Davenport and Sayer, 1986). Higher concentrations have been measured for air breathing fish, many of which are predominantly ureotelic. The urea molecule is adipole and behaves similarly to water molecules when in aqueous solution. It has a low oil-water partition coefficient and penetrates cell membranes through aqueous pores rather than through the lipid protein component. The diffusion coefficient of urea is similar to that of ammonia, although ammonia passes through most biological membranes, faster then urea, due to high lipid solubility of the un-ionised form. Urea production is usually considered to be energy expensive, although this view is contested by Vilstrup, (1989), who maintained that the "synthesis of urea can be more self sufficient in energy terms".

The two major routes for urea synthesis in fish are believed to be:

- Uricolysis, ie. the oxygen dependent conversion of uric acid, derived from purine metabolites to urea.
- Argenolysis of arginine to urea by the enzyme arginase (Mommsen and Walsh, 1991; Wilkie and Word, 1994; Wright, 1995).

In addition, there could be two other routes of urea production in fish such as,

- The urea cycle, reported recently in some teleosts and
- The γ–guanidino urea hydrolase pathway (Saha and Ratna, 1989).

Tilapia mossambica, herbivore, digests carbohydrates more efficiently, and proteins less efficiently, than carnivorous fish such as *Perca fluviatilis* (Fish, 1960). The excretion of nitrogen soon declines if mammals are given a diet free from proteins, and after a while it reaches a steady level known as the

'endogenous nitrogen excretion' (Folin, 1905). At first sight, excretion in fish appears to follow a similar path. Pora and Precoop, (1960) found that *Cyprinus carpio* starved for 90 days excreted less organic nitrogen per day than fed ones, and Sano, (1962), who measured the urea output of *Anguilla japonica* during starvation, found that it dropped sharply during the first five days and then remained steady upto 90 days.

The nitrogen excretion of the same species according to the Inui and Ohshima, (1966) showed asymptotic level in summer caught fish than winter caught, presumably because of higher temperature and metabolic rate. When *Cyprinus carpio* was starved by Vellas and Serfaty, (1967), the excretion of urea, via both gills and urine, was observed to decrease at first, but when starved for 5 months it increased again. This could be due to mobilisation of carbohydrates and lipids, rather than proteins. It seems likely therefore, the preliminary drop in the nitrogen excretion marks the end of the ingested nitrogen circulating in the blood, while the secondary rise is brought about by the rapid mobilization of structural protein after the other reserves have been utilised.

1.6 NITROGENOUS BIOMOLECULES

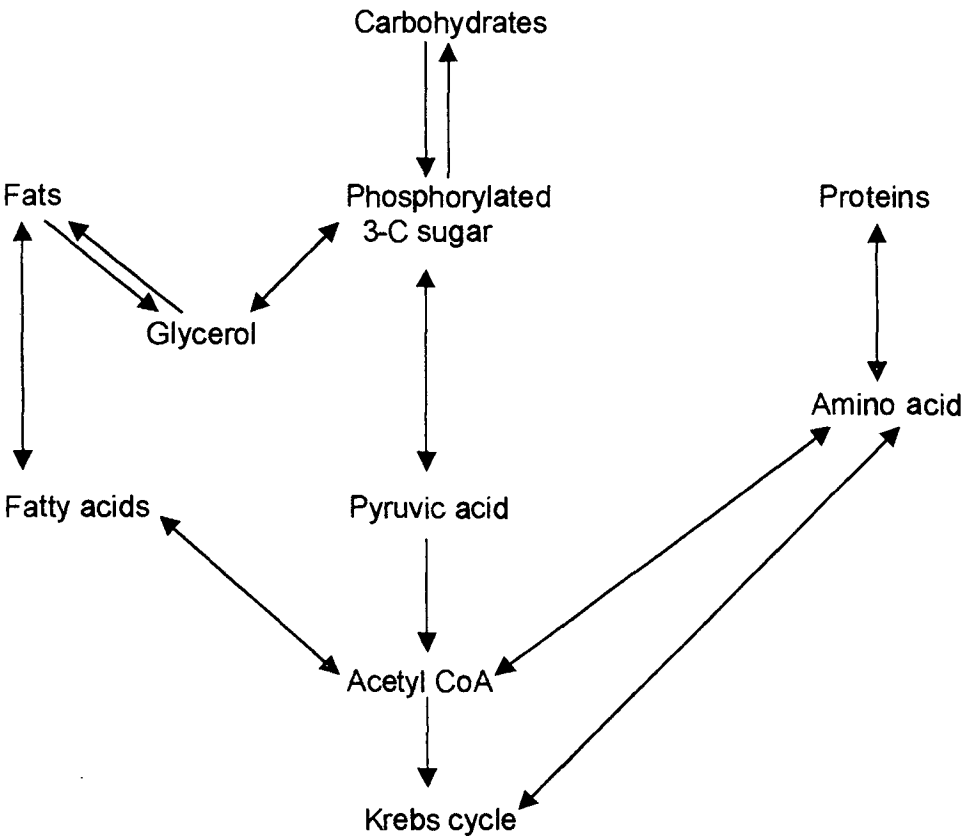
The complex physiology of any life basically depends upon the various biochemical interactions of various biomolecules, which includes proteins, carbohydrates, lipids, aminoacids, which are made of major constituents which include carbon, hydrogen, oxygen and nitrogen atoms. Life is surrounded by biotic and abiotic components of the environment. Catabolism and anabolism reactions in physiology is a must to maintain in a constant homeostasis /homophasic or homeoviscous state. *Oreochromis mossambicus*

can tolerate salt stress, thermal fluctuations and oxygen depleted environment, through osmoregulation process, brought about by the membrane physical state or homeoviscous adaptation. The exact mechanism of micro viscosity brought about by the membrane and the exact physiological and biochemical mechanisms to environmental perturbations is to be explored in fishes of tropical and subtropical waters. Hence there is dearth of information on this tropical, exotic, non-migratory and hardy fish *Oreochromis mossambicus*.

Carbohydrates and fats are primary source of energy, while proteins are required for growth and repair work of the body. However if carbohydrates and fats are in short supply, tissue proteins can be converted into carbohydrates to supply the energy need for the body. In order to maintain life, organisms need source of energy. For most organisms this is provided by the oxidation of foodstuff by molecular oxygen. In animals and certain other organisms food is obtained from exogenous sources in organic form (heterotropic nutrition). Green plants can however synthesize their own food from inorganic source (autotropic nutrition). Any organism problem does not end after the food and oxygen have been taken into the body, because these have to be transported to the constituent cells by active or passive transport mechanisms. Energy is released from the foodstuff when they are broken down, to simpler and thermodynamically more stable molecules such as carbon dioxide and water.

Fish, (1960) showed that the digestion of carbohydrates is more efficient in *Tilapia mossambica*, a herbivore, then in *Perca fluviatilis,* a carnivore, and vice versa with the proteins. Gastric digestive activity was observed to be

reduced in starving *Tilapia mossambica*, Fish (1960), but much of this finding was mearly the result of a deficiency of chloride, for which Tilapia relies heavily on its food. Nagase, (1964), working on *Tilapia mossambica*, has described it as omnivorous and possessing a full complement of enzymes. Tissue proteins are used as a source of energy in abnormal circumstances only, as normally a certain amount of energy is always derived from the excess dietary proteins. Initially dietary proteins are hydrolysed into their constituent amino acids, which undergoes deamination and the residual carbon skeleton then enters the carbohydrate metabolism with subsequent release of energy. The amino acid residue may be converted into acyl CoA, pyruvic acid or a kreb's cycle intermediate (ketoglutarate, succinate or oxaloacetate). The reactions are reversible so that certain amino acids, can be resynthesised from carbohydrate and carbohydrate can be resynthesised from amino acid. The reactions by which the intermediates of the cycle are drained off and formed remains in a state of dynamic equilibrium so that the concentration of the intermediates in the mitochondria remains constant. It is clear that the kreb's cycle not only supplies energy but also provides the opportunity for metabolic interconversions between carbohydrates, fats and proteins as shown below. The liver plays an important role in controlling these metabolic interconversions.



Fats can be utilised in releasing energy for the synthesis of ATP. When demands of energy are greater, or carbohydrate is in short supply, fats are hydrolysed into glycerol and fatty acids. The former is phosphorylated and converted into 3C sugar, which is then converted into pyruvic acid and recycled into the kreb cycle. Meanwhile the amino acid component goes through a series of reactions in which carbon atoms are lost, two at a time. Each of the two carbon atom units forms a molecule of acetyl CoA that enters krebs cycle. The oxidation of fatty acids takes place in mitochondrial matrix. The successive oxidative removal of acetyl CoA units from the chain of fatty acids is called beta oxidation. A complete cycle involves a number of steps, at the end of which one acetyl CoA molecule unit is released. For complete

oxidation of a 16C palmitic acid, seven oxidative cycles will be required yielding eight molecules of acetyl CoA units.

About 48% of the standard free energy are recovered from the palmitic acid molecule in the form of phosphate bond energy ATP. The number of ATP molecules produced by the complete oxidation of fatty acid depends upon the number of carbon atoms it contains.

Under any given conditions there is a great variation in the number of total different molecules in cell. It may be concluded that there must be certain devices to ensure selective synthesis of protein molecules, so that the cell can respond to varying nutritional and environmental conditions. In all living organisms, a rapid adaptation to changes in environment is facilitated by meticulous regulation of the cells metabolic activities in more ways than one.

Energy utilized by organisms comes either from the sun or from exergonic oxidative chemical reactions. Similarly, the matter absorbed by organisms may be very simple, in the form of inorganic chemical substances or ions, or complex organic molecules. Thus, the way the organisms derive their nutrition and utilize energy may form an important basis for structural specialization.

Proteins in an animal are being constantly degraded and resynthesised from the free amino acid pool in tissues. A dynamic steady state always prevail between these two opposite processes of protein catabolism and anabolism. Although protein degradative systems in fish, has been broadly classified as lysosomal and non-lysosomal proteolytic systems (Saha and Das, 1994).

Several molecular mechanisms in eukaryotic cells are known to be essential adaptation to fast changes in environmental conditions. These

mechanisms include the synthesis of new polypeptides, the activation of proteolytic mechanisms able to remove the damaged or unfolded proteins generated by stress condition (Ang *et al.*, 1991). Variation in biochemical constituents like protein, carbohydrate, lipid, seems to be mainly influenced by reproductive cycle and availability of food (Jayabal and Kalyani, 1986).

1.7 LIPID BIOMOLECULES

A family of biomolecules, the lipids are water insoluble, oily or greasy organic substances that are extractable from cells and tissues by non polar solvents such as chloroform or ether have even number of carbon atoms. The simplest and most abundant lipids containing the fatty acids as building blocks are the triglycerol, also referred to as fats, neutral fats or triglycerides, (TG), which are esters of alcohol, glycerol with three fatty acid molecules. They are mainly storage fats found in fat cells or adipose tissues of animals and are convenient source of energy, not found in the membranes, non-polar and hydrophobic molecule. Those containing a single type of fatty acids esterified to all three positions of glycerol are simple TG. Fatty acids could be stearic acid, palmitic acid oleic acid. The trivial and more commonly used names of these simple TG are tristearin, tripalmitin and triolein. Presence of two or more different fatty acids is mixed TG, present in natural fats such as olive oil, butter, having different chain length and degree of saturation. Saturated fatty acids such as 16C palmitic acid and 18C stearic acid, have no double bond in their hydrocarbon chain, while 18C oleic acid has double bound and hence unsaturated which is biologically important as it increases the flexibility of the hydrocarbon chain as well the fluidity of the biological structure. TG is either

liquids or solids. Unsaturated fatty acids are liquids at room temperature while saturated fatty acids are solids.

Leela *et al.*, (2000) suggests, lipids play a very important role in the architectural dynamics of the cell and transport mechanisms across the cell membranes. Any stress is found to change the course of events associated with the lipid synthesis. Lipids also contribute to energy production as they are having high calorific value. Lipids play a vital role during the biochemical adaptations of animals to stress conditions.

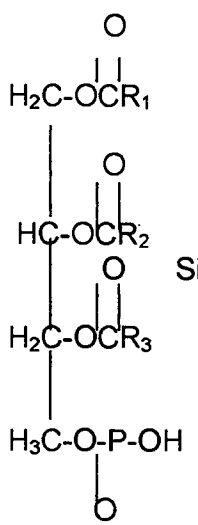
Fish is highly nutritious, easily digestible and much sought after food. Nutritional value of fish depends on their biochemical composition, which is affected by water pollution, and alterations in biochemical constituents in tissues, may reflect redistribution of carbohydrates, proteins and lipids. Hence the present study was undertaken to investigate the impact of naturally occurring pollutant, viz., sodium chloride, in freshwater on biomolecules concentration, in various tissues, seasonally. The most abundant membrane lipid are the phospholipid (PL), also called as phosphoglycerides, which contain two fatty acid molecules, esterified to the first and the second hydroxyl group of glycerol, while the third hydroxyl group is esterified to the phosphoric acid. The PL contains two hydrophobic fatty acid hydrocarbon chain forming tail (non-polar) and hydrophilic phosphate head (polar). Thus PL are amphipatic. The most important and abundant PL are the phosphatidylcholine and phosphatidylethanolamine which contains the alcohol's chloine and ethanolamine respectively on their polar heads. Cholesterol (CH) is synthesized in all animal tissues. The liver accounts for 90% of overall endogenic CH and its esters (Jyothi and Narayan, 2001).

Cholesterol is the major sterol in all animal tissues, important component of cell membrane and is a precursor of many steroids. CH also called as Cholest-5en-3B-ol or cholesterin C_{27} $H_{46}O$), molecular weight of 386.64 with C=83.87%, H=11.99% and O=4.14%. Cholesterol from animal organs always contain cholestanol (dihydrocholesterol) and other saturated sterols. CH when anhydrous MP=148^{0}C, practically insoluble in water and slightly soluble in alcohol. (1.29% w/w at 20^{0}C and more soluble in hot alcohol. It is precipitated by digitonin, (Martha, 1976).

CH_2-CO-R^1

CH-O-CO-R^{11}Simple triglyceride. R^1 R^{11} $R^{111}$are fatty acid hydrocarbon chain.

CH_2OH-CO-R^{111}



Simple phospholipid. R^1 R^{11} $R^{111}$are fatty acid hydrocarbon chain.

Considering the fluid mosaic model of membrane structure, Singer and Nicholson, (1972), which predicts that the physical state of membrane depends on the membrane lipid composition which itself is very sensitive to environmental perturbations and the membrane are in liquid crystalline state LCS at the body temperature which in turn may change in case of

poikilothermic animals. With the rapid fluctuations of environmental temperature and salinity, during summer and winter, the poikilothermic animals, like fishes, are facing a great challenge to maintain the homeostasis with respect to membrane bounded cellular activities. Adaptation of membrane composition (lipid bilayer) and physical state (fluidity/ microviscocity) of the membrane during cold adaptation, winter acclimatization /low temperature acclimation in laboratory in fresh water teleost, have been studied scatterdly by several workers. Increase in the relative concentration of unsaturated fatty acids, and alteration of polar head groups of phospholipid due to cold adaptation is reported for several fishes (Dey *et al.*, 1993; Farkas and Roy, 1989; Hazel and Zebra 1986; Hazel and Carpenter, 1985; Cossins and Lee, 1985). These alterations are achieved by the activation of different enzymes viz, phospholipase A, fatty acid synthetasse, acyltransferase, desaturase (Hazel, 1995; Buda *et al.*, 1994; Cossins and Raynard 1987). This generality is based on the studies on fishes of cold and temperate climate. However the entire mechanism of how the membrane fluidity or microviscocity is regulated by membrane lipid bilayer in a perturbed environment is not fully understood. Moreover there is absolute dearth of information in this respect with regards to tropical Indian freshwater fishes.

Physiological and biochemical reactions in poikilotherms are subjected to Vant Hoff's or Arrhenius rate temperature laws. Compensation for temperature of vital physiological functions has been reviewed by Percht *et al.*, (1973).

Lipids are the first to be mobilised during starvation. Once the lipid reserve decreases to a certain point, the carbohydrate and protein start to decline

Bahuguna and Rawat, (1994). The sequential mobilisation of protein, lipid and carbohydrate being different, in different fishes during starvation Love, (1970; 1980). Starvation causes a progressive reduction in lipid reserves, which must reach a critical low value before proteins to be utilized. It has been shown that fish starve more slowly at lower temperatures due to reduction in their metabolic activities and less oxygen consumption. Gradual increase in temperature may encourage more swimming activity and increase in oxygen consumption.

1.8 HISTOLOGY

Man's industrial and agricultural activities often produce water quality alterations, particularly as a result of contamination by heavy metals. Many studies have dealt with toxic effects of these metals on fish chemoreception and chemical communication. Since chemosensory receptors are directly exposed to aquatic environment, these therefore appear to be the prime targets for interactions with pollutants (Sutterlin, 1974). Among the chemosensory channels, the olfactory system has received particular attention in toxicity experiments. Alteration in the multifunctional organ, the gill, that constitutes the main site of respiratory gas exchange, may reflect evolutionary adaptation to different environment and modes of life (Hughes 1966, 1982). Lot of work has been carried out on the histopathology of gill tissue organization to assess the effect of the pesticide on the respiratory organ (Dalela *et al.*, 1979 and Evans 1987).

Histology is the term derived from the Greek 'histo' meaning tissue and 'logia' meaning 'the study of' or 'knowledge' or science of tissue of of both plants and animals, (Carleton and Short, 1954). It is the German anatomist

who coined the term histology in 1819 and later in 1852 English microscopist Quekett described histology as 'science of microscopic anatomy of animals and plants' (Leeson and Lesson, 1981). Histology is the science that deals with detailed structure of animals and plants and in its broad aspect correlates structural features with function. We are aware that the vital functional units in a living organism are called as cells, and they not only exhibit variations with regard to form and structural contents, but also may vary in physiological status.

Many researchers have worked on histopathology based on histological techniques for eg. Chakraborty and Mandal, (1992), studied the histopathological changes in liver and kidney of mice fed with pure Karanja oil and its different fractions. While Bhonde, (1997) studied the factors influencing induction and reversal of virus induced diabetes by making the use of histology and his method of study. Rajinikukreja and Khan, (1997) studied selenium deficiency and its supplementation on lymphoid organ like spleen, thymus in albino rats using histological techniques. Histopathological studies on cardiac lesions in mice was studied by Kurvilla *et al.*, (1998).

Apart from the above, array of histopathological examinations has been carried out using rabbit as a model, Sato *et al.*, (1994), Harima *et al.*, (1996). Toxicity studies were conducted by following histological techniques by Nair *et al.*, (1998) and Kandarkar *et al.*, (1998). Sacco *et al.*, (1991) used histology as a tecnique to understand immunological responses in squirrel monkey. However, it is surprising to see not much histological work has been carried out in one of the most important membeer of order Perciformis, namely *Tilapia mossambique* (*Oreochromis mossambicus*). Hence to fill the lacunae of

knowledge, the present work was undertaken in a view, not only to study and understand histological aspects in various tissues such as gills, liver, large intestine and testes, but also to find the alterations in the histology with STT and MTT for 10ppt and 20ppt sodium chloride concentrations.

The performance of an organism is the crucial link between its phenotype and its ecological success. When does an organisms morphology, affect its performance? Quantitative mechanistic analyses of how function depends on biological form have shown that the relationship between morphology and performance can be non-linear, context dependent and sometimes surprising. In some cases, small changes in morphology or simple changes in size can lead to novel functions, while in other cases changes in the form can occur without performance consequences. Furthermore, the effect of specific change in morphology can depend on size, shape, stiffness or habitat of an organism. Likewise a particular change in posture or behaviour can produce opposite effects when performed by bodies with different morphologies. These mechanistic studies not only reveal potential misconception that can arise from the descriptive statistical analyses often used in ecological and evolutionary research, but they also show how new functions, and novel consequences of changes in morphology, can arise simply as the result of changes in size or habitat. Such organismal level mechanistic research can be used in concert with other tools to gain insight about issues in ecology (eg. foraging, competition, disturbance, keystone species, functional groups) and evolution (eg. adaptation, interpretation of fossils and origin of novelty) (Koehl, 1996).

The histopathological studies on fish is a noteworthy and promising field to understand the extent to which changes in the structural organization occurs in the organs due to the pollutants in the environment. Most information about the environmental pollution on aquatic animals has been obtained from mortality studies (Singh *et al.*, 1990). Very little is known about the damage to different internal organs through physiological and biochemical fluctuations occurring in the organisms, consequent on the exposure to environmental poison. The structural change sin the organs at microscopic, cellular and organ level leads to alterations of the function systems (Jeelani and Shaffi, 1988).

STATEMENT OF PROBLEM

The state of Goa is located in the Konkan region along the west coast of India along $14^0 51^1$ N latitude and 73^0E longitude, with a length of 105km, from north to south and is 60km wide, from east to west. The area amounts to 3701km^2 (MPEDA, 2000). Besides being sanctuaries for the estuarine fisheries along 104km coast line, the rivers afford potential 12000ha khazan or khar lands along the banks, of which around 4500ha is totally marshy, can be gainfully developed for fish farming or pisciculture and prawn farming. There are estimated 400 tidal fed prawn filtration farms along the banks or creeks and riverlets bordering the rivers in the tidal zone. Around 100ha area has been developed for prawn farming during the last five years of 250km inland water line.

The freshwater resources of Goa state are comparatively very limited, and over major part of the estuaries in the tidal zone, the traditional fishing is carried out by bag nets. The marine fish production, which was around 17,000

million tonnes until 1963, has been steadily increasing to 96039million tonnes by 1996. This includes around 3302million tonnes approximately from estuaries, creeks and prawn farming areas. Inspite of increase in the total fish production, it is not satisfying the demand of fish for the increasing population.

95% of Goan population consume fish as their main protein source. Since 1975, the frozen prawn export picked up but under stiff competition and changing international import tariffs there was a setback after 1986, but the export market has picked up since 1991. As against 4429 tonnes of exports of marine products, valued 17.6crores in 1992, the same increased to 11908tonnes, valued 60.03 crores in 1996.

The notable feature is the export of fish species like Thread fin bream, Ribbon fish, Seer fish, Promfrets and sea-foods like Squids, Cuttle fish, Crabs. This is over and above the traditional export of frozen shrimps which constitutes 35.8% in value for the year 1996. Fishes caught in Goa are marketed as fresh fish or sun dried fish or salted fish and a small quantity is used as manure. Of the fishes caught mackerels accounts for the maximum catch followed by sardines. The other major fish caught are Mackeral 21741 tonnes, Sardines 17120 tonnes, Prawns 4174 tonnes, Promfrets 1191 tonnes, Seerfish 1143 tonnes, Silverbelly 4558 tonnes, Shark 964 tonnes, Cuttlefish 5159 tonnes, Ribbonfish 3720 tonnes, Scianoids 4150 tonnes, Threadfin bream 4648 tonnes, others 22709 tonnes, during 1997 (MPEDA, 1998a,b).

In the early 80's ICAR have reported that, Goa has lots of scope for aquaculture, and hence bivalves (oysters) and prawn culture boosted up drastically in 18,000 hectare khazan lands. There is lack of knowledge in boosting of pisciculture in these khazan lands since most of the fishes are

sensitive to slight environmental fluctuations. Also most of these khazan lands are not utilized for cultivation of crops, due to infertility of land and high cost of nitrogen fertilizers. Also very little is known about this exotic fish in Indian waters.

Although seasonal fluctuation of water temperature is the most important environmental stimulus for triggering adaptive physiological and biochemical changes in an annual cycle in the aquatic animals, like fish, thermal independence of rhythmic oscillatory changes in fishes are quiet unknown (Hoar and Robertson 1959; Beamish, 1964).

A rise in temperature increases metabolic rate, which in turn accelerates growth of eggs and fish. The velocity of development of *Salvelinus fontinalis* and *Salmo gairdnerii* embryos increases with increasing water temperature (Garside 1966a,b). A similar situation exists in *Gadus morhua* and *Perca fluviatilis*, where the size of the generation increases in a season of unusually high temperature (Moiseev, 1955).

To fill the lacunae on the above information, and also to make an sincere attempt to analyse and recommend a suitable species of fish for aquacultural purposes, particularly in khazan lands, where salinities is high, the present work was undertaken.

AIM

The objective of the present study was to obtain the basic information on physiology, ethology, biochemical and histological adjustments of *Oreochromis mossambicus* towards environmental changes. The extent of participation of facultative air breathing machinery, osmosis, nitrogenous excretion, behaviour, tissue nitrogenous and tissue lipid biomolecules, and the survival mechanism of the fish during safe and sublethal dose of salinity exposure, constitute an important part of the work.

The following specific aspects were investigated.

1. To find the LC_{50} value of fish, for sodium chloride.
2. To study behavioural adaptations during increasing salinity treatment.
3. To study seasonal dynamics in oxygen consumption during different salinity treatments for varying exposure periods.
4. To study the seasonal change in the water content of different tissues during different salinity treatments and for varied exposure periods.
5. To study seasonal relationship between nitrogenous ammonia and urea excretion during different salinity treatments for varied exposure periods.
6. To study the seasonal dynamics of tissue nitrogenous macromolecules during varied salinity treatments for different exposure periods.
7. To study the seasonal dynamics of some lipid macromolecules during different salinity treatments for varied exposure periods.
8. To study the histology and histomorphological changes if any, in different tissues during different salinity treatment and for varied exposure periods.

SCOPE

- If the physiological mechanisms of adaptation brought about by this fish, with respect to nitrogen excretion, in different seasonal temperatures and salinity concentration is known, then this hardy exotic fish could be introduced in the semiaquatic environment of saline khazan lands for obtaining expensive nitrogenous fertilizers like urea, for crops.
- If the physiological mechanisms of adaptation brought about by this fish, with respect to oxygen consumption and behaviour, in different seasonal temperatures and salinity concentration is known, then this hardy exotic fish could be introduced in any aquatic medium (fresh, brackish or saline water) for aquacultural purpose and could be used in home aquarium, without aerator.
- If the biochemical mechanisms of adaptation brought about by this fish, with respect to nitrogen macromolecules like protein, ammonia and urea content in different seasonal temperatures and salinity concentration, in different tissues is known, then this proteinaceous fish could be aquacultured, to meet the nitrogenous protein demand, especially in growing children population suffering from kwashiorkor's syndrome, (the deficiency of dietary protein causes some organs to become watery any bloated, due to inadequate synthesis of serum albumin, required to maintain water balance. Kwashiorkor-African word that means "weaning disease") and also in the adults.
- To prevent retarded growth and anaemia, followed by degeneration of liver, kidney and pancreas, which is caused due to watery and bloated

tissue due to upset of normal distribution of water between tissues and blood in protein deficient children.

- If the biochemical mechanisms of adaptation brought about by this fish, with respect to lipid macromolecules like cholesterol, triglyceride and phospholipid content in different seasonal temperatures and salinity concentration, in different tissues is known, then this low caloric value fish could be consumed safely by heart patients to some extent.
- The economy and revenue of this state can be boosted up by exporting this aqua cultured proteinaceous fish, which grows to a maximum size of 35cms, to neighbouring state.
- Thus there is a future scope of boosting pisciculture of the desired exotic and hardy fish species at any environment in Goa.

PURPOSE FOR CHOOSING *OREOCHROMIS MOSSAMBICUS* FOR THE PRESENT RESEARCH

- In Goa, khazan lands are temporarily or permanently filled with water bodies (freshwater and /or saline water), during different seasons. Also, these khazan lands shows environmental perturbations, due to temperature and salinity fluctuations, during different seasons. Hence hardy fish was the only organism, which could be introduced in such khazan lands (figure 1).
- Freshwater and exotic *Oreochromis mossambicus* (F:Chichlidae) is a hardy, eurythermal poikilotherm which can tolerate temperature ranging from 16^0C-40^0C, and very low dissolved oxygen content of water. Hence this fish was chosen which was easy to handle and easy to maintain at any temperature and at any salinity. Hence my research

work aims to find out the various mechanisms (behavioural, physiological, biochemical and histological evolved by this fish to survive in different temperature and salinity perturbations, since there is lack of information with respect to tropical, non-migratory, exotic and hardy fish.

- In short it was easy to handle, maintain and experiment on this eurythermal, euryhaline, non-migratory, exotic, hardy, freshwater poikilotherm.

ORIGIN OF FISH

History of *Oreochromis mossambicus* can be traced back in 1952, when severe malarial conditions prevailed in the states of Tamil Nadu and Kerela, due to heavy rainfall and floods. There by, as a means of biological control, exotic and hardy fish were imported from three freshwater lakes of South-East Africa, (figure 2.) which kept the mosquito larval population under control (Sterba, 1989). Since then, though a lot of work has been carried out on this fish with respect to toxic chemicals, pollutants and industrial effluents. Hardly any information is available with respect to role of sodium chloride, a natural perturbant of freshwater in these fishes.

Oreochromis mossambicus, commenly called Tilapia, is basically a planktivore and herbivore, feeding on Chlorophyceae, Myxophyceae and Bacillariophyceae. When vegetable food is scarce, it becomes omnivorous Datta *et al.*, (1993), and in extreme conditions, cannibalistic too. These cichlid are voracious feeders, feeding on benthic algae, phytoplanktons, dead and decaying materials, and are usually caught using bag net or cast net. Hence we can conclude that this fish is microphagus and macrophagus, and feeding usually presents no difficulty. Large live food of any kind is greedily accepted. Young fishes are peaceful towards one another in schools, they become more independent with increasing size and even cannibalistic. Grown fishes are often extraordinarily hostile towards one another, and a strong fish may kill all its companions of the same species. This fish grows upto 35cms in length.

This fish belongs to Phylum: Chordata, Subphylum: Vertebrata, Class: Pisces, Super class: Osteichthyes, Order: Perciformes, Super order:

Teleostei, Family: Cichlidae, Genus: *Oreochromis* and Species: *mossambicus*. Once diagnosed, *Oreochromis* shows elongated body, laterally compressed. Mouth large and terminal. Dorsal fin inserted above the base of pectoral fin with 15 or 16 spines and 10-12 soft rays. Anal fin with 3 spines and 10-11 soft rays. Caudal fin truncate, often with rounded corners. Body is covered with cycloid scales.

This fish can be easily aquacultured in the temperate and sub-tropical region due to its eurythermal and euryhaline nature. Although a freshwater fish, it can tolerate a wide range of salinity and temperature for quiet some time. Ideal temperature for culture of this fish is 23-25^{0}C. It can survive in adverse conditions like low dissolved oxygen content of water. Mortality occurs if this fish is kept in LDO=0.3mg/L for 6hours. Although the ideal oxygen level for *Oreochromis* is 4-5mg/L. It can tolerate pH range from 6.5-11, but ideal being 8-9.

Males grow faster than females during breeding and shows distinct dimorphism in colour and other features. Mouth and snout are much more enlarged, and lips becomes more fleshy. Body and fins becomes darker, usually black. Although they are not seasonal breeders, *Oreochromis mossambicus* spawns three or four times in one season and 6-11 times per year. Prespawning in these cichlids involves the pair circling each other with fins spread and there may be mutual nudging in the head region. Both excavate shallow depression in the substrate. Spawning is between 22-40 days and is influenced by climatic and other biotic and abiotic factors. During each spawning, the female lays 75-1000 eggs in the excavated shallow depression in 3-8 rows in chosen place, followed immediately by the males

who fertilizes the eggs, by gliding down upon them with extended genital papillae. This process is repeated several times. After fertilization, the female who is a mouth brooder, will take the eggs in the mouth and will take care of the eggs by buccal incubation. All this process of spawning completes within a minute. The fertilized eggs are elliptical in shape, with a maximum length-wise diameter of 2.5mm. Yolk is yellow in colour. Eggs hatch in mouth within 60hrs. at temperature of 24-26^{0}C. The young fish measures 4mm in length immediately after hatching. Yolk bearing stage lasts for 3-5 days. The alevins (post larvae) stays for another 3-5 days in the buccal cavity of mother. During brooding the female is aggressive. From the alevins emerges early juveniles which possess a black ovate blotch at the base of the first few dorsal rays which disappears as the specimen reaches the juvenile and adult stage. The early juvenile and juvenile feeds on phytoplanktons, diatoms, filamentous algae and the adult feeds on phytoplanktons, diatoms, algae, fresh and decaying aquatic angiosperms and gymnosperms in natural waters. Also feeds on animal matter (worms, insects, crustaceans, fish larvae) and detritus bacteria. This species is bold enough to grab the dirt from the feet of people bathing from rural temple tanks. Feeding intensity is maximum between 12.00 noon and 4.00pm.

2.0 MATERIAL AND METHOD

2.1 PHYSICO CHEMICAL PARAMETERS

The physico-chemical factors that are of major significance are Turbidity, Colour, Smell, pH, Chloride, Alkalinity, Hardness, Calcium, Magnesium, Phosphate, Sulphate and Iron. The interaction of these factors, make the aquatic habitat a unique one, which favours the living of

the organisms (Pillai, 1986). Water quality affects productivity and physiological conditions, apart from various other aspects. Therefore, the study of physico-chemical parameters are of prime importance.

2.1.1 Turbidity, Colour and Smell

The method followed in the present investigation to analyse turbidity was by Barium sulphate method, Colour and Smell was assessed by visible characteristics and by presence or absence of odour (APHA, 1985).

2.1.2 Test for pH

It was done by following the instructions as provided in the water testing kit, procured from C.P.R. foundation, Chennai (C.P.R. foundation 1998).

2.1.3 Test for Chlorides

To test chloride concentration in the water used for maintaining *Oreochromis mossambicus*, for present experiments, was analysed by following the methods provided in water testing kit produced from C.P.R. Environmental Education, Chennai, (C.P.R. foundation, 1998), and was confirmed further by following Argenometric Method (APHA, 1985).

2.1.4 Test for Alkalinity

Alkalinity estimation was done by following the methods provided in water testing kit produced from C.P.R. Environmental Education, Chennai (C.P.R. foundation, 1998), and was confirmed further by following Electrometric Titrator Method (APHA, 1985).

2.1.5 Test for Hardness

Hardness of water used to maintain the experimental animal, was investigated by following the methods provided in water testing kit

produced from C.P.R. Environmental Education, Chennai (C.P.R. foundation, 1998), and was confirmed further by following EDTA Titrimetric Method (APHA, 1985).

2.1.6 Test for Calcium

It was done following the procedure provided in the water testing kit procured from C. P. R. Environmental Education, Chennai, (C.P.R. foundation, 1998), and was confirmed further by following EDTA Titrimetric method (APHA, 1985).

2.1.7 Test for Magnesium

The magnesium content in the water used for maintaining *Oreochromis mossambicus* for various experiments was analysed by following the method from C.P.R. Environmental Education, Chennai, (C.P.R. foundation, 1998), and was confirmed further by following Atomic Absorption Spectrometric Method (APHA, 1985).

2.1.8 Test for Phosphate

It was done following the procedure provided in the water testing kit procured from C.P.R. Environmental Education, Chennai, (C.P.R. foundation, 1998), and was confirmed further by following Ascorbic Acid Method (APHA, 1985).

2.1.9 Test for Sulphate

Sulphate content in the water used for maintaining *Oreochromis mossambicus* for present experiments was analysed by following the method from C.P.R. Environmental Education, Chennai, (C.P.R. foundation, 1998), and was confirmed further by following Turbidimetric Method (APHA, 1985).

2.1.10 Test for Iron

Iron concentration in the water used for maintaining *Oreochromis mossambicus* for present experiments was analysed by following the method from C.P.R. Environmental Education, Chennai, (C.P.R. foundation, 1998), and was confirmed further by following Atomic Absorbtion Spectrometric Method (APHA, 1985).

2.2 LC_{50} AND BEHAVIOUR

2.2.1 LC_{50}

Toxicant concentration produced in death of test organism usually defined as (Median 50% lethal concentration) LC_{50} ie. Concentration killing 50% of exposed organisms at a specific time of observation like 24h LC_{50}, 48h LC_{50}, 72h LC_{50}, etc.

Median Tolerance Limit (TLm) is the test material concentration of which 50% of the test organisms survive for a specific exposure time. This term has been superseded by median lethal concentration (LC_{50}). In the present investigation various salinities were prepared as per (Golterman *et al.,* 1978).

[A] APPARATUS AND CHEMICALS

1) ACCLIMATIZING TANK

The test animal species were acclimatized or adapted to the laboratory environmental conditions in cemented tank of (140 x 140 x 100 cms), filled with 1000 litres of tap water after they were procured from the fish farm. The temperature of the tank water was same as the test temperature.

2) TEST CONTAINER

10 glass aquariums, which were chemically clean, of similar size (46 x 26 x 26 cms), were taken in which 10 fishes were exposed to test media in each aquarium.

3) CHEMICALS

The chemical pollutant used was local white sodium chloride (AR grade) from Sigma.

[B] SELECTION AND PREPARATION OF TEST ANIMAL

The specimen used for the study was *Oreochromis mossambicus* (family: Chichlidae) due to its hardy nature, it is easily adaptable to laboratory conditions of temperature perturbation, feeding and handling. Also availability of adequate supply of healthy fish of desirable and uniform size was possible. Moreover these fishes are not deemed important locally.

1) SOURCE

These fishes were obtained from Ela Fishery Farm, Old Goa.

2) SIZE

The test specimen were almost of equal size, ranging 10–12 cms. length, and weighing 15–25 gms.

3) STOCKS

The stock of test fishes, were kept in the acclimatizing tank with tap water.

4) ACCLIMATIZING AND FEEDING

Test animals were acclimatized to laboratory without food for 2 days and later fed *ad libitum* with wheat bran. Initially they were not fed for a period of 2 days prior to the test, as it could tend to increase the rate of respiratory

metabolism and tend to increase excretory and other waste products, which may influence the toxicity of test solution.

[C] SELECTION OF THE POLLUTANT

Sodium chloride is present in all water bodies of Goa, in small or large quantity, which in turn changes salinity. Hence the aqueous solution of sodium chloride was selected as a pollutant of choice.

[D] OTHER BIO – ESSAY CONDITIONS

TEMPERATURE: Air temperature: 25–35^0 C.
Water temperature: 22-33^0 C/16L.

pH : pH of medium was approximately 7.2–7.4

WATER IN AQUARIUM: It was 16L/aquarium.

NUMBER OF TEST SPECIMENS: 10 fishes for each test were used.

WEIGHT OF INDIVIDUAL FISH: Average weight of 15–25gms were used.

[E] OBSERVATION DURING TEST

The number of fish, which had died in each aquarium was observed regularly.

PROCEDURE.

The concentration and dilution of liquid pollutant are expressed as % volume of that solution and that of a solution of non-aqueous pollutant are expressed in terms of gm/l or ppt by weight of substance added to experimental water. Different concentrations of the pollutant were tested to find out the exact concentration at which 50% lethality appears. The dissolved oxygen concentration well above the minimal tolerable range was maintained throughout the course of each test.

To evaluate the toxicity, different concentrations was tested so that the concentration lethal to 50% of test animals for a prescribed test period (72 hours) was found out directly. But this kind of direct measurement of TLm is

often imperfect and impractical. Thus the test range should fall between highest concentration at which all fishes die and lowest concentration all fishes survive at same time period. The dose selected, were from distilled water, tap water, sea water, and 2gms/16L upto 800gms/16L which were prepared from stock solutions as described in table7. The fishes were then transferred from acclimatizing tank to the experimental solution as soon as possible. Regular renewal of experimental water was done to avoid an increase of toxicity of the medium by accumulation of metabolic wastes of fish.

2.2.2 BEHAVIOUR

METHOD DEVELOPED TO STUDY THE BEHAVIOUR IN *OREOCHROMIS MOSSAMBICUS* WERE

A) SAMPLING METHODS (Gadagkar, 2001)

i) AD LIBITUM OBSERVATION.

This means that one has to observe everything qualitatively without any specific rules or any bias. Data from this method cannot be used to measure behaviour but such data are essential prerequisite to plan appropriate sampling for any quantitative ethological study.

ii) FOCAL ANIMAL / GROUP SAMPLING.

Here attention is entirely focused on one or a small group of animals at any given time. If the decision as to when, which animal or which group is to be observed is made randomly and the observation sessions are governed by time contingent rules of starting and stopping, almost all sources of bias are minimized.

iii) FOCAL BEHAVIOUR / GROUP OF BEHAVIOURS SAMPLING.

Here atleast a few behaviours are almost observed simultaneously. As the name implies, it is the number of behaviours recorded at any given time that is reduced. For exactly the same reason as above, most sources of bias are minimized.

iv) INSTANTANEOUS SAMPLING.

- Choose animals and behaviours randomly, rather than, because they are occurring at a particular time.
- Use a predetermined duration of observation, rather than, decide it during the observation, so that we don't (even subconsciously) observe longer when something interesting and unusual is happening or stop sooner, if it turns out to be a dull and boring session.
- Use time contingent rules of starting and stopping.
- Odd it may seen, observe less.

B) BEHAVIOURAL METHODS (Santhakumar *et al.*, 2000)

BEHAVIOUR	DISCRIPTION
➢ Fanning:	Non-stop movement of pectoral fin.
➢ Burst Swimming:	Sudden and rapid movement.
➢ Nudge:	Movement of fish towards another.
➢ Nip:	Biting.
➢ Cough:	Rapid opening and closing of mouth and opercular coverings.
➢ Motionless:	No movement.

2.3 OSMOSIS

For measurement of water content in some selected tissues in control, and experimental animals, after exposing them for 7 and 21 days, each individual fish was weighed, and then carefully wrapped around with tissue paper to remove the excess water. Later they were dissected in live condition. 2gms of each of the selected wet tissues viz, gill filament, large intestine and liver were carefully removed and placed on separate pre-weighed, oven dried glass slide. Later, these tissues were blotted dry, and weighed on separate pre-weighed, oven dried glass slides. The water content in various tissues of the control and treated fishes, were calculated as the difference between the wet and dry weights, and expressed as gm. wet wt. / gm. tissue wt. respectively as per Storey, (1970). Later the results were subjected to percentage calculation.

2.4 RESPIRATION

Fishes are known to follow mainly aerial respiration and aquatic respiration.

2.4.1 AERIAL RESPIRATION

A pre-weighed fish comprising of control, 10ppt and 20ppt sodium chloride treated, exposed for 6h, 12h, 24h, 7days and 21 days, was introduced in a small rectangular jar of 750ml and 720ml capacities filled with tap water, 10ppt and 20ppt NaCl saline water. Immediately after introduction, and between the period of 5 minutes, the amount of time spend by the fish at the surface for aerial respiration was noted using stop watch before the fish could be transferred for aquatic respiration. Then based on the results of aquatic

respiration expressed in ml O_2/gm/hr, aerial respiration was also calculated in ml O_2/gm/hr only for 7 and 21 days treatment.

CALCULATION FORMULA

1minute = 60 seconds----------equation 1.

5 minutes = 300 seconds----------equation 2.

1hour = 3600 seconds----------equation 3.

For 5 minutes aerial respiration observed was -------'X' seconds --- equation 4.

Therefore, for 300 seconds, aerial respiration---'X'seconds --- equations 2 & 4.

Therefore, for 3600 seconds, aerial respiration------?

$$? = \frac{3600 \times \text{'X'}}{300}$$

Therefore for 1 hour, fish showed ? seconds of aerial respiration.

Calculating in terms of aquatic respiration,

In 3600seconds, aquatic respiration was-------'Y' ml oxygen/gm/hr.

Therefore, in ? seconds, aerial respiration was--------

$$= \frac{? \times \text{'Y'}}{3600} \text{ ml oxygen/gm/hr.}$$

2.4.2 AQUATIC RESPIRATION. (Welsh and Smith, 1960)

PRINCIPLE

The physically dissolved oxygen from the measured volume of sample water is chemically bound with manganous hydroxide. In strong alkali medium, the dissolved oxygen reacts with manganous hydroxide to form manganese hydroxide. The formation of manganese hydroxide is marked by the formation of cloudy brownish precipitate. Upon acidifying this precipitate of manganese hydroxide to a pH less than 2.5, manganic ions are liberated.

These manganic ion in an acidic medium, oxidizes the iodides that had been previously added, to liberate iodine. The liberated iodine is neutralized with standardized sodium thiosulphate solution from the burette using starch as the indicator. The end point is marked by a change in colour from blue to colourless.

CHEMICALS AND REAGENT PREPARATION

1) 40% Manganous chloride.

a) 40gms of manganous chloride.

b)100ml distilled water.

This reagent should be freshly prepared.

2) Alkaline potassium iodide solution.

a) 33gms of sodium hydroxide.

b) 10gms potassium iodide.

c) 100ml distilled water.

Mix (a), (b) and (c) and refrigerate.

3) Sodium thiosulphate solution (N/80).

a) 155mg sodium thiosulphate.

b) 100ml distilled water.

Mix (a) and (b) and refrigerate.

4) 1% Starch indicator.

a) 1gm starch.

b) 100ml distilled water.

c) Pinch of sodium chloride.

Freshly mix (a), (b) and (c) and boil slightly to dissolve.

5) Concentrated ortho phosphoric acid.

PROCEDURE

[a] SAMPLING

A pre-weighed fish comprising of control, 10ppt and 20ppt NaCl treated, batches were exposed for 6hr, 12hr, 7days and 21days, were introduced in small rectangular glass jars (15x10x30cms) of 720ml and 750ml capacities, overfilled with tap water, 10ppt and 20ppt NaCl solution respectively. The time of introduction of fish was noted. Immediately the mouth of the overflowing glass rectangular jars was covered with glass lid by sliding it horizontally over the over flowing water sample. Utmost care was taken to see that there was no turbulence while filling the sample jar and no air space left between the water surface and the glass lid. After half an hour interval, 120ml of water sample was collected by siphon system into 120ml capacity amber coloured bottle without causing turbulence. The collected water was fixed immediately.

[b] FIXATION OF OXYGEN

To the collected 120ml siphoned water sample, 1ml of winkler's A and 1ml of winkler's B reagents was added by inserting the pipette deep inside the amber coloured bottles. After stoppering, the amber coloured bottles were incubated for 20 minutes in the dark. Later 2.5ml concentrated ortho-phosphoric acid was added, to dissolve the brown precipitate.

[c] TITRATION

25ml of fixed water sample from the amber coloured bottle is initially titrated with few drops of N/80 sodium thiosulphate from the burette till the colour changes from dark yellow to light yellow. 1ml of freshly prepared starch is added to the water sample; and titration with sodium thio-sulphate is

continued till the end point is reached which is indicated by the colour change from blue to colourless.

CALCULATION FORMULA

1ml of 1N sodium thiosulphate = 5.6ml dissolved oxygen at N.T.P.

Therefore 1/80N sodium thiosulphate = ?

$$\text{Rate of aquatic resp.} = \frac{(a-f) \times 5.6 \times N \times \text{volume of sample in rectangular jar} \times 60}{\text{Volume of sample taken for titration in ml} \times \text{fish weight in gms} \times \text{time exposure in minutes.}}$$

Where, a = mean titration of tap water, 10ppt and 20ppt saline water before fish is introduced.

f = mean titration of tap water, 10ppt and 20ppt saline water after the fish is introduced.

N = normality of sodium thiosulphate used in titration.

2.5 EXCRETION

Though fish excretes a number of waste products, ammonia and urea are the most important ones.

2.5.1 AMMONIA. (Chaney and Marbach, 1962)

PRINCIPLE

Ammonia in solution becomes ammonium ion, which reacts with phenol to produce indophenol, which is blue in colour in alkaline medium. The presence of the alkali in this medium is very important because indophenol is blue in colour only in alkaline medium. But normally it is dark brown in colour. The intensity of colour varies according to the concentration of the ammonium ion present in the sample and is measured at 650nm.

CHEMICALS AND REAGENT PREPARATION

1) Alkaline hypochlorite reagent.

a) 32ml of 1N sodium hydroxide.

b) 2.1ml of 5% sodium hypochlorite.

c) 67.9ml distilled water.

Mix (a), (b) and (c) in amber coloured bottle and refrigerate.

2) Phenol colour reagent.

2.7ml phenol weighed in crucible.

12.5ml sodium nitroprusside.

84.8ml distilled water.

Mix (a), (b) and (c) in amber coloured bottle and refrigerate.

3) Standard ammonia-N solution.

(Concentration: Stock solution of 100μg/ml

: Working solution of 10μg/ml)

0.019gms ammonium chloride-stock solution of 100μg/ml.

50ml distilled water ammonia free.

1ml of stock solution was mixed with 9ml distilled water and deep freezed-working solution.

PROCEDURE

For measurement of ammonia excretion in control, 10ppt and 20ppt NaCl concentrations, after exposure for 7, 21 and 30 days fish, individual fish were weighed and then placed in glass specimen rectangular jars of 3000ml capacity, containing 1500ml tap water, 10ppt and 20ppt NaCl saline water without food for 24hrs. Later water sample was filtered using whatmann's filter 1, and 1ml sample aliquot for each assay was used. Ammonia estimation in

excreted water sample was followed as by the above method using alkaline hypochlorite to produce blue colour, which was read at 650nm using spectrophotometer using blank, with ammonium chloride as standard.

$$\text{Excretion rate} = \frac{\text{vol. of sample in rectangular jar x excreted ammonia from standard graph in } \mu g.}{\text{sample aliquot for ammonia excretion in ml x fish weight in gms x time interval for excretion measurement.}}$$

2.5.2 UREA. (Ashley, 1968)

PRINCIPLE

In acidic medium, and in the presence of ferric ion, diactyl monoxime reacts with urea and gives a pink coloured complex called diacyl monoxime urea complex. The intensity of the colour depends on the amount of urea-N present and is measured at 520nm. In the presence of acid only, it is a yellowish to dark yellowish substance.

CHEMICAL AND REAGENT PREPARATION

1)Acid mixture.

a) 0.01gms ferric chloride.

b) 100ml of 85% orthophosphoric acid.

Mix (a) and (b) in amber coloured bottle and refrigerate.

2) Diacetyl monoxime reagent (2,3 butane iodine monoxime c.p.).

a) 2.5gms diactyl monoxime.

b) 0.12gms thio semicarbazide.

c) 100ml distilled water.

Mix (a), (b) and (c) in amber coloured bottle and refrigerate.

3) Urea-N standard solution.

(Concentration: Stock solution of 100μg/ml

: Working solution of 10μg/ml)

3mg urea-stock solution of 100μg/ml.

50ml distilled water free.

1ml of stock solution was mixed with 9ml distilled water and deep freezed-working solution.

PROCEDURE

For measurement of urea excretion in control, 10ppt and 20ppt NaCl concentrations, after exposure for 7, 21 and 30 days fish, individual fish were weighed and then placed in glass specimen rectangular jars of 3000ml capacity, containing 1500ml tap water, 10ppt and 20ppt NaCl saline water without food for 24hrs. Later water sample was filtered using whatmann's filter 1, and 1ml sample aliquot for each assay was used. Urea estimation in excreted water sample was followed as by the above method of using diactyl monoxime to produce pink colour, which was read at 520nm using spectrophotometer using blank, with urea as standard.

$$\text{Excretion rate} = \frac{\text{vol. of sample in rectangular jar x excreted urea from standard graph in } \mu g.}{\text{sample aliquot for urea excretion in ml x fish weight in gms x time interval for excretion measurement.}}$$

2.6 NITROGENOUS BIOMOLECULES

2.6.1 PROTEINS. (Lowrey *et al.*, 1951)

PRINCIPLE

Proteins react with folin-ciocalteau reagent to give a coloured complex. The colour so formed is due to the reduction of alkaline copper with proteins and reduction of phosphomolybdate by tyrosine and tryptophan, present in the protein.

CHEMICAL AND REAGENT PREPARATION

1) Alkaline solution.

4% sodium carbonate solution.

2% copper sulphate solution.

4% sodium potassium tartarate.

490ml of (a), 5ml of (b) and 3ml of (c) was prepared on the day of use and stored in refrigerator.

2) 1N sodium hydroxide.

1gm sodium hydroxide was dissolved in 25ml distilled water.

3) Standard bovine serum albumin solution (BSA).

(Concentration 250μg/ml of 1N sodium hydroxide).

1mg BSA in 4ml of 1N sodium hydroxide.

4) Folin ciocalteau reagent.

PROCEDURE

The tissues (gill filament, large intestine and liver) from control, 10ppt and 20ppt NaCl concentrations exposed for 7days, 21days and 30 days fish, were dissected out freshly and transferred immediately in a icepan in petridishes. A known amount of 100mg was homogenized separately with 2ml distilled water

using glass teflon homoginiser. The homoginate was centrifuged at 2000 rpm for 10mins. The residue was discarded, and 20µl of supernatent aliquot was used. Protein content of tissue homogenates were determined by the above method using crystalline BSA as the standard, and phenol ciocalteau reagent to produce blue coloured complex, which was read at 690nm using spectrophotometer against a blank solution.

$$\text{Concentration of proteins in tissue} = \frac{\text{total vol. of supernatant in ml. x amount of protein from standard graph in } \mu g.}{\text{vol. of sample taken for protein estimation in } \mu l \text{ x total weight of tissue in mg.}}$$

2.6.2 AMMONIA-N. (Chaney and Marbach, 1962)

PROCEDURE

The tissues (gill filament, large intestine and liver) from control, 10ppt and 20ppt NaCl concentrations exposed for 7days, 21days and 30 days fish, were dissected out freshly and transferred immediately in a icepan in petridishes. A known amount of 100mg was homogenized separately with 2ml distilled water using type glass teflon homoginiser. 0.5ml of 5% zinc sulphate and o.5ml of 0.3N barium hydroxide was added to each homoginate, vortexed on cyclomixer and centrifuged at 2000rpm for 10mins. Supernatent aliquot volume was noted and 0.2ml of aliquot sample was used for ammonia estimation, by the above method using ammonium chloride as the standard, and alkaline hypochlorite reagent to produce blue coloured complex, which was read at 650nm using spectrophotometer against a blank solution.

total vol. of supernatant in ml. x amount

of ammonia from standard graph in μg.

Concentration of ammonia in tissue = ----------

sample aliquot for ammonia

estimation in μl x total weight of tissue in mg.

2.6.3 UREA-N. (Ashley, 1968)

PROCEDURE

The tissues (gill filament, large intestine and liver) from control, 10ppt and 20ppt NaCl concentrations exposed for 7days, 21days and 30 days fish, were dissected out freshly and transferred immediately in a icepan in petridishes. A known amount of 100mg was homogenized separately with 2ml distilled water using type glass teflon homoginiser. 0.5ml of 5% zinc sulphate and o.5ml of 0.3(N) barium hydroxide was added to each homoginate, vortexed on cyclomixer and centrifuged at 2000rpm for 10mins. Supernatent aliquot volume was noted and 0.2ml of aliquot sample was used for urea estimation, by the above method using urea as the standard, and diactyl monoxime reagent to produce pink coloured complex, which was read at 520nm using spectrophotometer against a blank solution.

total vol. of supernatant in ml. x amount

of urea from standard graph in μg.

Concentration of urea in tissue = ----------

sample aliquot taken for urea

estimation in μl x total weight of tissue in mg.

2.7 LIPID BIOMOLECULES. (Kate's, 1968)

EXTRACTION OF LIPIDS

The tissues (gill filament, large intestine and liver) from control, 10ppt and 20ppt NaCl concentrations exposed for 7days, 21days and 30 days fish, were dissected out, and a known amount of 0.2gm of each tissue, for various types of lipid quantification, were pooled. Later homogenised separately with 4ml of 2:1 chloroform:methanol solvent, using teflon glass homoginiser. This was then filtered and the residue was re-extracted for remaining lipids using 2ml of the same solvent. To the pooled filterate, 2.5ml of 0.2(N) sodium chloride was added, vortexed and kept overnight in the refrigerator for complete phase separation. The solvent phase was discarded and the lipid phase was filtered into another test tube using filter paper and activated sodium sulphate for complete absorption of any trace of water molecule from lipid sample. Then dried in small glass vials using nitrogen gas cylinder with accessories and 1ml benzene/BHT was added to it and then transferred in ependroff tubes. 100µl (0.1ml) of aliquot was used for each of the different types of lipid estimation (Floch *et al.*, 1957).

2.7.1 CHOLESTEROL

PRINCIPLE

The cholesterol has a hydroxyl group, which is hydrophilic. This hydroxyl group of cholesterol forms ester bonds with hot solution of diluted ferric chloride and acetic acid to give a brown colour. The intensity of brown colour determines the amount of cholesterol present in the sample and is measured at 550.

CHEMICALS AND REAGENT PREPARATION

1) Ferric chloride reagent (stock solution of 2.5%).

a) 2.5gms ferric chloride.

b) 100ml orthophosphoric acid.

2) Dilute ferric chloride solution (working standard solution).

a) 4ml of stock solution.

b) 46ml concentrated sulphuric acid.

3) Standard cholesterol solution.

a) 3mg cholesterol.

b) 6ml hexane.

PROCEDURE

A lipid aliquot was taken and dried on a hot plate. To it, 6ml of acetic acid and 4ml of dilute ferric chloride solution were added. The mixture was then incubated at 25^0C ice bath for 15mins. The intensity of the colour was measured against a suitable blank at 550nm. Finally, the quantification of sterol content of the sample was calculated with the help of a prepared standard curve for cholesterol.

$$\text{Tissue sterol concentration} = \frac{\text{total vol. of extracted lipid in test-tube vial made upto 1ml} \times \text{sterol concentration from standard graph in } \mu g.}{\text{vol. of sample taken for cholesterol estimation in } \mu l \times \text{total weight of tissue in mg.}}$$

2.7.2 PHOSPHOLIPID

PRINCIPLE

The phospholipid present in the lipid sample reacts with 10% alcoholic magnesium nitrate and ammonium molybdate in acidic medium to give dark brown colour. The intensity of this brown precipitate, marks the amount of phospholipid present in the sample, which is quantified at 820nm using a spectrophotometer.

CHEMICALS AND REAGENT PREPARATION

1) 10% alcoholic magnesium nitrate.

a) 10gms magnesium nitrate.

b) 100ml ethyl alcohol.

2) 0.5N hydrochloric acid.

a) 4.1ml hydrochloric acid.

b) 95.9ml distilled water.

3) 0.42% ammonium molybdate solution.

a) 0.42gms ammonium molybdate.

b) 100ml of 1N concentrated sulphuric acid.

4) 1N sulphuric acid.

a) 10.9ml sulphuric acid.

b) 89.1ml distilled water.

5) 10% aqueous ascorbic acid.

a) 10gms ascorbic acid.

b) 100ml distilled water.

This solution must be freshly prepared.

PROCEDURE

A lipid aliquot was taken, and evaporated on a water bath. After evaporation, 0.2ml of 10% of alcoholic magnesium nitrate was added, and the mixture charred by keeping over a Bunsen flame at 300^0C till a white powder was formed. This powder was dissolved in 1.5ml of 0.5N hydrochloric acid, and the mixture was kept on the water bath for 30mins. Later, 3.5ml of ammonium molybdate reagent was added and again kept on water bath for colour development. The intensity of the colour was then measured against the blank at 820nm. The quantification of phospholipid content of the sample was calculated with the help of a prepared standard curve for ATP.

$$\text{Tissue phospholipid concentration} = \frac{\text{total vol. of extracted lipid in test-tube vial made upto 1ml} \times \text{PL concentration from standard graph in } \mu g.}{\text{sample vol. for phospholipid estimation in } \mu l \times \text{total weight of tissue in mg.}}$$

2.7.3 TRIGLYCERIDE

PRINCIPLE

Triglyceride can be estimated at 570nm, with sodium per iodate, sodium arsenate and chromotropic acid reagent. A brown complex is formed and the intensity of brown colour is the measure of amount of triglyceride present within the sample.

CHEMICALS AND REAGENT PREPARATION

1)0.4% ethanolic potassium hydroxide.

a) 0.4gm potassium hydroxide.

b) 100ml ethanol.

2) 0.25N sulphuric acid.

a) 2.7ml sulphuric acid.

b) 97.3ml distilled water.

3) 0.05M sodium arsenate solution.

a) 3.12gms sodium arsenate.

b) 20ml distilled water.

Mix (a) and (b) and refrigerate.

4) 0.5(M) sodium arsenate solution.

a) 3.12gms of sodium arsenate.

b) 20ml of distilled water.

5) Chromotropic acid reagent.

a) 0.448gm sodium salt chromotropic acid.

b) 40ml distilled water.

c) 120ml ice chilled sulphuric acid.

This reagent should be freshly prepared.

PROCEDURE

A lipid aliquot was taken and dried in a glass stoppered vial on a water bath. The lipid was saponified at 60-70^{0}C for 30mins, with 0.5ml of 0.4% ethanolic potassium hydroxide solution. The mixture was then cooled and neutralized by adding 0.5ml of o.o25N sulphuric acid, and the ethanol was completely removed on a water bath. Next, 0.1ml of 0.05M sodium per iodate was added. After 10mins, 0.1ml of 0.5M sodium arsenate was added and was allowed to stand for another 10mins. Then 5ml of freshly prepared chromotropic acid was added and equilibrated to 30mins for developing the colour on a boiling water bath. The test tubes were then ice cooled, and the

intensity was measured against a blank at 570nm. The quantification of triglyceride content of the sample was calculated with the help of a prepared curve for tripalmitin.

$$\text{Tissue triglyceride concentration} = \frac{\text{total vol. of extracted lipid in testtube vial made upto 1ml} \times \text{TG concentration from standard graph in } \mu g.}{\text{sample vol. for triglyceride estimation in } \mu l \times \text{total weight of tissue in mg.}}$$

2.8 HISTOLOGY

It is presumable that structural change and physiological activity are closely linked. Thus any structural change will therefore be easily reflected in the altered functional integrity of the tissue in question.

In the study of histology, the most important consideration with regard to methodology is the preparation of the tissues or organs in a manner suitable for viewing under the Olympus Bx-40 trinocular stereo-microscope as per (Pearse, 1972). In the present study paraffin technique using microtome were employed for standardization and cryostat technique was used for the preparation of permanent histological slides. *Oreochromis mossambicus* were dissected live, and different tissues / organs was removed and washed in fish saline (0.85% NaCl), to rid the tissues of superficial blood. The tissues were sliced into small pieces (5mm or 0.5cm thick) and placed in aqueous bouin's fixative for 48 hours (saturated solution of picric acid in distilled water + 20ml formaldehyde + 5ml glacial acetic acid), which kills and fixes the tissues without any structural and chemical damage. The tubular tissues like the intestine were flushed with the fixative, with the help of a micro-syringe. The principle behind this operation is to flush out the contents and also to fix the inner cells. After fixation, the tissues were washed in running tap water for 24hrs.

Before the tissues were embedded, they were thoroughly dehydrated by using upgraded strengths of ethyl alcohol, the final grade being 100% (absolute). Further, dehydration was enhanced by passing the tissues through acetone, the duration being 1hour in each dehydrating agent.

The tissues were then cleared in benzene, which has two advantages, one causing minimum of shrinking of tissues, and secondly it penetrates the tissues and subsequently evaporates from them when placed in melted paraffin bath. The tissues were placed in pure melted paraffin (M.P. 100^0C). Wax blocks were made by using iron L-blocks. The wax blocks were trimmed using a knife into a cubical block. Further, cubical blocks were firmly sealed to the microtome holder and sections of 5-6 micron, were cut using a rotatory microtome and the paraffin ribbon containing the tissue was placed onto a clean slide smeared with Mayer's albumin (1:1 ratio of egg albumin and glycerol + small thymol crystal as preservative).

Flattening of the ribbons was accomplished on a hot plate at temperature of 50^0 C. the sections thus spread with the aid of water as a media to float the ribbons were dried overnight in the incubator set at 36.5^0C. the slides now ready for double staining were dehydrated in down graded series of alcohol and stained regressively with Ehrich's haematoxylin. The slides was later dipped in acid alcohol (1ml concentrated HCl + 99ml of 70% alcohol) for precise staining of the nuclei. The sections were counter stained in alcoholic eosin (cytoplasmic stain). After dehydrating the sections in alcohol the slides was cleared in xylene and the stained sections were mounted in DPX. Photomicrography was carried out by using Olympus Bx-40 trinocular microscope.

2.9 SOURCE, COLLECTION AND MAINTAINENCE OF LIVE ANIMALS

2.9.1 SOURCE

Fish, *Oreochromis mossambicus* weighing 15-30gm were procured in large number (150 fishes) in each season, from its acclimatized Ella State Fishery Farm, Directorate of Fisheries, Government of India, Old Goa, before they were transferred in glass aquariums (46 x 26 x 26cm) containing 16L of tap water that was previously stored overnight, to expel chlorine for further acclimatizing in the laboratory. Usually 1-2 months old fish were collected weighing approximately 18-30gms for experimental purpose, as they are easy to maintain in a laboratory aquarium.

The different seasons for study included,

- Post winter (January, Feburary 27 Mean ± 2^{0}C) years:1999,2000,2001, 2002.
- Summer (March, April, May 33 Mean ± 2^{0}C) years:1999,2000,2001, 2002.
- Monsoon (June, July, August 23 Mean ± 2^{0}C) years:1999,2000,2001, 2002.
- Post monsoon (September, October Mean 24 ± 2^{0}C) years:1999,2000,2001, 2002.
- Winter (November, December 20 Mean ± 2^{0}C) years:1999,2000,2001, 2002.

Fishes were first acclimatized to laboratory condition without food for three days prior to any experimental treatments. Food comprising of wheat bran was fed *ad libitum* from fourth day onwards routinely. They were subjected to natural day and night cycle. The changing of water from the aquarium and aeration of water was done regularly using aerator stone pump.

On the seventh day they were divided into three groups simultaneously. One group was the control, second group was treated with 10ppt NaCl (160g /16L), and the third group was treated with 20ppt NaCl (320g /16L). The treated fish was also exposed to short term treatment (STT=7days), medium term treatment (MTT=21days) and long term treatment (LTT=30days).

At the end of STT, MTT, LTT for both groups (10ppt and 20ppt), six fishes from each group and each treatment, along with the control six fishes were sacrificed to remove selected tissues namely gill filament, large intestine, liver and testis to study the physiological, biochemical and histological parameters.

2.9.2 ISOLATION OF TISSUES

Oreochromis, 1-2 months old, were fished out from the aquarium using a net and washed under running tap water to remove any superficial impurities. They were then sacrificed by a rapid incision using angular sharp scissors from the anal aperture and extended to the mouth along the ventral side to expose the abdominal cavity. Using a pointed and blunt forceps, gill filament, liver and large intestine were carefully excised with minimum damage and kept on tissue paper in watch glass in ice-bath can.

2.9.3 PROCUREMENT OF CHEMICAL

The chemicals like organic solvents namely chloroform, methanol, ether, benzene etc. and acids like sulphuric acid, hydrochloric acid, acetic acid etc. were procured from SDS Fine Chemicals. Other chemicals namely sodium chloride, potassium chloride, sodium sulphate, sodium hydroxide, ferric chloride, silver nitrate, ammonium molybdate, magnesium nitrate etc. were obtained from E Merck and BDH.

Standard chemicals like cholesterol, tripalmitin, lipid standards were procured from M /s Sigma chemicals, USA. Most of the chemicals used for my work were either in AR or GR grade.

The required glass wares like beakers, pipettes, test tubes, funnels, conical flasks, burette, round bottom flask etc. were procured from Borosil and other lab wares like plastic storage bottles, centrifuge tubes, pipette stand, ependroff tubes were obtained from Tarson. All the glass-wares and lab wares were properly washed and cleaned with tepol detergent and dried in incubator thoroughly before and after use.

2.9.4 STATISTICAL ANALYSIS

Throughout the present study, unless otherwise mentioned, the control and experimental values, are the replicates of atleast six independent biochemical observations. The X±SD or X±SE was calculated wherever necessary. Whenever needed, estimates of degree of statistical significance of difference between groups were made. The significance deviation from the control was determined using paired student's 't' test (Zar, 1984). All calculations were done using HP-Pavilion Computer using MS-Word-2000 and Excel-2000 softwares. Standard statistical techniques were adopted using an Electronic Programmable Fx–100_D (super Fx-casio) calculator.

$$\text{Standard error (SE)} = \frac{SD}{n}$$

$$\text{Student 't' test} = \frac{Xc - Xt}{\sqrt{(SEc)^2} + \sqrt{(SEt)^2}}$$

where, SD = Standard deviation.

n = Number of fishes. = 6.

Xc = Mean value of control.

Xt = Mean value of treated experimental.

SEc = Mean standard area of control.

SEt = Mean standard area of treated.

PART iii

3.0 RESULTS

3.1 PHYSICO-CHEMICAL PARAMETERS

Table 1. provides information on the quality of potable water, as specified by Bureau of Indian Standard.

Analysis of tap water (control) used in the present investigations showed the following results.

During post winter, the temperature was 27±1°C, pH 7.5, Alkalinity 28 ppm, Chlorides 10 ppm. The water did show any Turbidity, Smell and Colour. Apart from the above Hardness, Calcium, Magnesium, Phosphate and Sulphates were present in very low quantities as indicated in Table 2.

Similarly, Table 7. provides the role of various concentration of NaCl on life of *Oreochromis mossambicus*. Table indicates 450mg/10ml as LC_{50} for 72 hours. Hence for the convineance sake 200mg/10ml and 100mg/10ml concentrations were taken as sub-lethal and safe concentration for the present studies.

3.2 LC_{50} AND BEHAVIOUR

3.2.1 LC_{50}

The results of LC_{50} on *Oreochromis mossambicus*, for 72 hours, is presented in Table 7.

3.2.2 BEHAVIOUR

Subsequent to LC_{50} value exposure, the observed behavioural changes were, erratic swimming, hyper and hypoactivity, imbalance in posture, increase surfacing activity with gradual decrease in opercular movement, gradual loss in equilibrium, spreading of mucous all over the body surface, finally followed by sluggishness and death. These findings are in conformity

with Sadhu, (1993); Pravakar *et al.*, (1993); Sabita *et al.*, (1995); Santkumar and Balaji, (2000).

From 30.508‰ - 33.559‰, fish exhibited rapid, jerky movements, followed by hyperactive and lethargic state at 38.222‰ - 41.012‰. Restlessness, cough, burst swimming, nudge, nip, as described in page 75, were observed in all concentrations above LC_{50} values.

Beyond 45.762‰, lethargy and a tendency for the fish to settle motionless on the bottom of the aquarium was observed.

The presence or absence of a particular behaviour in 50% of the test animal is indicated in Table 8.

The percentage increase of the various behaviours under study was almost directly proportional to the concentration, which is duly represented in Table 9. In distilled water, Fanning was 20%, Burst Swimming was 12%, Nudge was 16%, while Nip, Cough, Motionless was not observed.

While at 75mg/10ml, the fishes showed, 66% Fanning, 49% Burst Swimming, 46% Nudge, 38% Nip, and 35% Cough, though Motionless percentage was zero.

At the highest concentration tested (600mg/10ml), all the fishes (100%) exhibited all the behaviours under study.

3.3 OSMOSIS

Table 10. provides the data of water content in gill-filament, in both, control and treated groups, during post winter, summer, monsoon, post monsoon and winter seasons.

The water content in gill-filament ranges from 0.155 wet wt./g/tissue wt. (winter) to 0.250 wet wt./g/tissue wt. (summer) in control.

At 10ppt treated water, for 7 days exposure period, the water content in gill-filament ranges from 0.160 wet wt./g/tissue wt. (winter) to 0.255 wet wt./g/tissue wt. (summer).

At 10ppt experimental water, for 21 days exposure period, the water content in gill-filament ranges from 0.165 wet wt./g/tissue wt. (winter) to 0.260 wet wt./g/tissue wt. (summer).

At 20ppt treated water, for 7 days exposure period, the water content in gill-filament ranges from 0.170 wet wt./g/tissue wt. (winter) to 0.260 wet wt./g/tissue wt. (summer).

At 20ppt experimental water, for 21 days exposure period, the water content in gill-filament ranges from 0.175 wet wt./g/tissue wt. (winter) to 0.275 wet wt./g/tissue wt. (summer).

Table 11. provides the data of water content in large-intestine, in both, control and treated groups, during post winter, summer, monsoon, post monsoon and winter seasons.

The water content in large-intestine ranges from 0.350 wet wt./g/tissue wt. (winter) to 0.400 wet wt./g/tissue wt. (summer) in control.

At 10ppt treated water, for 7 days exposure period, the water content in large-intestine ranges from 0.355 wet wt./g/tissue wt. (winter) to 0.445 wet wt./g/tissue wt. (summer).

At 10ppt experimental water, for 21 days exposure period, the water content in large-intestine ranges from 0.360 wet wt./g/tissue wt. (winter) to 0.450 wet wt./g/tissue wt. (summer).

At 20ppt treated water, for 7 days exposure period, the water content in large-intestine ranges from 0.365 wet wt./g/tissue wt. (winter) to 0.500 wet wt./g/tissue wt. (summer).

At 20ppt experimental water, for 21 days exposure period, the water content in large-intestine ranges from 0.370 wet wt./g/tissue wt. (winter) to 0.550 wet wt./g/tissue wt. (summer).

Table 12. provides the data of water content in liver, in both, control and treated groups, during post winter, summer, monsoon, post monsoon and winter seasons.

The water content in liver, ranges from 0.250 wet wt./g/tissue wt. (winter) to 0.350 wet wt./g/tissue wt. (summer) in control.

At 10ppt treated water, for 7 days exposure period, the water content in liver, ranges from 0.255 wet wt./g/tissue wt. (winter) to 0.400 wet wt./g/tissue wt. (summer).

At 10ppt experimental water, for 21 days exposure period, the water content in liver, ranges from 0.260 wet wt./g/tissue wt. (winter) to 0.450 wet wt./g/tissue wt. (summer).

At 20ppt treated water, for 7 days exposure period, the water content in liver, ranges from 0.265 wet wt./g/tissue wt. (winter) to 0.465 wet wt./g/tissue wt. (summer).

At 20ppt experimental water, for 21 days exposure period, the water content in liver, ranges from 0.270 wet wt./g/tissue wt. (winter) to 0.470 wet wt./g/tissue wt. (summer).

Table 13. shows, the percentile data of water content in gill-filament, large-intestine and liver, in both, control and treated groups, during post winter, summer, monsoon, post monsoon and winter seasons.

In control group, percentile water content ranges from 15.50% (gill-filament), 35.00% (large-intestine), 25.00% (liver) during winter, to 25.00% (gill-filament), 40.00 % (large-intestine), 35.00% (liver), during summer.

At 10ppt treated water, for 7 days exposure, percentile water content ranges from 16.00% (gill-filament), 35.50% (large-intestine), 25.50% (liver) during winter, to 25.50% (gill-filament), 44.50 % (large-intestine), 40.00% (liver) during summer.

At 10ppt treated water, for 21 days exposure, percentile water content ranges from 16.50% (gill-filament), 36.00% (large-intestine), 26.00% (liver) during winter, to 26.00% (gill-filament), 45.00 % (large-intestine), 45.00% (liver) during summer.

At 20ppt treated water, for 7 days exposure, percentile water content ranges from 17.00% (gill-filament), 36.50% (large-intestine), 26.50% (liver) during winter, to 26.00% (gill-filament), 50.00 % (large-intestine), 46.50% (liver) during summer.

At 20ppt treated water, for 21 days exposure, percentile water content ranges from 17.50% (gill-filament), 37.00% (large-intestine), 27.00% (liver) during winter, to 27.50% (gill-filament), 55.00 % (large-intestine), 47.00% (liver) during summer.

3.4 RESPIRATION

To have a comparative picture about the survival of some of the air breathing fishes out of water has been provided in Table. 14. which indicates,

Boleophthalmus boddaerti survives for about 724 hours out of water, while *Oreochromis mossambicus* can live upto 3-5 hours out of water.

Similarly circadian rhythm peaks of bimodal oxygen uptake of some air-breathing fishes has been depicted in Table 15.

Table 16. provides information on asphyxiation time of some of air-breathing fishes, which ranges from 15 minutes in *Anabas testudineus* to 120 days in *Channa gachua*.

Circadian rhythm of bimodal oxygen uptake is exhibited in Table 17, which indicates its range from 2.30 ml O_2 gm^{-1} hr^{-1} during late noon and late evening to 5.67 ml O_2 gm^{-1} hr^{-1} during early dawn.

Table 18. depicts circadian rhythm of bimodal oxygen uptake, which indicates its range from 2.59 ml O_2 gm^{-1} hr^{-1} during early evening to 3.83 ml O_2 gm^{-1} hr^{-1} during early dawn.

Table 19. shows circadian rhythm of bimodal oxygen uptake, which indicates its range from 2.73 ml O_2 gm^{-1} hr^{-1} during late evening to 4.23 ml O_2 gm^{-1} hr^{-1} during early dawn.

Table 20. depicts circadian rhythm of bimodal oxygen uptake, which indicates its range from 2.89 ml O_2 gm^{-1} hr^{-1} during early evening to 4.00 ml O_2 gm^{-1} hr^{-1} during early dawn.

Table 21. depicts circadian rhythm of bimodal oxygen uptake, which indicates its range from 2.84 ml O_2 gm^{-1} hr^{-1} during late evening to 4.06 ml O_2 gm^{-1} hr^{-1} during early dawn.

Table 22. depicts circadian rhythm of bimodal oxygen uptake, which indicates its range from 2.85 ml O_2 gm^{-1} hr^{-1} during early evening to 4.03 ml O_2 gm^{-1} hr^{-1} during early dawn.

Table 23. depicts circadian rhythm of bimodal oxygen uptake, which indicates its range from 1.06 ml O_2 gm^{-1} hr^{-1} during morning to 1.44 ml O_2 gm^{-1} hr^{-1} during evening.

Table 24. depicts circadian rhythm of bimodal oxygen uptake, which indicates its range from 3.02 ml O_2 gm^{-1} hr^{-1} during early evening to 4.26 ml O_2 gm^{-1} hr^{-1} during early dawn.

Table 25. depicts circadian rhythm of bimodal oxygen uptake, which indicates its range from 1.17 ml O_2 gm^{-1} hr^{-1} during early evening to 1.56 ml O_2 gm^{-1} hr^{-1} during evening.

Table 26. depicts circadian rhythm of bimodal oxygen uptake, which indicates its range from 3.37 ml O_2 gm^{-1} hr^{-1} during early evening to 5.14 ml O_2 gm^{-1} hr^{-1} during noon.

Table 27. depicts circadian rhythm of bimodal oxygen uptake, which indicates its range from 2.91 ml O_2 gm^{-1} hr^{-1} during early evening to 3.70 ml O_2 gm^{-1} hr^{-1} during evening.

Table 28. depicts circadian rhythm of bimodal oxygen uptake, which indicates its range from 3.68 ml O_2 gm^{-1} hr^{-1} during early evening to 4.30 ml O_2 gm^{-1} hr^{-1} during early dawn.

Seasonal variation with regard to aquatic respiration in *Oreochromis mossambicus* at 10ppt and 20ppt is provided in Table 29. Control groups exhibited a range from 0.050±0.02 (winter) to 0.250±0.03 (summer), which is statistically significant at 0.05% level.

At 10ppt concentration, 6 hours duration exhibited aquatic respiration to be 0.059±0.02 (winter) and 0.265±0.03 (summer) as extremes.

While for 12 hours duration, results were similar, in winter showing minimum 0.055±0.02, and summer the maximum 0.268±0.03.

For 7 days duration, aquatic respiration was found to be minimum 0.049±0.02 (winter), and maximum 0.240±0.03 (summer).

For 21 days duration, aquatic respiration was found to be minimum 0.047±0.02 (winter), and maximum 0.195±0.03 (summer).

At 20ppt concentration, 7 days duration exhibited aquatic respiration to be 0.048±0.02 (winter) and 0.220±0.03 (summer) as extremes.

For 21 days duration, aquatic respiration was found to be minimum 0.046±0.004(winter), and maximum 0.192±0.02 (summer).

Seasonal variation with regard to aerial respiration in *Oreochromis mossambicus* at 10ppt and 20ppt is provided in Table 30. Control groups exhibited a range from $1.0x10^{-4}$±0.00 (winter) to $5.8x10^{-4}$±0.03 (summer), which is statistically significant at 0.05% level.

At 10ppt concentration, 7 days duration, exhibited aerial respiration to be $1.0 x10^{-4}$±0.01 (winter) and $6.4x10^{-4}$±0.02 (summer) as extremes.

For 21 days duration, aerial respiration was found to be minimum$1.0x10^{-4}$±0.00 (winter), and maximum $5.9x10^{-4}$±0.03 (summer).

At 20ppt concentration, 7 days duration exhibited aerial respiration to be $1.0 x10^{-4}$±0.00 (winter) and $5.8x10^{-4}$±0.02 (summer) as extremes.

For 21 days duration, aerial respiration was found to be minimum $1.1x10^{-4}$±0.000 (winter), and maximum $5.7x10^{-4}$±0.03 (summer).

Seasonal total bimodal respiration by *Oreochromis mossambicus* for control, 10ppt and 20ppt concentration, during various seasons and exposure period is shown in Table 31.

It's well known when the fish survives out of water, looses body weight continuously. In the present experiments it is seen in Table 32, fish weighing 2.21g, surviving for about 2h out of water looses 90mg/hr, while fish weighing 14.06g survives for about 4h, looses about 47.7mg/hr, indicating average percentage loss of body weight is around 18.00 to 21.76%.

In control, as in Table 33, when an analysis was made to understand the correlation between bimodal respiration, with that of body weight, it showed the same is ranging from 2.43ml O_2 gm^{-1} hr^{-1} to 2.64ml O_2 gm^{-1} hr^{-1} indicating statistically insignificant variation.

Similarly, at 10ppt, as shown in Table 34. correlation between bimodal respiration with that of body weight, showed the same ranging from 2.30ml O_2 gm^{-1} hr^{-1} to 5.67ml O_2 gm^{-1} hr^{-1}.

At 20ppt, as shown in Table 35. correlation between bimodal respiration with that of body weight, ranged from 2.59ml O_2 gm^{-1} hr^{-1} to 3.83ml O_2 gm^{-1} hr^{-1}.

3.5 EXCRETION

Nitrogen excretion in some facultative air-breathing fishes is provided in Table 36, which includes the present investigation too.

3.5.1 AMMONIA

The data of ammonia excretion, in both, control and treated groups, during post winter, summer, monsoon, post monsoon and winter seasons is provided in Table.37.

Ammonia excretion ranges from, 55.38±0.43µg gm body wt $^{-1}$ hr^{-1} (winter) to 212.67±2.42µg gm body wt $^{-1}$ hr^{-1} (summer) in control.

At 10ppt, for STT, ammonia excretion ranges from, 46.18±0.72µg gm body wt $^{-1}$ hr^{-1} (winter) to 162.91±2.01µg gm body wt $^{-1}$ hr^{-1} (summer).

At 10ppt, for MTT, ammonia excretion ranges from, 26.62±0.19µg gm body wt $^{-1}$ hr^{-1} (winter) to 160.07±2.40µg gm body wt $^{-1}$ hr^{-1} (summer).

At 10ppt, for LTT, ammonia excretion ranges from, 22.43±0.11µg gm body wt $^{-1}$ hr^{-1} (winter) to 80.00±1.40µg gm body wt $^{-1}$ hr^{-1} (summer).

At 20ppt, for STT, ammonia excretion ranges from, 40.48±0.57µg gm body wt $^{-1}$ hr^{-1} (winter) to 130.72±1.60µg gm body wt $^{-1}$ hr^{-1} (summer).

At 20ppt, for MTT, ammonia excretion ranges from, 24.70±0.17µg gm body wt $^{-1}$ hr^{-1} (winter) to 85.00±1.20 g gm body wt $^{-1}$ hr^{-1} (summer).

At 20ppt, for LTT, ammonia excretion ranges from, 20.71±0.10µg gm body wt $^{-1}$ hr^{-1} (winter) to 52.00±0.80µg gm body wt $^{-1}$ hr^{-1} (summer).

3.5.2 UREA

The data of urea excretion, in both, control and treated groups, during post winter, summer, monsoon, post monsoon and winter seasons is provided in Table 38.

Urea excretion ranges from, 40.13±0.49µg gm body wt $^{-1}$ hr^{-1} (winter) to 76.24±1.20µg gm body wt $^{-1}$ hr^{-1} (summer) in control.

At 10ppt, for STT, urea excretion ranges from, 28.90±0.56µg gm body wt $^{-1}$ hr^{-1} (winter) to 67.47±0.67µg gm body wt $^{-1}$ hr^{-1} (summer).

At 10ppt, for MTT, urea excretion ranges from, 36.86±0.87µg gm body wt $^{-1}$ hr^{-1} (winter) to 70.85±0.81µg gm body wt $^{-1}$ hr^{-1} (summer).

At 10ppt, for LTT, urea excretion ranges from, 80.88±1.00µg gm body wt $^{-1}$ hr^{-1} (winter) to 102.99±0.85µg gm body wt $^{-1}$ hr^{-1} (summer).

At 20ppt, for STT, urea excretion ranges from, 41.09±0.15µg gm body wt $^{-1}$ hr^{-1} (winter) to 72.94±0.82µg gm body wt $^{-1}$ hr^{-1} (summer).

At 20ppt, for MTT, urea excretion ranges from, 45.92±0.37µg gm body wt $^{-1}$ hr^{-1} (winter) to 88.45±0.50µg gm body wt $^{-1}$ hr^{-1} (summer).

At 20ppt, for LTT, urea excretion ranges from, 90.11±0.44µg gm body wt $^{-1}$ hr^{-1} (winter) to 122.00±1.10µg gm body wt $^{-1}$ hr^{-1} (summer).

3.6 NITROGENOUS BIOMOLECULES

3.6.1 PROTEINS

Table 39. provides the data of protein concentration, in both, control and treated groups, during post winter, summer, monsoon, post monsoon and winter seasons. Protein concentration in gill-filament ranges from, 8146.45±1.00µg 100mg tissue wt $^{-1}$ (winter) to 4923.76±1.00µg 100mg tissue wt $^{-1}$ (summer) in control.

At 10ppt, for STT, protein concentration in gill-filament, ranges from, 7017.63±0.64µg 100mg tissue wt $^{-1}$ (winter) to 3583.11±1.78µg 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for MTT, protein concentration in gill-filament, ranges from, 6602.58±0.69µg 100mg tissue wt $^{-1}$ (winter) to 3323.55±0.07µg 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for LTT, protein concentration in gill-filament, ranges from, 6601.55±0.94µg 100mg tissue wt $^{-1}$ (winter) to 3321.50±0.69µg 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for STT, protein concentration in gill-filament, ranges from, 5963.84±0.42µg 100mg tissue wt $^{-1}$ (winter) to 3182.93±0.79µg 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for MTT, protein concentration in gill-filament, ranges from, 5385.40±1.00µg 100mg tissue wt $^{-1}$ (winter) to 3067.60±1.92µg 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for LTT, protein concentration in gill-filament, ranges from, 5384.11±0.60µg 100mg tissue wt $^{-1}$ (winter) to 3066.00±0.24µg 100mg tissue wt $^{-1}$ (summer).

Table 40. provides the data of protein concentration, in both, control and treated groups, during post winter, summer, monsoon, post monsoon and winter seasons. Protein concentration in large-intestine, ranges from, 9711.00±1.11µg 100mg tissue wt $^{-1}$ (winter) to 4958.46±1.70µg 100mg tissue wt $^{-1}$ (summer) in control.

At 10ppt, for STT, protein concentration in large-intestine, ranges from, 7499.05±0.13µg 100mg tissue wt $^{-1}$ (winter) to 4382.05±0.86µg 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for MTT, protein concentration in large-intestine, ranges from, 7007.49±0.06µg 100mg tissue wt $^{-1}$ (winter) to 4341.05±1.79µg 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for LTT, protein concentration in large-intestine, ranges from, 7002.44±0.64µg 100mg tissue wt $^{-1}$ (winter) to 4340.11±0.57µg 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for STT, protein concentration in large-intestine, ranges from, 6402.06±0.07µg 100mg tissue wt $^{-1}$ (winter) to 3622.78±0.15µg 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for MTT, protein concentration in large-intestine, ranges from, 5628.78±0.17µg 100mg tissue wt $^{-1}$ (winter) to 3333.98±1.69µg 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for LTT, protein concentration in large-intestine, ranges from, 5624.77±0.61µg 100mg tissue wt $^{-1}$ (winter) to 3333.11±0.61µg 100mg tissue wt $^{-1}$ (summer).

Table 41. provides the data of protein concentration, in both, control and treated groups, during post winter, summer, monsoon, post monsoon and winter seasons. Protein concentration in liver, ranges from, 9998.16±2.00µg 100mg tissue wt $^{-1}$ (winter) to 5632.63±1.10µg 100mg tissue wt $^{-1}$ (summer) in control.

At 10ppt, for STT, protein concentration in liver, ranges from, 9988.13±0.84µg gm body wt $^{-1}$ hr^{-1} (winter) to 5560.70±0.10µg 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for MTT, protein concentration in liver, ranges from, 9665.07±1.83µg 100mg tissue wt $^{-1}$ (winter) to 4459.86±0.63µg 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for LTT, protein concentration in liver, ranges from, 9664.02±1.71µg 100mg tissue wt $^{-1}$ (winter) to 4458.16±1.71µg 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for STT, protein concentration in liver, ranges from, 7319.21±0.33µg 100mg tissue wt $^{-1}$ (winter) to 4294.88±1.71µg 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for MTT, protein concentration in liver, ranges from, 6999.60±0.02µg 100mg tissue wt $^{-1}$ (winter) to 4294.10±1.48µg 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for LTT, protein concentration in liver, ranges from, 5989.57±0.17µg 100mg tissue wt $^{-1}$ (winter) to 4293.99±0.24µg 100mg tissue wt $^{-1}$ (summer).

3.6.2 AMMONIA

Table 42. provides the data of nitrogenous ammonia, in both, control and treated groups, during post winter, summer, monsoon, post monsoon and winter seasons. Ammonia concentration in gill-filament tissue ranges from 45.78±0.90µg 100mg tissue wt $^{-1}$ (winter) to 10.66±0.20 µg 100mg tissue wt $^{-1}$ (summer) in control.

At 10ppt, for STT, ammonia concentration in gill-filament tissue ranges from, 36.57±0.26µg 100mg tissue wt $^{-1}$ (winter) to 12.24±1.20µg 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for MTT, ammonia concentration in gill-filament tissue ranges from, 35.86±0.10µg 100mg tissue wt $^{-1}$ (winter) to 12.02±0.60µg 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for LTT, ammonia concentration in gill-filament tissue ranges from, 35.62±0.28µg 100mg tissue wt $^{-1}$ (winter) to 12.01±1.20µg 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for STT, ammonia concentration in gill-filament tissue ranges from, 32.68±1.42µg 100mg tissue wt $^{-1}$ (winter) to 10.50±0.66µg 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for MTT, ammonia concentration in gill-filament tissue ranges from, 25.05±0.95µg 100mg tissue wt $^{-1}$ (winter) to 10.32±0.76µg100mg tissue wt $^{-1\ 1}$ (summer).

At 20ppt, for LTT, ammonia concentration in gill-filament tissue ranges from, 25.01±0.70µg 100mg tissue wt $^{-1}$ (winter) to 10.23±0.33µg 100mg tissue wt $^{-1}$ (summer).

Table 43. provides the data of nitrogenous ammonia, in both, control and treated groups, during post winter, summer, monsoon, post monsoon and winter seasons. Ammonia concentration in large-intestine tissue ranges from 143.80±1.10µg 100mg tissue wt $^{-1}$ (winter) to 56.09±0.80µg 100mg tissue wt $^{-1}$ (summer) in control.

At 10ppt, for STT, ammonia concentration in large-intestine tissue ranges from, 138.43±1.10µg100mg tissue wt $^{-1}$ (winter) to 10.29±0.33µg 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for MTT, ammonia concentration in large-intestine tissue ranges from, 137.80±1.27µg 100mg tissue wt $^{-1}$ ^{1} (winter) to 11.22±0.22µg 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for LTT, ammonia concentration in large-intestine tissue ranges from, 137.00±0.99µg 100mg tissue wt $^{-1}$ (winter) to 11.20±0.90µg 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for STT, ammonia concentration in large-intestine tissue ranges from, 127.16±1.40µg 100mg tissue wt $^{-1}$ (winter) to 10.00±0.33µg 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for MTT, ammonia concentration in large-intestine tissue ranges from, 114.84±1.37µg 100mg tissue wt $^{-1}$ (winter) to 8.55±0.03µg 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for LTT, ammonia concentration in large-intestine tissue ranges from, 114.48±0.79µg 100mg tissue wt $^{-1}$ (winter) to 8.54±0.15µg 100mg tissue wt $^{-1}$ (summer).

Table 44. provides the data of nitrogenous ammonia, in both, control and treated groups, during post winter, summer, monsoon, post monsoon and winter seasons. Ammonia concentration in liver tissue, ranges from, 86.57±1.06µg 100mg tissue wt $^{-1}$ (winter) to 14.10±1.00µg gm 100mg tissue wt $^{-1}$ (summer) in control.

At 10ppt, for STT, ammonia concentration in liver tissue ranges from, 73.96±0.67µg 100mg tissue wt $^{-1}$ (winter) to 11.26±0.32µg 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for MTT, ammonia concentration in liver tissue ranges from, 62.18±0.55µg 100mg tissue wt $^{-1}$ (winter) to 8.77±0.04µg 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for LTT, ammonia concentration in liver tissue ranges from, 62.15±0.96µg 100mg tissue wt $^{-1}$ (winter) to 8.76±0.21µg 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for STT, ammonia concentration in liver tissue ranges from, 53.36±0.40µg 100mg tissue wt $^{-1\ 1}$ (winter) to 9.75±0.30µg 100mg tissue wt $^{-1\ 1}$ (summer).

At 20ppt, for MTT, ammonia concentration in liver tissue ranges from, 52.98±0.43µg 100mg tissue wt $^{-1}$ (winter) to 8.00±0.04µg 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for LTT, ammonia concentration in liver tissue ranges from, 52.89±0.38µg 100mg tissue wt $^{-1}$ (winter) to 7.80±0.15µg 100mg tissue wt $^{-1}$ (summer).

3.6.3 UREA

Table 45. provides the data of urea concentration, in both, control and treated groups, during post winter, summer, monsoon, post monsoon and winter seasons. Urea concentration in gill-filament ranges from 104.0±1.11µg 100mg tissue wt $^{-1}$ (winter) to 11.40±0.12µg 100mg tissue wt $^{-1}$ (summer) in control.

At 10ppt, for STT, urea concentration in gill-filament ranges from, 27.09±0.40µg 100mg tissue wt $^{-1}$ (winter) to 10.92±0.68µg 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for MTT, urea concentration in gill-filament ranges from, 22.66±0.11µg 100mg tissue wt $^{-1}$ (winter) to 10.98±0.27µg 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for LTT, urea concentration in gill-filament ranges from, 22.65±0.07µg 100mg tissue wt $^{-1}$ (winter) to 10.98±0.21µg 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for STT, urea concentration in gill-filament ranges from, 19.68±0.87µg 100mg tissue wt $^{-1}$ (winter) to 4.52±0.01µg 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for MTT, urea concentration in gill-filament ranges from, 17.80±0.18µg 100mg tissue wt $^{-1}$ (winter) to 4.51±0.02µg 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for LTT, urea concentration in gill-filament ranges from, 17.70±0.04µg 100mg tissue wt $^{-1}$ (winter) to 4.50±0.08µg 100mg tissue wt $^{-1}$ (summer).

Table 46. provides the data of urea concentration, in both, control and treated groups, during post winter, summer, monsoon, post monsoon and winter seasons. Urea concentration in large-intestine, ranges from, 126.63±1.60µg 100mg tissue wt $^{-1}$ (winter) to 17.29±0.10µg 100mg tissue wt $^{-1}$ (summer) in control.

At 10ppt, for STT, urea concentration in large-intestine, ranges from, 47.36±0.60µg 100mg tissue wt $^{-1}$ (winter) to 16.38±0.12µg 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for MTT, urea concentration in large-intestine, ranges from, 39.19±0.02µg 100mg tissue wt $^{-1}$ (winter) to 13.56±0.21µg 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for LTT, urea concentration in large-intestine, ranges from, 39.18±0.40µg 100mg tissue wt $^{-1}$ (winter) to 13.55±0.12µg 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for STT, urea concentration in large-intestine ranges from, 32.02±0.16µg 100mg tissue wt $^{-1}$ (winter) to 10.10±0.02 µg 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for MTT, urea concentration in large-intestine, ranges from, 28.68±0.22µg 100mg tissue wt $^{-1}$ (winter) to 7.83±0.14µg 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for LTT, urea concentration in large-intestine, ranges from, 28.67±0.12µg 100mg tissue wt $^{-1}$ (winter) to 7.83±0.12µg 100mg tissue wt $^{-1}$ (summer).

Table 47. provides the data of urea concentration, in both, control and treated groups, during post winter, summer, monsoon, post monsoon and winter seasons. Urea concentration in liver, ranges from, 108.55±1.11µg 100mg tissue wt $^{-1}$ (winter) to 15.28±0.01µg 100mg tissue wt $^{-1}$ (summer) in control.

At 10ppt, for STT, urea concentration in liver, ranges from, 28.62±0.01µg 100mg tissue wt $^{-1}$ (winter) to 14.90±0.08µg 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for MTT, urea concentration in liver, ranges from, 28.11±0.02µg 100mg tissue wt $^{-1}$ (winter) to 13.38±0.60µg 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for LTT, urea concentration in liver, ranges from, 28.10±0.01µg 100mg tissue wt $^{-1}$ (winter) to 13.37±0.04µg 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for STT, urea concentration in liver, ranges from, 22.23±0.03µg 100mg tissue wt $^{-1}$ (winter) to 8.36±0.10µg 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for MTT, urea concentration in liver, ranges from, 21.80±0.06µg 100mg tissue wt $^{-1}$ (winter) to 5.02±0.02µg 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for LTT, urea concentration in liver, ranges from, 21.80±0.01µg 100mg tissue wt $^{-1}$ (winter) to 4.50±0.08µg 100mg tissue wt $^{-1}$ (summer).

3.7 LIPID BIOMOLECULES

3.7.1 CHOLESTEROL

Table 48. provides the data of cholesterol concentration, in both, control and treated groups, during post winter, summer, monsoon, post monsoon and winter. seasons. Cholesterol concentration in gill-filament, ranges from, 9.16±0.21µM 100mg tissue wt $^{-1}$ (winter) to 0.12±0.00µM 100mg tissue wt $^{-1}$ (summer) in control.

At 10ppt, for STT, cholesterol concentration in gill-filament, ranges from, 8.53±0.20µM 100mg tissue wt $^{-1}$ (winter) to 0.10±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for MTT, cholesterol concentration in gill-filament, ranges from, 8.175±0.81µM 100mg tissue wt $^{-1}$ (winter) to 0.09±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for LTT, cholesterol concentration in gill-filament, ranges from, 8.00±0.12µM 100mg tissue wt $^{-1}$ (winter) to 0.09±0.02µM 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for STT, cholesterol concentration in gill-filament, ranges from, 7.665±0.91µM 100mg tissue wt $^{-1}$ (winter) to 0.105±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for MTT, cholesterol concentration in gill-filament, ranges from, 5.53±0.44µM 100mg tissue wt $^{-1}$ (winter) to 0.075±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for LTT, cholesterol concentration in gill-filament, ranges from, 5.535±0.24µM 100mg tissue wt $^{-1}$ (winter) to 0.075±0.00µM 100mg tissue wt $^{-1}$ (summer).

Table 38. provides the data of cholesterol concentration, in both, control and treated groups, during post winter, summer, monsoon, post monsoon and winter seasons. Cholesterol concentration in large-intestine, ranges from, 11.085±0.39µM 100mg tissue wt $^{-1}$ (winter) to 0.265±0.01µM 100mg tissue wt $^{-1}$ (summer) in control.

At 10ppt, for STT, cholesterol concentration in large-intestine, ranges from, 9.85±0.20µM 100mg tissue wt $^{-1}$ (winter) to 0.135±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for MTT, cholesterol concentration in large-intestine, ranges from, 8.75±0.10µM 100mg tissue wt $^{-1}$ (winter) to 0.125±0.03µM 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for LTT, cholesterol concentration in large-intestine, ranges from, 8.75±0.00µM 100mg tissue wt $^{-1}$ (winter) to 0.125±0.00µM 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for STT, cholesterol concentration in large-intestine, ranges from, 8.00±0.15µM 100mg tissue wt $^{-1}$ (winter) to 0.115±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for MTT, cholesterol concentration in large-intestine, ranges from, 6.60±0.20µM 100mg tissue wt $^{-1}$ (winter) to 0.085±0.04µM 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for LTT, cholesterol concentration in large-intestine, ranges from, 6.60±0.10µM 100mg tissue wt $^{-1}$ (winter) to 0.085±0.01µM 100mg tissue wt $^{-1}$ (summer).

Table 50. provides the data of cholesterol concentration, in both, control and treated groups, during post winter, summer, monsoon, post monsoon and winter seasons. Cholesterol concentration in liver, ranges from, 13.28±1.10µM 100mg tissue wt $^{-1}$ (winter) to 0.385±0.01µM 100mg tissue wt $^{-1}$ (summer) in control.

At 10ppt, for STT, cholesterol concentration in liver, ranges from, 12.7±0.12µM 100mg tissue wt $^{-1}$ (winter) to 0.295±0.01µM 100mg tissue wt $^{-1}$ (summer).

At10ppt, for MTT, cholesterol concentration in liver, ranges from, 12.23±0.49µM 100mg tissue wt $^{-1}$ (winter) to 0.275±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for LTT, cholesterol concentration in liver, ranges from, 12.25±0.10µM 100mg tissue wt $^{-1}$ (winter) to 0.275±0.00µM 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for STT, cholesterol concentration in liver, ranges from, 8.43±0.03µg gm body wt $^{-1}$ hr^{-1} (winter) to 0.215±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for MTT, cholesterol concentration in liver, ranges from, 8.065±0.01 µM 100mg tissue wt $^{-1}$ (winter) to 0.09±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for LTT, cholesterol concentration in liver, ranges from, 8.06±0.01 µM 100mg tissue wt $^{-1}$ (winter) to 0.09±0.01µM 100mg tissue wt $^{-1}$ (summer).

3.7.2 TRIGLYCERIDE

Table 51. provides the data of triglyceride concentration, in both, control and treated groups, during post winter, summer, monsoon, post monsoon and winter seasons. Triglyceride concentration in gill-filament, ranges from, 6.76±0.05µM 100mg tissue wt $^{-1}$ (winter) to 0.535±0.02µM 100mg tissue wt $^{-1}$ summer) in control.

At 10ppt, for STT, triglyceride concentration in gill-filament, ranges from, 3.17±0.03µM 100mg tissue wt $^{-1}$ (winter) to 0.48±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for MTT, triglyceride concentration in gill-filament, ranges from, 1.785±0.02µM 100mg tissue wt $^{-1}$ (winter) to 0.38±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for LTT, triglyceride concentration in gill-filament, ranges from, 1.775±0.01µM 100mg tissue wt $^{-1}$ (winter) to 0.38±0.00µM 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for STT, triglyceride concentration in gill-filament, ranges from, 1.725±0.01µM 100mg tissue wt $^{-1}$ (winter) to 0.365±0.02µM 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for MTT, triglyceride concentration in gill-filament, ranges from, 1.34±0.02µM 100mg tissue wt $^{-1}$ (winter) to 0.36±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for LTT, triglyceride concentration in gill-filament, ranges from, 1.3±0.01µM 100mg tissue wt $^{-1}$ (winter) to 0.35±0.01µM 100mg tissue wt $^{-1}$ (summer).

Table 38. provides the data of triglyceride concentration, in both, control and treated groups, during post winter, summer, monsoon, post monsoon and winter seasons. Triglyceride concentration in large-intestine, ranges from, 7.205±0.05µM 100mg tissue wt $^{-1}$ (winter) to 0.795±0.02µM 100mg tissue wt $^{-1}$ (summer) in control.

At 10ppt, for STT, triglyceride concentration in large-intestine, ranges from, 4.745±0.03µM 100mg tissue wt $^{-1}$ (winter) to 0.695±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for MTT, triglyceride concentration in large-intestine, ranges from, 2.455±0.02µM 100mg tissue wt $^{-1}$ (winter) to 0.50±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for LTT, triglyceride concentration in large-intestine, ranges from, 2.45±0.01µM 100mg tissue wt $^{-1}$ (winter) to 0.50±0.00µM 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for STT, triglyceride concentration in large-intestine, ranges from, 2.185±0.03µM 100mg tissue wt $^{-1}$ (winter) to 0.49±0.00µM 100mg tissue wt $^{-1}$ ^{1} (summer).

At 20ppt, for MTT, triglyceride concentration in large-intestine, ranges from, 1.53±0.02µM 100mg tissue wt $^{-1}$ (winter) to 0.475±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for LTT, triglyceride concentration in large-intestine, ranges from, 1.50±0.01µM 100mg tissue wt $^{-1}$ (winter) to 0.45±0.02µM 100mg tissue wt $^{-1}$ (summer).

Table 53. provides the data of triglyceride concentration, in both, control and treated groups, during post winter, summer, monsoon, post monsoon and winter seasons. Triglyceride concentration in liver, ranges from, 9.345±1.10µM 100mg tissue wt $^{-1}$ (winter) to 2.31±0.33µM 100mg tissue wt $^{-1}$ (summer) in control.

At 10ppt, for STT, triglyceride concentration in liver, ranges from, 6.00±0.09µM 100mg tissue wt $^{-1}$ ^{1} (winter) to 1.575±0.02µM 100mg tissue wt $^{-}$ ^{1} (summer).

At 10ppt, for MTT, triglyceride concentration in liver, ranges from, 4.29±0.05µM 100mg tissue wt $^{-1}$ (winter) to 1.335±0.10µM 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for LTT, triglyceride concentration in liver, ranges from, 4.275±0.03µM 100mg tissue wt $^{-1}$ (winter) to 1.34±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for STT, triglyceride concentration in liver, ranges from, 3.695±0.02µM 100mg tissue wt $^{-1}$ (winter) to 0.705±0.03µM 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for MTT, triglyceride concentration in liver, ranges from, 1.81±0.01 µM 100mg tissue wt $^{-1}$ (winter) to 0.65±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for LTT, triglyceride concentration in liver, ranges from, 1.805±0.01 µM 100mg tissue wt $^{-1}$ (winter) to 0.65±0.00 µM 100mg tissue wt $^{-1}$ (summer).

3.7.3 PHOSPHOLIPID

Table 38. provides the data of phospholipid concentration, in both, control and treated groups, during post winter, summer, monsoon, post monsoon and winter seasons. Phospholipid concentration in gill-filament, ranges from, 1.095±0.01µg gm body wt $^{-1}$ hr^{-1} (winter) to 0.28±0.01µg gm body wt $^{-1}$ hr^{-1} (summer) in control.

At 10ppt, for STT, phospholipid concentration in gill-filament, ranges from, 1.055±0.03µM 100mg tissue wt $^{-1}$ (winter) to 0.275±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for MTT, phospholipid concentration in gill-filament, ranges from, 0.885±0.02µM 100mg tissue wt $^{-1}$ (winter) to 0.27±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for LTT, phospholipid concentration in gill-filament, ranges from, 0.88±0.01µM 100mg tissue wt $^{-1}$ (winter) to 0.27±0.00µM 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for STT, phospholipid concentration in gill-filament, ranges from, 0.815±0.01µM 100mg tissue wt $^{-1}$ (winter) to 0.26±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for MTT, phospholipid concentration in gill-filament, ranges from, 0.79±0.01µM 100mg tissue wt $^{-1}$ (winter) to 0.25±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for LTT, phospholipid concentration in gill-filament, ranges from, 0.79±0.00µM 100mg tissue wt $^{-1}$ (winter) to 0.25±0.01µM 100mg tissue wt $^{-1}$ (summer).

Table 55. provides the data of phospholipid concentration, in both, control and treated groups, during post winter, summer, monsoon, post monsoon and winter seasons. Phospholipid concentration in large-intestine, ranges from, 1.28±0.11µM 100mg tissue wt $^{-1}$ (winter) to 0.325±0.01µM 100mg tissue wt $^{-1}$ (summer) in control.

At 10ppt, for STT, phospholipid concentration in large-intestine, ranges from, 1.07±0.02µM 100mg tissue wt $^{-1}$ (winter) to 0.28±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for MTT, phospholipid concentration in large-intestine, ranges from, 1.055±0.01µM 100mg tissue wt $^{-1}$ (winter) to 0.275±0.00µM 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for LTT, phospholipid concentration in large-intestine, ranges from, 1.05±0.01µM 100mg tissue wt $^{-1}$ (winter) to 0.27±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for STT, phospholipid concentration in large-intestine, ranges from, 0.97±0.02µM 100mg tissue wt $^{-1}$ (winter) to 0.265±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for MTT, phospholipid concentration in large-intestine, ranges from, 0.715±0.02µM 100mg tissue wt $^{-1}$ (winter) to 0.255±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for LTT, phospholipid concentration in large-intestine, ranges from, 0.705±0.01µM 100mg tissue wt $^{-1}$ (winter) to 0.255±0.01µM 100mg tissue wt $^{-1}$ (summer).

Table 56. provides the data of phospholipid concentration, in both, control and treated groups, during post winter, summer, monsoon, post monsoon and winter seasons. Phospholipid concentration in liver, ranges from, 1.59±0.03µM 100mg tissue wt $^{-1}$ (winter) to 1.125±0.10µM 100mg tissue wt $^{-1}$ (summer) in control.

At 10ppt, for STT, phospholipid concentration in liver, ranges from, 1.56±0.02µM 100mg tissue wt $^{-1}$ (winter) to 0.48±0.01 µM 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for MTT, phospholipid concentration in liver, ranges from, 1.555±0.01µM 100mg tissue wt $^{-1}$ (winter) to 0.31±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 10ppt, for LTT, phospholipid concentration in liver, ranges from, 1.55±0.01 µM 100mg tissue wt $^{-1}$ (winter) to 0.30±0.01µM 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for STT, phospholipid concentration in liver, ranges from, 1.52±0.02µM 100mg tissue wt $^{-1}$ (winter) to 0.30±0.00µM 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for MTT, phospholipid concentration in liver, ranges from, 1.39±0.02µM 100mg tissue wt $^{-1}$ (winter) to 0.275±0.02µM 100mg tissue wt $^{-1}$ (summer).

At 20ppt, for LTT, phospholipid concentration in liver, ranges from, 1.375±0.01 µM 100mg tissue wt $^{-1}$ (winter) to 0.27±0.01µM 100mg tissue wt $^{-1}$ (summer).

3.8 HISTOLOGY

3.8.1 GILL FILAMENT

Plate1. depicts histological cryostat section of control gill filament tissue, of *Oreochromis mossambicus*, double stained by haematoxylin and eosin, showing, presence of uniform and parallel distribution of gill lamellae on one functional row of the gill filament, large interlamellar pore, presence of large number of blood cells.

Plate 2. figs. A and B depicts histological cryostat section of 10ppt (safe dose), 7 and 21 days treated gill filament tissue, of *Oreochromis mossambicus* double stained by haematoxylin and eosin showing, shrinkage of gill filament and disturbed parallel lamellae arrangement on one functional row of the gill filament, reduced interlamellar pore or water pore, decrease in blood cells.

Plate 3. figs. A and B depicts histological cryostat section of 20ppt (sub lethal dose), 7 and 21 days treated gill filament tissue, of *Oreochromis mossambicus* double stained by haematoxylin and eosin showing respiratory lamellae on one functional row of gill filament were frequently obliterated by hyperplasia of interlamellar epithelium (widening of the gill filament) suggesting markedly compressed respiratory ability, reduction of lamellae from one side and disturbed parallel arrangement of lamellae, presence of inflammatory epithelium, presence of interlamellar bridge.

3.8.2 LARGE INTESTINE.

The section of intestine tissue usually exhibits magnified segment. The four layers of the wall namely, the mucosa (MUS), the submucosa (subM), the muscularis externa (ME) and the serosa (S) are clearly visible. Finger like and leaf like projections extending into the intestinal lumen are called villi (V).

Plate 4. depicts histological cryostat section of control large intestine tissue, of *Oreochromis mossambicus,* double stained by haematoxylin and eosin showing, the columnar /squamous cells, devoid of excessive mucous secretion over the columnar /squamous cells, absence of cell lesions.

Plate 5. figs. A and B depicts histological cryostat section of10ppt (safe dose), 7 and 21 days treated large intestine tissue, of *Oreochromis mossambicus,* double stained by haematoxylin and eosin showing, minor anatomical changes with respect to loosening of columnar cells, mucous secretion over the columnar / squamous cells were clearly visible, few cell lesions visible.

Plate 6. figs. A and B depicts histological cryostat section of 20ppt (sub lethal dose), 7 and 21 days treated large intestine tissue, of *Oreochromis mossambicus,* double stained by haematoxylin and eosin showing,

a) Major anatomical changes with respect to loosening of columnar epithelial cells with a disturbed boundary, excessive mucous secretion over the columnar /squamous cells were clearly visible, further distorted with further damage and loss of identity, excessive and prominent cell lesions visible.

3.8.3 LIVER

A classical structure and histological cross section of control liver tissue shows a number of lobes and lobules. Each polyhedral lobule has a central vein (CV) in the centre. The interlobular vein is present in middle of each lobule. Hepatocytes radiate from the central vein, which appears to be uniformly dispersed throughout. The hepatic cells appear as inter connecting cords of one or more cell thick. The sinusoids appear as the light areas between the cords of cells and they are also radially arranged surrounding the central vein. Distinguishing feature of sinusoid is that it penetrates the wall of the vein.

Plate 7. depicts histological cryostat section of control liver tissue, of *Oreochromis mossambicus,* double stained by haematoxylin and eosin showing, hexagonal shaped hepatic cells /hepatocytes, intact fibrous wall and accumulation of blood cells /blood sinusoids in hepatic lobules.

Plate 8. figs. A and B depicts histological cryostat section of10ppt (safe dose), 7 and 21 days treated liver tissue, of *Oreochromis mossambicus,* double stained by haematoxylin and eosin showing, swollen hepatocytes, hepatocytes showed conspicuous small vacuolation which marks the initial stage of degenerative damage, large number of necrotic region seen and pyknosis of necrotic cells, blood sinusoids infiltered with half damaged blood cells and disentigration of sinusoids.

Plate 9. figs. A and B depicts histological cryostat section of 20ppt (sub lethal dose), 7 and 21 days treated liver tissue, of *Oreochromis mossambicus,* double stained by haematoxylin and eosin showing, extensive damage to the liver marked by hyperplasia and hypertrophy, hepatic cell arrangement was

disturbed, hepatocytes were filled with large vacuoles due to degeneration of cytoplasm of hepatic cells, pathological lesions included infilteration of blood cells, hypertrophy and degeneration of hepatocytes.

3.8.4 TESTIS.

Plate 10. depicts histological cryostat section of control testis tissue of *Oreochromis mossambicus,* double stained by haematoxylin and eosin showing, intact, undamaged sertoli cell (Sc) with testicular limiting membrane, normal distribution of sperms, presence of nutritive cells, some spermatogonia are yet to develop while spermatozoa are in the process of detachment from sertoli cells.

Plate 11. figs. A and B depicts histological cryostat section of10ppt (safe dose), 7 and 21 days treated testis tissue, of *Oreochromis mossambicus,* double stained by haematoxylin and eosin showing, degenerating spermatids (DS) and clumping of sperms.

Plate 12. figs. A and B depicts histological cryostat section of 20ppt (sub lethal dose), 7 and 21 days treated testis tissue, of *Oreochromis mossambicus,* double stained by haematoxylin and eosin showing, sertoli cells appeared deshaped and vacuolized. Their nucleus disappeared and individual cells lost their identity and became fragmented, severe clumping of sperms, vacuolized tissue, nurse cells and nutritive cells got degenerated.

Table 1. Bureau of Indian standards for drinking water (IS: 10500-1983).

S. No.	Substance or characteristics	Requirement /desirable limit
1	Colour, Hazen units, Max.	10
2	Odour	Unobjectionable
3	Taste	Agreeable
4	Turbidity, NTU, Max.	10
5	pH value	6.5-8.5
6	Total hardness (as $CaCo_3$) mg/l	300
7	Calcium (as Ca), mg/l., Max.	75
8	Magnesium (as Mg), mg/l., Max.	30
9	Copper (as Cu), mg/l., Max.	0.05
10	Iron (as Fe), mg/l., Max.	0.3
11	Manganeae (as Mn), mg/l., Max.	0.1
12	Chlorides (as Cl), mg/l., Max.	250
13	Sulphates (as So_4), mg/l., Max.	150
14	Nitrates (as No_3), mg/l., Max.	45
15	Fluoride (as F), mg/l., Max.	0.6-1.2
16	Phenolics (as C_6H_5OH), mg/l., Max.	0.001
17	Mercury (as Hg), mg/l., Max.	0.001
18	Cadmium (as Cd), mg/l., Max.	0.01
19	Selenium (as Se), mg/l., Max.	0.01
20	Arscenic (as As), mg/l., Max.	0.05
21	Cyanide (as CN), mg/l., Max.	0.05
22	Lead (as Pb), mg/l., Max.	0.1
23	Zinc (as Zn), mg/l., Max.	5.0
24	Anionic Detergents (as MBAS), mg/l., Max.	0.2
25	Chromium (as Cr^{6+}), mg/l., Max.	0.05
26	Polynuclear Aromatic Hydrocarbons (as PAH), mg/l., Max.	Nil

Table 2. Physico-chemical parameters of tap water during post winter.

Parameters	Post winter 31-1-2000 January to February	Post winter 30-2-2001 January to February
Temperature	27 mean±1^{0}C	27 mean±1^{0}C
PH	7.5	7.0
Turbidity (NTU)	0.8	1.0
Colour	-	-
Smell	-	-
Alkalinity (ppm)	28	27
Hardness (ppm)	70	72
Chloride (ppm)	10	7.0
Calcium (ppm)	1.1	1.0
Magnesium (ppm)	0.4	0.2
Iron (ppm)	-	-
Phosphate (ppm)	-	-
Sulphate (ppm)	1.0	1.3

Table 3. Physico-chemical parameters of tap water during summer.

Parameters	Summer 15-4-2000 March to May	Summer 15-5-2001
Temperature	33 mean±1^{0}C	32 mean±1^{0}C
PH	7.0	7.0
Turbidity (NTU)	1.1	1.0
Colour	-	-
Smell	-	-
Alkalinity (ppm)	25	26
Hardness (ppm)	73	74
Chloride (ppm)	11	10
Calcium (ppm)	1.3	1.2
Magnesium (ppm)	0.4	0.3
Iron (ppm)	0.001	-
Phosphate (ppm)	-	-
Sulphate (ppm)	-	-

Table 4. Physico-chemical parameters of tap water during monsoon.

Parameters	Monsoon 18-6-2000 June to August	Monsoon 12-7-2001 June to August
Temperature	23 mean±1^{0}C	23 mean±1^{0}C
PH	7.5	7.5
Turbidity (NTU)	0.8	0.9
Colour	-	-
Smell	-	-
Alkalinity (ppm)	70	70
Hardness (ppm)	73	74
Chloride (ppm)	5.0	6.0
Calcium (ppm)	1.0	1.0
Magnesium (ppm)	0.2	0.3
Iron (ppm)	0.001	-
Phosphate (ppm)	0.003	0.006
Sulphate (ppm)	1.2	1.2

Table 5. Physico-chemical parameters of tap water during post monsoon.

Parameters	Post monsoon 14-9-2000 September to October	Post monsoon 14-10-2001
Temperature	25 mean±1^{0}C	26 mean±1^{0}C
PH	7.5	7.0
Turbidity (NTU)	0.8	0.8
Colour	-	-
Smell	-	-
Alkalinity (ppm)	60	50
Hardness (ppm)	73	72
Chloride (ppm)	7.0	5.0
Calcium (ppm)	1.0	1.0
Magnesium (ppm)	0.3	0.3
Iron (ppm)	-	-
Phosphate (ppm)	0.001	0.002
Sulphate (ppm)	1.1	1.2

Table 6. Physico-chemical parameters of tap water during winter.

Parameters	Winter 9-11-2000 November to December	Winter 10-12-2001
Temperature	20 mean±1^{0}C	20 mean±1^{0}C
PH	7.5	7.0
Turbidity (NTU)	0.8	0.9
Colour	-	-
Smell	-	-
Alkalinity (ppm)	27	27
Hardness (ppm)	72	74
Chloride (ppm)	8.0	9.0
Calcium (ppm)	1.0	1.0
Magnesium (ppm)	0.3	0.3
Iron (ppm)	-	-
Phosphate (ppm)	-	-
Sulphate (ppm)	1.2	1.0

Table 7. Estimation of LC_{50} value in *Oreochromis mossambicus* for 72h during monsoon, 23 mean±2^{0}C, June-August.

NaCl stock sol.	Salt conc. gm/16L	Vol. of $AgNo_3$ 'Vb'	Chlorinity C%	Salinity ppt	Fish mortality
DW	DW	0.4	0.007	0.013	0
TW	TW	0.77	0.015	0.027	0
SW	SW	19.55	15.093	27.266	2
2.5mg/100ml TW	2g/16L	6.47	0.125	0.226	0
5mg/100ml TW	2.4g/16L	7.43	0.143	0.259	0
20mg/100ml TW	3.2g/16L	9.83	0.190	0.343	0
25mg/100ml TW	4.0g/16L	12.5	0.241	0.436	0
30mg/100ml TW	4.8g/16L	15.13	0.292	0.528	0
35mg/100ml TW	5.6g/16L	16.37	0.316	0.570	0
37.5mg/100ml TW	6.0g/16L	17.20	0.332	0.600	0
40mg/100ml TW	6.4g/16L	17.63	0.340	0.615	0
45mg/100ml TW	7.2g/16L	21.50	0.415	0.750	0
50mg/100ml TW	8.0g/16L	23.40	0.452	0.816	0
55mg/100ml TW	8.8g/16L	26.00	0.501	0.907	0
60mg/100ml TW	9.6g/16L	27.57	0.532	0.961	0
10mg/10ml TW(DF=5)	16g/16L	6.50	0.625	1.129	0
30mg/10ml TW(DF=5)	48g/16L	17.10	1.650	2.981	0
50mg/10ml TW(DF=5)	80g/16L	29.50	2.845	5.143	0
75mg/10ml TW(DF=8.33)	120g/16L	26.65	4.284	7.740	0
100mg/10ml TW(DF=8.33)	160g/16L	35.10	5.643	10.194	0
125mg/10ml TW(DF=8.33)	200g/16L	43.50	6.993	12.634	0
150mg/10ml TW(DF=8.33)	240g/16L	50.50	8.119	14.667	1

200mg/10ml TW(DF=12.5)	320g/16L	46.50	11.218	20.266	1
250mg/10ml TW(DF=12.5)	400g/16L	59.00	14.233	25.714	2
300mg/10ml TW(DF=12.5)	480g/16L	70.00	16.888	30.508	3
350mg/10ml TW(DF=12.5)	560g/16L	77.00	18.576	33.559	4
400mg/10ml TW(DF=12.5)	640g/16L	87.70	21.158	38.222	4
450mg/10ml TW(DF=12.5)	720g/16L	94.10	22.702	41.012	5
500mg/10ml TW(DF=12.5)	800g/16L	105.0	25.331	45.762	6

DW=distilled water. 'Vb'=volume of silver nitrate. DF=dilution factor.
TW=tap water. Cl%=chlorinity.
SW=sea water. 'ppt'=parts per thousand.

Table 8. Behavioural dynamics in fish *Oreochromis mossambicus* with increasing salinity concentration.

NaCl stock sol.	Salt conc. gm/16L	Salinity ppt	Different behaviour observed in fish					
			fa	bs	nu	ni	co	mo
DW	DW	0.013	+	+	+	-	-	-
TW	TW	0.027	+	+	+	-	-	-
SW	SW	27.266	+	+	+	+	+	+
2.5mg/100ml TW	2g/16L	0.226	+	+	+	-	-	-
5mg/100ml TW	2.4g/16L	0.259	+	+	+	-	-	-
20mg/100ml TW	3.2g/16L	0.343	+	+	+	-	-	-
25mg/100ml TW	4.0g/16L	0.436	+	+	+	-	-	-
30mg/100ml TW	4.8g/16L	0.528	+	+	+	-	-	-
35mg/100ml TW	5.6g/16L	0.570	+	+	+	-	-	-
37.5mg/100ml TW	6.0g/16L	0.600	+	+	+	-	-	-
40mg/100ml TW	6.4g/16L	0.615	+	+	+	-	-	-
45mg/100ml TW	7.2g/16L	0.750	+	+	+	-	-	-
50mg/100ml TW	8.0g/16L	0.816	+	+	+	-	-	-
55mg/100ml TW	8.8g/16L	0.907	+	+	+	-	-	-
60mg/100ml TW	9.6g/16L	0.961	+	+	+	-	-	-
10mg/10ml TW(DF=5)	16g/16L	1.129	+	+	+	-	-	-
30mg/10ml TW(DF=5)	48g/16L	2.981	+	+	+	-	-	-
50mg/10ml TW(DF=5)	80g/16L	5.143	+	+	+	-	+	-
75mg/10ml TW(DF=8.33)	120g/16L	7.740	+	+	+	+	+	-
100mg/10ml TW(DF=8.33)	160g/16L	10.194	+	+	+	+	+	-
125mg/10ml TW(DF=8.33)	200g/16L	12.634	+	+	+	+	+	-
150mg/10ml TW(DF=8.33)	240g/16L	14.667	+	+	+	+	+	+
200mg/10ml TW(DF=12.5)	320g/16L	20.266	+	+	+	+	+	+
250mg/10ml TW(DF=12.5)	400g/16L	25.714	+	+	+	+	+	+
300mg/10ml TW(DF=12.5)	480g/16L	30.508	+	+	+	+	+	+
350mg/10ml TW(DF=12.5)	560g/16L	33.559	+	+	+	+	+	+
400mg/10ml TW(DF=12.5)	640g/16L	38.222	+	+	+	+	+	+
450mg/10ml TW(DF=12.5)	720g/16L	41.012	+	+	+	+	+	+
500mg/10ml TW(DF=12.5)	800g/16L	45.762	+	+	+	+	+	+

DF=dilution factor. '+'=behaviour observed. '-'=behaviour absent.
TW=tap water. DW=distilled water. SW=sea water.
fa=fanning
bs=burst swimming
nu=nudge
ni=nip
mo=motionless
co=cough

Table 9. Percentile behavioural dynamics in fish *Oreochromis mossambicus* with increasing salinity concentration.

NaCl stock sol.	Salt conc. gm/16L	Salinity ppt	Percentile behaviour observed in fish					
			fa	bs	nu	ni	co	mo
DW	DW	0.013	20%	12%	15%	0%	0%	0%
TW	TW	0.027	22%	18%	17%	0%	0%	0%
SW	SW	27.266	89%	85%	81%	77%	72%	50%
2.5mg/100ml TW	2g/16L	0.226	23%	19%	20%	0%	0%	0%
5mg/100ml TW	2.4g/16L	0.259	23%	20%	20%	0%	0%	0%
20mg/100ml TW	3.2g/16L	0.343	25%	23%	21%	0%	0%	0%
25mg/100ml TW	4.0g/16L	0.436	25%	24%	22%	0%	0%	0%
30mg/100ml TW	4.8g/16L	0.528	25%	24%	26%	0%	0%	0%
35mg/100ml TW	5.6g/16L	0.570	27%	24%	24%	0%	0%	0%
37.5mg/100ml TW	6.0g/16L	0.600	28%	23%	23%	0%	0%	0%
40mg/100ml TW	6.4g/16L	0.615	32%	28%	25%	0%	0%	0%
45mg/100ml TW	7.2g/16L	0.750	35%	30%	28%	0%	0%	0%
50mg/100ml TW	8.0g/16L	0.816	35%	32%	30%	0%	0%	0%
55mg/100ml TW	8.8g/16L	0.907	40%	38%	33%	0%	0%	0%
60mg/100ml TW	9.6g/16L	0.961	40%	39%	35%	0%	0%	0%
10mg/10ml TW(DF=5)	16g/16L	1.129	40%	40%	40%	0%	0%	0%
30mg/10ml TW(DF=5)	48g/16L	2.981	45%	43%	42%	0%	0%	0%
50mg/10ml TW(DF=5)	80g/16L	5.143	60%	45%	44%	0%	16%	0%
75mg/10ml TW(DF=8.33)	120g/16L	7.740	65%	49%	45%	38%	35%	0%
100mg/10ml TW(DF=8.33)	160g/16L	10.194	66%	50%	45%	43%	40%	0%
125mg/10ml TW(DF=8.33)	200g/16L	12.6	58%	55%	63%	54%	58%	16%

150mg/10ml TW(DF=8.33) 240g/16L 14.667 65% 64% 62% 59% 58% 32%

200mg/10ml TW(DF=12.5) 320g/16L 20.266 79% 78% 73% 70% 63% 57%

250mg/10ml TW(DF=12.5) 400g/16L 25.714 88% 81% 77% 75% 69% 62%

300mg/10ml TW(DF=12.5) 480g/16L 30.508 92% 90% 82% 80% 77% 71%

350mg/10ml TW(DF=12.5)560g/16L 33.559 100% 95% 90% 88% 83% 84%

400mg/10ml TW(DF=12.5)640g/16L 38.222 100%100%100%100%100%94%

450mg/10ml TW(DF=12.5)720g/16L41.012100%100%100%100%100%100%

500mg/10ml TW(DF=12.5)800g/16L45.762100%100%100%100%100%100%

DF=dilution factor. TW=tap water. DW=distilled water. SW=sea water.
fa=fanning
bs=burst swimming
nu=nudge
ni=nip
mo=motionless
co=cough

Table 10. Seasonal hydrodynamics in gill filament tissue as gm. wet wt. gm $^{-1}$ tissue wt.

season	month	temp	C	C	10ppt 7day	10ppt 7day	10ppt 21day	10ppt 21day	20ppt 7day	20ppt 7day	20ppt 21day	20ppt 21day
			DWGF	WC	DWGF	WC	DWGF	WC	DWGF	WC	DWGF	WC
Post	Jan.	27±2^{0}C	.785	.215	.78	.222*	.775	.225*	.77	.230*	.76	.240*
winter	Feb.			±0.001		±0.001		±0.001		±0.001		±0.001
Summer	Mar.	33±2^{0}C	.75	.25	.745	.255*	.74	.260*	.74	.260*	.725	.275*
	May			±0.001		±0.001		±0.001		±0.001		±0.001
Monsoon	Jun.	23±2^{0}C	.815	.185	.81	.19*	.805	.195*	.80	.20*	.79	.21*
	Aug.			±0.001		±0.001		±0.001		±0.001		±0.001
Post	Sept.	24±2^{0}C	.81	.19	.805	.195*	.80	.20*	.79	.21*	.785	.215*
Monsoon	Oct.			±0.001		±0.001		±0.001		±0.001		±0.001
Winter	Nov.	20±2^{0}C	.845	.155	.84	.16*	.835	.165*	.83	.17*	.825	.175*
	Dec.			±0.001		±0.001		±0.001		±0.001		±0.001

values represent mean±SE of six fishes. * = P<0.05% level of significance.
C=control, DWGF=dry weight of gill filament, WC=water content.

Table 11. Seasonal hydrodynamics in large intestine tissue as gm. wet wt. gm $^{-1}$ tissue wt.

season	month	temp	C	C	10ppt 7day	10ppt 7day	10ppt 21day	10ppt 21day	20ppt 7day	20ppt 7day	20ppt 21day	20ppt 21day
			DWLI	WC	DWLI	WC	DWLI	WC	DWLI	WC	DWLI	WC
Post	Jan.	27±2^{0}C	.625	.375	.62	.38*	.615	.385*	.61	.39*	.605	.395*
Winter	Feb.			±0.001		±0.001		±0.001		±0.001		±0.001
Summer	Mar.	33±2^{0}C	.60	.40	.555	.445*	.55	.45*	.50	.50*	.45	.55*
	May			±0.001		±0.001		±0.001		±0.001		±0.001
Monsoon	Jun.	23±2^{0}C	.645	.355	.64	.36*	.365	.365*	.63	.37*	.625	.375*
	Aug.			±0.001		±0.001		±0.001		±0.001		±0.001
Post	Sept.	24±2^{0}C	.64	.36	.635	.365*	.63	.37*	.625	.375*	.615	.385*
Monsoon	Oct.			±0.001		±0.001		±0.001		±0.001		±0.001
Winter	Nov.	20±2^{0}C	.65	.35	.645	.355*	.64	.36*	.635	.365*	.63	.37*
	Dec.			±0.001		±0.001		±0.001		±0.001		±0.001

values represent mean±SE of six fishes. * = P<0.05% level of significance.
C=control, DWLI=dry weight of large intestine, WC=water content.

Table 12. Seasonal hydrodynamics in liver tissue as gm. wet wt. gm $^{-1}$ tissue wt.

season	month	temp	C	C	10ppt 7day	10ppt 7day	10ppt 21day	10ppt 21day	20ppt 7day	20ppt 7day	20ppt 21day	20ppt 21day
			DWLi	WC	DWLi	WC	DWLi	WC	DWLi	WC	DWLi	WC
Post	Jan.	27±2^{0}C	.71	.29	.70	.30*	.695	.305*	.685	.315*	.68	.32*
Winter	Feb.			±0.001		±0.001		±0.001		±0.001		±0.001
Summer	Mar.	33±2^{0}C	.65	.35	.60	.40*	.55	.45*	.535	.465*	.53	.47*
	May			±0.001		±0.001		±0.001		±0.001		±0.001
Monsoon	Jun.	23±2^{0}C	.73	.27	.275	.725*	.72	.28*	.715	.285*	.705	.295*
	Aug.			±0.001		±0.001		±0.001		±0.001		±0.001
Post	Sept.	24±2^{0}C	.725	.275	.72	.28*	.715	.285*	.705	.295*	.70	.30*
Monsoon	Oct.			±0.001		±0.001		±0.001		±0.001		±0.001
Winter	Nov.	20±2^{0}C	.75	.25	.745	.255*	.74	.26*	.735	.265*	.73	.27*
	Dec.			±0.001		±0.001		±0.001		±0.001		±0.001

values represent mean±SE of six fishes. * = P<0.05% level of significance.
C=control, DWLi=dry weight of liver, WC=water content.

Table 13. Percentile seasonal water content in *Oreochromis mossambicus*.

season	month	temp	tissue	C	10ppt 7day	10ppt 21day	20ppt 7day	20ppt 21day
				%WC	%WC	%WC	%WC	%WC
Post	Jan.	27±2°C	GF	21.50	22.00	22.50	23.00	24.00
Winter	Feb.		LI	37.50	38.00	38.50	39.00	39.50
			Liv.	29.00	30.00	30.50	31.50	32.00
Summer	Mar.	33±2°C	GF	25.00	25.50	26.00	26.00	27.50
	May		LI	40.00	44.50	45.00	50.00	55.00
			Liv.	35.00	40.00	45.00	46.50	47.00
Monsoon	Jun.	23±2°C	GF	18.50	19.00	19.50	20.00	21.00
	Aug.		LI	35.50	36.00	36.50	37.00	37.50
			Liv.	27.00	27.50	28.00	28.50	29.50
Post	Sept.	24±2°C	GF	19.00	19.50	20.00	21.00	21.50
Monsoon	Oct		LI	36.00	36.50	37.00	37.50	38.50
			Liv.	27.50	28.00	28.50	29.50	30.00
Winter	Nov.	20±2°C	GF	15.50	16.00	16.50	27.00	17.50
	Dec.		LI	35.00	35.50	36.00	36.50	37.00
			Liv.	25.00	25.50	26.00	26.50	27.00

GF= gill filament.
LI= large intestine.
Liv.= liver.

Table 14. Summary of observations on the survival out of water in a few air breathing fishes.

Fish species	Total survival out of water (h)	Reference
Amia calva	72-120	McKenzie and Randall, 1990.
Amphipnous cuchia	60	Das, 1927.
Boleophthalmus boddaerti	724	Biswas *et al.*, 1979.
Channa punctatus	7	Sinha and Munshi, 1981.
Clarias batracus	18	Das, 1927.
Clinocottus analis	24	Martin, 1991.
Colisa lalia	3	Ramaswamy and Reddy, 1979.
Erepetoichthys calabaricus	6-8	Pettit and Beitinger, 1985.
Lepisosteus oculatus	7.8-60	Smatresk and Cameron, 1982.
Synbranchus marmoratus	5-10	Bicudo and Johansen, 1979.
Trigogaster tricopterus	4-6	Burggren, 1979.
Notopterus notopterus	3-7	Vijayalaxhmi, 1996.
Macrognathus aculeatus	3	Sumathiral, 1996.
Mystus punctatus	$3^{1/2}$	Devika, 1999.
Mystus gulio	2-6	Raveendran, 2000.
Oreochromis mossambicus	3-5	Present work.

Table 15. Circadian rhythm of peaks of bimodal oxygen uptake of some air-breathing fishes.

Species	Peak periods of bimodal O_2 uptake	Reference
Anabas scandens	24:00	Natarajan, 1972.
Channa gachua	Early parts of night	Munshi *et al.*, 1979.
Channa marulies	24:00	Munshi *et al.*, 1979.
Channa punctata	Dusk	Munshi *et al.*, 1979.
Channa straita	18:00	Rani, 1994
Hoplosternum littorale	Dusk (Aquatic resp.)	Boujard *et al.*, 1990.
Lepidocephalis thermalis	06:00	Natarajan, 1981.
Osphronemus olfax	24:00	Natarajan, 1984.
Macropodus cupanus	06:00	Natarajan, 1987.
Monoptrus cuchia	16:00-18:00	Patra *et al.*, 1979.
	04:00-06:00	
Notopterus notopterus	24:00	Vijayalakshmi, 1996.
.*Oreochromis mossambicus*	3:00-4:00 (early morning)	Present work.
	17:00-18:00 (early evening)	

Table 16. Published observations on the asphyxiation time of some air-breathing fishes.

Fish species	Asphyxiation time (min)	Reference
Amia calva	45-60	Daxboeck *et al.*, 1981.
Amphipnous cuchia	300	Lomholt and Johansen, 1976.
Anabas scandens	60	Reddy and Natarajan, 1970.
Anabas testudineus	15	Hoar, 1935.
Brochis splendus	7 days	Gee and Graham, 1978.
Channa gachua	120 days	Ramasamy and Reddy, 1978.
Channa marulius	240	Ojha *et al.*, 1979.
Clarias	120	Das, 1927.
Clarias batrachus	5 days	Munshi *et al.*, 1976.
Clarias macrocephalus	25 days	Bevan and Kramer, 1987.
Gillichithys mirabilis	120-1980	Todd and Ebeling, 1966.
Heteropneustes fossilis	360-720	Hughes and Singh, 1971.
Lepidocephalus thermalis	40	Natarajan, 1981.
Osphromenus olfax	60	Natarajan, 1980.
Pseudapocryptes	900-1200	Das, 1927.
Tricogaster	240	Das, 1927.
Notoptrus notopterus	10-15days	Vijayalaxkshmi, 1996.
Mystus gulio	12-16days	Raveendran, 2000.
Macrognathus aculeatus	15 days	Sumathiral, 1996.
Mystus punctatus	20 days	Devika, 1999.
Oreochromis mossambicus	80-100	Present work

Table 17. Circadian rhythm of bimodal oxygen uptake (ml O_2 k^{-1} h^{-1}) of *Oreochromis mossambicus*, STT, (5.3-6.2g; N=6 fishes) at summer (33±2^{0}C), II days.

Time of the day	Aquatic R.	Aerial R.	Total	%Aquatic	% Aerial	Aquatic / Aerial
06:00	100.11±5.50	31.23±6.22	131.34±5.0	75.01	24.99	3.00
09:00	98.21±5.10	29.62±7.00	127.34±5.2	74.55	25.45	2.93
12:00	99.36±7.30	31.00±5.78	130.36±6.3	74.98	25.02	2.30
15:00	95.28±6.00	31.59±8.01	126.87±7.0	73.11	26.89	2.72
18:00	99.00±6.21	40.33±7.73	139.33±6.5	74.80	25.02	2.97
21:00	99.97±5.55	26.73±5.55	126.70±5.8	74.99	25.01	2.30
00:00	115.03±7.8	25.65±5.11	140.68±6.5	80.00	20.00	4.00
03:00	135.22±9.1	31.77±3.99	166.99±5.1	85.00	15.00	5.67

N=number of fishes, R=respiration, X±S.E. of 6 determinations.

Table 18. Circadian rhythm of bimodal oxygen uptake (ml O_2 k^{-1} h^{-1}) of *Oreochromis mossambicus*, STT, (5.3-6.2g; N=6 fishes) at winter (20±2^{0}C), II days.

Time of the day	Aquatic R.	Aerial R.	Total	%Aquatic	%Aerial	Aquatic / Aerial
06:00	52.31±4.3	10.11±1.1	62.42±3.5	77.78	22.02	3.54
09:00	49.73±4.3	9.25±1.2	58.98±3.2	75.11	24.89	3.02
12:00	50.11±4.1	10.01±1.5	60.12±3.0	76.87	23.13	3.32
15:00	45.78±3.7	10.30±1.0	56.08±2.9	72.13	27.87	2.59
18:00	48.00±3.5	11.12±2.0	59.12±2.7	74.66	25.34	2.95
21:00	49.99±3.6	5.43±0.9	55.42±2.2	75.77	24.23	3.13
00:00	52.55±5.1	4.99±0.5	57.54±4.3	78.00	22.00	3.56
03:00	53.67±5.3	11.00±1.5	67.67±3.3	79.31	20.69	3.83

N=number of fishes, R=respiration, X±S.E. of 6 determinations.

Table 19. Circadian rhythm of bimodal oxygen uptake (ml O_2 k^{-1} h^{-1}) of *Oreochromis mossambicus*, STT, (5.3-6.2g; N=6 fishes) at summer (33±2°C), III days.

Time of the day	Aquatic R.	Aerial R.	Total	%Aquatic	%Aerial	Aquatic / Aerial
06:00	103.72±6.00	33.00±6.80	136.72±4.3	75.20	24.80	3.03
09:00	100.11±5.70	31.71±6.40	131.82±5.2	75.60	24.40	3.10
12:00	98.71±3.830	30.80±7.10	129.51±5.7	76.11	23.89	3.19
15:00	99.20±7.00	31.99±9.06	131.82±7.7	73.75	26.25	2.18
18:00	103.94±5.09	38.76±9.02	142.70±7.1	73.18	26.82	2.73
21:00	101.00±4.98	30.00±7.42	131.0±6.33	76.44	23.56	3.24
00:00	110.43±7.41	29.76±6.10	140.19±6.5	78.40	21.60	3.63
03:00	122.77±6.10	30.08±8.02	152.85±7.5	80.84	19.16	4.23

N=number of fishes, R=respiration, X±S.E. of 6 determinations.

Table 20. Circadian rhythm of bimodal oxygen uptake (ml O_2 k^{-1} h^{-1}) of *Oreochromis mossambicus*, STT, (5.3-6.2g; N=6 fishes) at winter (20±2°C), III days.

Time of the day	Aquatic R.	Aerial R.	Total	%Aquatic	%Aerial	Aquatic / Aerial
06:00	50.81±4.72	10.00±5.6	60.81±4.2	79.23	20.77	3.81
09:00	47.22±7.16	9.20±3.92	56.42±4.5	75.98	24.04	3.16
12:00	48.00±8.06	8.70±2.44	56.70±4.5	77.11	22.89	3.37
15:00	44.73±7.09	9.33±2.08	54.06±3.9	74.23	25.77	2.89
18:00	46.22±8.14	10.11±3.06	52.33±4.2	75.24	24.76	3.04
21:00	47.99±9.01	4.32±1.10	52.31±5.2	77.73	22.27	3.49
00:00	49.32±9.71	4.00±1.00	53.32±5.5	79.16	20.84	3.80
03:00	51.00±8.80	12.00±2.45	63.00±4.8	80.00	20.00	4.00

N=number of fishes, R=respiration, X±S.E. of 6 determinations.

Table 21. Circadian rhythm of bimodal oxygen uptake (ml O_2 k^{-1} h^{-1}) of *Oreochromis mossambicus*, STT, (10-15.5g; N=6 fishes) at summer (33±2^{0}C), II days.

Time of the day	Aquatic R.	Aerial R.	Total	%Aquatic	%Aerial	Aquatic / Aerial
06:00	128.31±5.10	57.11±7.1	185.42±6.1	75.51	24.49	3.08
09:00	120.00±6.17	50.23±3.4	170.23±5.1	75.88	24.12	3.15
12:00	118.33±7.08	55.79±5.1	174.12±5.3	77.00	23.00	3.35
15:00	119.11±6.92	52.22±8.0	171.33±7.0	74.31	25.69	2.89
18:00	129.72±5.90	59.45±8.0	189.17±6.6	73.99	26.01	2.84
21:00	125.51±7.62	56.68±9.0	182.11±8.2	77.48	22.52	3.44
00:00	122.21±8.50	54.22±9.22	176.43±8.9	70.11	20.89	3.79
03:00	115.73±9.70	55.55±7.1	171.28±8.1	80.25	19.75	4.06

N=number of fishes, R=respiration, X±S.E. of 6 determinations.

Table 22. Circadian rhythm of bimodal oxygen uptake (ml O_2 k^{-1} h^{-1}) of *Oreochromis mossambicus*, STT, (10-15.5g; N=6 fishes) at winter (20±2°C), II days.

Time of the day	Aquatic R.	Aerial R.	Total	%Aquatic	%Aerial	Aquatic / Aerial
06:00	55.27±5.4	11.98±3.1	67.25±4.1	77.18	22.82	3.38
09:00	48.11±5.3	11.00±3.0	59.11±4.3	76.17	23.83	3.20
12:00	49.76±4.8	10.76±3.0	60.52±4.3	78.15	21.85	3.58
15:00	46.98±4.5	10.98±2.8	57.96±4.0	74.00	26.00	2.85
18:00	47.13±4.2	10.99±2.9	58.12±3.8	75.99	24.01	3.16
21:00	48.99±4.1	8.40±1.3	57.39±2.9	79.73	20.27	3.93
00:00	52.32±4.6	7.60±4.0	59.92±4.3	79.00	21.00	3.76
03:00	53.11±5.5	10.00±3.2	63.11±3.9	80.11	19.89	4.03

N=number of fishes, R=respiration, X±S.E. of 6 determinations.

Table 23. Circadian rhythm of bimodal oxygen uptake (ml O_2 k^{-1} h^{-1}) of *Oreochromis mossambicus*, STT, (10-15.5g; N=6 fishes) at summer (33±2°C), III days.

Time of the day	Aquatic R.	Aerial R.	Total	%Aquatic	%Aerial	Aquatic / Aerial
06:00	132.0±7.81	59.73±4.13	191.73±5.3	57.32	42.68	1.34
09:00	131.0±6.73	55.11±6.32	186.11±6.3	51.44	48.56	1.06
12:00	130.0±9.85	53.32±5.75	183.32±6.2	57.80	42.20	1.37
15:00	131.92±8.47	51.0±4.34	182.92±5.0	53.11	46.89	1.13
18:00	135.73±7.39	62.11±7.16	197.84±7.0	59.00	41.00	1.44
21:00	133.64±7.21	58.79±7.05	192.43±6.9	55.78	44.22	1.36
00:00	130.98±8.13	52.11±4.88	183.09±5.13	55.56	44.44	1.25
03:00	131.21±6.35	54.46±4.99	185.67±5.04	56.77	43.23	1.31

N=number of fishes, R=respiration, X±S.E. of 6 determinations.

Table 24. Circadian rhythm of bimodal oxygen uptake (ml O_2 k^{-1} h^{-1}) of *Oreochromis mossambicus*, STT, (10-15.5g; N=6 fishes) at winter (20±2^{0}C), III days.

Time of the day	Aquatic R.	Aerial R.	Total	%Aquatic	%Aerial	Aquatic / Aerial
06:00	58.11±4.12	57.13±3.66	115.24±3.5	77.15	22.85	3.38
09:00	55.10±3.23	54.74±3.72	109.84±3.2	76.15	23.85	3.19
12:00	47.20±3.54	52.98±2.73	100.18±2.8	79.30	20.70	3.83
15:00	48.27±7.67	52.00±2.11	100.27±4.5	75.13	24.87	3.02
18:00	49.09±5.89	59.98±4.14	109.07±4.1	76.44	23.56	3.24
21:00	50.77±4.11	56.63±3.13	107.50±3.9	78.15	21.85	3.58
00:00	52.92±4.33	51.77±4.17	104.69±4.7	79.00	21.00	3.76
03:00	54.76±4.00	53.20±4.19	107.96±3.8	80.99	19.01	4.26

N=number of fishes, R=respiration, X±S.E. of 6 determinations.

Table 25. Circadian rhythm of bimodal oxygen uptake (ml O_2 k^{-1} h^{-1}) of *Oreochromis mossambicus*, STT, (22-25.5g; N=6 fishes) at summer (33±2^{0}C), II days.

Time of the day	Aquatic R.	Aerial R.	Total	%Aquatic	%Aerial	Aquatic / Aerial
06:00	130.98±8.17	59.73±5.10	190.71±6.3	59.73	40.27	1.48
09:00	129.11±9.11	55.11±4.23	184.22±5.5	55.82	44.18	1.26
12:00	125.05±7.12	53.20±4.11	178.25±5.7	58.11	41.89	1.39
15:00	126.77±6.15	50.11±4.13	176.88±5.3	54.00	46.00	1.17
18:00	135.00±7.17	59.99±4.17	194.99±6.3	60.99	39.01	1.56
21:00	130.11±7.19	57.03±4.14	187.14±5.3	57.83	42.17	1.37
00:00	125.00±6.21	53.12±3.73	178.12±4.4	56.00	44.00	1.27
03:00	119.73±6.00	55.00±5.74	174.73±5.9	57.00	43.00	1.33

N=number of fishes, R=respiration, X±S.E. of 6 determinations.

Table 26. Circadian rhythm of bimodal oxygen uptake (ml O_2 k^{-1} h^{-1}) of *Oreochromis mossambicus*, STT, (22-25.5g; N=6 fishes) at winter (20±2^{0}C), II days.

Time of the day	Aquatic R.	Aerial R.	Total	%Aquatic	%Aerial	Aquatic / Aerial
06:00	56.11±4.32	13.00±3.11	69.11±3.7	79.99	20.01	4.00
09:00	43.00±4.13	12.00±2.12	55.00±3.5	77.13	22.87	3.37
12:00	50.17±4.12	11.11±1.15	61.28±2.9	83.71	16.29	5.14
15:00	47.15±4.12	11.98±1.17	59.13±3.7	77.11	22.89	3.37
18:00	48.12±5.11	11.19±1.94	59.31±2.5	78.00	22.00	3.55
21:00	49.57±5.00	9.55±1.74	59.12±3.0	79.93	20.07	3.98
00:00	53.63±5.09	8.78±1.74	62.41±4.4	80.41	19.59	4.10
03:00	53.93±5.01	14.44±2.00	68.37±3.0	81.74	18.26	4.48

N=number of fishes, R=respiration, X±S.E. of 6 determinations.

Table 27. Circadian rhythm of bimodal oxygen uptake (ml O_2 k^{-1} h^{-1}) of *Oreochromis mossambicus*, STT, (22-25.5g; N=6 fishes) at summer (33±2^{0}C), III days.

Time of the day	Aquatic R.	Aerial R.	Total	%Aquatic	%Aerial	Aquatic / Aerial
06:00	137.73±8.00	59.98±4.14	195.71±6.1	78.13	21.87	3.57
09:00	133.22±9.12	55.73±4.17	188.95±5.5	75.14	24.86	3.02
12:00	132.17±7.77	54.22±4.37	186.39±5.7	77.77	22.23	3.50
15:00	131.99±7.12	52.11±3.13	184.10±5.3	74.43	25.57	2.91
18:00	137.00±7.50	59.00±5.15	196.00±6.1	78.73	21.27	3.70
21:00	134.11±7.11	58.73±4.75	192.84±4.9	76.13	23.87	3.19
00:00	130.21±7.22	52.00±4.74	182.21±5.2	75.14	24.86	3.02
03:00	133.79±7.14	55.10±4.34	188.89±5.0	77.74	22.26	3.50

N=number of fishes, R=respiration, X±S.E. of 6 determinations.

Table 28. Circadian rhythm of bimodal oxygen uptake (ml O_2 k^{-1} h^{-1}) of *Oreochromis mossambicus*, STT, (22-25.5g; N=6 fishes) at winter (20±2^{0}C), III days.

Time of the day	Aquatic R.	Aerial R.	Total	%Aquatic	%Aerial	Aquatic / Aerial
06:00	59.23±5.31	19.11±3.12	78.34±4.4	80.13	19.87	4.03
09:00	56.56±6.66	15.00±2.15	71.56±5.1	78.98	21.02	3.76
12:00	45.21±2.10	14.13±2.17	59.34±2.1	79.77	20.23	3.94
15:00	48.48±3.00	13.22±2.18	61.70±2.5	78.63	21.37	3.68
18:00	49.90±3.00	13.97±2.20	63.87±2.7	79.74	20.26	3.94
21:00	53.22±4.90	12.88±1.14	66.10±3.0	80.14	19.86	4.04
00:00	54.31±5.50	10.36±1.13	64.67±3.7	80.00	20.00	4.00
03:00	57.73±5.70	19.10±2.73	76.83±3.8	81.13	18.87	4.30

N=number of fishes, R=respiration, X±S.E. of 6 determinations.

Table 29. Seasonal aquatic respiration in *Oreochromis mossambicus* at 10ppt and 20ppt NaCl exposure in ml O_2 k^{-1} h^{-1}.

season	month	temp	control	10ppt 6h	10ppt 12h	10ppt 7day	10ppt 21day	20ppt 7day	20ppt 21day
Post	Jan.	27±2°C	0.240	0.250*	0.248*	0.234*	0.200*	0.231**	0.180*
winter	Feb.		±0.03	±0.03	±0.03	±0.03	±0.03	±0.03	±0.02
Summer	Mar.	33±2°C	0.250	0.265*	0.268*	0.240*	0.197N	0.220*	0.192*
	May		±0.03	±0.03	±0.03	±0.03	±0.03	±0.03	±0.02
Monsoon	Jun.	23±2°C	0.144	0.152*	0.148*	0.143*	0.121*	0.142*	0.380N
	Aug.		±0.03	±0.03	±0.03	±0.03	±0.02	±0.02	±0.02
Post	Sept.	24±2°C	0.190	0.197*	0.195*	0.170*	0.150*	0.160*	0.150*
Monsoon	Oct.		±0.03	±0.03	±0.02	±0.03	±0.02	±0.02	±0.02
Winter	Nov.	20±2°C	0.050	0.059*	0.055*	0.049*	0.047*	0.048*	0.046*
	Dec.		±0.02	±0.02	±0.02	±0.02	±0.02	±0.02	±0.004

Values represent mean±SD of six fishes.
*=P<0.05% level of significance.
**=P<0.01% level of significance.
N=not significant.

Table 30. Seasonal aerial respiration in *Oreochromis mossambicus* at 10ppt and 20ppt NaCl exposure in ml O_2 k^{-1} h^{-1}.

season	month	temp	control	10ppt 7day	10ppt 21day	20ppt 7day	20ppt 21day
Post	Jan.	27±2°C	$5.4x10^{-4}$	*$5.4x10^{-4}$	*$5.0x10^{-4}$	*$5.6x10^{-4}$	*$4.8x10^{-4}$
winter	Feb.		±0.03	±0.03	±0.03	±0.02	±0.03
Summer	Mar.	33±2°C	$5.8x10^{-4}$	*$6.4x10^{-4}$	*$5.9x10^{-4}$	*$5.8x10^{-4}$	*$5.7x10^{-4}$
	May		±0.03	±0.02	±0.03	±0.02	±0.03
Monsoon	Jun.	23±2°C	$3.1x10^{-4}$	*$3.1x10^{-4}$	*$2.7x10^{-4}$	*$3.2x10^{-4}$	*$9.3x10^{-4}$
	Aug.		±0.02	±0.03	±0.03	±0.02	±0.02
Post	Sept.	24±2°C	$4.1x10^{-4}$	*$3.7x10^{-4}$	*$3.4x10^{-4}$	*$3.7x10^{-4}$	*$3.7x10^{-4}$
Monsoon	Oct.		±0.01	±0.03	±0.03	±0.02	±0.03
Winter	Nov.	20±2°C	$5.4x10^{-4}$	*$5.4x10^{-4}$	*$5.4x10^{-4}$	*$5.4x10^{-4}$	*$5.4x10^{-4}$
	Dec.		±0.00	±0.01	±0.00	±0.00	±0.00

Values represent mean±SD of six fishes.
*=P<0.05% level of significance.
**=P<0.01% level of significance.
N=not significant.

Table 31. Seasonal total bimodal oxygen uptake by *Oreochromis mossambicus* in ml O_2 k^{-1} h^{-1}.

seasonal temp	control	10ppt 7day	10ppt 21day	20ppt 7day	20ppt 21day
Post winter 27 ± 2^0C Jan. Feb.	$5.64x10^{-4}$	$5.65x10^{-4}$	$5.20x10^{-4}$	$5.83x10^{-4}$	$4.98x10^{-4}$
Summer 33 ± 2^0C Mar. May	$6.05x10^{-4}$	$6.64x10^{-4}$	$6.09x10^{-4}$	$6.02x10^{-4}$	$5.89x10^{-4}$
Monsoon 23 ± 2^0C Jun. Aug.	$3.14x10^{-4}$	$3.24x10^{-4}$	$2.82x10^{-4}$	$3.34x10^{-4}$	$9.68x10^{-4}$
Post monsoon 24 ± 2^0C Sept. Oct.	$4.29x10^{-4}$	$3.87x10^{-4}$	$3.55x10^{-4}$	$3.86x10^{-4}$	$3.85x10^{-4}$
Winter 20 ± 2^0C Nov. Dec.	$1.05x10^{-4}$	$1.04x10^{-4}$	$1.04x10^{-4}$	$1.04x10^{-4}$	$1.06x10^{-4}$

Values represent mean±SD of six fishes.
TR=Total respiration.

Table 32. Survival time (h) out of water and % loss of body weight.

Fish wt. (g)	Survival time (h)	Total % loss of body wt.	Loss of wt. in mg/g/h
2.21	2.05	18.00	90.00
3.40	3.00	17.62	58.80
3.80	3.00	18.10	60.65
4.70	3.00	18.33	60.10
5.45	3.00	18.40	62.00
6.70	3.00	17.50	58.56
6.90	4.00	19.90	50.00
7.92	4.00	20.80	51.05
8.00	3.00	18.40	61.00
10.20	4.00	21.40	52.09
12.36	5.00	21.76	43.52
14.06	4.00	19.00	47.70

Table 33. Control respiration in ml O_2 k^{-1} h^{-1} by *Oreochromis mossambicus*.

fish wt. (g)	aquatic	aerial	total	%aquatic	%aerial	aquatic /aerial
5.50	120.21	45.11	165.32	72.22	27.78	2.60
7.00	90.76	38.52	129.28	72.54	27.46	2.64
8.10	111.00	41.55	152.55	72.05	27.95	2.58
8.75	100.50	40.01	140.01	71.54	28.46	2.51
9.56	105.00	38.00	143.00	73.74	26.26	2.81
10.32	89.54	36.32	125.86	70.83	29.17	2.43
11.50	92.10	36.44	128.54	71.22	28.78	2.47
12.61	85.20	34.72	119.92	71.82	28.18	2.53

Table 34. 10ppt respiration in ml O_2 k^{-1} h^{-1} by *Oreochromis mossambicus*.

fish wt. (g)	aquatic	aerial	total	%aquatic	%aerial	aquatic /aerial
5.50	100.11	31.23	131.34	75.01	24.99	3.00
5.10	98.21	29.62	127.83	74.55	25.45	2.93
7.30	99.36	31.00	130.36	74.98	25.02	2.30
6.00	95.28	31.59	126.87	73.11	26.89	2.72
6.21	99.00	40.33	139.33	74.80	25.20	2.97
5.55	99.97	26.73	126.70	74.99	25.01	2.30
7.80	115.03	25.65	140.68	80.00	20.00	4.00
9.10	135.22	31.77	166.99	85.00	15.00	5.67

Table 35. 20ppt respiration in ml O_2 k^{-1} h^{-1} by *Oreochromis mossambicus*.

fish wt. (g)	aquatic	aerial	total	%aquatic	%aerial	aquatic /aerial
6.22	52.31	10.11	62.42	77.98	22.02	3.54
7.00	49.73	9.25	58.98	75.11	24.89	3.02
5.78	50.11	10.01	60.12	76.87	23.13	3.32
8.01	45.78	10.30	56.08	72.13	27.87	2.59
7.73	48.00	11.12	59.12	74.66	25.34	2.95
5.55	49.99	5.43	55.42	75.77	24.23	3.13
5.11	52.55	4.99	57.54	78.00	22.00	3.56
3.99	53.67	11.00	64.67	79.31	20.69	3.83

Table 36. Nitrogen excretion in some facultative air breathing fishes (Ravendran, 2000).

species	environmental conditions	ammonia-N	urea-N	urea as total nitrogen excreted	reference
Acipenser baeri	12^{0}C	6.2	3.1	33.33	Gershanovich and Pototskiji, 1995.
Amia calva	freshwater	607[3]	60	9	
Alticus kirki	immersed	0.52[1]	0.62	545	Rozemeijer
	5h immersed	0.30	0.39	56.4	and Plaut, 1993.
	24h immersed	0.15	0.18	55.0	
Blennius pholis	starved 6 days	13	0.3	18.7	Davenport and Sayer, 1986.
Boleophthalamus pectinirostris	normal MW	34.26[1]	3.91	9.97	Morii *et al.*, 1978.
Channa punctatus	freshwater	585[3]	71	10.82	Saha and Ratha, 1989.
Clarias batracus	freshwater	421[3]	74	14.95	Saha and Ratha, 1989.
Clarias mossambicus	freshwater	0.26	0.04	13.3	Eddy *et al.*, 1980.
	3$^{1/2}$h air exposed	0.76	0.41	36.14	
Heteropneustes fossilis	freshwater	254[3]	48	15.89	Saha and Ratha, 1989.
Mystus vittatus	normal FW	10.6	7.8	42.4	Ramaswamy & Reddy,
	5h air exposed	37.77	18.29	32.6	1983.
Ophioceophalus punctatus	normal FW	0.36	0.48	57.0	Natarajan, 1979a.
	12h air exposed	0.68	1.33	66.2	
Periopthalmus cantonesis	starved 9days	6.3[1]	7.4	54.0	Gordon *et al.*, 1978.
	normal	106.16	10.65	8.80	Morii *et al.*, 1978.
Periopthalmus expeditionium	normal	259[2]	129	26.48	Gregory, 1977.
Periopthalmus gracilis	normal	184[2]	32	14.4	Gregory, 1977.
Periopthalmus sobrinus	starved 24h	6.9[1]	10.1	59.1	Gordon *et al.*, 1969.

Scartelaos histophorus	normal	156[2]	37	19.17	Gregory, 1977.
Sicyases sanguineus	starved 24h	5.3[1]	7.6	58.9	Gordon *et al.*, 1970.
Notopterus notopterus	normal FW	78.32[4]	21.6	21.62	Vijayalakshmi, 1996.
	4h air exposed	117	124	51.45	
	6h air exposed	125	135	51.92	
Mystus gulilo	normal FW	12.50[4]	5.3	29.78	Raveendran, 2000.
	50% SW	11.4	4.8	29.63	
	100% SW	8.2	3.71	31.15	
Clarias gariepinus	larvae 28^{0}C	-	-	19.7%	Terjesen etal., 1997.
	post fertilized	-	-	44.13%	
Oreochromis mossambicus	normal	refer present thesis			
	7day 10ppt	refer present thesis			
	21day 10ppt	refer present thesis			
	30day 10ppt	refer present thesis			
	7day 20ppt	refer present thesis			
	21day 20ppt	refer present thesis			
	30day 20ppt	refer present thesis			

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1 values in μg N g^{-1} /fish wet weight /h 2 values in μg N /24h g^{-1} /fish

3 values in μ mol kg^{-1} body wt. h^{-1} 4 *Mystus gulio* mg N kg^{-1} h^{-1}

All other values are in mg kg^{-1} h^{-1}

Table 37. Seasonal ammonia excretion in μg gm body weight^{-1} hr^{-1} in *Oreochromis mossambicus* under the influence of NaCl.

season	month	temp	control	10ppt 7day STT	10ppt 21day MTT	10ppt 30day LTT	20ppt 7day STT	20ppt 21day MTT	20ppt 30day LTT
Post winter	Jan. Feb.	27±2°C	181.96 ±2.50	109.61* ±3.60	106.04* ±2.50	75.00* ±0.60	102.14* ±1.20	56.00* ±0.50	42.00* ±0.30
Summer	Mar. May	33±2°C	212.67 ±2.42	162.91* ±2.01	160.07* ±2.40	80.00* ±1.40	130.72* ±1.60	85.00** ±1.20	52.00^{N} ±0.80
Monsoon	Jun. Aug.	23±2°C	73.41 ±1.10	53.33* ±0.43	35.76* ±0.79	32.00* ±1.20	58.28* ±0.49	24.63^{N} ±0.20	22.99* ±0.24
Post Monsoon	Sept. Oct.	24±2°C	82.53 ±1.10	57.29* ±0.98	48.48* ±0.44	40.00* ±0.62	52.35* ±0.59	46.69* ±0.37	38.00* ±0.20
Winter	Nov. Dec.	20±2°C	55.38 ±0.43	46.18* ±0.72	26.62* ±0.19	22.43^{N} ±0.11	40.48* ±0.57	24.70* ±0.17	20.71* ±0.10

Values represent mean±SD of six fishes.
*=P<0.05% level of significance.
**=P<0.01% level of significance.
N=not significant.

Table 38. Seasonal urea excretion in μg gm body weight^{-1} hr^{-1} in *Oreochromis mossambicus* under the influence of NaCl.

season	month	temp	control	10ppt 7day STT	10ppt 21day MTT	10ppt 30day LTT	20ppt 7day STT	20ppt 21day MTT	20ppt 30day LTT
Post winter	Jan. Feb.	27±2°C	74.43 ±0.71	65.26* ±0.56	70.71* ±1.10	98.88* ±0.45	72.90* ±0.43	75.05* ±0.93	120.50* ±1.50
Summer	Mar. May	33±2°C	76.24 ±1.20	67.47* ±0.67	70.85* ±0.81	102.99* ±0.85	72.94* ±0.82	88.45* ±0.50	122.00* ±1.10
Monsoon	Jun. Aug.	23±2°C	43.22 ±0.49	42.73* ±0.50	43.13* ±0.71	89.71** ±0.43	44.80* ±0.64	46.75* ±0.53	100.75* ±0.86
Post Monsoon	Sept. Oct.	24±2°C	57.28 ±0.24	53.65* ±0.59	55.72* ±1.09	95.77* ±1.10	52.41* ±0.79	69.23* ±0.64	109.90* ±1.10
Winter	Nov. Dec.	20±2°C	40.13 ±0.49	28.90* ±0.56	36.86* ±0.87	80.88* ±1.00	41.09* ±0.15	45.92* ±0.37	90.11* ±0.44

Values represent mean±SD of six fishes.
*=P<0.05% level of significance.
**=P<0.01% level of significance.
N=not significant.

Table 39. Seasonal protein concentration in μg 100mg body weight^{-1} in *Oreochromis mossambicus* under the influence of NaCl.

season month temp	tissue	control	10ppt 7day	10ppt 21day	10ppt 30day	20ppt 7day	20ppt 21day	20ppt 30day
			STT	MTT	LTT	STT	MTT	LTT
Post win. Jan. Feb. 27±2^{0}C	GF	5217.87 ±1.50	4963.4* ±0.12	4912.78* ±0.67	4911.89* ±0.64	4405.35* ±0.26	3353.56 ±0.42	*3351.52* ±0.02
Summer Mar. May 33±2^0 C	GF	4923.76 ±1.00	3583.11* ±1.78	3323.55* ±0.07	3321.5* ±0.69	3182.93* ±0.79	3067.6* ±1.92	3066.0* ±0.24
Monsoon Jun. Aug. 23±2^{0}C	GF	7443.46 ±1.10	6633.6* ±0.50	6481.52* ±2.69	6480.12* ±4.27	5404.91* ±0.24	4280.03* ±0.61	4279.99* ±0.32
Post mon. Oct. Sept. 24±2^{0}C	GF	6622.2 ±0.90	6566.56* ±0.27	5503.1* ±1.71	5501.98* ±0.69	5282.19* ±0.32	4203.26* ±2.24	4201.66* ±0.61
Winter Nov. Dec. 20±2^{0}C	GF	8146.45 ±1.00	7017.63* ±0.64	6602.58* ±0.69	6601.55* ±0.94	6963.84* ±0.42	5385.4* ±1.00	5384.11* ±0.60

Values represent mean±SD of six fishes. GF=gill filament
*=P<0.05% level of significance.
**=P<0.01% level of significance.
N=not significant.
STT=short term treatment.
MTT=medium term treatment.
LTT=long term treatment.

Table 40. Seasonal protein concentration in μg 100mg body weight^{-1} in *Oreochromis mossambicus* under the influence of NaCl.

season month temp	tissue	control	10ppt 7day	10ppt 21day	10ppt 30day	20ppt 7day	20ppt 21day	20ppt 30day
			STT	MTT	LTT	STT	MTT	LTT
Post win. Jan. Feb. 27±2°C	LI	6141.36 ±1.38	5466.93* ±1.38	5155.15* ±1.06	5154.22* ±1.83	4819.53* ±0.23	4136.16* ±0.40	4135.99* ±0.42
Summer Mar. May 33±2° C	LI	4958.96 ±1.70	4382.05* ±0.86	4341.05* ±1.79	4340.11* ±0.57	3622.78* ±0.15	3333.98* ±1.69	3333.11* ±0.61
Monsoon Jun. Aug. 23±2°C	LI	7891.72 ±1.15	7249.01* ±0.15	6590.33* ±0.66	6588.98* ±3.60	6241.56* ±0.57	5341.05* ±0.35	5341.0* ±0.35
Post mon. Oct. Sept. 24±2°C	LI	7858.71 ±2.70	7067.45* ±2.25	6067.45* ±0.94	6066.98* ±0.06	6194.93* ±0.70	5278.08* ±0.61	5276.19* ±0.24
Winter Nov. Dec. 20±2°C	LI	9711.0 ±1.11	7499.05* ±0.13	7007.49* ±0.06	7002.44* ±0.64	6402.06* ±0.07	5628.78* ±0.17	5624.77* ±0.61

Values represent mean±SD of six fishes. LI=large intestine.
*=P<0.05% level of significance.
**=P<0.01% level of significance.
N=not significant.
STT=short term treatment.
MTT=medium term treatment.
LTT=long term treatment.

Table 41. Seasonal protein concentration in μg 100mg body weight^{-1} in *Oreochromis mossambicus* under the influence of NaCl.

season month temp	tissue	control	10ppt 7day	10ppt 21day	10ppt 30day	20ppt 7day	20ppt 21day	20ppt 30day
			STT	MTT	LTT	STT	MTT	LTT
Post win. Jan. Feb. 27±2°C	Liv	6391.01 ±1.11	5989.19* ±1.80	5341.05* ±1.61	5340.1* ±1.83	5067.45* ±1.21	5037.74* ±1.25	5036.11* ±1.25
Summer Mar. May 33±2° C	Liv	5632.63 ±1.10	5560.7* ±0.10	4459.86* ±0.63	4458.16* ±1.17	4294.88* ±1.71	4294.1* ±1.48	4293.99* ±0.24
Monsoon Jun. Aug. 23±2°C	Liv	7930.65 ±1.00	7303.93* ±1.23	7283.25* ±0.92	7282.99* ±1.83	6488.8* ±0.60	5980.52* ±0.60	5979.99* ±0.33
Post mon. Oct. Sept. 24±2°C	Liv	7930.65 ±0.20	7215.65* ±0.31	7058.13* ±0.60	7056.21* ±0.64	6464.68* ±0.79	5902.86* ±1.92	5900.98* ±0.60
Winter Nov. Dec. 20±2°C	Liv	9998.16 ±2.00	9988.13* ±0.84	9665.07* ±1.83	9664.02* ±1.71	7319.21* ±0.33	6999.6* ±0.02	5989.57* ±0.17

Values represent mean±SD of six fishes. Liv=liver.
*=P<0.05% level of significance.
**=P<0.01% level of significance.
N=not significant.
STT=short term treatment.
MTT=medium term treatment.
LTT=long term treatment.

Table 42. Seasonal ammonia concentration in μg 100mg body weight^{-1} in *Oreochromis mossambicus* under the influence of NaCl.

season	month	temp	tissue	control	10ppt 7day STT	10ppt 21day MTT	10ppt 30day LTT	20ppt 7day STT	20ppt 21day MTT	20ppt 30day LTT
Post winter	Jan. Feb.	27±2°C	GF	13.32 ±1.10	9.69* ±0.90	9.35* ±0.96	9.34* ±0.79	8.50* ±0.94	6.87* ±0.18	6.86* ±0.03
Summer	Mar. May	33±2°C	GF	10.66 ±0.20	12.24** ±1.20	12.02** ±0.60	12.01** ±1.20	10.50* ±0.66	10.32* ±0.76	10.23* ±0.33
Monsoon	Jun. Aug.	23±2°C	GF	26.00 ±1.02	25.13* ±0.28	24.43* ±0.27	24.43* ±1.03	23.83* ±0.47	20.02* ±0.15	20.00* ±0.38
Post Monsoon	Sept. Oct.	24±2°C	GF	25.54 ±0.90	24.89* ±0.26	21.41* ±0.10	21.01* ±0.28	13.25* ±0.42	12.46* ±1.95	12.40* ±0.70
Winter	Nov. Dec.	20±2°C	GF	45.78 ±0.90	36.57* ±0.26	35.86* ±0.10	35.62* ±0.28	32.68* ±1.42	25.05* ±0.95	25.01* ±0.70

Values represent mean±SD of six fishes. GF=gill filament
*=P<0.05% level of significance.
**=P<0.01% level of significance.
N=not significant.
STT=short term treatment.
MTT=medium term treatment.
LTT=long term treatment.

Table 43. Seasonal ammonia concentration in μg 100mg body weight^{-1} in *Oreochromis mossambicus* under the influence of NaCl.

season	month	temp	tissue	control	10ppt 7day	10ppt 21day	10ppt 30day	20ppt 7day	20ppt 21day	20ppt 30day
					STT	MTT	LTT	STT	MTT	LTT
Post winter	Jan. Feb.	27±2°C	LI	59.46 ±0.23	51.45* ±0.17	25.63* ±1.31	25.60* ±0.37	22.46* ±0.25	20.09* ±0.49	20.00* ±0.67
Summer	Mar. May	33±2°C	LI	56.09 ±0.80	10.29* ±0.33	11.22* ±0.22	11.20* ±0.90	10.00* ±0.33	8.55* ±0.03	8.54* ±0.15
Monsoon	Jun. Aug.	23±2°C	LI	94.57 ±0.90	60.31* ±0.73	46.04* ±1.30	46.00* ±0.12	42.25* ±0.46	31.95* ±0.63	31.05* ±0.38
Post Monsoon	Sept. Oct.	24±2°C	LI	64.36 ±1.00	36.81* ±0.39	28.10* ±0.09	28.00* ±0.99	32.71* ±1.21	17.41* ±0.58	17.29* ±0.41
Winter	Nov. Dec.	20±2°C	LI	143.80 ±0.02	138.43** ±0.02	137.80* ±0.02	137.00* ±0.02	127.16* ±0.02	114.84* ±0.02	114.48 N ±0.004

Values represent mean±SD of six fishes. LI=large intestine.
*=P<0.05% level of significance.
**=P<0.01% level of significance.
N=not significant.
STT=short term treatment.
MTT=medium term treatment.
LTT=long term treatment.

Table 44. Seasonal ammonia concentration in μg 100mg body weight^{-1} in *Oreochromis mossambicus* under the influence of NaCl.

season	month	temp	tissue	control	10ppt 7day STT	10ppt 21day MTT	10ppt 30day LTT	20ppt 7day STT	20ppt 21day MTT	20ppt 30day LTT
Post winter	Jan. Feb.	27±2°C	Liv	21.15 ±0.22	17.06* ±0.35	16.45* ±0.33	16.24* ±0.27	12.06* ±0.34	11.98* ±0.41	11.82* ±0.32
Summer	Mar. May	33±2°C	Liv	14.10 ±1.00	11.26* ±0.32	8.77* ±0.04	8.76* ±0.21	9.75* ±0.30	8.00* ±0.04	7.80* ±0.15
Monsoon	Jun. Aug.	23±2°C	Liv	53.31 ±0.90	50.52* ±0.74	49.73* ±0.52	49.70* ±0.73	38.20* ±0.44	32.39* ±0.33	33.09^{N} ±0.49
Post Monsoon	Sept. Oct.	24±2°C	Liv	33.04 ±1.10	28.85* ±0.70	26.43* ±0.12	26.34* ±0.36	20.97* ±0.67	16.86* ±0.38	16.66* ±0.40
Winter	Nov. Dec.	20±2°C	Liv	86.57 ±1.06	73.96* ±0.67	62.18* ±0.55	62.15* ±0.96	53.36* ±0.40	52.98* ±0.43	52.89** ±0.38

Values represent mean±SD of six fishes. Liv=liver
*=P<0.05% level of significance.
**=P<0.01% level of significance.
N=not significant.
STT=short term treatment.
MTT=medium term treatment.
LTT=long term treatment.

Table 45. Seasonal urea concentration in μg 100mg body weight^{-1} in *Oreochromis mossambicus* under the influence of NaCl.

season	month	temp	tissue	control	10ppt 7day STT	10ppt 21day MTT	10ppt 30day LTT	20ppt 7day STT	20ppt 21day MTT	20ppt 30day LTT
Post winter	Jan. Feb.	27±2°C	GF	14.09 ±0.10	12.03* ±0.70	11.12* ±0.47	11.11* ±0.20	7.32* ±0.06	6.78* ±0.02	6.77* ±0.02
Summer	Mar. May	33±2°C	GF	11.40 ±0.12	10.92* ±0.68	10.98^{N} ±0.27	10.98^{N} ±0.21	4.52* ±0.01	4.51* ±0.02	4.50** ±0.08
Monsoon	Jun. Aug.	23±2°C	GF	18.60 ±0.10	17.04* ±0.15	16.79* ±0.11	16.78* ±0.15	11.86* ±0.22	11.22* ±0.27	11.22^{N} ±0.07
Post Monsoon	Sept. Oct.	24±2°C	GF	15.51* ±0.60	13.56* ±0.44	12.05* ±0.12	12.04* ±0.17	8.84* ±0.19	8.41* ±0.21	8.40* ±0.15
Winter	Nov. Dec.	20±2°C	GF	104.0 ±1.11	27.09* ±0.40	22.66* ±0.11	22.65* ±0.07	19.68* ±0.87	17.80* ±0.18	17.70* ±0.04

Values represent mean±SD of six fishes. GF=gill filament
*=P<0.05% level of significance.
**=P<0.01% level of significance.
N=not significant.
STT=short term treatment.
MTT=medium term treatment.
LTT=long term treatment.

Table 46. Seasonal urea concentration in μg 100mg body weight^{-1} in *Oreochromis mossambicus* under the influence of NaCl.

season	month	temp	tissue	control	10ppt 7day STT	10ppt 21day MTT	10ppt 30day LTT	20ppt 7day STT	20ppt 21day MTT	20ppt 30day LTT
Post winter	Jan. Feb.	27±2°C	LI	39.60 ±0.11	26.85* ±0.71	26.37^{N} ±0.09	26.37* ±0.02	13.91* ±0.29	11.08* ±0.06	11.05* ±0.06
Summer	Mar. May	33±2°C	LI	17.29 ±0.10	16.38* ±0.12	13.56* ±0.21	13.55* ±0.12	10.10* ±0.02	7.83* ±0.14	7.83* ±0.12
Monsoon	Jun. Aug.	23±2°C	LI	100.55 ±1.73	42.20* ±0.70	37.87* ±0.26	37.87* ±0.70	32.24* ±0.06	15.61* ±0.25	15.60* ±0.22
Post Monsoon	Sept. Oct.	24±2°C	LI	43.66 ±1.30	38.83* ±0.10	27.28* ±0.16	27.27* ±0.20	22.32* ±0.08	14.76* ±0.05	14.76** ±0.69
Winter	Nov. Dec.	20±2°C	LI	126.63 ±1.60	47.36* ±0.60	39.19* ±0.02	39.18* ±0.40	32.02* ±0.16	28.68* ±0.22	28.67* ±0.12

Values represent mean±SD of six fishes. LI=large intestine.
*=P<0.05% level of significance.
**=P<0.01% level of significance.
N=not significant.
STT=short term treatment.
MTT=medium term treatment.
LTT=long term treatment.

Table 47. Seasonal urea concentration in μg 100mg body weight^{-1} in *Oreochromis mossambicus* under the influence of NaCl.

season	month	temp	tissue	control	10ppt 7day STT	10ppt 21day MTT	10ppt 30day LTT	20ppt 7day STT	20ppt 21day MTT	20ppt 30day LTT
Post winter	Jan. Feb.	27±2°C	Liv	24.64 ±0.10	18.25* ±0.20	14.64* ±0.29	14.36* ±0.19	12.99* ±0.50	8.30* ±0.02	8.28* ±0.03
Summer	Mar. May	33±2°C	Liv	15.28 ±0.01	14.90* ±0.08	13.38* ±0.60	13.37* ±0.04	8.36* ±0.10	5.02* ±0.02	4.50* ±0.08
Monsoon	Jun. Aug.	23±2°C	Liv	28.70 ±0.12	27.64* ±0.03	21.45* ±0.41	21.44* ±0.12	19.87* ±0.06	12.15* ±0.30	12.14** ±0.19
Post Monsoon	Sept. Oct.	24±2°C	Liv	28.08 ±0.11	22.83* ±0.30	19.71* ±0.20	19.70* ±0.07	17.42* ±0.40	10.14* ±0.04	10.14* ±0.03
Winter	Nov. Dec.	20±2°C	Liv	108.55 ±1.11	28.62* ±0.01	28.11* ±0.02	28.10* ±0.01	22.23* ±0.03	21.80* ±0.06	21.80* ±0.01

Values represent mean±SD of six fishes. Liv =liver.
*=P<0.05% level of significance.
**=P<0.01% level of significance.
N=not significant.
STT=short term treatment.
MTT=medium term treatment.
LTT=long term treatment.

Table 48. Seasonal cholesterol concentration in μM 100mg tissue weight^{-1} in gill filament of *Oreochromis mossambicus* under the influence of NaCl.

season	month	temp	control	10ppt 7day STT	10ppt 21day MTT	10ppt 30day LTT	20ppt 7day STT	20ppt 21day MTT	20ppt 30day LTT
Post winter	Jan. Feb.	27±2°C	3.185 ±0.07	0.21* ±0.02	0.15* ±0.02	0.15* ±0.03	0.26* ±0.02	0.303* ±0.01	0.30* ±0.02
Summer	Mar. May	33±2°C	0.12 ±0.00	0.10* ±0.01	0.09* ±0.01	0.09* ±0.02	0.105 N ±0.01	0.075* ±0.01	0.075** ±0.00
Monsoon	Jun. Aug.	23±2°C	6.465 ±0.10	5.80* ±0.42	5.38* ±0.02	5.375* ±0.02	5.44* ±0.06	0.495 N ±0.01	0.495* ±0.00
Post Monsoon	Sept. Oct.	24±2°C	0.13 ±0.01	0.11* ±0.00	0.10* ±0.01	0.095* ±0.01	0.10* ±0.00	0.085* ±0.02	0.08* ±0.01
Winter	Nov. Dec.	20±2°C	9.16 ±0.21	8.53* ±0.20	8.175* ±0.81	8.00* ±0.12	7.665* ±0.91	5.53** ±0.44	5.525* ±0.24

Values represent mean±SD of six fishes.
*=P<0.05% level of significance.
**=P<0.01% level of significance.
N=not significant.
STT=short term treatment.
MTT=medium term treatment.
LTT=long term treatment.

Table 49. Seasonal cholesterol concentration in μM 100mg tissue weight^{-1} in large intestine of *Oreochromis mossambicus* under the influence of NaCl.

season	month	temp	control	10ppt 7day STT	10ppt 21day MTT	10ppt 30day LTT	20ppt 7day STT	20ppt 21day MTT	20ppt 30day LTT
Post winter	Jan. Feb.	27±2°C	6.465 ±0.10	5.505* ±0.08	5.065* ±0.36	5.065* ±0.40	5.33** ±0.72	2.31* ±0.04	2.305* ±0.02
Summer	Mar. May	33±2°C	0.265 ±0.01	0.135* ±0.01	0.125* ±0.03	0.125* ±0.00	0.115* ±0.01	0.085* ±0.04	0.085^{N} ±0.01
Monsoon	Jun. Aug.	23±2°C	7.835 ±0.11	6.065* ±0.20	5.865* ±0.09	5.86* ±0.05	6.00* ±0.18	5.3* ±0.02	5.3** ±0.01
Post Monsoon	Sept. Oct.	24±2°C	0.275 ±0.02	0.14* ±0.01	0.135* ±0.01	0.13* ±0.01	0.12* ±0.00	0.10* ±0.01	0.095* ±0.00
Winter	Nov. Dec.	20±2°C	11.085 ±0.39	9.85* ±0.20	8.75* ±0.10	8.75* ±0.00	8.00* ±0.15	6.60* ±0.20	6.60* ±0.10

Values represent mean±SD of six fishes.
*=P<0.05% level of significance.
**=P<0.01% level of significance.
N=not significant.
STT=short term treatment.
MTT=medium term treatment.
LTT=long term treatment.

Table 50. Seasonal cholesterol concentration in μM 100mg tissue weight^{-1} in liver of *Oreochromis mossambicus* under the influence of NaCl.

season	month	temp	control	10ppt 7day STT	10ppt 21day MTT	10ppt 30day LTT	20ppt 7day STT	20ppt 21day MTT	20ppt 30day LTT
Post winter	Jan. Feb.	27±2°C	11.69 ±1.00	9.50* ±1.10	8.03* ±0.40	8.00* ±0.10	5.80* ±0.30	5.195* ±0.10	5.15* ±0.10
Summer	Mar. May	33±2°C	0.385 ±0.01	0.295* ±0.01	0.275* ±0.01	0.275* ±0.00	0.215* ±0.01	0.09* ±0.01	0.09^{N} ±0.01
Monsoon	Jun. Aug.	23±2°C	12.03 ±0.10	10.655* ±0.07	10.31* ±0.09	10.305* ±0.05	6.99* ±0.08	5.70* ±0.05	5.65* ±0.02
Post Monsoon	Sept. Oct.	24±2°C	0.75 ±0.01	0.39* ±0.01	0.33* ±0.01	0.325* ±0.01	0.295* ±0.01	0.245* ±0.01	0.24* ±0.00
Winter	Nov. Dec.	20±2°C	13.28 ±1.10	12.70* ±0.12	12.23* ±0.49	12.25** ±0.10	8.43* ±0.03	8.065* ±0.01	8.06** ±0.01

Values represent mean±SD of six fishes.
*=P<0.05% level of significance.
**=P<0.01% level of significance.
N=not significant.
STT=short term treatment.
MTT=medium term treatment.
LTT=long term treatment.

Table 51. Seasonal triglyceride concentration in μM 100mg tissue weight^{-1} in gill filament of *Oreochromis mossambicus* under the influence of NaCl.

season	month	temp	control	10ppt 7day STT	10ppt 21day MTT	10ppt 30day LTT	20ppt 7day STT	20ppt 21day MTT	20ppt 30day LTT
Post winter	Jan. Feb.	27±2°C	0.995 ±0.01	0.585* ±0.01	0.565* ±0.02	0.56* ±0.01	0.55* ±0.01	0.43* ±0.02	0.43* ±0.01
Summer	Mar. May	33±2°C	0.535 ±0.02	0.48* ±0.01	0.38* ±0.01	0.38* ±0.00	0.365* ±0.02	0.36* ±0.01	0.35* ±0.01
Monsoon	Jun. Aug.	23±2°C	3.385 ±1.10	0.635* ±0.02	0.65* ±0.01	0.675* ±0.01	0.57* ±0.02	0.495* ±0.01	0.495 N ±0.00
Post Monsoon	Sept. Oct.	24±2°C	0.545 ±0.03	0.495* ±0.01	0.395* ±0.02	0.39** ±0.01	0.35* ±0.02	0.375* ±0.02	0.365* ±0.01
Winter	Nov. Dec.	20±2°C	6.76 ±0.05	3.17* ±0.03	1.785* ±0.02	1.775* ±0.01	1.725* ±0.01	1.34* ±0.02	1.3* ±0.01

Values represent mean±SD of six fishes.
*=P<0.05% level of significance.
**=P<0.01% level of significance.
N=not significant.
STT=short term treatment.
MTT=medium term treatment.
LTT=long term treatment.

Table 52. Seasonal triglyceride concentration in μM 100mg tissue weight^{-1} in large intestine of *Oreochromis mossambicus* under the influence of NaCl.

season	month	temp	control	10ppt 7day STT	10ppt 21day MTT	10ppt 30day LTT	20ppt 7day STT	20ppt 21day MTT	20ppt 30day LTT
Post winter	Jan. Feb.	27±2°C	2.245 ±0.11	.75* ±0.10	1.115* ±0.10	1.11* ±0.09	1.055* ±0.09	0.63* ±0.09	0.625* ±0.01
Summer	Mar. May	33±2°C	0.795 ±0.02	0.695* ±0.01	0.50* ±0.01	0.50* ±0.00	0.49* ±0.00	0.475* ±0.01	0.45* ±0.02
Monsoon	Jun. Aug.	23±2°C	5.575 ±0.03	2.315* ±0.01	2.30^{N} ±0.01	2.30* ±0.00	1.84* ±0.01	1.82* ±0.01	1.81* ±0.01
Post Monsoon	Sept. Oct.	24±2°C	0.81 ±0.03	0.725* ±0.01	0.525* ±0.02	0.52** ±0.01	0.4955* ±0.01	0.485* ±0.01	0.46* ±0.01
Winter	Nov. Dec.	20±2°C	7.205 ±0.05	4.745** ±0.03	2.455* ±0.02	2.45* ±0.01	2.185* ±0.03	1.53* ±0.02	1.50* ±0.01

Values represent mean±SD of six fishes.
*=P<0.05% level of significance.
**=P<0.01% level of significance.
N=not significant.
STT=short term treatment.
MTT=medium term treatment.
LTT=long term treatment.

Table 53. Seasonal triglyceride concentration in μM 100mg tissue weight^{-1} in liver of *Oreochromis mossambicus* under the influence of NaCl.

season	month	temp	control	10ppt 7day STT	10ppt 21day MTT	10ppt 30day LTT	20ppt 7day STT	20ppt 21day MTT	20ppt 30day LTT
Post winter	Jan. Feb.	27±2°C	3.295 ±0.11	2.61* ±0.10	2.105* ±0.10	1.975* ±0.08	1.975^{N} ±0.05	1.165* ±0.01	1.16* ±0.01
Summer	Mar. May	33±2°C	2.31 ±0.33	1.575* ±0.02	1.335* ±0.10	1.34^{N} ±0.01	0.705* ±0.03	0.65* ±0.01	0.65* ±0.00
Monsoon	Jun. Aug.	23±2°C	5.075 ±1.00	2.975* ±0.02	2.895* ±0.01	2.89* ±0.01	2.28* ±0.02	2.175* ±0.01	2.16* ±0.01
Post Monsoon	Sept. Oct.	24±2°C	2.35 ±0.11	1.77** ±0.01	1.485* ±0.02	1.48* ±0.01	0.725* ±0.03	0.69* ±0.01	0.665* ±0.02
Winter	Nov. Dec.	20±2°C	9.345 ±0.02	6.00* ±0.02	4.29* ±0.02	4.275* ±0.02	3.695* ±0.02	1.81* ±0.02	1.805* ±0.004

Values represent mean±SD of six fishes.
*=P<0.05% level of significance.
**=P<0.01% level of significance.
N=not significant.
STT=short term treatment.
MTT=medium term treatment.
LTT=long term treatment.

Table 54. Seasonal phospholipid concentration in μM 100mg tissue weight^{-1} in gill filament of *Oreochromis mossambicus* under the influence of NaCl.

season	month	temp	control	10ppt 7day STT	10ppt 21day MTT	10ppt 30day LTT	20ppt 7day STT	20ppt 21day MTT	20ppt 30day LTT
Post winter	Jan. Feb.	27±2°C	0.705 ±0.01	0.61* ±0.03	0.545* ±0.04	0.545 N ±0.05	0.49* ±0.01	0.475* ±0.01	0.45* ±0.01
Summer	Mar. May	33±2°C	0.28 ±0.01	0.275* ±0.01	0.27* ±0.01	0.27* ±0.01	0.26* ±0.01	0.25* ±0.01	0.25 N ±0.01
Monsoon	Jun. Aug.	23±2°C	0.31 ±0.02	0.92* ±0.01	0.885* ±0.03	0.885 N ±0.01	0.75* ±0.01	0.58* ±0.01	0.55* ±0.00
Post Monsoon	Sept. Oct.	24±2°C	0.31 ±0.01	0.30* ±0.01	0.285* ±0.02	0.285** ±0.01	0.275* ±0.01	0.27* ±0.00	0.265* ±0.01
Winter	Nov. Dec.	20±2°C	1.095 ±0.01	1.055* ±0.03	0.885** ±0.02	0.88* ±0.01	0.815* ±0.01	0.79* ±0.01	0.79* ±0.00

Values represent mean±SD of six fishes.
*=P<0.05% level of significance.
**=P<0.01% level of significance.
N=not significant.
STT=short term treatment.
MTT=medium term treatment.
LTT=long term treatment.

Table 55. Seasonal phospholipid concentration in μM 100mg tissue weight^{-1} in large intestine of *Oreochromis mossambicus* under the influence of NaCl.

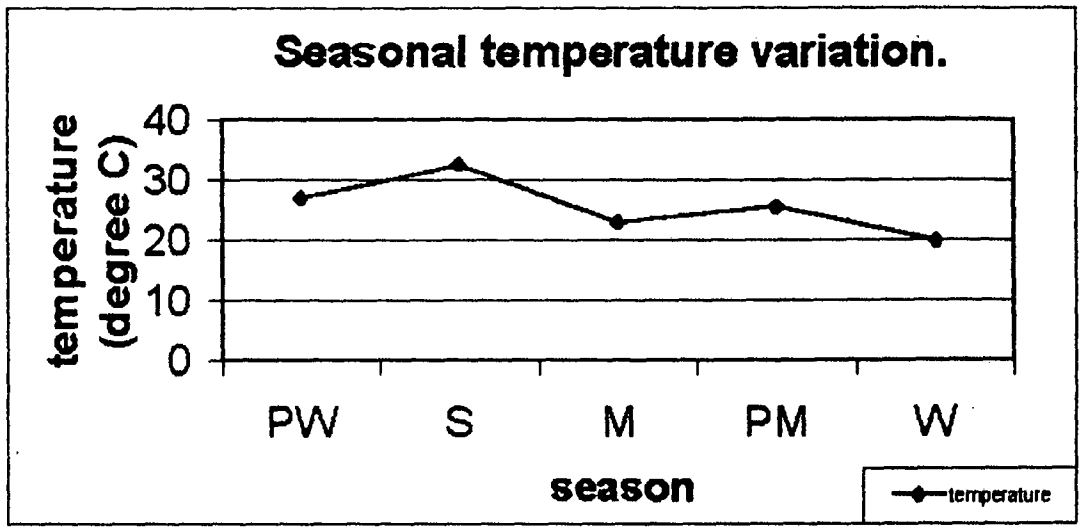
season	month	temp	control	10ppt 7day STT	10ppt 21day MTT	10ppt 30day LTT	20ppt 7day STT	20ppt 21day MTT	20ppt 30day LTT
Post winter	Jan. Feb.	27±2°C	0.855 ±0.03	0.46* ±0.01	0.455* ±0.01	0.45* ±0.01	0.435* ±0.01	0.425* ±0.01	0.415* ±0.01
Summer	Mar. May	33±2°C	0.325 ±0.01	0.28* ±0.01	0.275* ±0.01	0.27* ±0.01	0.265* ±0.01	0.255* ±0.01	0.255** ±0.01
Monsoon	Jun. Aug.	23±2°C	1.005 ±0.04	0.715* ±0.01	0.71* ±0.01	0.70* ±0.01	0.51* ±0.02	0.46* ±0.02	0.46 N ±0.01
Post Monsoon	Sept. Oct.	24±2°C	0.36 ±0.00	0.355* ±0.01	0.355* ±0.00	0.35* ±0.00	0.34* ±0.01	0.325* ±0.01	0.31* ±0.01
Winter	Nov. Dec.	20±2°C	1.28 ±0.11	1.07* ±0.02	1.055* ±0.01	1.05* ±0.01	0.97* ±0.02	0.715** ±0.02	0.725* ±0.01

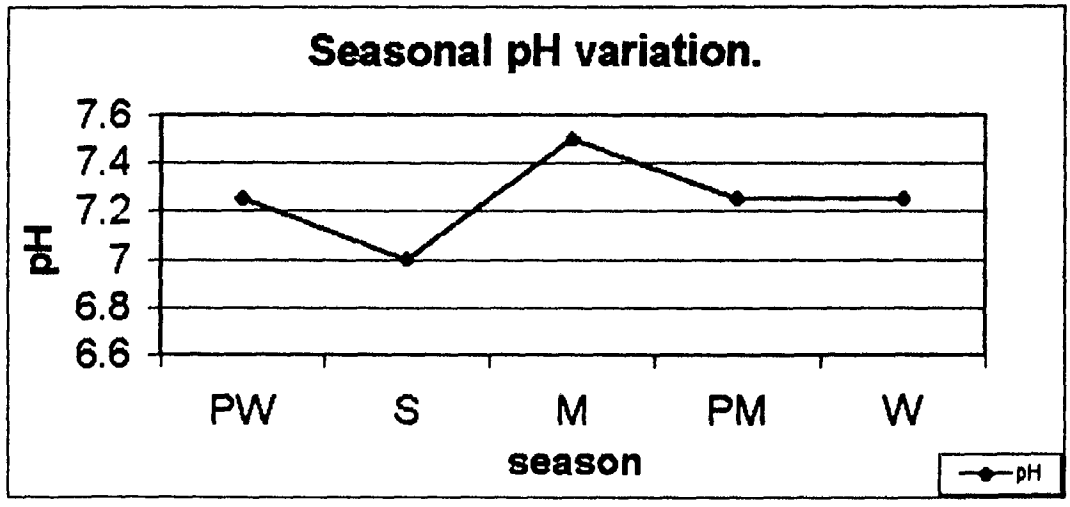
Values represent mean±SD of six fishes.
*=P<0.05% level of significance.
**=P<0.01% level of significance.
N=not significant.
STT=short term treatment.
MTT=medium term treatment.
LTT=long term treatment.

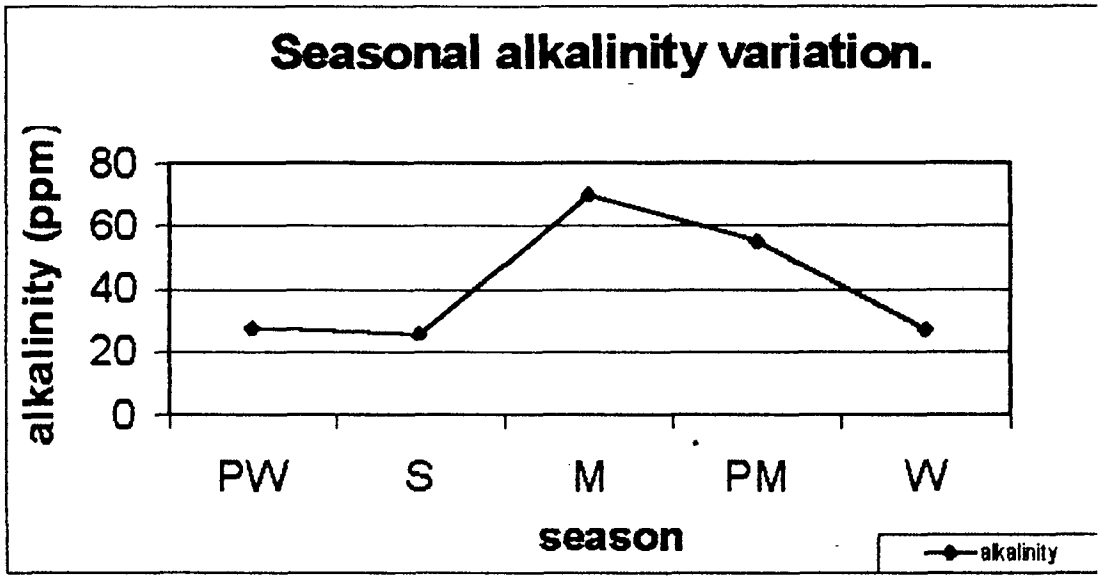
Table 56. Seasonal phospholipid concentration in μM 100mg tissue weight^{-1} in liver of *Oreochromis mossambicus* under the influence of NaCl.

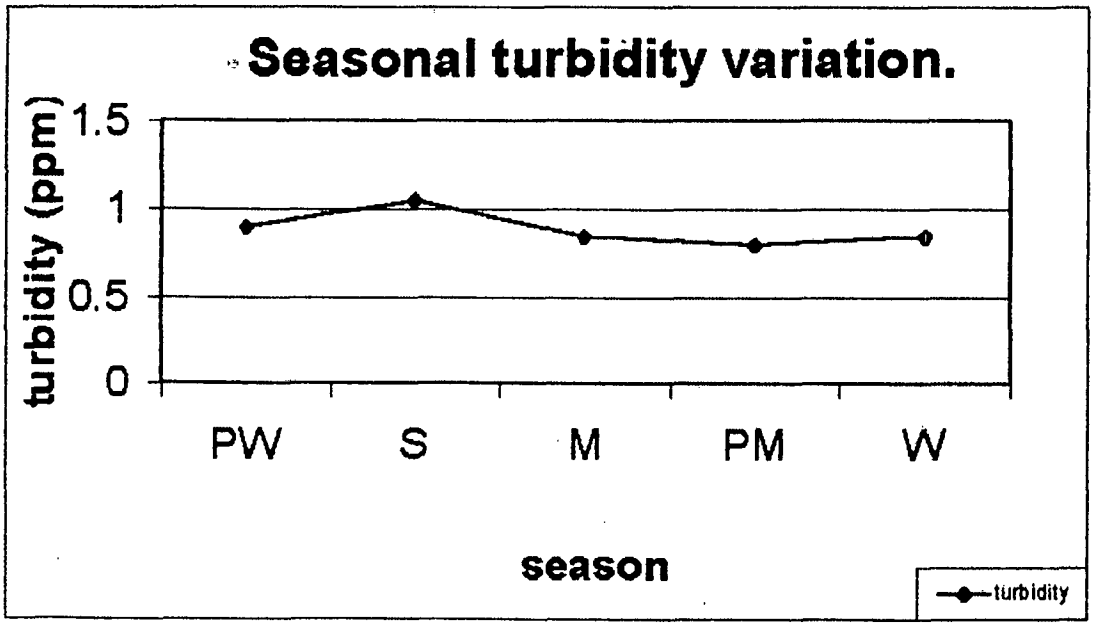
season	month	temp	control	10ppt 7day STT	10ppt 21day MTT	10ppt 30day LTT	20ppt 7day STT	20ppt 21day MTT	20ppt 30day LTT
Post winter	Jan. Feb.	27±2°C	1.265 ±0.03	0.995* ±0.02	0.695* ±0.01	0.69* ±0.01	0.66* ±0.02	0.63* ±0.02	0.55* ±0.01
Summer	Mar. May	33±2°C	1.125 ±0.10	0.48^{N} ±0.01	0.31* ±0.01	0.30* ±0.01	0.30^{N} ±0.01	0.275* ±0.02	0.27* ±0.01
Monsoon	Jun. Aug.	23±2°C	1.395 ±0.04	1.055* ±0.02	0.715* ±0.01	0.71* ±0.01	0.68* ±0.01	0.565* ±0.01	0.56* ±0.01
Post Monsoon	Sept. Oct.	24±2°C	1.14 ±0.09	0.445* ±0.01	0.30* ±0.01	0.29* ±0.02	0.285* ±0.02	0.27** ±0.01	0.265* ±0.01
Winter	Nov. Dec.	20±2°C	1.59 ±0.03	1.56* ±0.02	1.555* ±0.01	1.55* ±0.01	1.52* ±0.02	1.39* ±0.02	1.375* ±0.01

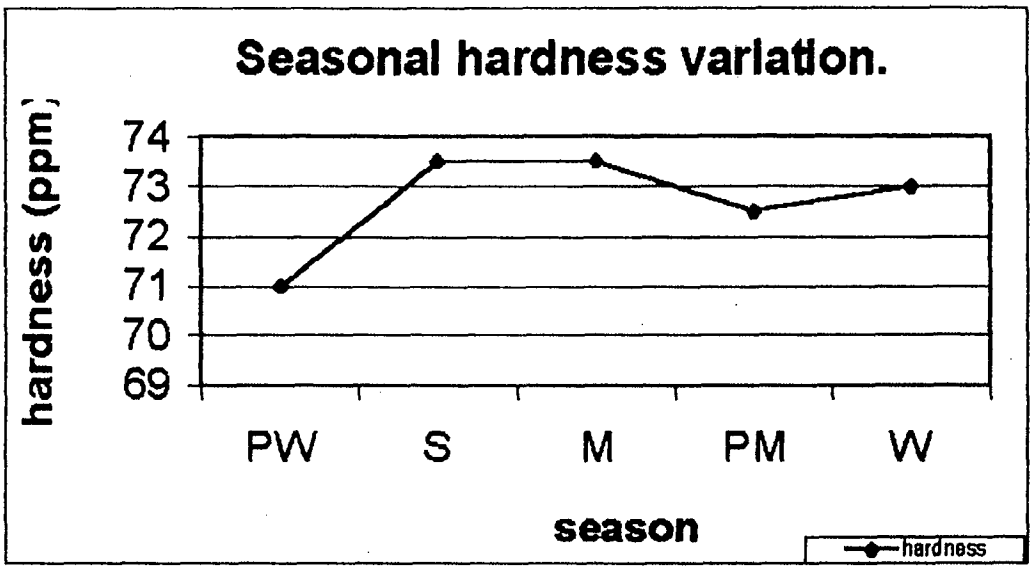
Values represent mean±SD of six fishes.
*=P<0.05% level of significance.
**=P<0.01% level of significance.
N=not significant.
STT=short term treatment.
MTT=medium term treatment.
LTT=long term treatment.

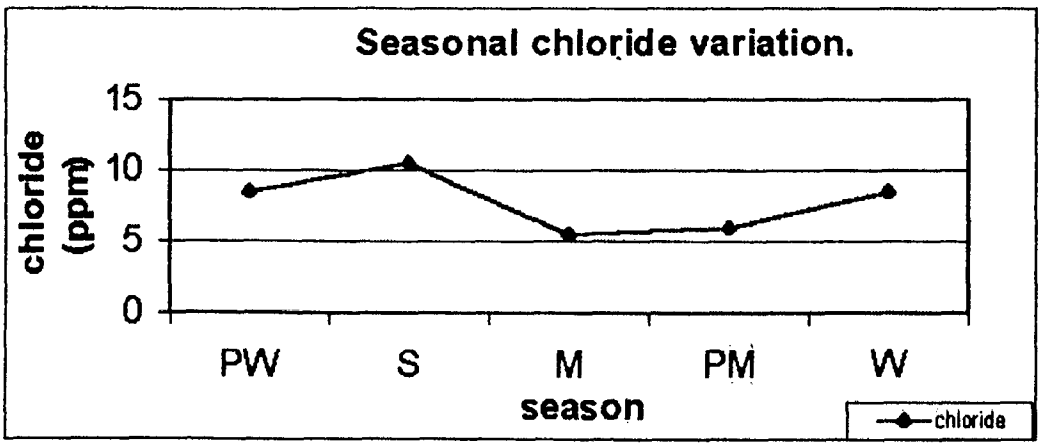

Seasonal temperature variation.
temperature (degree C)
40
30
20
10
0
PW
S
M
PM
W
season
temperature

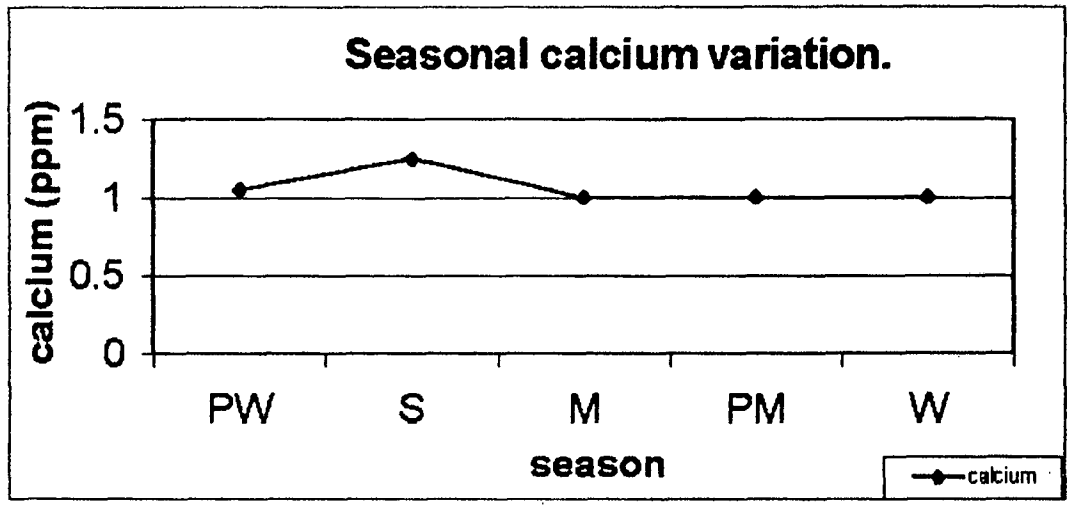

Seasonal pH variation.
pH
7.6
7.4
7.2
7
6.8
6.6
PW
S
M
PM
W
season
pH

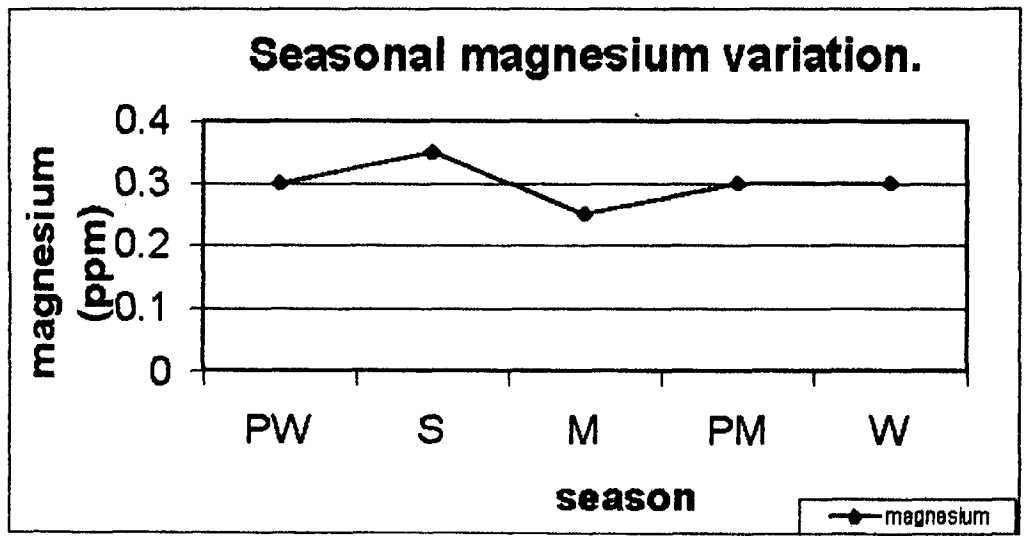

Seasonal alkalinity variation.
alkalinity (ppm)
80
60
40
20
0
PW
S
M
PM
W
season
alkalinity


Seasonal turbidity variation.
turbidity (ppm)
1.5
1
0.5
0
PW
S
M
PM
W
season
turbidity


Seasonal hardness variation.
hardness (ppm)
74
73
72
71
70
69
PW
S
M
PM
W
season
hardness


Seasonal chloride variation.
chloride (ppm)
15
10
5
0
PW
S
M
PM
W
season
chloride


Seasonal calcium variation.
calcium (ppm)
1.5
1
0.5
0
PW
S
M
PM
W
season
calcium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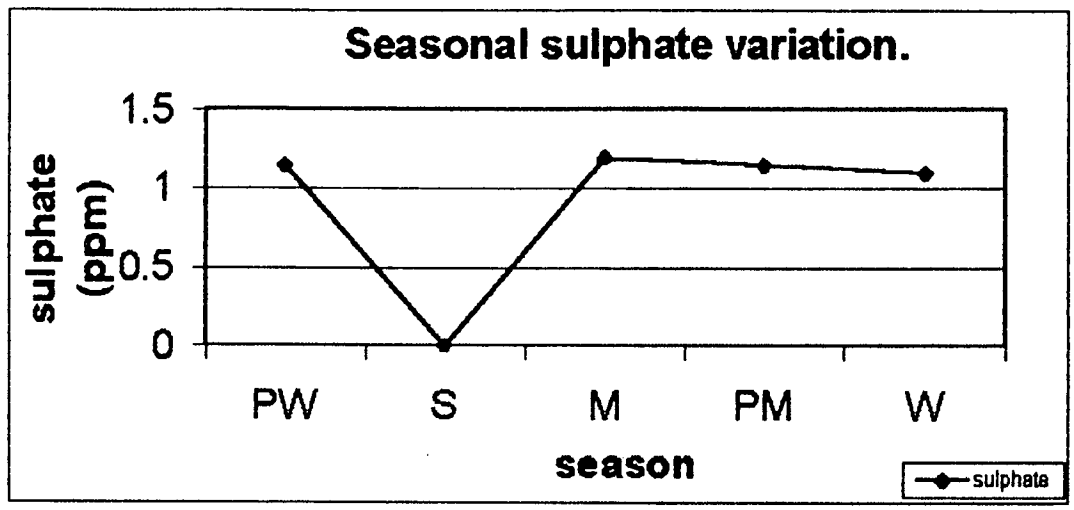
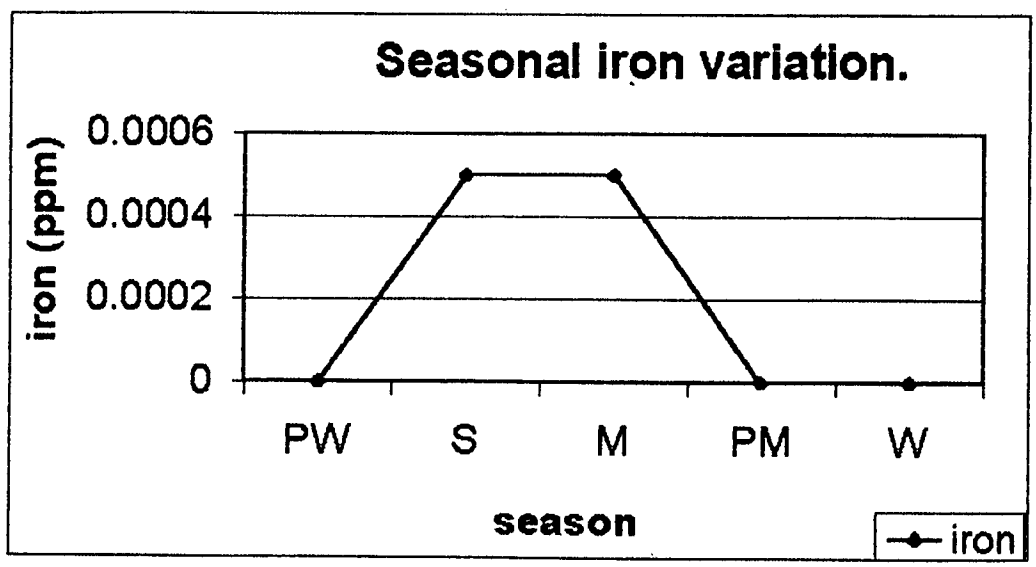
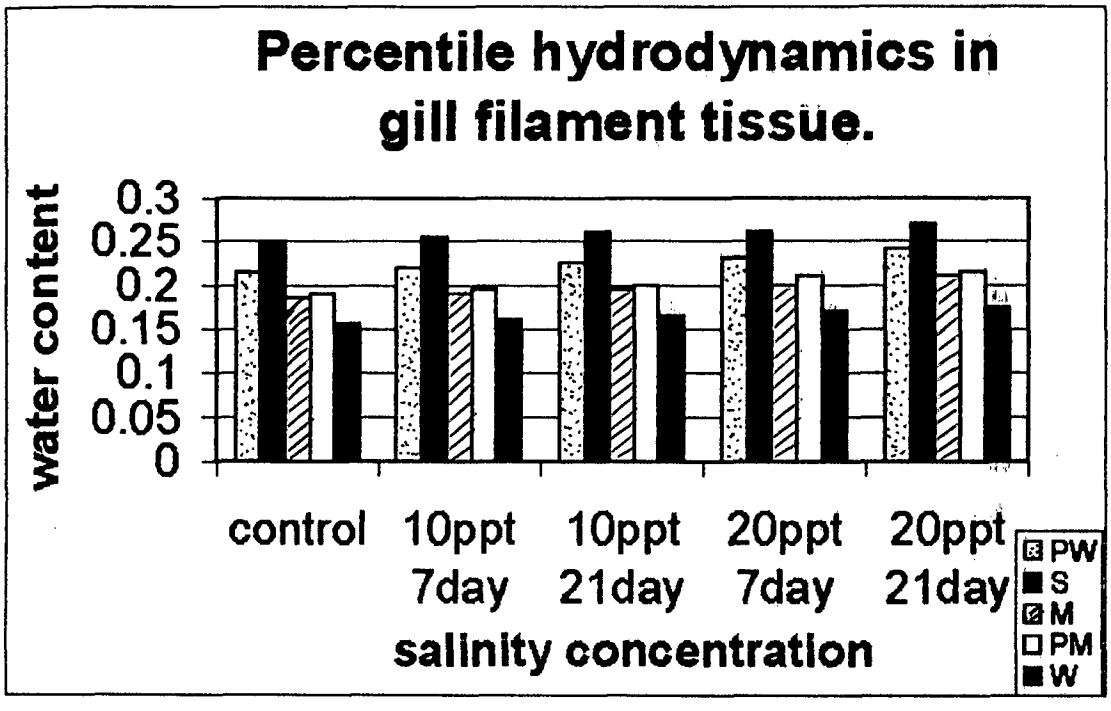
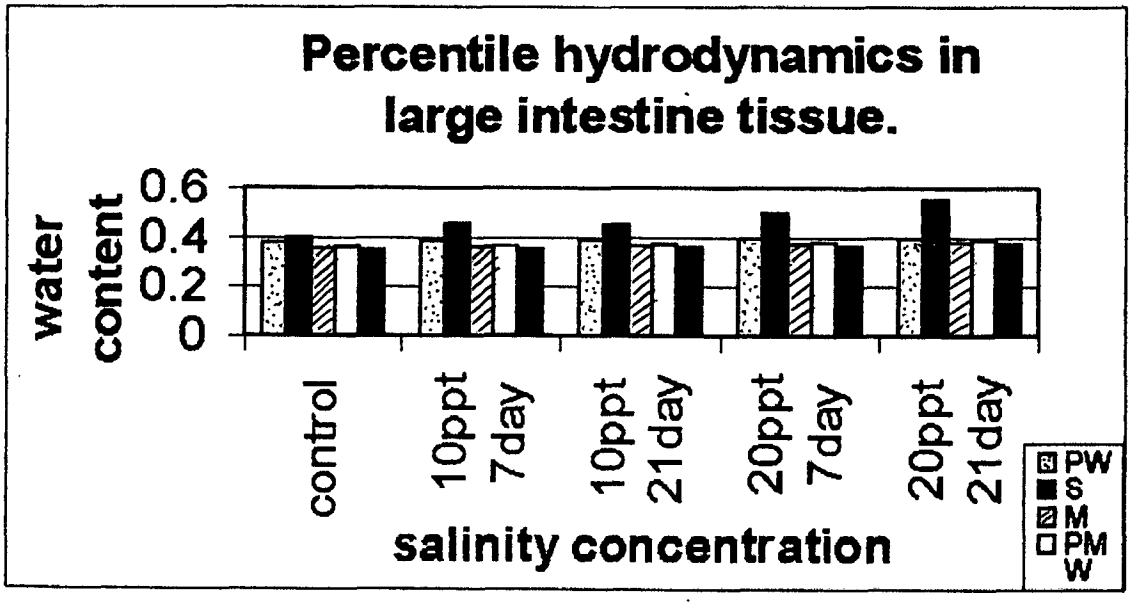
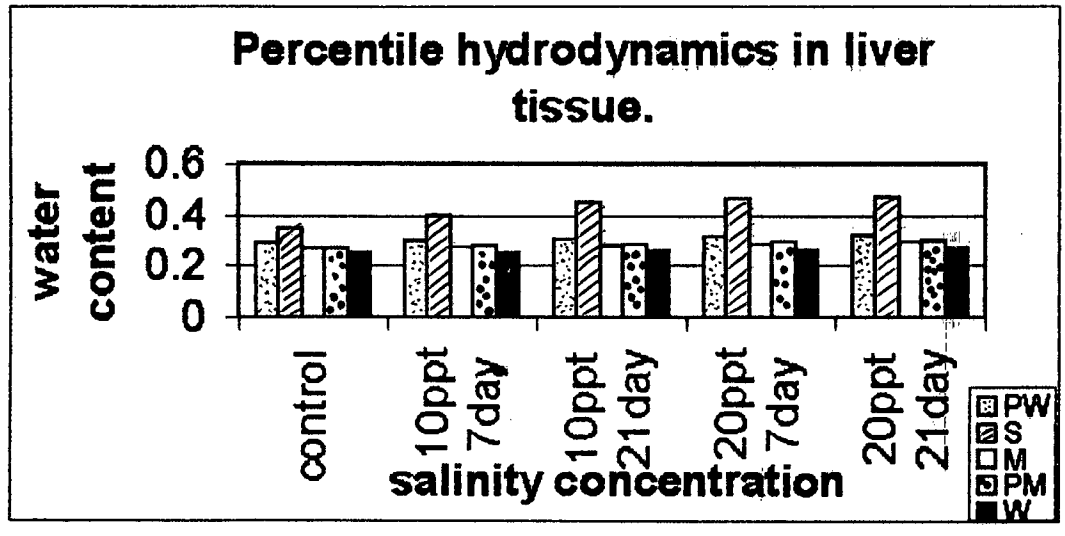
0.3
0.2
0.1
0
PW
S
M
PM
W
season
magnesium

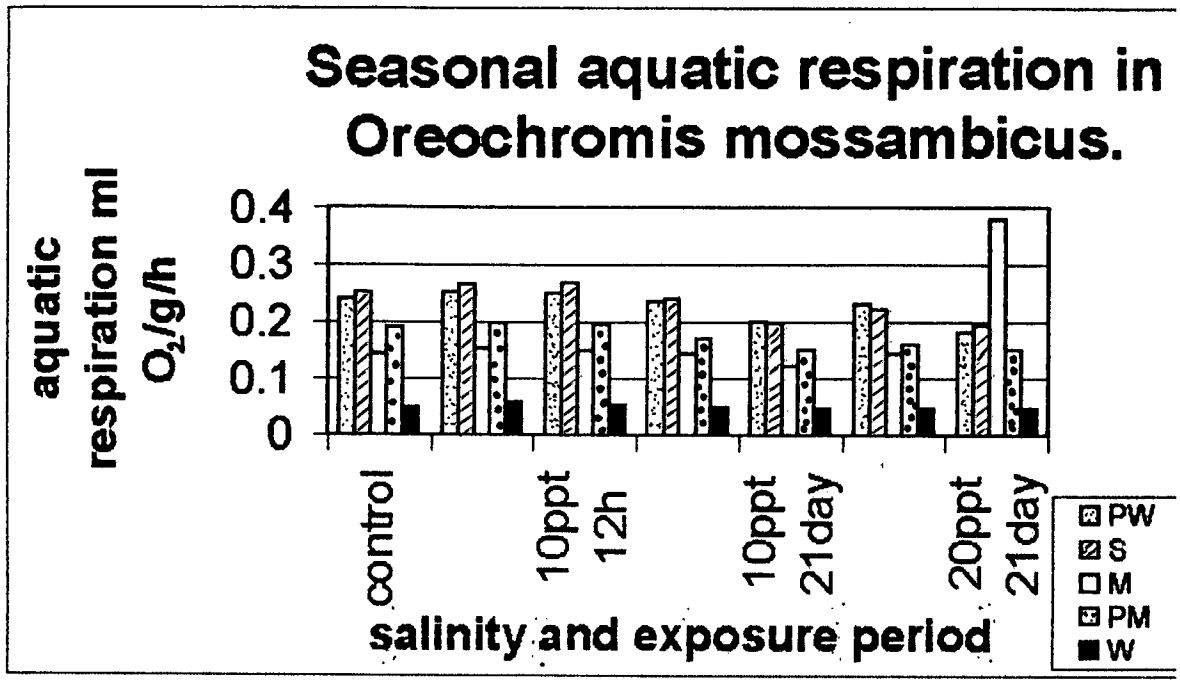

Seasonal sulphate variation.
sulphate (ppm)
1.5
1
0.5
0
PW
S
M
PM
W
season
sulphate

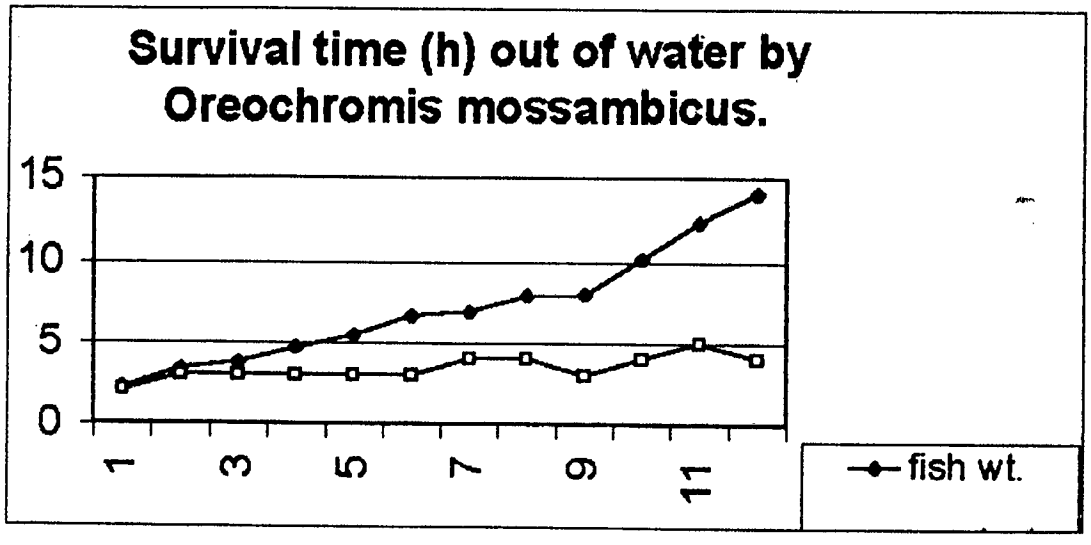

Seasonal iron variation.
iron (ppm)
0.0006
0.0004
0.0002
0
PW
S
M
PM
W
season
iron

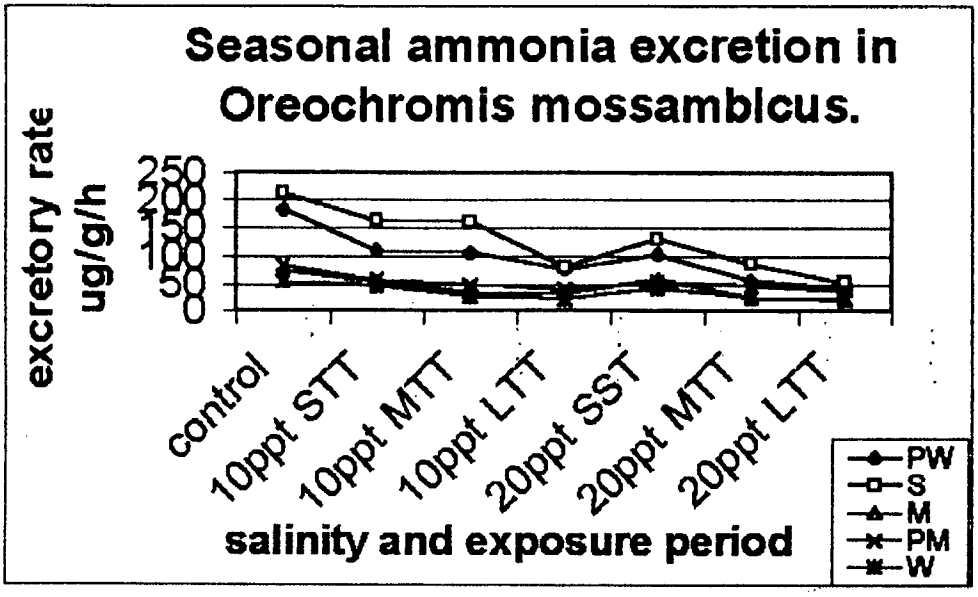

Percentile hydrodynamics in gill filament tissue.
water content
0.3
0.25
0.2
0.15
0.1
0.05
0
control
10ppt 7day
10ppt 21day
20ppt 7day
20ppt 21day
salinity concentration
PW
S
M
PM
W

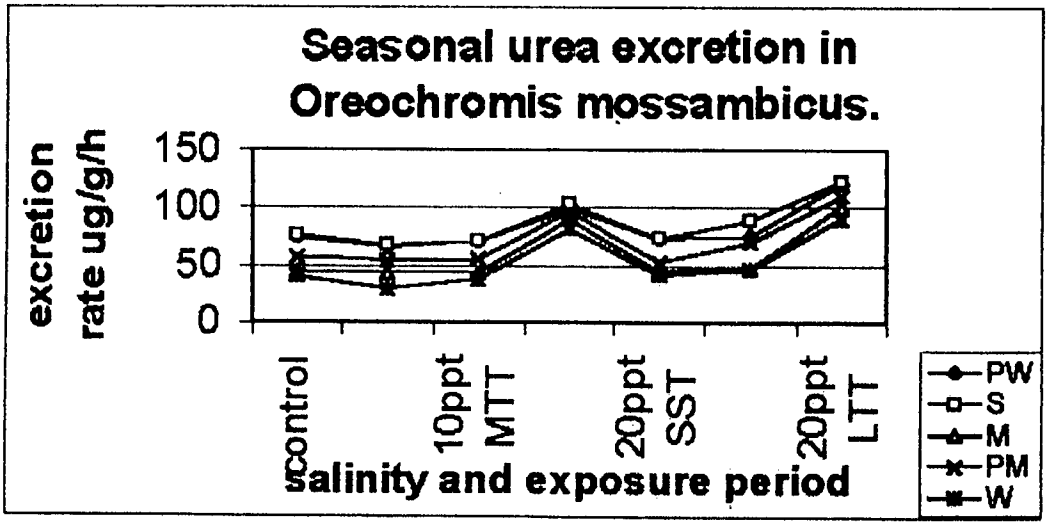

Percentile hydrodynamics in large intestine tissue.
water content
0.6
0.4
0.2
0
control
10ppt 7day
10ppt 21day
20ppt 7day
20ppt 21day
salinity concentration
PW
S
M
PM
W

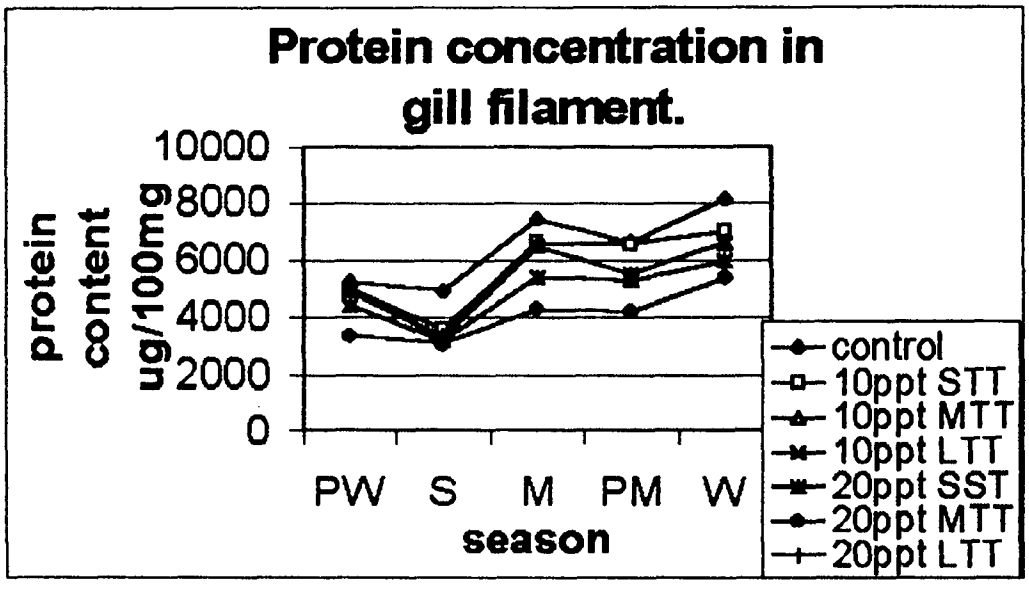

Percentile hydrodynamics in liver tissue.
water content
0.6
0.4
0.2
0
control
10ppt 7day
10ppt 21day
20ppt 7day
20ppt 21day
salinity concentration
PW
S
M
PM
W

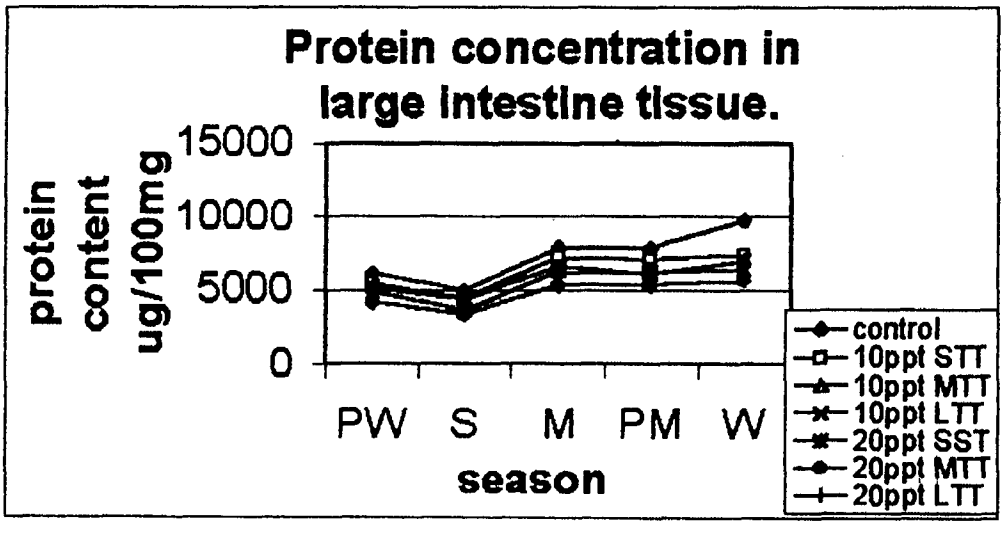

Seasonal aquatic respiration in Oreochromis mossambicus.
aquatic respiration ml O_2/g/h
0.4
0.3
0.2
0.1
0
control
10ppt 12h
10ppt 21day
20ppt 21day
salinity and exposure period
PW
S
M
PM
W

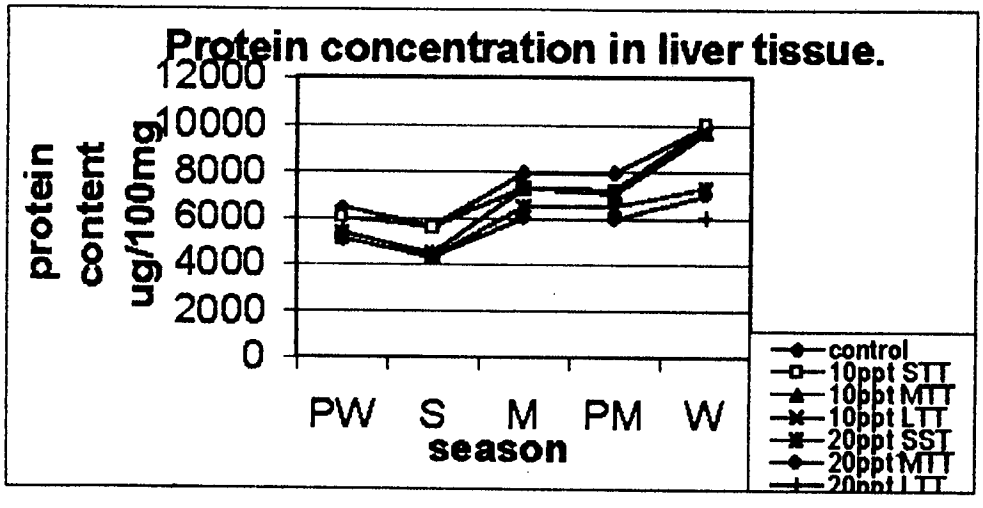

Survival time (h) out of water by Oreochromis mossambicus.
15
10
5
0
1
3
5
7
9
11
fish wt.

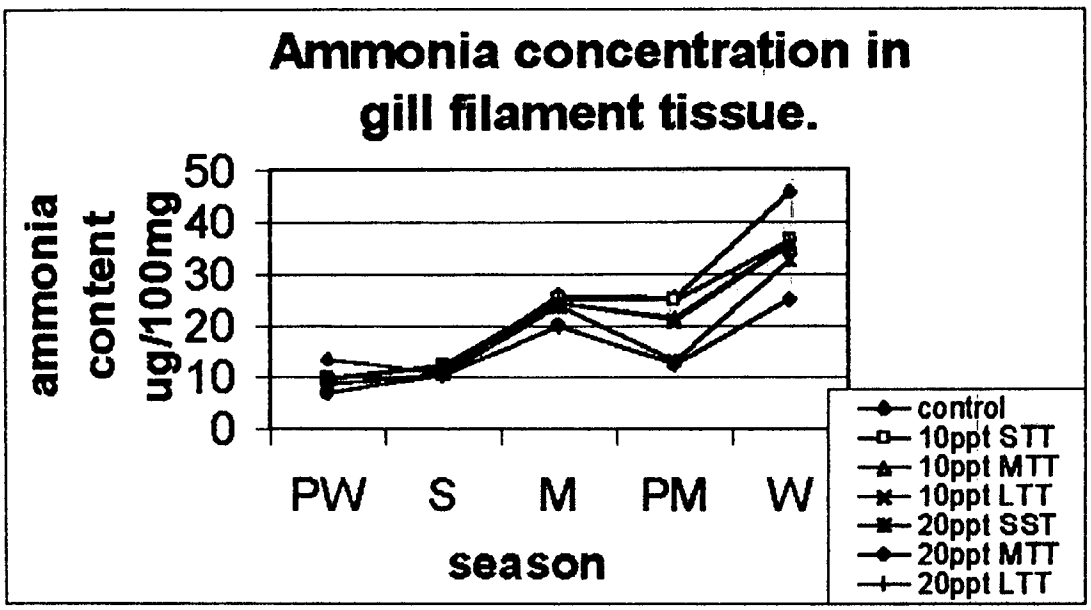

Seasonal ammonia excretion in Oreochromis mossambicus.
excretory rate ug/g/h
250
200
150
100
50
0
control
10ppt STT
10ppt MTT
10ppt LTT
20ppt SST
20ppt MTT
20ppt LTT
salinity and exposure period
PW
S
M
PM
W

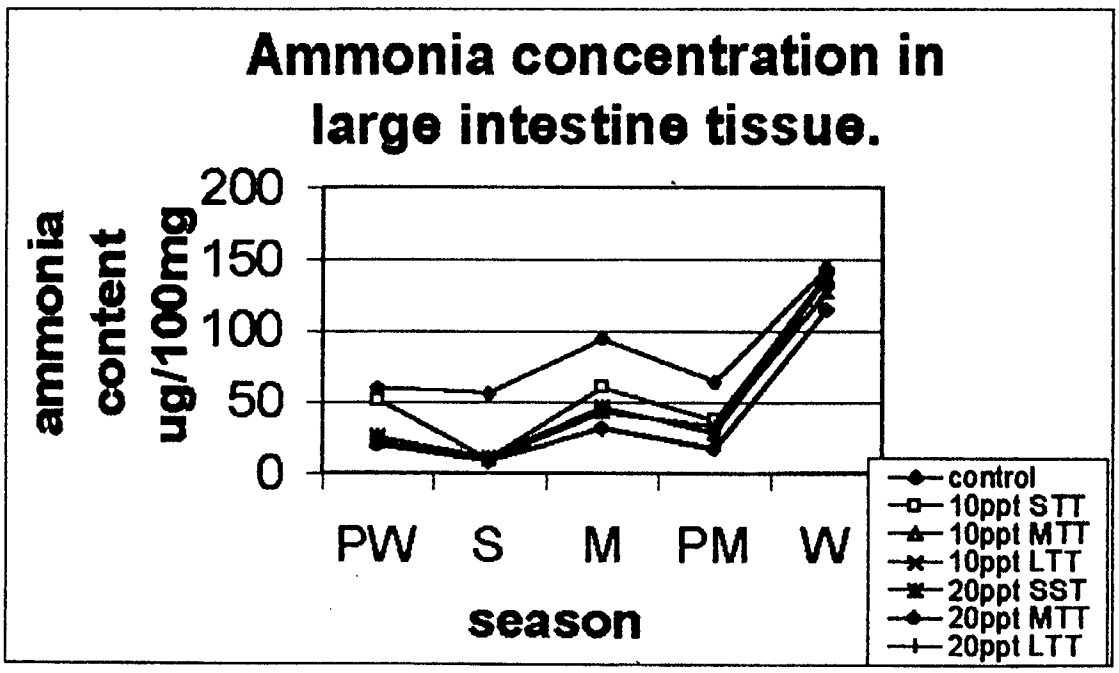

Seasonal urea excretion in Oreochromis mossambicus.
excretion rate ug/g/h
150
100
50
0
control
10ppt MTT
20ppt SST
20ppt LTT
salinity and exposure period
PW
S
M
PM
W

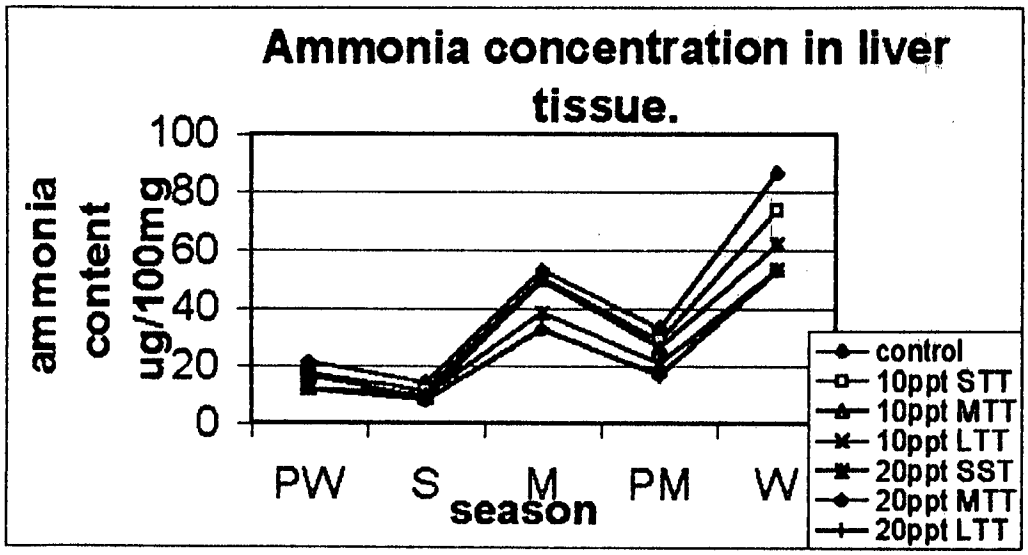

Protein concentration in gill filament.
protein content ug/100mg
10000
8000
6000
4000
2000
0
PW
S
M
PM
W
season
control
10ppt STT
10ppt MTT
10ppt LTT
20ppt SST
20ppt MTT
20ppt LTT

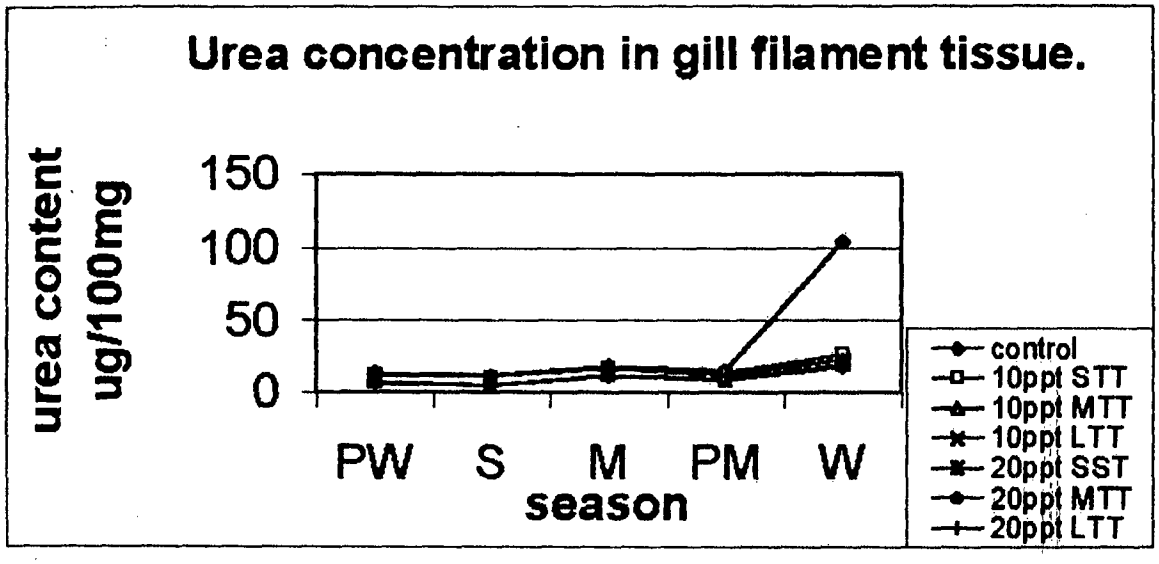

Protein concentration in large intestine tissue.
protein content ug/100mg
15000
10000
5000
0
PW
S
M
PM
W
season
control
10ppt STT
10ppt MTT
10ppt LTT
20ppt SST
20ppt MTT
20ppt LTT

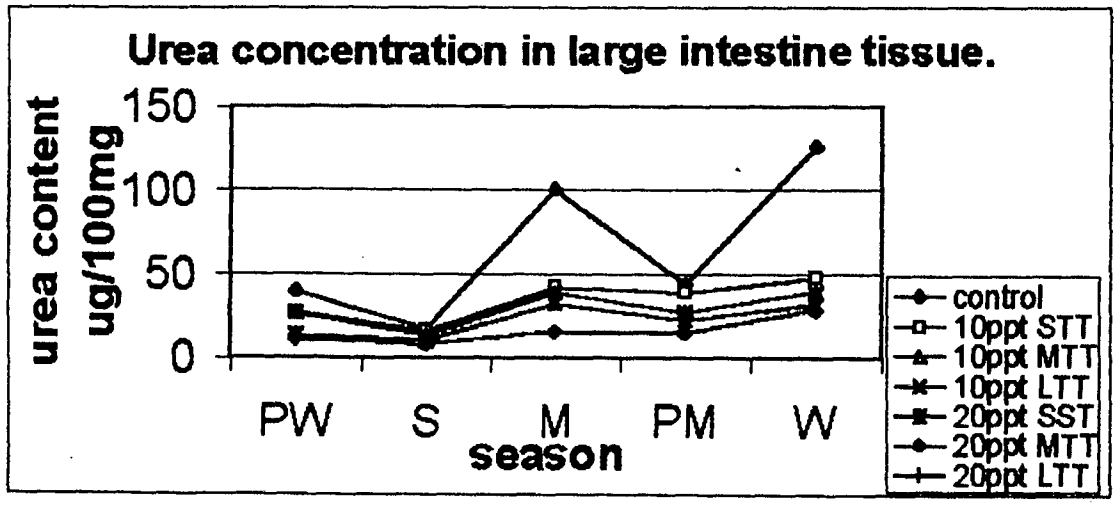

Protein concentration in liver tissue.
protein content ug/100mg
12000
10000
8000
6000
4000
2000
0
PW
S
M
PM
W
season
control
10ppt STT
10ppt MTT
10ppt LTT
20ppt SST
20ppt MTT
20ppt LTT

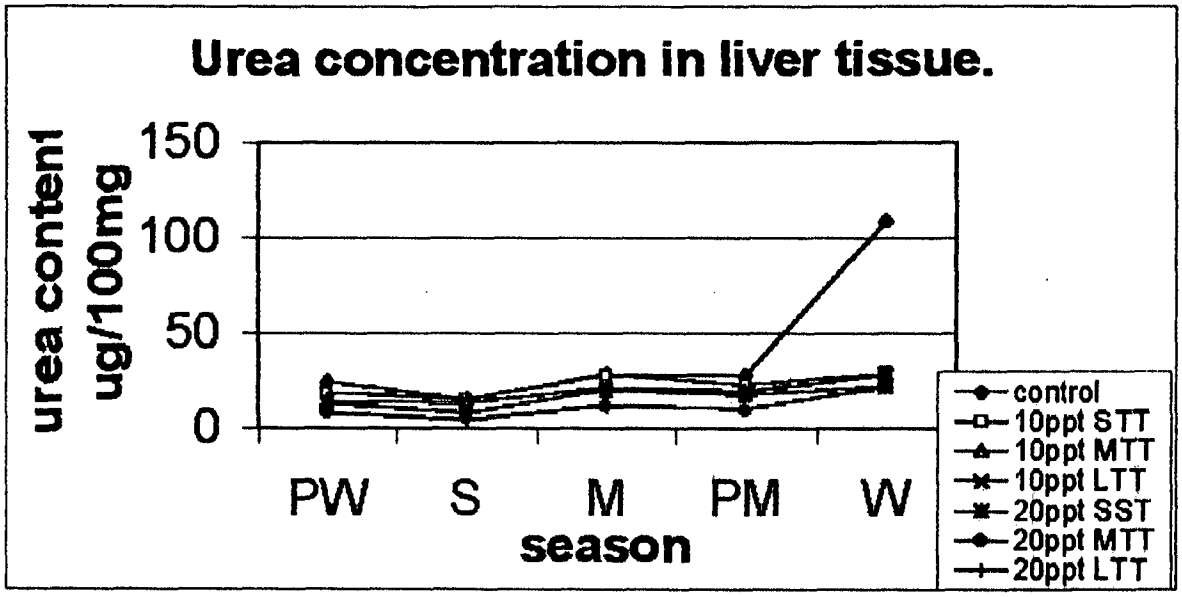

Ammonia concentration in gill filament tissue.
ammonia content ug/100mg
50
40
30
20
10
0
PW
S
M
PM
W
season
control
10ppt STT
10ppt MTT
10ppt LTT
20ppt SST
20ppt MTT
20ppt LTT

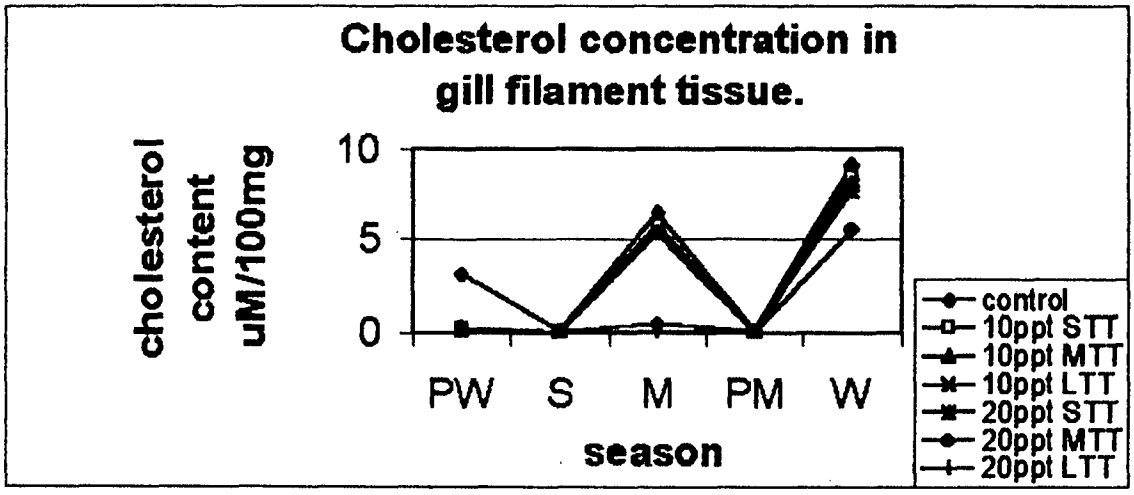

Ammonia concentration in large intestine tissue.
ammonia content ug/100mg
200
150
100
50
0
PW
S
M
PM
W
season
control
10ppt STT
10ppt MTT
10ppt LTT
20ppt SST
20ppt MTT
20ppt LTT

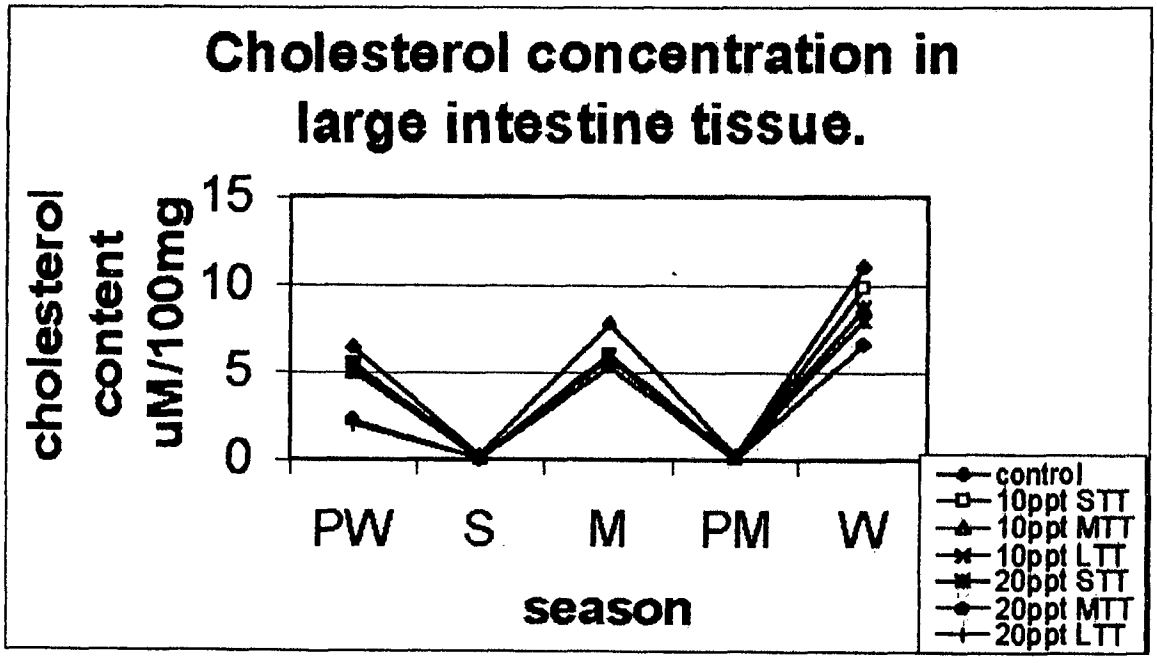

Ammonia concentration in liver tissue.
ammonia content ug/100mg
100
80
60
40
20
0
PW
S
M
PM
W
season
control
10ppt STT
10ppt MTT
10ppt LTT
20ppt SST
20ppt MTT
20ppt LTT

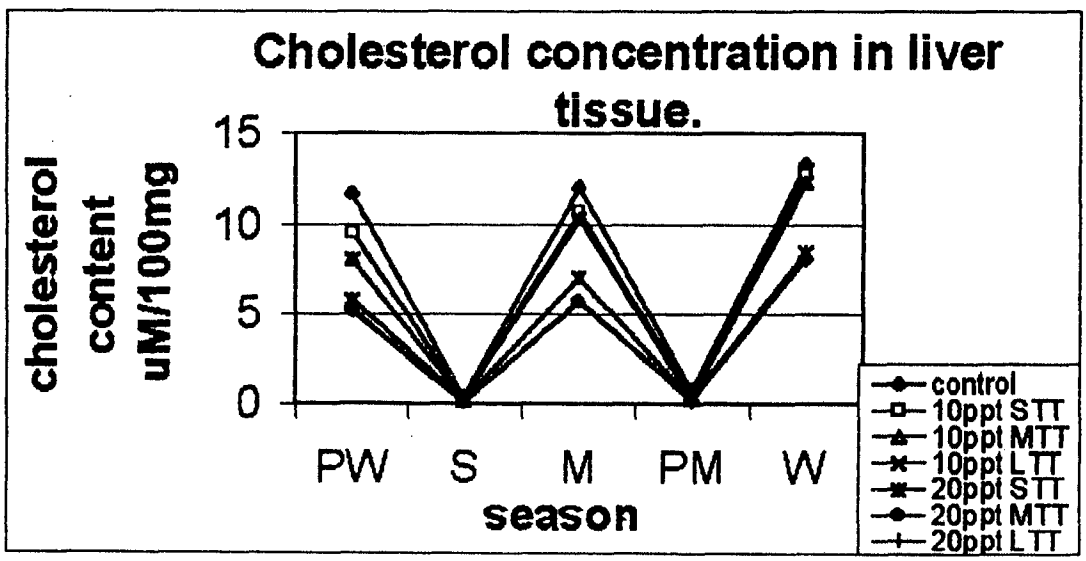

Urea concentration in gill filament tissue.
urea content ug/100mg
150
100
50
0
PW
S
M
PM
W
season
control
10ppt STT
10ppt MTT
10ppt LTT
20ppt SST
20ppt MTT
20ppt LTT

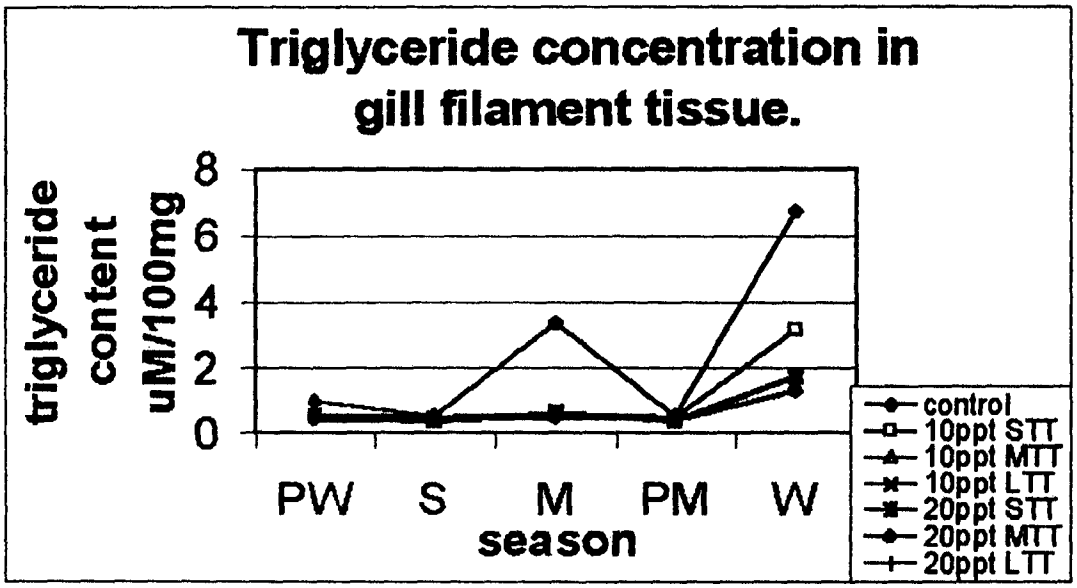

Urea concentration in large intestine tissue.
urea content ug/100mg
150
100
50
0
PW
S
M
PM
W
season
control
10ppt STT
10ppt MTT
10ppt LTT
20ppt SST
20ppt MTT
20ppt LTT

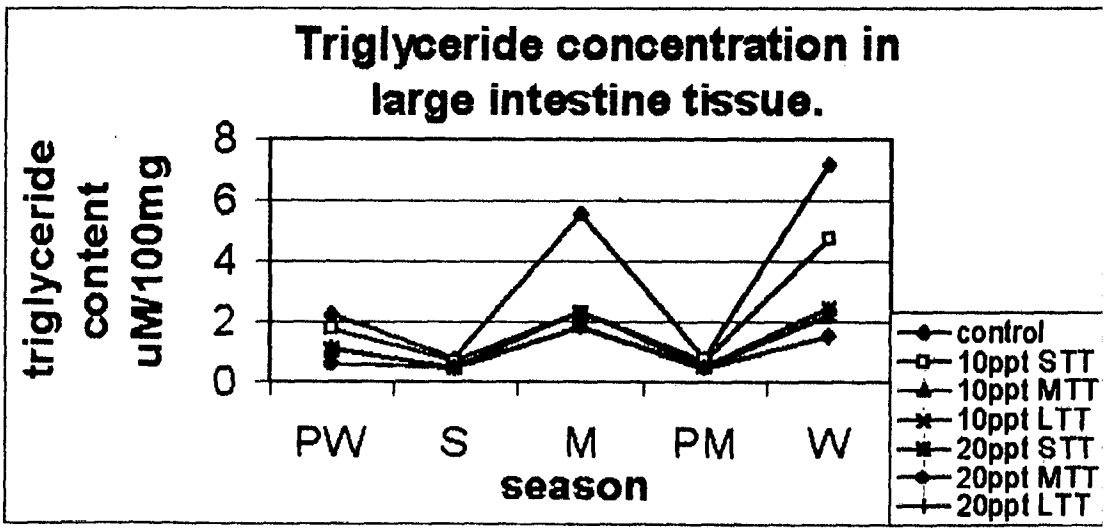

Urea concentration in liver tissue.
urea content ug/100mg
150
100
50
0
PW
S
M
PM
W
season
control
10ppt STT
10ppt MTT
10ppt LTT
20ppt SST
20ppt MTT
20ppt LTT

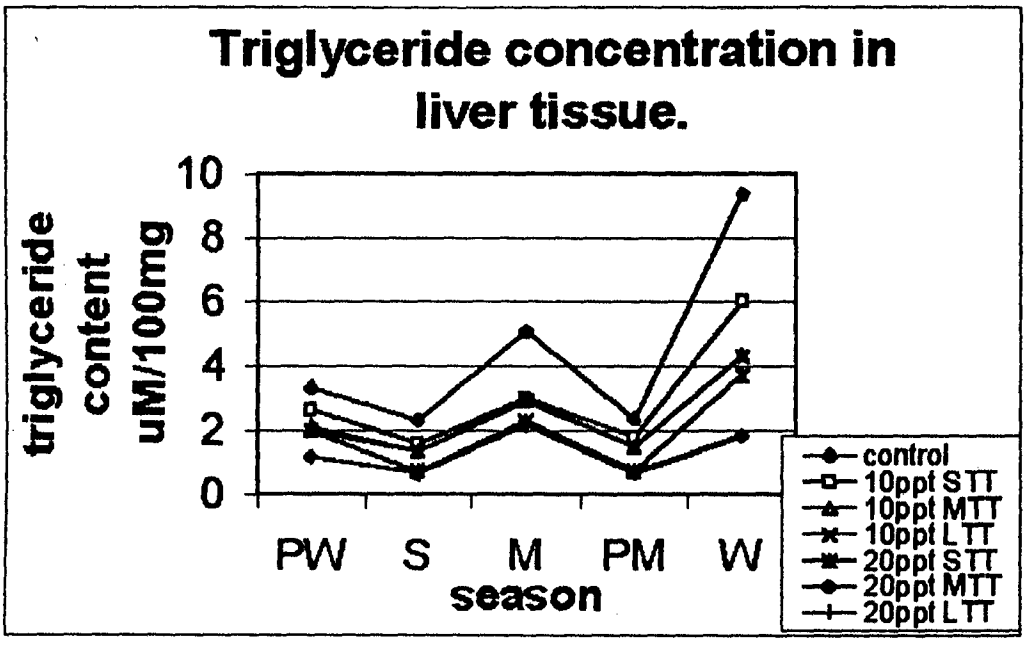

Cholesterol concentration in gill filament tissue.
cholesterol content uM/100mg
10
5
0
PW
S
M
PM
W
season
control
10ppt STT
10ppt MTT
10ppt LTT
20ppt STT
20ppt MTT
20ppt LTT

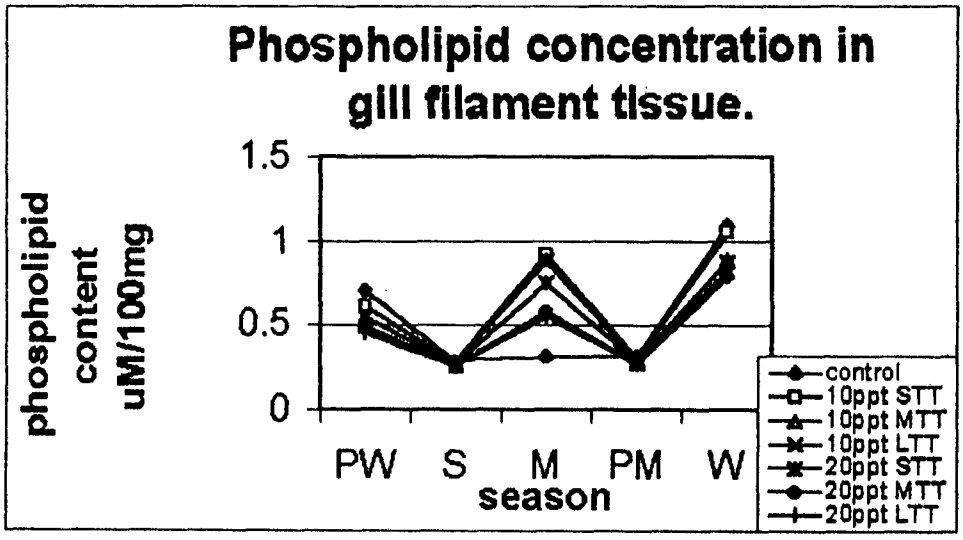

Cholesterol concentration in large intestine tissue.
cholesterol content uM/100mg
15
10
5
0
PW
S
M
PM
W
season
control
10ppt STT
10ppt MTT
10ppt LTT
20ppt STT
20ppt MTT
20ppt LTT

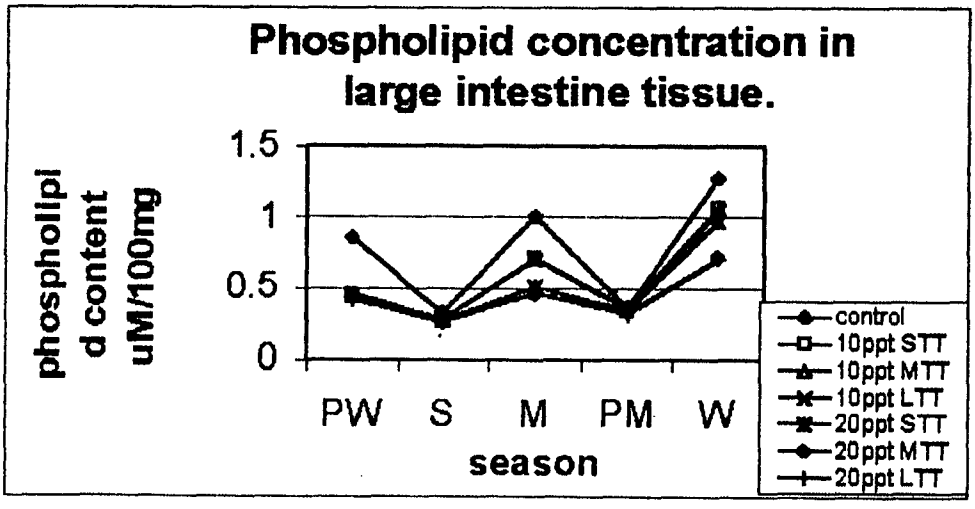

Cholesterol concentration in liver tissue.
cholesterol content uM/100mg
15
10
5
0
PW
S
M
PM
W
season
control
10ppt STT
10ppt MTT
10ppt LTT
20ppt STT
20ppt MTT
20ppt LTT

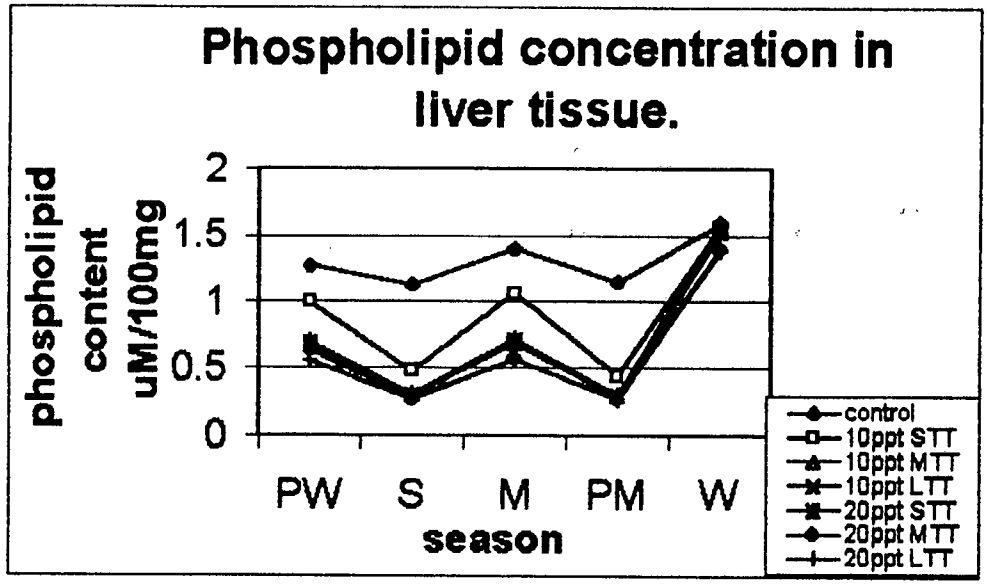

Triglyceride concentration in gill filament tissue.
triglyceride content uM/100mg
8
6
4
2
0
PW
S
M
PM
W
season
control
10ppt STT
10ppt MTT
10ppt LTT
20ppt STT
20ppt MTT
20ppt LTT


Triglyceride concentration in large intestine tissue.
triglyceride content uM/100mg
8
6
4
2
0
PW
S
M
PM
W
season
control
10ppt STT
10ppt MTT
10ppt LTT
20ppt STT
20ppt MTT
20ppt LTT


Triglyceride concentration in liver tissue.
triglyceride content uM/100mg
10
8
6
4
2
0
PW
S
M
PM
W
season
control
10ppt STT
10ppt MTT
10ppt LTT
20ppt STT
20ppt MTT
20ppt LTT


Phospholipid concentration in gill filament tissue.
phospholipid content uM/100mg
1.5
1
0.5
0
PW
S
M
PM
W
season
control
10ppt STT
10ppt MTT
10ppt LTT
20ppt STT
20ppt MTT
20ppt LTT


Phospholipid concentration in large intestine tissue.
phospholipid content uM/100mg
1.5
1
0.5
0
PW
S
M
PM
W
season
control
10ppt STT
10ppt MTT
10ppt LTT
20ppt STT
20ppt MTT
20ppt LTT


Phospholipid concentration in liver tissue.
phospholipid content uM/100mg
2
1.5
1
0.5
0
PW
S
M
PM
W
season
control
10ppt STT
10ppt MTT
10ppt LTT
20ppt STT
20ppt MTT
20ppt LTT

HISTOLOGICAL SECTION OF CONTROL GILL FILAMENT TISSUE OF *OREOCHROMIS MOSSAMBICUS*, DOUBLE STAINED BY HAEMATOXYLIN AND EOSIN (X 300).

PLATE I.

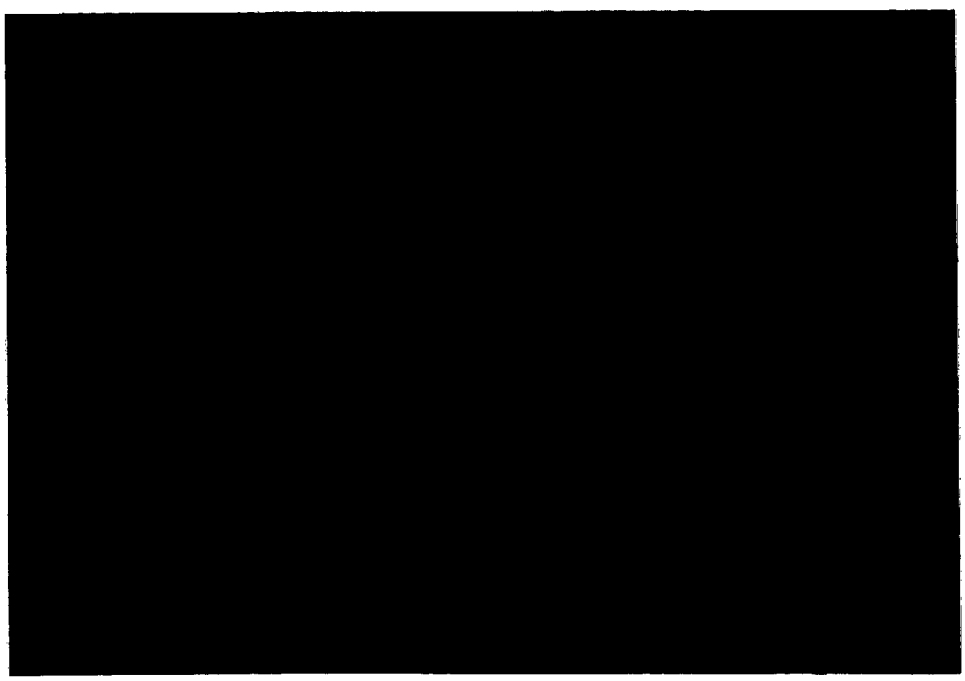
(A): HISTOLOGICAL SECTION OF 10ppt (SAFE DOSE), 7 DAYS TREATED, GILL FILAMENT TISSUE OF *OREOCHROMIS MOSSAMBICUS*, DOUBLE STAINED BY HAEMATOXYLIN AND EOSIN (X 300).

(B): HISTOLOGICAL SECTION OF 10ppt (SAFE DOSE), 21 DAYS TREATED, GILL FILAMENT TISSUE OF *OREOCHROMIS MOSSAMBICUS*, DOUBLE STAINED BY HAEMATOXYLIN AND EOSIN (X 300).

PLATE 2

A

B



(A): HISTOLOGICAL SECTION OF 20ppt (SUB LETHAL DOSE), 7 DAYS TREATED, GILL FILAMENT TISSUE OF *OREOCHROMIS MOSSAMBICUS*, DOUBLE STAINED BY HAEMATOXYLIN AND EOSIN (X 300).

(B): HISTOLOGICAL SECTION OF 20ppt (SUB LETHAL DOSE), 21 DAYS TREATED, GILL FILAMENT TISSUE OF *OREOCHROMIS MOSSAMBICUS*, DOUBLE STAINED BY HAEMATOXYLIN AND EOSIN (X 300).

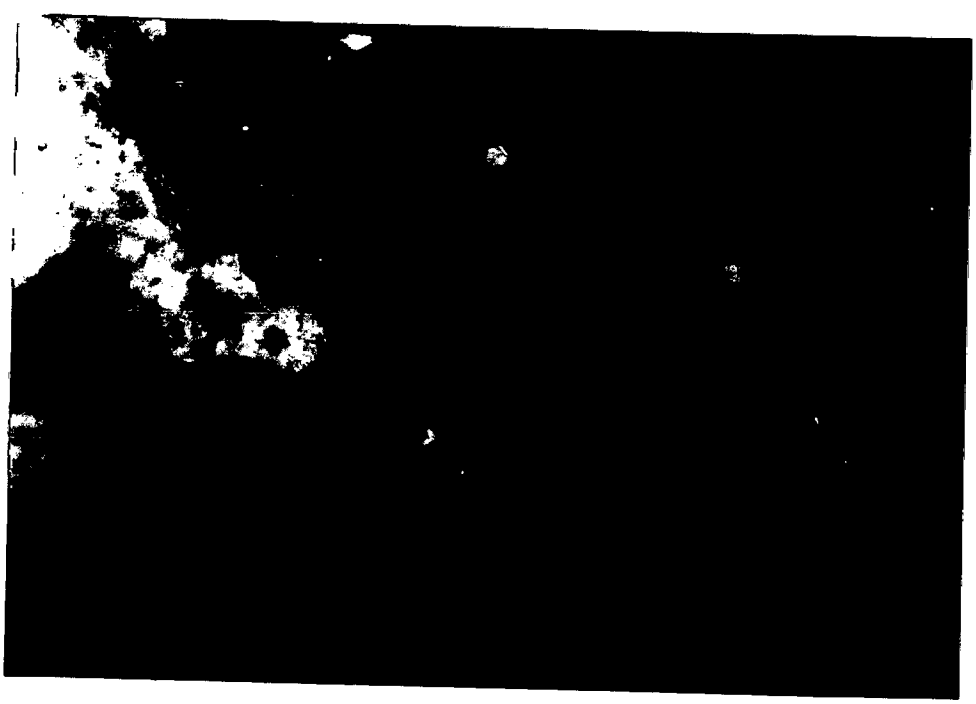
PLATE 3

A

B

HISTOLOGICAL SECTION OF CONTROL LARGE INTESTINE TISSUE OF *OREOCHROMIS MOSSAMBICUS*, DOUBLE STAINED BY HAEMATOXYLIN AND EOSIN (X 300).

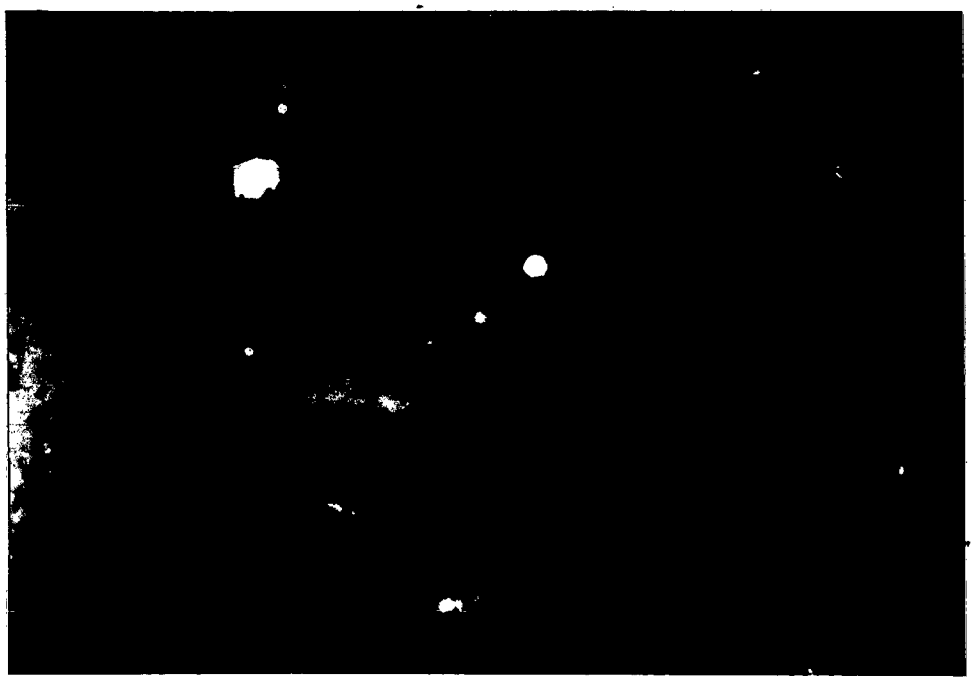
PLATE 4



(A): HISTOLOGICAL SECTION OF 10ppt (SAFE DOSE), 7 DAYS TREATED, LARGE INTESTINE TISSUE OF *OREOCHROMIS MOSSAMBICUS*, DOUBLE STAINED BY HAEMATOXYLIN AND EOSIN (X 300).

(B): HISTOLOGICAL SECTION OF 10ppt (SAFE DOSE), 21 DAYS TREATED, LARGE INTESTINE TISSUE OF *OREOCHROMIS MOSSAMBICUS*, DOUBLE STAINED BY HAEMATOXYLIN AND EOSIN (X 300).

PLATE 5



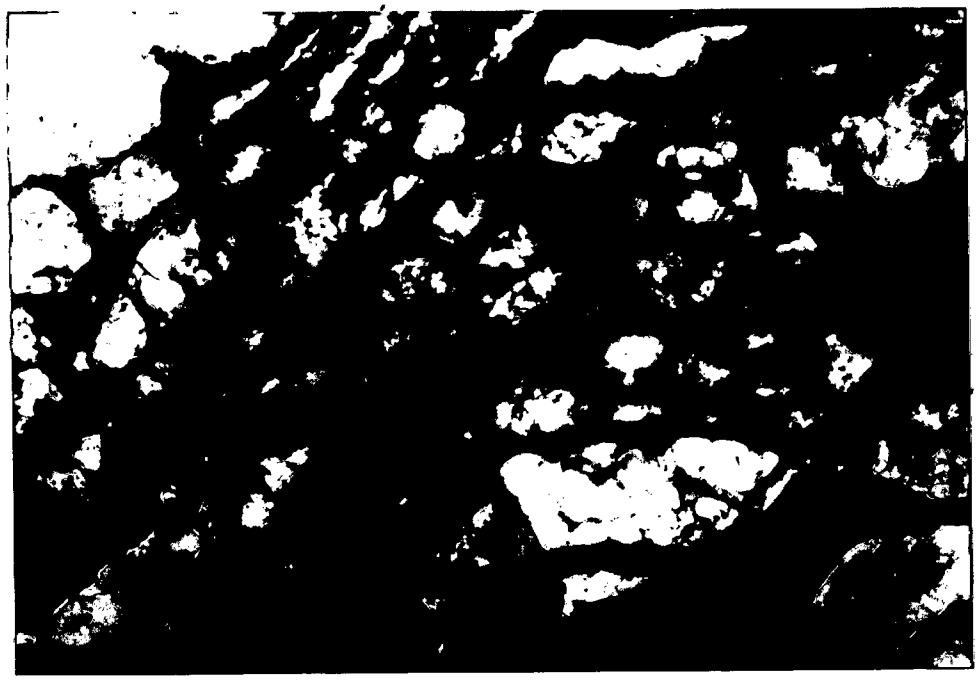
A

B

(A): HISTOLOGICAL SECTION OF 20ppt (SUB LETHAL DOSE), 7 DAYS TREATED, LARGE INTESTINE TISSUE OF *OREOCHROMIS MOSSAMBICUS*, DOUBLE STAINED BY HAEMATOXYLIN AND EOSIN (X 300).

(B): HISTOLOGICAL SECTION OF 20ppt (SUB LETHAL DOSE), 21 DAYS TREATED, LARGE INTESTINE TISSUE OF *OREOCHROMIS MOSSAMBICUS*, DOUBLE STAINED BY HAEMATOXYLIN AND EOSIN (X 300).

PLATE 6

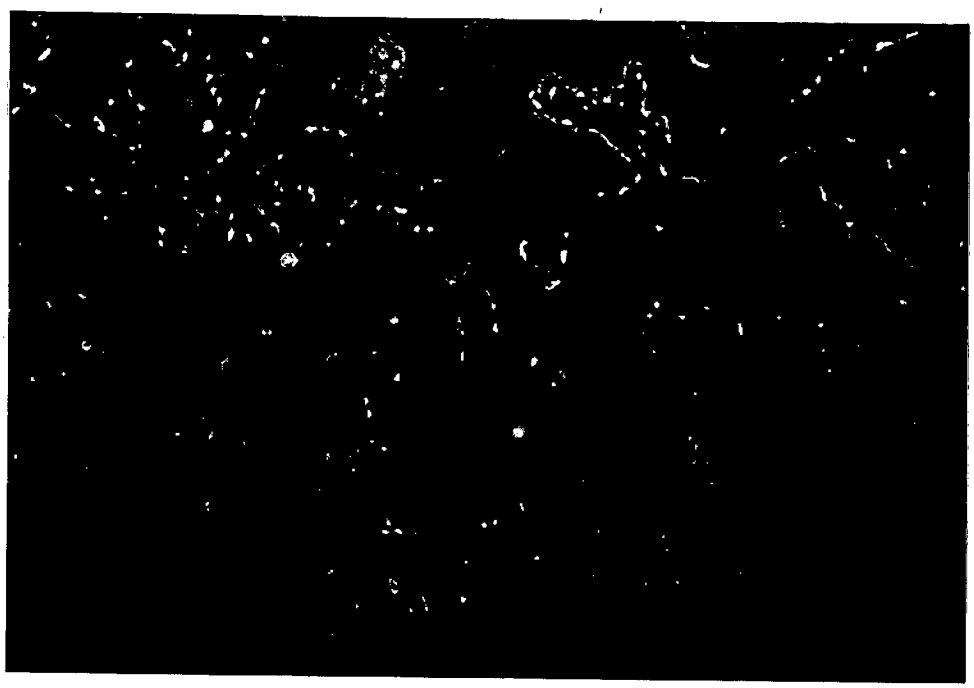


A

B

HISTOLOGICAL SECTION OF CONTROL LIVER TISSUE OF *OREOCHROMIS MOSSAMBICUS*, DOUBLE STAINED BY HAEMATOXYLIN AND EOSIN (X 300).

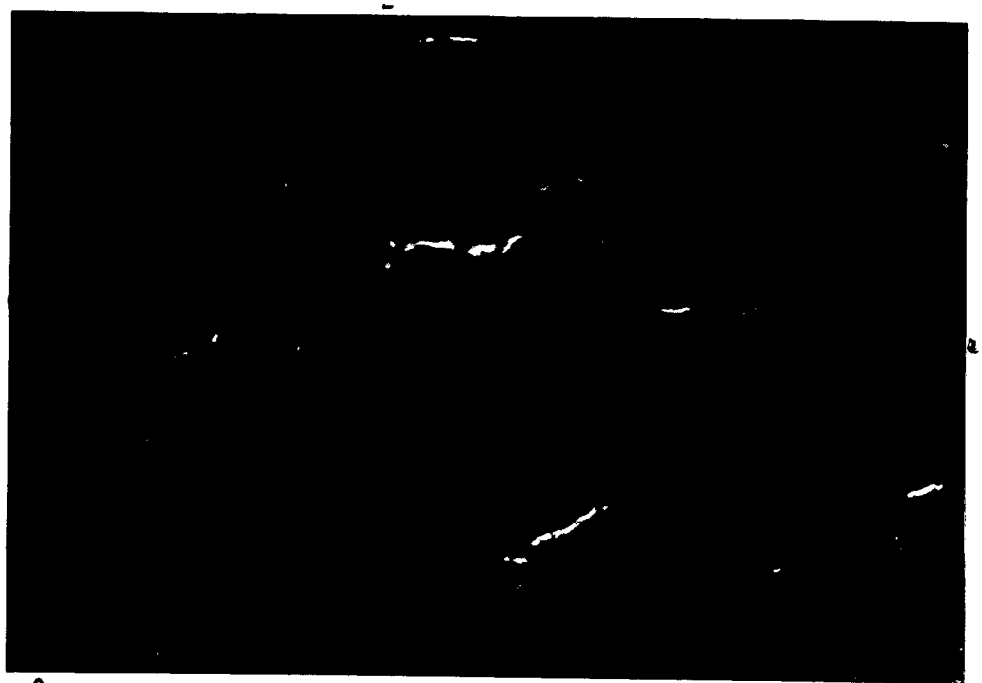
PLATE 7



(A): HISTOLOGICAL SECTION OF 10ppt (SAFE DOSE), 7 DAYS TREATED, LIVER TISSUE OF *OREOCHROMIS MOSSAMBICUS*, DOUBLE STAINED BY HAEMATOXYLIN AND EOSIN (X 300).

(B): HISTOLOGICAL SECTION OF 10ppt (SAFE DOSE), 21 DAYS TREATED, LIVER TISSUE OF *OREOCHROMIS MOSSAMBICUS*, DOUBLE STAINED BY HAEMATOXYLIN AND EOSIN (X 300).

PLATE 8



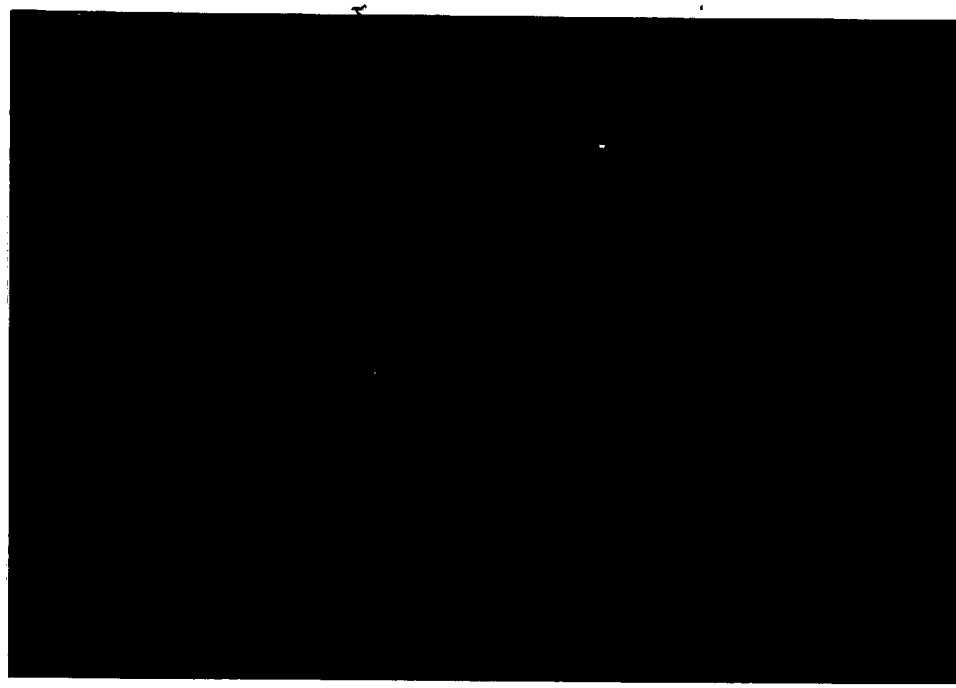
A

B

(A): HISTOLOGICAL SECTION OF 20ppt (SUB LETHAL DOSE), 7 DAYS TREATED, LIVER TISSUE OF *OREOCHROMIS MOSSAMBICUS*, DOUBLE STAINED BY HAEMATOXYLIN AND EOSIN (X 300).

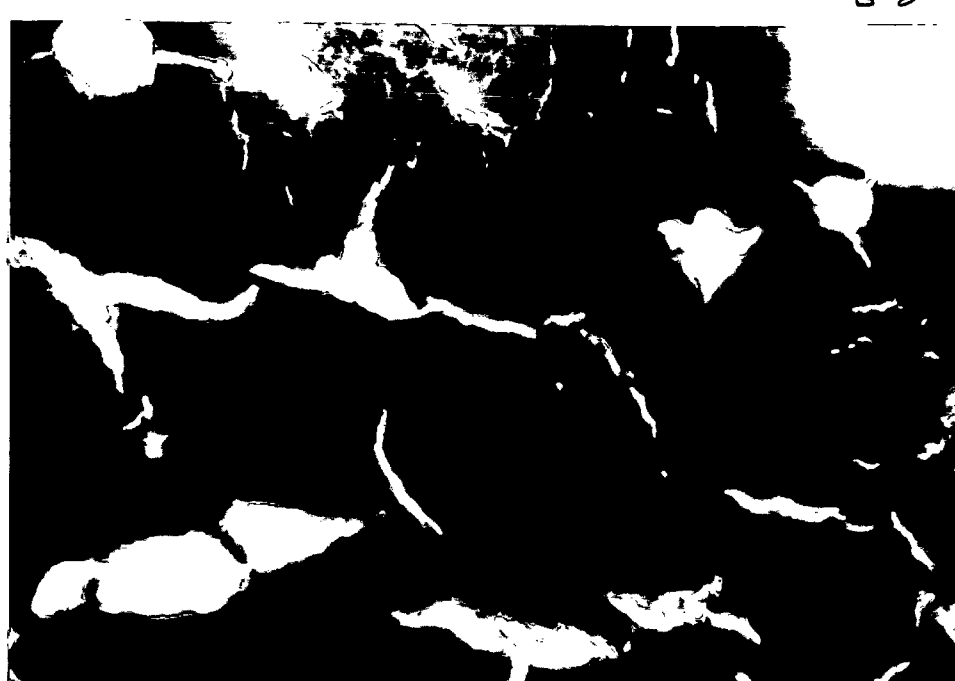
(B): HISTOLOGICAL SECTION OF 20ppt (SUB LETHAL DOSE), 21 DAYS TREATED, LIVER TISSUE OF *OREOCHROMIS MOSSAMBICUS*, DOUBLE STAINED BY HAEMATOXYLIN AND EOSIN (X 300).

PLATE 3



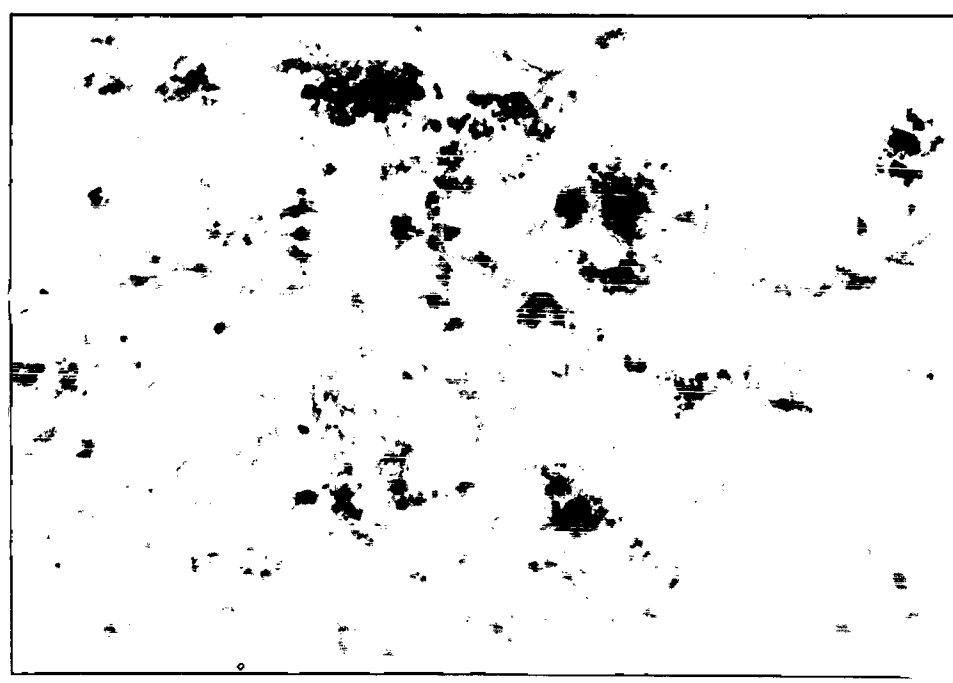
A

B



HISTOLOGICAL SECTION OF CONTROL TESTIS TISSUE OF *OREOCHROMIS MOSSAMBICUS*, DOUBLE STAINED BY HAEMATOXYLIN AND EOSIN (X 300).

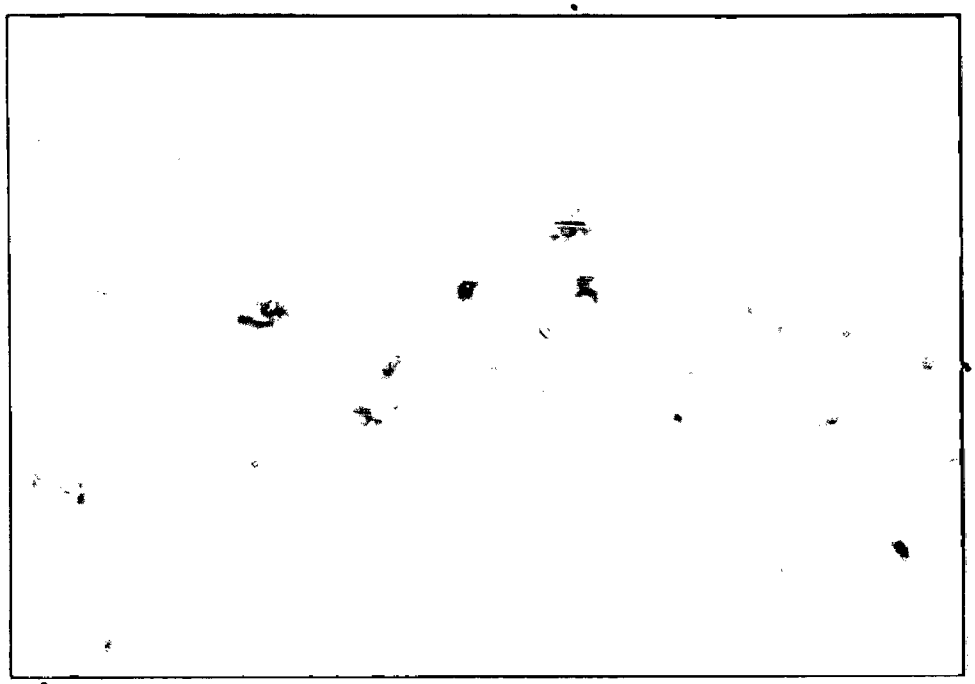
PLATE 10



(A): HISTOLOGICAL SECTION OF 10ppt (SAFE DOSE), 7 DAYS TREATED, TESTIS TISSUE OF *OREOCHROMIS MOSSAMBICUS*, DOUBLE STAINED BY HAEMATOXYLIN AND EOSIN (X 300).

(B): HISTOLOGICAL SECTION OF 10ppt (SAFE DOSE), 21 DAYS TREATED, TESTIS TISSUE OF *OREOCHROMIS MOSSAMBICUS*, DOUBLE STAINED BY HAEMATOXYLIN AND EOSIN (X 300).

PLATE 11



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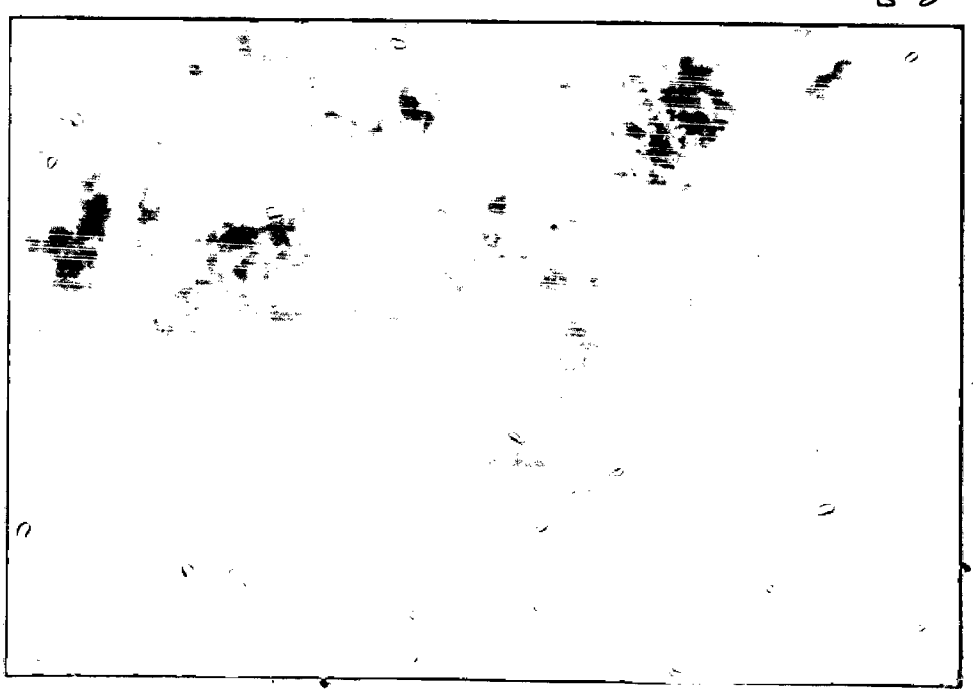
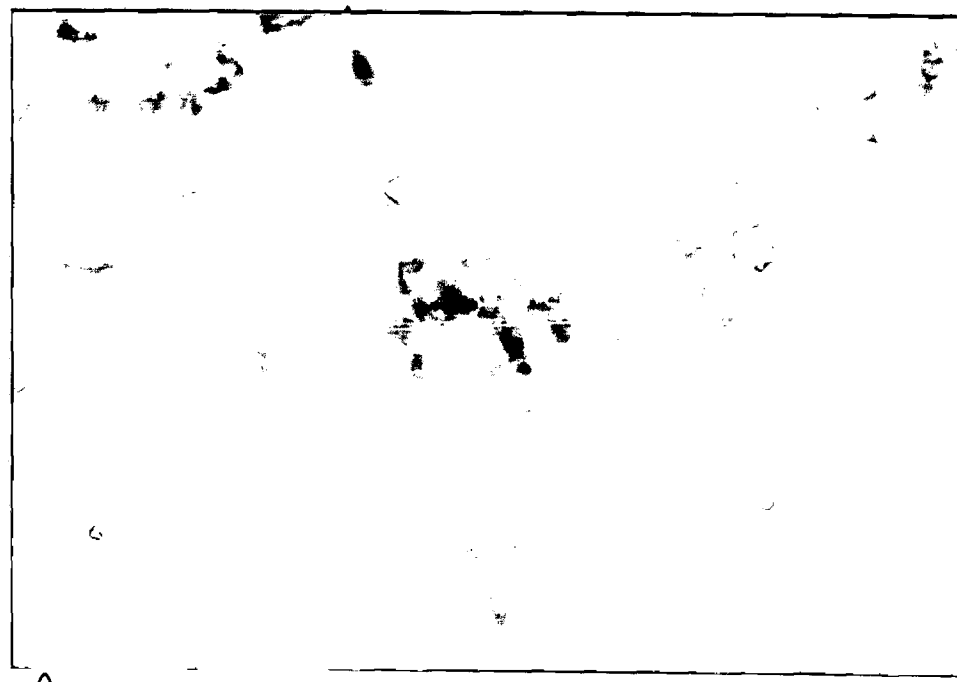


(A): HISTOLOGICAL SECTION OF 20ppt (SUB LETHAL DOSE), 7 DAYS TREATED, TESTIS TISSUE OF *OREOCHROMIS MOSSAMBICUS*, DOUBLE STAINED BY HAEMATOXYLIN AND EOSIN (X 300).

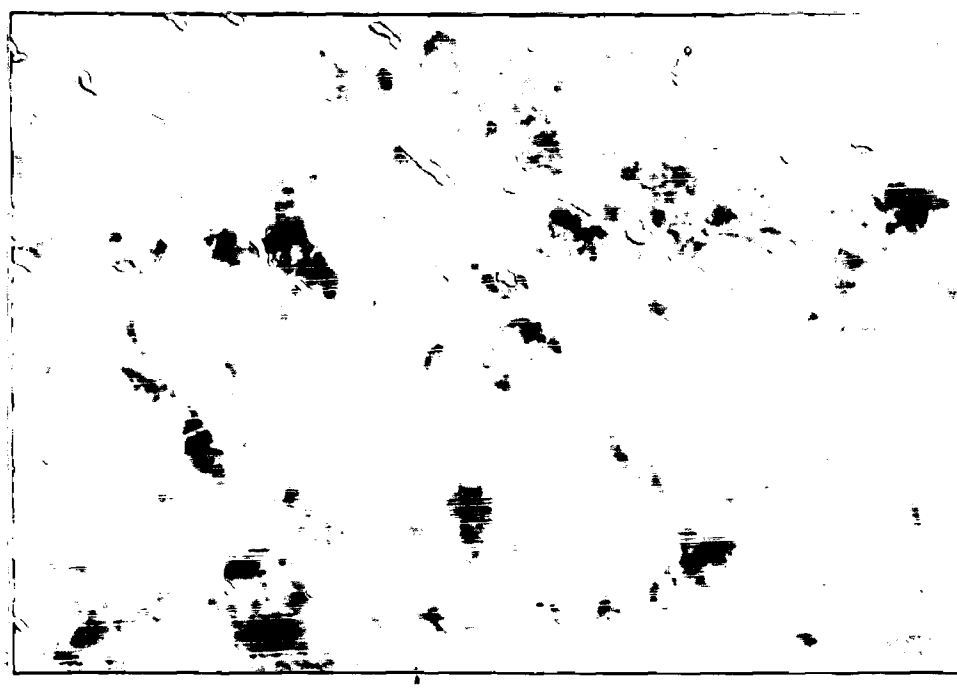
(B): HISTOLOGICAL SECTION OF 20ppt (SUB LETHAL DOSE), 21 DAYS TREATED, TESTIS TISSUE OF *OREOCHROMIS MOSSAMBICUS*, DOUBLE STAINED BY HAEMATOXYLIN AND EOSIN (X 300).

PLATE 12



A

B



PART IV

4.0 DISCUSSION

4.1 PHYSICO CHEMICAL PARAMETERS

About 71% of the surface of this planet is covered by water body, with average depth of 3.8km, a volume of 1370 x 10^6 km^3, and animal life of all phyla exists throughout this immense volume. Further, it is also reported that, 73 animal classes live in sea, 35 in freshwater and 33 on land Nicol, (1971), for which possible reasons are listed by (May, 1994). All, however, are subjected to the properties of seawater that surrounds them and many features common to these plants or animals, results from adaptations to watery medium and its movement. The water and its contents do have a major role to play in the associations and assemblages of organisms (Nybakken, 1997). To under stand the above one needs to examine physico chemical conditions of water before introducing the experimental animal. It is reported that, the water contains a variety of solids and gases in dissolved condition, which could be toxic and which could alter the physiological, behavioural, biochemical and histological parameters. It is reported that, a thousand-gram sample of sea water will contain about 35gm of dissolved components collectively called salts. In other words, 96.5% of sea water is water and 3.5% is dissolved substance, which include Cl^- (55.04%), Na^+ (30.61%), SO_4^{-2} (55.04%), Mg^{+2} (3.69%), Ca^{+2} (1.16%), K^+ (1.10%) as major constituents and HCO_3^- (0.41%), Br_2 (0.19%), H_2BO_4 (0.07%), Sr^{+2} (0.04%), apart from 0.01% of dissolved substances of several other inorganic salts needed for living aquatic organisms.

India is having vast freshwater resources, but major areas are still to be brought under control for fish production. To employ scientific methods for

rearing fish, through understanding of the environment, is essential. In India, the knowledge of the ecology of freshwater nature, fish-ponds, is limited. Ponds being small water bodies attracted the attention of limnologists, after considerable work on lakes. Study of physicochemical parameter of any freshwater body, enables us, to understand, whether the water is portable for any living organism or not.

Temperature is the chief factor influencing the other abiotic as well as biotic parameters of any aquatic ecosystem. The measurements of water temperature, is of vital importance for knowing the solubility of other chemicals and respiratory gases. Water temperature within the tolerable limit of 21-34^{0}C, is most ideal for cultivable fishes (Sakhre and Joshi, 1992). In the present investigation, maximum temperature (33±1^{0}C) was recorded during summers (March-May), and minimum temperature (20±1^{0}C) was recorded during winters (Nov.-Dec.). While other seasons, post winter (Jan.-Feb.) showed (27±1^{0}C), monsoon (June-Aug.) showed (23±1^{0}C), post monsoon showed (26±1^{0}C), all ranging, intermediately.

Similar trends of temperature were recorded in freshwater bodies by Praksan and Johnson, (1992), and (Kumar, 2002). Also the wide range of temperature variations in freshwater reservoir are in conformity with the findings of Bohra, (1976) in Padmasagar and Ranisagar; Mishra, *et al.*, (1978) in Balsamand; Vyas and Nama, (1991) in Akaraj-ji-ka talab of Jodhpur. The fluctuations of water temperature with seasonal trends has been observed by Rao, (1955) and (Saha and Pandit, 1986).

Keeping in view of the above, in the present investigation, tap water was used for maintaining *Oreochromis mossambicus*, was highly potable and

productive, since freshwater reservoirs having water temperature more than 22^0C are found to be highly productive (Jhingran and Sugunan, 1990; Sugunan, 1995).

Transparency and turbidity is a physical variable, significant to productivity. The poor light penetration may be due to high material/ matters like silt and clay or due to high density of living organisms inhabiting the water column.

In the present study, turbidity was very very low, approximately equal to nil, while colour and odour of the freshwater used to maintain the live fish was completely absent. Turbidity ranged from 0.8 to 1.1 NTU and transparency was inversely proportional to turbidity.

Similar observations has been made by Sinha and Sinha, (1990) and Sakhre and Joshi, (2002), who recorded Secchi disc transparency ranging from 73-117cm. The minimum transparency values were recorded in August while maximum in January. Present findings corroborate with findings of Singh *et al.*, (1993), who have reported the lowest transparency values in rainy seasons. The lowest values recorded during rainy season may be due to the heavy rains and winds of high velocity. Similarly, high values were recorded during winter and summer period in Palas-Ni-Legaon reservoir, in Osmanabad district, Maharashtra by (Sakhre and Joshi, 2002). High values during winter and summer period can be attributed to low moderate wind velocity. According to Das, (1996), high Secchi disc transparency values indicate low primary productivity by phytoplankton and consequent low fish production. The turbidity increases during bathing, which may be due to disturbance of water sediment particles during bathing, like the sand and clay particles (Sinha *et al.*,1991).

pH is a important parameter under study, which tells us whether the water is acidic or basic and which in turn influences other physico-chemical parameters of water body.

In the present investigation, the pH of the tap water sample, during different seasons was found to be more or less constant, ranging from 7.0-7.5. Lowest pH was recorded during March to May (7.0) and highest during June-Aug. (7.5), indicating, tap water to be in a narrow alkaline range. This may be due to the strong buffering (CO_3^- – HCO_3^-) capacity of water (Sinha *et al.*, 1991).

Similarly the pH value was found alkaline of Unapdeo thermal spring freshwater of Maharashtra, (Nandan, 2002). The above observations are in conformity with the observations reported by Singh (1960), Verma (1964), Adoni (1975), Choudhary *et al.*, (1979), Ramani (1980), and (Bose, 1984). Rawat and Jakher, (2002) has shown lowest and highest peaks at July (7.2) and June (8.15) respectively. Nair, (2000) reported pH to vary from 7.8 to 9, being lower in summer, and higher in winters. The highest values at summer seasons / temperature were may be due to higher photosynthetic activity (Zutshi and Vass, 1978). Or, due to decrease in the water level caused by the evaporation of water (Adoni, 1975; Bose, 1984). According to Freiser and Fernando, (1966), the pH differences among lakes, probably results from shifts in major buffering systems. The pH is affected, not only by level of carbon dioxide, but also by other organic and inorganic components of water.

Optimum growth of aquatic plants and animals will be subject to various kinds of stresses, and the diurnal fluctuations of pH should remain in the range of 6.4 to 8.5 in order to support the optimum fish growth (Das, 1996).

The average pH value (7.6) of Palas-Nilegaon is suitable for optimum fish growth. According to Jhingran and Sugunan, (1990), the pH range between 6.0 to 8.5 indicates medium productive nature of the reservoir, more than 8.5 highly productive and less than 6 as low productive reservoir.

Chloride is one of the important constituent which imparts saline condition of freshwater body. During the present investigations, the concentration of chloride was found to vary within 5 to 11ppm during various seasons. Highest was attributed during March to May (10-11ppm) and lowest during June to Aug. (5-6ppm).

Similar trend in variations, in chlorides were recorded by Kumar, (2000) in Mayurakshi river; Sandwar and Prasad, (2000) in river Ganga. Concentration of chloride varied within 79-179mg/l of surface water source of Akola city, Maharashtra (Musaddiq and Fokmare, 2002).

Higher chloride values can be attributed to, reduced flow of river and large amount of sewage being carried into it, or maximum evaporation. Low chloride concentration might be due to the dilution of river water, and rapid flow of water (Palhary *et al.*, 1993). Or, minimum evaporation (Sandwar and Prasad, 2000).

The alkalinity is a measure of capacity of water to neutralize a strong acid. Alkalinity is generally imparted by the salts of carbonates, bicarbonates, phosphates, nitrates, borates, silicates etc., together with the hydroxyl ions in free state. However, most of the waters are rich in carbonates and bicarbonates, with lower concentration of other alkalinity imparting ions (Trivedy and Goel, 1984). Waters having 40mg/l or more levels of total

alkalinities are considered to be more productive than waters of lower alkalinities (Moyle, 1945; Maris, 1960).

During the present investigation, the total alkalinity ranged from 26-70ppm, thereby indicating the tap water to be highly productive during monsoon and post monsoon.

Sakhre and Joshi, (2002) has shown alkalinity to be 100mg/l of the reservoir, indicating water to be highly productive. Outbreaks of Epizootic Ulcerative Syndrome (EUS), was more severe with waters having alkalinities below 20mg/l (Das and Das, 1995). The observed values of total alkalinity were found to be 169.60mg/l, 250.84mg/l and 169.24mg/l in Morna and Purna rivers, and Kapshi lake respectively. Thermal spring water was found to be alkaline (Nandan, 2002). Nair, (2000) reported alkalinity to be ranging between 30.4 to 106ppm, being maximum in summer, and minimum in monsoons of a village pond. These observations support the opinion of Saha and Pandit, (1986) and Munawar, (1970) that, the accumulation of bicarbonate in summer may be due to increased rate of decomposition.

Higher values of total alkalinity might be due to presence of excess free carbon dioxide produced as a result of decomposition process coupled with mixing of sewage and industrial effluents (Chatterjee and Raziuddin, 2002; Palharya *et al.*, 1993; Singh, 2000; Mohanta and Patra, 2000).

Hardness is governed by the contents of calcium and magnesium salts largely combined (a) with bicarbonates and carbonates giving temperate hardness and (b) sulphates, chlorides and other anions of mineral acids causing permanent hardness. In the present study, total hardness of water varied between 70 to 74ppm. Sakhre and Joshi, (2002) has shown total

hardness of reservoir varying between 62 to 188.6mg/l. Fishes have been found to be susceptible to diseases when hardness is below 20mg/l. EUS outbreak has been observed to be more frequent in waters with lower values of hardness (Das and Das, 1995). Productive waters generally have hardness above 20mg/l. Very high level of hardness of water (> 300mg/l) affects fish production, because of higher pH. The optimum hardness ranging between 75 to 150mg/l supports fish productivity (Das, 1996). Hardness values were recorded within 87 to 442mg/l in Morna, Purna rivers and Kapshi lake. Higher values of hardness can be attributed to low water level, and high rate of evaporation. Similar observations were recorded by Ajmal and Raziuddin, (1988) in Hindon and Kali river. Mohanta and Patra, (2000) opined that addition of sewage, detergents and large scale human use might be the cause of elevation of hardness.

Relative hardness of Unapdeo thermal spring, was due to decomposition of leaves thrown into the tank, and also contamination of mankind activities as it is considered as a holy place (Nandan, 2002). Thus the values of total hardness recorded during present study, ranged around as mentioned above, thus making tap water suitable for *Oreochromis mossambicus* fishery.

Phosphate ranged between 0.001 to 0.006ppm, being higher during monsoon and post monsoon in the present investigation. Similar results were obtained by Nair, (2000), where the phosphate content of the village pond ranged between 0.073 to 0.126ppm, being higher in the monsoon and post monsoon, which is probably due to the influx through rain water (Michael, 1969; Munawar, 1970; Singh and Sahai, 1979). Phosphates showed positive correlations with turbidity, conductivity, pH, dissolved oxygen and negative

correlations with water temperature, transparency, hardness, alkalinity, free carbon dioxide and chloride (Nair, 2000). Singh, (1986) in his studies on lakes has also reported negative correlations of phosphate with water temperature, transparency, pH, alkalinity, hardness and positive correlation with dissolved oxygen.

Sulphate ranged from 0.0 to 1.3ppm, being maximum during post winter and minimum during summer and winter. Nair, (2000), has reported sulphate ranging between 4.1 and 16.6ppm, which showed positive correlations with water temperature, turbidity, conductivity, chloride, ammonia, phosphate, nitrate and negative correlations with transparency, pH, hardness, total alkalinity, dissolved oxygen and free carbon dioxide. Munawar, (1970), has mentioned an inverse relationship between sulphate and pH.

4.2 BEHAVIOUR

The special branch of biology that deals with the study of animal behaviour is called ethology. It is a relatively new area of scientific investigation. The study of animal behaviour involves various approaches, of which three approaches are of significant value as far as historical, evolutionary analysis of modern field of ethology is concerned. These three approaches are the psychological, the physiological and the zoological. These three approaches have so much intermingled with one another that the study of animal behaviour cannot be done in isolation, taking any one of these approaches into consideration. No animal lives alone, few of them are solitary. Not all aggregations of animals are social.

The study of behaviour begins with observations of an animal/ animals movement, posture and so forth. Very often an animal may appear doing

nothing, even if its surroundings changes. This is because it fails to perceive the changes, but it is equally possible that its response to the changes may be to remain still. It is only by close and repeated observations of animals, in natural or near natural surroundings, it is possible to recognise behaviour, and begin to see how it is related to the environmental stimuli. The observations and description provide the foundation upon which the study of behaviour is built. Ethology deals with fascination and excitement of the latest discoveries and interpretations, chiefly emphasizing Territoriality, Aggression, Conflict behaviour, Learning, Feeding, Anti-predatory behaviour. Biological Communication and Orientation, which involves, selection of habitat, pattern of behaviour, motivation, sexual behaviour, biological clock, fighting behaviour, parental care and social behaviour (Arora, 2001).

From evolutionary viewpoint, most behaviours that is performed at reproductive or survival "cost" by one animal and that "benefits" a recipient by increasing its chances of survival, is thought to be done at some direct, unusually immediate, cost of the actor (Altmann, 1986).

Though the scientific study of animal behaviour has its origin in the work of eighteen-century naturalists, such as Gilbert White (1720-1793), and Charles Leroy (1723-1789), it is Charles Darwin (1802-1882), who is regarded as the father of scientific study of animal behaviour (McFarland, 1985).

Technically speaking, every behaviour, can be thought of as an event or a state. Behaviour, which are of long duration, are more appropriately thought of as state, while short duration behaviours, are more appropriately thought as events. Focal behaviour sampling, convenient and widely used method for treating behaviour as events, keeps a continuous records of all behaviours,

shown by the focal animal, during specific duration. While scan sampling, convenient and widey used method for treating behaviour as state, keeps record of association behaviours, between different animals of same / different species, during specific duration (Gadakar, 2001).

Lots of information is available on behavioural studies of various organisms. Some of them are provided below.

The fruit fly *Drosophila melanogaster*, popularly known as Cindrella of Genetics, as it has contributed a lot for the study of principles of genetics, has also contributed significantly in ethological experiments, (Speith, 1952; Manning, 1965; VonSchilcher, 1976; Patterson, 1980; Hedge and Krishna, 1997; Sheeba *et al.*, 2001) where in, various studies on specific behaviours like sexual behaviour, eclosion behaviour, chemically induced behavioural variations etc. were studied.

Communication pattern during courtship by field criket, *Teleogryllus oceanicus* which is known to be multimodal, involving a minimum of both acoustic and chemical cues leading to courtship song, which is necessary to elicit normal levels of female mountings was studied by (Balakrishnan and Pollack, 1997). They have also analysed the chemically suppressed activity, which established contact chemosensory behaviour in *Teleogryllus oceanicus*.

Studies was done by Roy and Md. Mushahidunnabi, (2001) on courtship and egg laying of *Tylototriton verrucosus* salamander, which is confined to higher altitudes of Eastern Himalayas.

Smitha *et al.*, (1999) probed into a not widely known behaviour of the animals, to appreciate the mental states of individual Bee eater birds, called

the 'theory of mind'. The results indicate that the bird can appreciate the visual perspective of the observer and take a decision based on the observer's vision, suggesting that the bird can probably differentiate what the observer knows and what he does not.

It was Marimuthu and Chandrashekaran, (1985), revealed from their experiments, that there is a communication and social synchronization of circadian rhythm in the flight activity of the microchiropteran cave-dwelling bat, *Hipposideros speoris*. Viswanathan and Chandrashekaran, (1984), studied complex locomotion behaviour serving foraging, exploration and territoriality by field mouse *Mus booduga*.

As far as fishes are concerned, they come only next to birds, with respect to studies on behaviour. Some of the common behaviour exhibited by both birds and fishes are migration, nest building, parental care etc.

The behaviour of a fish in response to a change in temperature may vary according to its biological conditions. The autumnal drop in the temperature of the Aral sea causes *Abramis brama* to cease feeding and commence winter migration (Nikolsky, 1963a,b). *Saratherodon mossambicus* is endowed with morphologic plasticity to cope up with the given physical and biological environment since it is exotically euryhaliune in nature. The behavioural changes are directly related to complex physiological needs by the animal to sustain homeostatic life for survival and hence often been used as a sensitive measure of stress syndrome in organisms experiencing them (Eisler, 1977). The opercular movements of the air breathing fish *Channa stiatus* ceases immediately following exposure to carbamate pesticide, sevin (Anbu and Ramaswamy, 1991). This drop in opercular movement and corresponding

increase in frequency in surfacing of fish in initial period, clearly indicates that, fish adaptively shifts to aerial respiration by gulping atmospheric air by surfacing, and the fish avoids contact with sevin through gill chambers. These findings are similar to Santhakumar and Balaji, (2000) who treated monochrotophos to *Anabas testudineus*. Increased surfacing in the initial period to malathion stress shows elevated rate of metabolism.

The above observations can be correlated with the present work, where non-stop movements of the pectoral fins (fanning), along with sudden and rapid movement (burst swimming), was observed in initial period, on contact with NaCl chemical, indicating that the fish adaptively tries to push the chemical away from its body.

Changes in the ventilation rate and surfacing frequencies are the general symptoms noticed in fish after exposure to pesticide and these activities helps the fish to avoid contact with poison and fight against the stress (Ray and Munshi, 1987). Movement of the fish towards another (nudge), biting (nip), rapid opening and closing of mouth and opercular coverings (cough), behaviours in the present study, is similar to the findings of Santhakumar and Balaji, (2000) in their study. Different locomotion and orientation patterns are found to be involved in various fish behaviour such as migration, mating, courtship and feeding which are altered under stress condition of environmental toxicant (Steel, 1983). Increased mucous secretion is an adaptive measure perhaps for preventing absorption of pollutant by general body surface. These findings, agrees with earlier findings of Sadhu, (1993); Sabita *et al.*, (1995) as in present studies.

The consequences of the behavioural abnormalities, depend on their severity and duration. Voyer *et al.*, (1982) described winter flonder larvae *Pseudopleuronectes americanus* incubated in cadmium concentrations of upto 100μg/l, that show reduced swimming activity only in low salinity (10‰ salinity). This response suggests a potential long-term effect on larval feeding, growth and susceptibility to predation. Similarly in the present work, at higher concentration salinities, no movement (motionless) was observed. At safe dose (10.194ppt), motionless was 16% while at LC_{50} dose (41.012ppt), motionless was 100%.

A number of studies have shown conclusively that amino acids acting singly and in combination, stimulate feeding behaviour in fishes (Hashimoto *et al.*, 1968; Sutterlin *et al.*, 1982). A comparison of the results obtained electrophysiologically, Marui *et al.*, (1983), and behaviourally, Adron and Mackie, (1978), in the rainbow trout, however is confusing. The L-isomers of tyrosine, phenylalanine and either lysine or histidine were the constituents of the simplest mixture tested that stimulated feeding activity. When this mixture was subdivided into two fractions, neither fraction was active. The release of any chemical /food into the water stimulates different types of behaviour only after the concentration exceeds the animal's threshold for perception. The first signs of detection are often quiet subtle, but with continued low level stimulation, more overt behavioural changes eventually surface. These are best observed in sedentary species because of their quiet life styles. Ictalurid catfish, for example, normally rest on the bottom during the daytime, in shelter, if available. Upon stimulation with a food extract /chemical they quickly increase their magnitude and rate of opercular pumping, twitch their

maxillary barbels, sway their head to-and-fro in a pronounced fashion, take one or more large gulps, and finally lunge forward to initiate their response (Olmsted, 1918; Bardach *et al.*, 1967; Atema, 1971; Holland and Teeter, 1981). In the present investigation, it was observed that, on exposure to the chemical, feeding ceases for some time, and the fish is seen to be restless and moving aimlessly for 10-20 minutes. Once *Oreochromis mossambicus* gets acclimated to the new environment, feeding restarts normally.

Other species show additional traits, including extensions and flicking of the fins, (especially those which serve as specialized chemoreceptors) muscular jerks, twitches and body quivers, rapid shifts of the eye, yawing or coughing, changes in body posture, and exaggerated lateral movements of the head and tail (Hoese and Hoese, 1967; Marusov, 1975; Pawson, 1977; Hodgson and Mathewson, 1978; Holland, 1978; Mearns, 1985, 1986). These signs of arousal show considerable plasticity. A single individual may display several simultaneously or in rapid succession, the entire display lasting from a few seconds to several minutes. If the level of stimulation rises rapidly, or if any individual has repeatedly experienced a given stimulus, then some or all of these behaviours can be abbreviated or bypassed altogether.

Largemouth bass did not respond to 0.79μM copper, which attracted goldfish and channel catfish (Timms *et al.*, 1972). Rainbow trout displays initial attraction to all lethal copper concentrations tested, with fish orientation towards the copper source (Pedder and Maly, 1985). The preference response was greatest at the highest copper concentrations tested, 0.05 and 0.06μM, which led to high mortality followed in survivors by a belated avoidance response. Hartwell *et al.*, (1989) studied the toxicity of five metals

(arsenic, cadmium, chromium, copper and selenium) and the avoidance responses of golden shiner, *Notemigonus crysoleucas*. They concluded that the most toxic metals may not elict an avoidance response. Selenium was not avoided at 44.2μM, a concentration approaching one half the 96h LC_{50} (concentration which kills 50% of the fish in 96h). Cadmium, the most toxic element tested in the golden shiner (96h LC_{50} = 0.03mM), was not avoided at 1.7μM, where as copper, the least toxic metal tested (96h LC_{50} = 1.33mM), was avoided at 0.41μM. McNicol and Scherer, (1991) reported that white fish *Coregonus clupeaformis* responded to concentrations of cadmium ≤ 0.01μM and ≥ 0.07μM. Similarly, *Oreochromis mossambicus* showed negative chemotropic orientation, immediately once the chemical was introduced in its natural acclimatized environment, before it got acclimated to the new environment to maintain homeostasis.

Detergents affect the feeding and locomotor behaviour of fish. Exposure to sublethal concentrations of branched alkylbenzene sulphonates impaired feeding behaviour of yellow bullhead and flagfish, *Jordanella floridae* (Foster *et al.*, 1966). *Plecoglossus altivelis*, avoided low concentrations but preffered high concentrations of linear alkylbenzene sulphonates (LAS) (Tatsukawa and Hidaka, 1978). Similarly Hidaka and Tatsukawa, (1989) observed that medaka, *Oryzias latipes*, avoided LAS at 10-30μg l^{-1} but not greater than 48μg l^{-1}. They speculate that the responses were due to adaptation to chemoreceptors to the lower concentration and to deterioration of detection ability, resulting from chemoreceptor damage at the higher concentration.

The range in avoidance concentrations determined for 18 fish species varies from 0.05 to 0.43mg l^{-1} total residue chlorine (TRC) (Cherry and Cairns,

1982). Salmonids are more sensitive, avoiding 0.05 to 0.10mg l^{-1} TRC, cyprinids as a group are more variable, showing avoidance thresholds as low as 0.05-0.2 mg l^{-1} by rosyface shiner, *Notropis rubella*, to as high as 0.210.43mg l^{-1} golden shiner. Fish avoidance of low concentrations of chlorine, Hidaka and Tatsukawa, (1985) is influenced by environmental factors such as acclimation temperature, salinity, pH, light intensity and combinations of these variables (Giattina and Garton, 1983). Similarly in the present study, it was observed, salinity concentration was directly proportional to fanning (fa), burst swimming (bs), nudge (nu), nip (ni), cough (co), motionless (mo). At 0.013ppt, fa=20%, bs=12%, nu=16%, ni=0%, co=0%, mo=0%. While at 41.012ppt, fa=100%, bs=100%, nu=100%, ni=100%, co=100%, mo=100% showing avoidance thresholds at higher concentrations.

Studies on the effect of pesticides on fish behaviour deal primarily with avoidance of lethal concentrations and have been summarized by (Sutterlin, 1974; Giattina and Garton, 1983; Hara *et al.*, 1983). Folmer, (1976) and Hidika *et al.*, (1984) found that fish will avoid many pesticides at sublethal levels but will not avoid others, even at lethal concentrations. Hall *et al.*, (1984) studied the avoidance response of striped bass, *Morone saxatilis*, and Atlantic menhaden, *Brevoorita tyrannus*, to an antifoulant material, bis (tri-N-butyltin) oxide. Atlantic menhaden and striped bass avoided concentrations of 5.5 and 14.7µg l^{-1} respectively, which were much higher than the highest recorded environmental concentration 0.92µg l^{-1} (Waldock and Miller, 1983). Therefore it is unlikely that these fish would avoid pesticide concentrations that they would normally encounter in the environment.

Adult female zebrafish, *Brachydanio rerio*, have a pheromonal sex attractant system that acts in attracting mature males to a spawning area. However, after nine days exposure to 0.08mM zinc, zebrafish failed to detect pheromone at concentrations that attracted control fish (Bloom *et al.*,1978). In a laboratory study Arctic charr accidentally exposed to > 19μg l^{-1} TRC failed to detect pheromone.

Santhakumar and Balaji, (2000) has shown two major behaviours, "hypoactivity" and "lethargy" exhibited by *Anabas testudineus*, an air breathing fish, exposed to different concentration of monocrotophos. Sharma *et al.*, (1983) has showed erractic swimming movements, followed by lethargy in *Clarias batrachus,* exposed to 0.25 to 2.00mg of malathion. Similar observations has been reported by Dutta *et al.*, (1994), in *Anabas testudineus* exposed to malathion. The lethargic condition that results due to chemical, would affect the fish in several ways. The results of the present study also indicate that, exposure of fish to various concentrations of sodium chloride in aquatic environment, brings about varid behavioural changes, or inability to continue a normal activity pattern for some time, which is necessary to search food and maintain homeostasis. Lethargy condition and no movement (motionless) behaviour exhibited, will not result in immediate death, but is a means, to bring about acclimation process to new environment.

Thus a variety of physiological and behavioural changes such as buoyancy, swimming ability and orientation occurs in *Salmo salar* and *Salmo gairdneri* during parr-smolt transformation, it seems likely that a variety of factors will be important in initiating migration (McCormic and Saunders, 1987).

4.3 OSMOSIS

The absolute amount of dry matter in a fish exhibits both inter and intraspecific variation. The percentage of dry weight and hence water content, is also variable. Following fertilization, egg water comes out under osmoregulatory control. The specific gravity of fish is inversely related to water content. Thus the buoyant nature of pelagic eggs is associated with higher water content relative to that of dense, less hydrated demersal eggs. The specific gravity of eggs and eleutheroembroys can decrease with time, eg. plaice, *Pleuronectes platessa*, Blaxter and Ehrlich, (1974), northern anchovy, *Engraulis mordax* (Hunter and Sanchez, 1976). These changes are a function of osmoregulatory adjustments and changes in the quantity and composition of yolk and tissues.

The percentage water content of yolk and tissue of various fishes, cannot be assumed to remain constant throughout development. The percentage water content of salmonid tissues has been found to increase during development Harvey, (1966); Escaffre and Bergot, (1984) or to remain unchanged (Peterson and Metcalfe, 1977). The documented changes are large in some cases eg. 20% increase in the relative water content of rainbow trout *Salmo gairdneri* yolk from hatch to complete yolk absorbtion, (Escaffre and Bergot, 1984).

Generally, at high density, low moisture yolk is converted into low density, high moisture tissues. There is decrease in yolk dry weight, and increase in tissue wet weight, and an increase in 'bulk water content eg. plaice *Pleuronectes platessa*, Blaxter and Ehrlich, (1974), suggested that the yolk of these species has a higher moisture content than the developing tissues.

Similarly in the present investigation it was seen that, there is decrease in tissue dry weight, compared to tissue wet weight, due to bulk water content in the tissues. Similarly water content was in the order of Large intestine > Liver > Gill filament, indicating intestine to play a very important role in water conservation during salinity stress. Similarly, at higher temperatures of summer, greater hydrodynamics were maintained by the tissues, as compared to lower temperature of winter, indicating the mechanism to maintain homeostasis by osmosis.

Plasma membrane permeability could vary considerably and inversely as a function of hydrostatic pressure. Temperature also is known to influence membrane permeability. Hansson and Lovtrup, (1974), pointed out that the effect of higher temperature can be encountered by an increase in plasma membrane tension as occurs when the egg is exposed to a more hypotonic medium.

Due to the catabolism of some yolk materials for energy production, the conversion of yolk to tissue is less than 100% efficient. As embryonic tissues and their associated maintenances costs grow, the amount of catabolic loss increases. This results in an increasing loss of bulk dry matter (yolk plus tissue). Tissue dry weight continues to increase, however, as yolk is absorbed and converted into tissues. As yolk reserves near exhaustion, the metabolic demands of maintenance and activity exceed the supply of yolk nutrients, and tissues begin to be catabolized for energy production. The resultant reduction in the body weight causes a maximum tissue weight to be reached before yolk absorption is completed. Wallace and Aasjord, (1984) found that Arctic char *Salvelinus alpinus* at 3^0C reached maximum tissue weight at complete yolk

absorption, whereby at 12^0C maximum tissue weight was reached with 1.5mg of dry yolk, remaining 12% of yolk reserves at hatching. The timing of maximum tissue weight, and hence the end of growth utilizing yolk alone, would appear to be influenced by temperature, occurring earlier in development at higher temperatures. This temperature dependency may reflect changes in maintenance costs or shifts in the metabolic rates of proteins and lipids mobilization from yolk. The temperature effect may also explain why a maximum tissue weight has not been evident.

The bulk wet weight of the embryo /eleutheroembryo increases during development despite the concurrent loss in bulk dry weight, because of the uptake of water. These early weight changes predicted that, dependent on metabolic costs, the bulk wet weight would reach a maximum before the tissue growth cycle was completed. Thus, towards the end of the endogenous nutrition period, eleutheroembryo wet weight can be expected to decrease despite a continued increase in tissue weight, the resultant maximum eleutheroembryo wet weight will be reached before the maximum tissue weight. Maximum eleutheroembryo wet weight is reached earlier in development when relative metabolic costs are elevated, such as at higher rearing temperatures Heming, (1982) or in smaller eggs of a given species (Smith, 1958; Escaffre and Bergot, 1984; Rombough, 1985).

While sodium is one of the ions most affected by a change of environment, the efforts of a fish to maintain a constant internal level are remarkable. An extreme case is to be found in *Tilapia mossambica*, which can survive over a wide range of salinities. Potts *et al.*, (1967), showed that although the ambient

sodium varies by a factor of 2500, the total body sodium is only 30% higher in double strength sea water than in freshwater.

An increase in tissue water during starvation is common to animals in general, and has for example, been observed in cockroaches Wharton *et al.*, (1965), mice Goldspink, (1966) and man, the latter either as a result of experiment Keys *et al.*, (1947) or in confinement in a prison camp Mollison, (1946). In the case of rats, however, there seems to be a slight decrease in body water when food is withheld for 168hrs, the animal having an unrestricted supply of water (Annegers, 1954).

In *Oncorhynchus nerka,* migration upstream, the water content of the whole body was observed by Idler and Bitners, (1958), to rise from 60% to 77%, but this was a 'fat water' relationship, and the sum of the two constituents remained at about 80%. In this species, the water content never seems to rise above 80% as it would be in non fatty species.

Values of 86% and 88% water have been measured in the muscle of starved *Gadus morhua* by Sutton, (1968) and Love, (1958) respectively, and Sorvachev, (1957), observed increased water content of *Cyprinus carpio* muscle by 88%, by starving them for three months in an aquarium at 12^0C to 15^0C. After 8 months at 20^0C the water content of these species has risen from 78.9% to 91%, Creach and Cournede, (1965), and that of *Carassius carassius* rose from 78.9% upto 93.3% after 105 days at 27^0C (Borek, 1958). The record appears to be held by *Hippoglossoides platessoides,* depleted naturally (Templeman and Andrews, 1956). These fish, found in the Great Bank area in water at -1^0C to 0^0C, become progressively more depleted as the size increases, and the muscle of the most 'jellied' specimen investigated

was found to consist of 2.83% protein, 0.06% lipid and the astounding value of 96.18% water. The above observations are in conformity with the present findings, which showed at 33±1^{0}C, maximum water content in controls, wherein gill filament showed 25%, in large intestine 40% and in liver 35%. While minimum water was seen at 20±1^{0}C, wherein gill filament showed 15.50%, in large intestine 35% and in liver 25%.

Survival in salt water after migration from fresh, appears to require increased metabolic activity in the liver. Isolated hepatic tissue from *Tinca tinca* and *Anguilla anguilla* was shown by Pequignot and Serfaty (1965), to consume more oxygen after the fish has been transferred from fresh to salt water. When *Cyprinus carpio* are acclimatized to various temperatures below 4^{0}C and 33^{0}C, the concentration of plasma chloride and calcium increases steadily with rise of temperature, and sodium rises to a maximum, at 27^{0}C (Houston and Madden, 1968). In *Petromyzon fluviatilis*, a reduction in temperature reduces the daily urine output and so presumably the permeability of the fish to freshwater (Wikgren, 1953).

Osmotic behaviour of marine fish at very low temperatures is evident due to an influx of ions, which can prevent them from being killed by freezing in polar waters. The blood of most fish freeze at about –0.8^{0}C, but sea water at the bottom of some fjords may be as cold as –1.7^{0}C or even less. If fish swimming in water are touched with ice-cube, they get into convulsions, stiffen and gradually become white as the ice propagates, although the surrounding water is not affected as seen in *Lycodes turneri*, *Liparis koefoedi* and *Icelus spatula* (Scholander, *et al.*, 1957). These workers found that fish

which usually inhabit the bottoms of cold fjords are supercooled, but are in little danger of freezing because the floating ice never reaches them.

The plasma of *Gadus ogac* from the same region freezes at $-1.47^{0}C$ in the winter time when the ice is about, but in the summer the plasma freezing point of both species rises to $-0.8^{0}C$ and with the onset of winter, Scholander and van Dam, (1957), suggested that non-protein nitrogen might help to prevent freezing in sub-zero sea water.

Elissen *et al.*, (1960) found that lowering the water temperature from $10^{0}C$ to $-1.5^{0}C$, causes a rise in the total plasma osmolarity and the chloride concentration of the plasma, both in the natural surroundings and in aquaria in *Cyclopterus lumpus*, *Anarrhichas minor*, *Drepanopsetta platessoides*. Umminger, (1968) acclimatized *Fundulus heteroclitus* to $20^{0}C$, $10^{0}C$, $2^{0}C$ and $-1^{0}C$, and found after a time at $2^{0}C$, there is 41% increase in the serum nitrogen, 44% in calcium, 73% in cholesterol, and the glucose, well known as an anti-freeze agent, shows no less than a 119% increase.

The water content of *Gadus morhua* increases during period of natural depletion, but if well-fed fish are staved artificially in an aquarium, the water content does not in fact rise until several weeks have elapsed. This is presumably because the fish at first consumes lipids from the liver, and starts to mobilize muscle protein only when this source of energy has been nearly used up. After that, as protein is utilized, water moves in to take its place (Love, 1970 unpublished).

Oncorhynchus kisutch showed branchial epithelium of typical teleostean population of mitochondria-rich MR cells which showed high levels of $Na^{+}K^{+}$-ATPase activity and these freshwater Cl^{-} uptake cells are lost in the sea water

acclimated fishes. *Salmo salar M.* showed decreased muscle lipid content, elevated gill Na^+K^+-ATPase activity, hypo-osmoregulatory ability and high muscle water content in freshwater. In Atlantic salmon, smolting and acquisition of salinity tolerance is under endocrine control. Growth hormone and cortisol have been found to increase gill Na^+K^+-ATPase activity and salinity tolerance. The morpho-physiological adaptations which permits tunas to achieve maximum metabolic rates MMR that are several times greater than those of other fishes, results in high water and ion flux rates across the gills and concoment high osmoregulatory costs. Thus high energy demanding fishes i.e., tunas and dolfin fish, has high gill and intestinal Na^+K^+-ATPase activity, to compensate for high rates of passive ion and water movements occuring across their exceptionally large, thin gills. Similarly gill and intestinal tissue from hybrid red tilapia (*Oreochromis mossambicus* x *Oreochromis niloticus*) showed increased Na^+K^+-ATPase activity, where ATPase activity per unit mass of intestinal tissue was higher in *tilapia* than gill tissue. Increase in gill H^+-ATPase activity in rainbow trout *Oncorhynchus mykiss* is known to accompany hypercapnic acidosis, which may reflect on reduced rate of Na^+uptake across the gills. Anadromous populations of Arctic charr, *Salvelinus alpinus* and Atlantic salmon, *Salmo salar* showed a pronounced seasonal pattern in sea water ability where the hypoosmoregulatory ability was high during spring to early summer followed by sharp decrease in seawater tolerance in late summer (Schmitz, 1992). The findings that landlocked Arctic charr from Northern Sweden improved their sea water tolerance from May to June, followed by marked decrease in August, agrees well with earlier studies on the Hornavan strain, (Schmitz, 1992; Schmitz and

Mayer, 1993). The loss of seawater performance in charr and salmon coincided with high ambient water temperature, which has a negative effect on seawater adaptability (Hoar, 1988). Finstad, (1989) observed marked decrease in sea water adaptability in charr in September, although water temperature was kept constant at 8^0C throughout summer and autumn, supporting the idea of a seasonal pattern in sea water adaptability. Evropeytseva, (1963), found that landlocked salmon from Lake Ladoga developed improved sea water adaptability during spring, whereas the sharp decline in hypoosmoregulatory ability during summer. Similarly the hydro-osmoregulation was maximum in the present study, during March to May and minimum during November to December which is in conformity with the above.

The smolting process in salmonids is temporally directed by photoperiod and to a lesser degree by temperature (Wagner, 1974). Smolt transformation can be shifted by several weeks by manipulation of these variables (Komourdjian *et al.*, 1976a,b; Saunders and Henderson, 1978; Wagner, 1974). In *Salmo salar L.*, DN (Natural rearing regime) fish had the typical smolt characteristics, a decrease in lipid and an increase in water content, while group DD (didromous) fish had the opposite responses of increased lipid and decreased water content (Barbour and Garsidae, 1983). However when the photoperiod was set in phase, both groups show a decrease in lipid and an increase in water content between days 50 and 170 after their respective winter solstices. The smoltification response of the freshwater salmon FD differed from that of the DD. When both the groups were reared in the delayed seasonal regimes of light and temperature, between days 50 and

170 after their respective winter solstices, lipid decreased and water content increased in group DD, the response typical of smoltification. However, group FD did not exhibit these changes, but maintained relatively constant lipid levels and slightly decreased water content, stating diadromous and freshwater salmon smolts respond differently to the same environmental stimuli.

The metabolism of animals increases when they are removed from their normal salinity and placed in a stressed medium (Nagabhushanam and Kulkarni, 1976). The freshwater salmon had a weaker tolerance to a 30% salinity in comparison with DD salmon. This can probably be attributed to the warmer temperatures, which may have inhibited Na^+K^+-ATPase activity (Saunders and Henderson, 1978). Reduction in smoltification is the result of an interruption in an endocrine link between the growth hormone postulated by Komourdjian *et al.*, (1976b), as the target organs of smoltification. Salinity tolerance Atlantic salmon *Salmo salar* alevins decreases as the water impermeable vitelline membrane decreases in favour of a water permeable epithelium (Parry, 1960; Talbot *et al.*, 1982). High level of gill Na^+K^+-ATPase activity, which for anadromous smolt, *Salmo salar* is considered to be the necessary preadaptive mechanism for coping with increase in salinity on entering the sea (Saunders and Henderson, 1978; McCormick *et al.*, 1987; Duston and Saunders, 1990).

Size, not age, is the primary determinant of increased sea water survival for steelhead *Salmo gairdneri* and brook trout *Salvilinus fontinalis*, which may be due to more favourable surface to volume ratio for larger fish, or to a progressive development of hypoosmoregulatory mechanisms with size, at

which, sea water survival occur in smallest for *Oncorhynchus* species, larger for *Salmo* species, and largest for *Salvelinus* species (McCormick *et al.*, 1987). Rainbow trout do not respond to seasonal cues with increased salinity tolerance until they are atleast 10cm long (Conte and Wagner, 1965). Considering the size dependent, degree of salinity tolerance, Atlantic and Coho salmon parr of 10-12cm can routinely tolerate (survive for many days) a salinity of 30‰ (Saunders and Henderson, 1969; Clarke and Nagahama, 1977). Smolt size 14-17cm Atlantic salmon that are denied seasonal cues through exposure to continuous light, can adapt 30‰ to seawater, but cannot survive in 40‰ as can normal smolts, and exhibits poor feeding and growth in sea water (Saunders, 1985; McCormic *et al.*, 1987). Increased drinking rate and absorption of water and salts across gut epithelia occurs following adaptation of euryhaline teleosts to seawater, as in present investigation. Collie and Bern, (1982) found that the capacity for net fluid absorption of the intestine increased twofold in freshwater adapted juvenile Coho salmon between March and May, and the high values in May were similar to those of salmon adapted to seawater. Reversion of intestinal net fluid absorption to prespring levels occurred in early autumn in fish held in freshwater.

Smolting and maturation are incompatible process, and low level of circulating testosterone inhibit smolting (Brit and Green, 1993). Smolting does not occur in intact, previously mature masou salmon, *Onchorhynchus masou* paar, yet castrated fish show clear evidence of smolting (Aida, 1984).

Landlocked salmon, *Salmo salar L.*, derived from wild population of stunted ouananiche and bred in the laboratory showed signs of smoltification, but adaptation to sea water was slow and limited to 11% of the fish tested.

Seawater survival was not related to initial size, within the range used, nor the sex, age (2+ or 3+ years) or spawning history. Freshwater ouananiche had fewer gill chloride cells than anadromous smolts of similar size in freshwater. Anadromous smolts showed an increase in chloride cells after adaptation to seawater and those ouananiche which successfully adapted to sea water had a great increased number of gill chloride cells (Burton and Idler, 1984).

Anadromous salmon *Salmo salar* grew more rapidly than nonanadromous salmon in the spring during the smolting period. Differences were not observed in seasonal levels of body moisture, plasma sodium and chloride, condition factor or salinity tolerance. Difference noted between groups are probably genetic in nature and suggest that the two forms of salmon in Gambo river represent separate breeding population (Brit *et al.*, 1991). In several salmonids, decreased condition factor is typically associated with smolting (Hoar, 1939; 1976; Farmer, 1978). Water content varies inversely with lipid content in fishes (Phillips, 1969). An elevated water content is usually observed in smolting salmon exhibiting decreased lipid contents. These changes are usually considered to result from metabolic activation and reorganization associated with expression of adaptations, appropriate for life in the marine environment. Smolting salmon typically exhibit a seasonal decrease in condition factor and increasing water content (Brit and Green, 1986).

The water content of muscle and liver of *Oreochromis mossambicus* and *Etroplus maculatus* of effluent treated fish showed significant increase when compared with the controls and there existed a positive correlation between water content of fish and the concentrations of the effluents used. The water

contents of the liver of both species of treated fishes were higher than that of muscles (Vijayamohanan and Nair, 2000). Similarly in the present investigation, water content showed significant increase over control. Water content in liver was greater than gill filament tissue and showed a positive correlation with the chemical used. Larsson *et al.*, (1980), reported that there is a leakage of sodium and chloride from extra cellular fluids of the exposed fish to the surrounding water and /or a decrease in the uptake of these ions through the gills on chemical exposure on flounders.

In the muscle, liver (female) and gonad of *Punctius filamentosus* (Val.) the variation of lipid and protein showed inverse relationship with the variation in water content. But the water content of the liver of male showed a direct relationship with lipid and protein content (Vijayakumar, 1987). Milroy (1908), observed a rapid rise in the percentage of fat in the tissue of herring prior to the rapid growth of ovary and testis and further decline in fat content at spawning with a simultaneous increase in water content.

Water content remained at a high level during Feb. to June. Protein content remained relatively high throughout the year with a decrease during breeding and maturation period (Nagabhushanam and Talikhedkar, 1977).

In the present investigation, a general trend is observed in gill filament, large intestine and liver tissue. There was increase in water balance with increasing smoltification. Water content was maximum in all three tissues at $33\pm1^{0}C$ and minimum during $20\pm1^{0}C$. This direct correlation between water content and salinity concentration could be due to accumulation of water in various tissues due to active uptake from the medium to maintain homeostasis or due to the interaction of the chemical with the neurosecretory

function of cerebral ganglia, which is known to regulate the hydrodynamics of osmoregulation. The present study is in conformity with all above observations.

4.4 RESPIRATION

Among the teleost fish, the air-breathing (AB) species are more than 140 in number and are found in 16 families (Bertin, 1958). However, a recent taxonomic survey reveals that there are currently over 370 known AB fish species occurring among 125 genera in 49 families. Fish bimodal respiration has likely evolved independently atleast 38 times and AB has appeared as many as 67 times (Graham, 1994). Direct measurement of oxygen consumption is difficult to assess because of the relatively high individual variation, dependence on temperature and size, and differential response to handling stress or confinement (McCormick and Saunders, 1987).

Pamila *et al.*, (1991) reported that, exposure of *Sarotherodon mossambicus* to sublethal concentration of chromium, elicted unequivocal changes in oxygen uptake for the first five days during exposure. The increased respiration suggests a stress induced phenomena. The above finding are in conformity with the present results, where *Oreochromis mossambicus* showed initial increase at 10ppt (6 hr.) exposure and further decrease at 10ppt (12 hr.) exposure to combat the chemical stress, to maintain homeostasis.

Maximum rates of consumption in active free swimming skipjack (2.5mg O_2/g/h) are more than twice as great as those reported for other teleosts (Stevens and Carey, 1981). The Asian catfish, *Clarias batrachus* consumes more oxygen from air (58.4%) than from water (48.6%) (Singh and Hughes,

1971). *Heteopneustes fossilis* obtains more than (60%) of oxygen from water than from air (40%) but in hypoxic water this ratio is reversed (Hughes and Singh, 1971).

In *Lepisosteus* the oxygen of the lung at 22^0C is about 73% of total oxygen demand Rahn *et al.*, (1971) and *Lepidosiren* 96% at 20^0C (Johanson, *et al.*, 1970). Hughes and Singh, (1970), reported that specimens of Anabas weighing about 29-51gm consume 53.6% of their total oxygen requirement through air breathing organs. In asphyxiation time when not allowed to breathe air, varies with the size of the fish, and as the body size increases, the asphyxiation time increases.

The average oxygen uptake from water is 105mg $h^{-1}kg^{-1}$ and 69 mg $h^{-1}kg^{-1}$ from air for *Hoplerythrinus*, 65 mg $h^{-1}kg^{-1}$ for water and 51 mg $h^{-1}kg^{-1}$ from air for *Erythrinids*. In normoxic water, *Hoplerythrinus* obtain from 0 to 56% of oxygen uptake (mean 31%) from air (Stevens and Holeton, 1978). *Hoplosternum thoracatum* and *Brochis splendus* are continuous but not obligate air breathers. Aquatic respiration in *H. thoracatum* decreased in hypoxic water (Gee and Graham, 1978).

Channa gaucha obtain 79% oxygen from air and 21% from water. In juvenile *Channa marulius*, of the total oxygen uptake, 136.1 + 8.92ml of oxygen kg^{-1} h^{-1}, about 82% (113.27+9.63ml of oxygen kg^{-1} h^{-1}) is contributed by air breathing organs and only 18% (22.83+2.27ml of oxygen kg^{-1} h^{-1}) by gills (Ojha *et al.*, 1979). The central mud minnow, *Umbra limi*, is a continuous facultative air breather whose respiration is primarily aquatic in normoxic water and primarily aerial in hypoxic water (Gee, 1980). Air breathing *Channa*

argus out of total oxygen uptake, about 60%-85% satisfied by air breathing in normoxic and hypoxic water (Itazawa and Ithimatsu, 1981).

Baraduc and Fontaine, (1955) found resting, weight specific oxygen consumption of wild Atlantic salmon parr at 8^0C was 25% lower than for wild smolts. Powar, (1959) working with Atlantic salmon from Arctic environment, found a temperature divergence in oxygen consumption than parr below 13.5^0C, but higher oxygen consumption above this temperature. Higgins, (1985) reported oxygen consumption as a function of differential growth and the parr smolt transformation in Atlantic salmon. Rapidly growing fish had a higher oxygen consumption at 7.5^0C than slower growing fish. Smolts (based on external appearance) had lower weight specific oxygen consumption than nonsmolts. Withey and Saunders, (1973) found that postsmolts Atlantic salmon had higher rates of oxygen consumption than nonsmolts.

In normally aerated water, at 20^0C gar, *Lepisosteus* oculatus accounted for 42% of their oxygen uptake from lungs, while in hypoxic water, entire oxygen uptake was from lungs and oxygen was not lost through gills. Elevation of temperature from 20^0C to 30^0C increased pulmonary ventilation (Smatresk and Cameron, 1982). Reed fish *Erpetoichthys calabaricus* acclimated to 33^0C had increase lung breathing compared with fish acclimated to 25^0C (Pettit and Beitinger, 1985). In *Notopterus chitala*, out of total oxygen uptake (131.1 ml oxygen /kg /hr) about 70% obtained through aerial route and remainder 30% through gills (Ghosh *et al.*, 1986). *Cebidichthys violaceus* consumed 0.54 ± 0.04 mg oxygen in water, which was significantly ($P<0.05$) more than the 0.041 ± 0.03 mg oxygen /hr consumed in air (Edward and Cech, 1990). Aerial respiration by rocky intertidal fishes of California and Oregon showed wide

variations though all species can respire aerially for extended periods of time (Yoshiyama and Cech, 1994). *Channa striata* ranging weight from 23-93gm extracts more oxygen from air 73% than water 27% (Rani, 1994).

Nitrogen bubbled in aquarium for 20mins reduced the water oxygen content to less than 60mm Hg, but this did not induce aerial respiration in *Saynbranchus marmoratus* (Johansen, 1966). Hypoxic conditions did not elevate aerial respiration in *Electrophorus* (Johansen and Lenfant, 1968). Hypoxic waters reduced the oxygen consumption in *Heteropneustes fossilis* (Hughes and Singh, 1971). When *Clarias* is kept in hypoxic waters with access to air breathing prevented, the gill breathing increases but the total oxygen uptake is reduced (Singh and Hughes, 1971). During progressive hypoxia, the frequency of air breathing increased more steeply as water oxygen content dropped below 100mm Hg in *Amphipnous* (Lomhalt and Johansen, 1974). Aerial respiration is 70% in hypoxic water in *Piabucina festae* (Graham, *et al.*, 1977). *Hoplerythrinus unitaeniatus* and *Erythrinids* breathe air in hypoxic water. Gill ventilation increases in hypoxic water if air breathing is prevented, but decreases if air breathing is permitted (Stevens and Holeton, 1978).

Channa argus surfaced at very irregular intervals both in normoxic and hypoxic water. In normoxic water 90% or air breathing intervals ABI ranged from 2.5mins, but in hypoxic water ABI became less variable, that is 90% of them distributed below 12.5mins (Itazawa and Ithimatsu, 1981). In *Ancistrus* and *Hypostomus*, air breathing is primarily facultative process to aquatic hypoxia (Graham and Baird, 1982). Aquatic respiration rate of *Clarias mossambicus* declined to 43% of the value for fish kept in aerated water

during hypoxia acclimation. 8hr hypoxic submergence increased the role of ABO 73% than gills 27% in *Channa striata* (Rani, 1994). Long term (150 days) hypoxic exposure reduced the aerial, aquatic and total respiration in *Notopterus notopterus* (Vijayalaxmi, 1996).

In *Synbranchus*, neither increased carbon dioxide content in the surrounding water nor the low pH (3.9) seems to accelerate the branchial water pumping (Johansen, 1966). *Electrophorus electricus* was irresponsive to carbon dioxide was 35mm Hg (Johansen and Lenfant, 1968). Carbon dioxide concentration between 1-2% in water caused a depression of water breathing in the Australian lung fish, *Neoceratodus* (Johansen *et al.*, 1967). In *Amia*, a bimodal breather carbon dioxide concentration upto 3% stimulated both branchial and aerial breathing. Where as higher concentrations depressed branchial breathing but further stimulated air breathing (Johansen *et al.*, 1970). In general, air breathing is increased and branchial respiration changes little in other air breathing fish exposed to hypercapnia. Gill ventilation when measured fell slightly for *Piabucina,* Graham, *et al.*, (1977) and *Heteropneustes,* (Hughes and Singh, 1971). Long term exposure to intermediate or high levels of hypercapnia can severely disrupt acid base balance and gas transfer across fish gills (Heisler, 1968). However the consequence of exposures to high levels of carbon dioxide for rates of oxygen consumption are disputable (Takeda, 1991). When combined with hypoxia, hypercapnia may add a further constraint on the oxygen demand and might hamper performance (Jensen *et al.*, 1993). Total respiration decreased by 28.57% in *Channa straita* exposed to 24hr hypercapnia conditions (Rani, 1994).

At 10^{0}C, A*mia* is relatively inactive and is exclusive water breather. With increasing water temperature and activity, the rate of oxygen depletion from air bladder decreases progressively, and their AB rate increases. At 30^{0}C, three times as much oxygen is taken from air as from water (Johansen, *et al.*,1970). Although many investigators have measured air /water partitioning in fishes, Natarajan, (1987), surprisingly few studies have addressed the role of temperature upon the bimodal respiration with lower water temperatures, the breathing rate (intervals) increased greatly, but the rate of increase is out of proportion to the anticipated change in the metabolic rate, suggesting that the gill function can adequately handle the oxygen uptake in gar fish *Lepisosteus osseus*. Rahn *et al.*, (1971). Then one might describe the gar as a fish that is a true gill breather at lower temperatures but which becomes more and more dependent upon aerial respiration as metabolic rate and water temperatures increases. In the sense, the gar is a facultative air breather, which can become an obligatory air breather under environmental stress.

Mud minnows *Umbra limi*, Gee, (1980) breathe air more frequently in hypoxic water at higher temperatures. In *Heteropneustes fossilis* aquatic breathing increases more rapidly than aerial breathing during rainy season (25^{0}C). However, during summer the fish relies more on air breathing (Munshi *et al.*, 1982). Elevation of temperature from 20^{0}C to 30^{0}C increased pulmonary oxygen consumption in *Lepisosteus oculatus,* (Smatresh and Cameron, 1982). Increase in temperature elevated aerial respiration in *Macropodus cupanus* (Natarajan, 1987). At 25^{0}C, *Channa striata* depends more on ABO (60%) than on gills (40%). However at 35^{0}C the fish relies more on aerial sources (69%) for respiration than on gills (31%) (Rani, 1994). At

20^0C, *Notoptererus notopterus* is increasingly aquatic (60.7%) than aerial (39.3%) (Vijayalaxmi, 1996). Compared to vast information available on obligatory air breathing, and the factors that controls the same, only very little is known about the facultative air breathing and its control (Takkasusuki *et al.*, 1998). In *Tiriopus brevicornis*, V-O_2 increased with increasing temperature over the range 5 to 30^0C. However, at 0 and 35^0C the rates of oxygen consumption was significantly lower than predicted. It appears that *Tiriopus brevicornis* is able to withstand exposure to low temperature and to serve hypoxia by entering a quiescent or dormant state during which its metabolic rate is significantly reduced (McAllen *et al.*, 1999).

In the present study circadian bimodal oxygen uptake, by *Oreochromis mossambicus*, for S.T.T. of fish weight ranging from (5.3-6.2g), (10-15.5g) and (22-25.5g), summer and winter temperatures, after 2 and 3 days, were studied in normoxic waters. It was observed that peak bimodal uptake averaged at 03:00hr. (maximum) early dawn, and 15:00-18:00hr. (minimum) early evening and evening, which may be due to the following reasons. Similar findings has been discussed below. It could be concluded that gill respiration is dominant over air bladder and hence facultative respiration was exhibited.

The mud eel, *Monopterus cuchia,* Patra *et al.*, (1978) exhibits circadian rhythm in the bimodal oxygen consumption. The peak hours of oxygen uptake were dusk (16-18hr) and the dawn (04-06hr). During all the periods, oxygen uptake through air breathing organs dominated over aquatic breathing. *Channa marulius* showed higher oxygen uptake during midnight, *Channa striata* and *Channa gachua* during early parts of night and *Channa punctata*

during dusk. In all species, the lowest rate of oxygen uptake was recorded at noon and during this period gill breathing dominated over aerial breathing (Munshi *et al.*, 1979).

The diurnal rhythm of oxygen consumption of *Anabas scandens* Reddy and Natarajan, (1970) shows two peaks, one around midnight and the other in the early hours of the day when the fish depends more on atmospheric air. In *Lepidocephalus thermalis,* Natarajan, (1981), the variation in oxygen consumption during different hours of the day may be due to the obligate air breathing habit, as the fish depends more on air breathing and their gills play a secondary role in oxygen consumption. In *Lepidocephalus thermalis,* Natarajan, (1984) the diurnal rhythm in oxygen consumption reveals two major peaks, one occurring at around midnight and the other in the early hours of the day. The oxygen obtained by air breathing organs is also maximum at these times. Minimum oxygen uptake is recorded during 12.00hr.

It is interesting to correlate the respiration rhythms in laboratory with that in the natural ecosystem where these fishes live. It has been recorded that the habitats of the air breathing fishes are often carbon dioxide rich swampy regions and is such senescent areas dissolved oxygen becomes practically nil and under such diverse ecological circumstances air breathing fish comes out of their niches to rest on the moist banks and rely on aerial breathing (Hora, 1935). In *M. cupanus,* Natarajan, (1987), exhibits a clear cut respiratory rhythm. Both juveniles and adult fishes exhibited a unimodal rhythm of maximum total oxygen uptake at 06.00hr.

The facultative air breathing *Notopterus notopterus,* Vijayalaxmi, (1996), shows a definite circadian rhythm of maximum oxygen uptake during

midnight. In *C. striata* Rani, (1994) a clear cut respiratory rhythm with the maximum oxygen uptake through bimodal oxygen exchange machinery is recorded at 18hr. Juveniles are more aquatic. *M. gulio* in the weight range 3.5 to 6.2gms display one major peak at 03.00hr.,10.5 to 15.3gm fish extracted more oxygen in early hours 03.00hr., fish in the weight range 22.2 to 24.6gm altered its unimodal peak to 18.00hr. Gill respiration is dominant at all times and it is strongly rhythemic.

Aquaculturists have routinely observed the feeding and behavioural responses of their fish in the morning and evening to evaluate fish health and activity. In the *Hoplesternum littorale,* Boujard *et al.*, (1990) respiratory activity progressively increased throughout the night and then fell abruptly at dawn. The European catfish, *Silurus glanis,* Boujard, (1995) shows strong nocturnal feeding activity. Oxygen consumption of catfish *Ictalurus punctatus* was generally highest in the first 12h after feeding (Jarboe, 1995). DeSilva *et al.*, (1986) recently reported no significant difference in earlier routine or standard (anesthetized) metabolic intensities of larvae of the euryhaline species *Oreochromis niloticus* reared at salinities between 0 and 18‰.

In the present investigation, with respect to aquatic respiration, it is observed that, with increase from 10ppt to 20ppt, resulted in initial increase at 10ppt (6 hours) followed by decrease at 10ppt (7days) and 20ppt (7days) over control, which is significant at $P<0.05\%$ level of significance. This is mainly due to increased or decreased need of organism to maintain homeostasis. Similar discussion is as follows.

Abrupt changes in salinity do affect rates of oxygen consumption. A 10fold increase in oxygen uptake by anesthetized herring embryos and larvae

abruptly transferred from 35‰ to 5‰. Rates gradually declined to the pre transfer level over a period of 6-8hr. Further, the period of elevated metabolism coincided with the length of time required to restore osmotic imbalance brought about by the abrupt change in salinity. The pattern of oxygen uptake in un-anesthetized larvae was quiet different. Transfer from 35‰ to both 15‰ and 5‰ resulted in a decrease in metabolic rate followed by a very gradual increase over the next 24hr to values typical of constant exposure to 35‰. (1964), attributed the initial reduction in $V\text{-}O_2$ to reduced activity associated with buoyancy changes (Holliday *et al.*, 1964).

Almater, (1984) similarly reported a reduction in oxygen uptake rates of herring and plaice yolksac larvae, transferred from seawater 32‰ to low salinities 5 and 12.7‰. In feeding larvae, however, metabolic rates were elevated on transfer to 12.7‰ compared with constant control larvae 32‰ and with larvae transferred to 5‰ and 40‰. Almater, (1984) attributed the apparent increase in $V\text{-}O_2$ to the fact that the larvae used in the 12.7‰ test were somewhat smaller than those used in the other tests.

Temperature variations within a narrow range 3 to 5^0C appear to have little effect in many species (Siefert *et al.*, 1974; Carlson and Siefert, 1974; Carlson *et al.*, 1974). There are some exceptions though. Increasing the temperature from 7 to 10^0C resulted in a significant increase in the sensitivity of young lake trout *Salvelinus namaycush* (Carlson and Siefert, 1974). Similarly, Arctic char embryos and larvae were less tolerant to hypoxia at 8 than at 4^0C (Gruber and Wieser, 1983). Both species are stenothermal, and in both studies the higher temperatures was near the upper limit of the zone of tolerance. Lethal levels for embryonic, Brooke and Colby, (1980) and larval Einsele, (1965)

coregonids increased with temperature, but again increases, were greatest at the higher temperatures. These results suggest that lethal oxygen concentrations may be relatively independent of temperature within the normal temperature range of a particular species but that of higher temperatures, there is a strong likelihood of an additive or synergistic interactions.

Salinity, like temperature, appears to have little effect on hypoxic tolerance within normal limit. Alderdice and Forrester, (1971) indicated that viable hatch of Pacific cod *Gadus macrocephalus* was largely independent of oxygen, provided levels were above 2-3mg l^{-1} within the optimal range of temperatures 3 to 4.5^{0}C and salinities 17 to 23‰. Oxygen requirements tended to increase at higher salinities. Similarly, embryonic survival of pilchard *Sardinops ocellata* was largely independent of oxygen levels greater than 2.1mg l^{-1} within the optimal range of temperature 16 to 21^{0}C and salinity 33 to 36‰ (King, 1977).

Marine species are generally less tolerant than freshwater species. Among marine species, De Silva and Tytler, (1973) attributed the greater resistance of newly hatched herring larvae compared with newly hatched plaice larvae to the fact that the former hatch found demersal eggs and thus more likely to encounter low oxygen levels. A glaring deficiency in the literature is the absence of any data on the hypoxia tolerance of tropical freshwater species, some of which spawn in virtually anoxic water.

Durborow and Avault, (1985) reported significant differences among full sibfamilies of channel catfish *Ictalurus punctatus* in larval resistance to hypoxia. This raises the possibility of selecting strains that are resistant to hypoxia for the use in aquaculture. Shepard, (1955) demonstrated that

acclimation to low oxygen levels increased the ability of juvenile brook trout to tolerate hypoxia. McDonald and McMohan, (1977,) reported that chronic exposure of Arctic char larvae to low oxygen 2.5mg l^{-1} compared with normoxic reared larvae.

Moderate levels of hypoxia have been reported to enhance survival of embryos, Gulidov, (1969); Gulidov, (1974); Gulidov and Popova, (1978) and larvae (Sylvester *et al.*, 1975). At higher concentrations (>300% air saturation), though, oxygen becomes toxic. Species vary in their ability to tolerate hypoxia. Gulidov, (1969) reported significant mortality in pike *Esox lucius* eggs incubated in 36.4mg l^{-1} (336% ASV). No pike embryos hatched in 45.3mg l^{-1} (418%ASV), apparently because of suppressed neuromuscular activity. Embryos of verkhovka *Leucospuis delineatus,* Gulidov, (1974) and roach *Rutilis rutilis* Gulidov and Popova, (1978), on the other hand both hatched successfully in about 40mg l^{-1}. In *Leucospuis delineatus*, hatching was delayed at high oxygen concentrations. Newly hatched larvae tended to have more body segments than normal, and no blood cells were present. The initial phase of erythropoeisis was not inhibited, and red blood cells appeared in circulation at about the normal time. The number of erythrocytes later declined, and red cells were absent at hatch. Absence of red blood cells did not adversely effect survival, which is not surprising given the high ambient oxygen concentration. Gulidov, (1974) linked the high tolerance of *Leucospuis delineatus* and roach to hypoxia to the fact that their eggs are frequently laid on vegetation and may thus be exposed to high oxygen concentrations in their natural habitat.

While a change in temperature usually alters the rate of metabolism in fish, an internal adjustment may take place to minimize the effect and keep the metabolism as near normal as possible. Stroganov, (1956), noted that *Gambusia holbrooki* which have adapted to low temperatures consume more oxygen than well adapted fish do when both groups are brought to an intermediate temperature. Scholander *et al.*, (1953), showed that the oxygen consumption of Arctic fish at their usual environmental temperature is only 4 to 10 times lower than that of tropical species at their normal temperature, if one extrapolates the values from the tropical forms down to 0^0C, it is clear that if the fish survived there would be a 30 to 40fold decrease, so the Arctic fish must modify their metabolic system.

Wohlschlag, (1963) showed that *Rhigophila dearborni*, an Antartic species from deep waters of the Mc Murdo Sound, has higher oxygen uptake at low temperatures than would be expected from studies in warmer waters, and Winberg, (1961) observed that fish habitually living at around 30^0C have an unexpectedly low rate of metabolism. Inspite of these modifications, fish in warmer waters do require more oxygen, and since less is available in solution, the oxygen capacity of the blood is improved by increasing the number of red cells per unit volume, and also the amount of haemoglobin in each cell, DeWilde and Houston, (1967) in *Salmo gairdnerii*.

Field and Peiss, (1949) showed that at every temperature studied, the oxygen consumption of the Arctic species is the greater of the two, and that below 10^0C, the rate of consumption in the temperate fish shows an additional retardation, that is, the curve becomes steeper, while that of the polar fish

shows a constant slope from 25^{0}C right down to 0^{0}C. Both the features in the Polar cod would be advantages in a cold environment.

4.5 EXCRETION

Most of the earlier investigations on the influence of ambient temperature on ammonia-N and urea-N excretion of fish, like those of Guerin-Ancy (1976) on *Dicentrarchus laboux*, Ray and Medda, (1973) and, Das and Das, (1985) on *Channa punctatus*, Arya (1979) on *Heteropneustes fossilis*, Jobling (1981) on *Pleuronectes platessa* and Das and Das, (1985) on *Labeo bata* etc., were not based on endogenous nitrogen excretion (ENE). ENE represents the protein maintenance level or the amount of protein (nitrogen) required by the fish to maintain nitrogen equilibrium (Gerking, 1955; Birkett, 1969; Savitz, 1971). It is often measured by the nitrogen excretory rate following a short term food- deprivation (Kaushik, 1980; Roy and Das, 1986).

On land the AB fish have to avoid built up of ammonia concentration to toxic levels. Many tropical air breathing teleost shift towards ureotelism when subjected to air exposure, Gorden *et al.*, (1969), (1970), (1978); Ramaswamy and Reddy, (1983); Natarajan, (1987); Rani, (1994); Vijayalaxmi, (1996), the waste nitrogen being accumulated in the body and released on return to water. *Oreochromis alcalicus grahami* excretes all nitrogenous wastes as urea (Randall *et al.*, 1989) and when exposed to neutral water, urea excretion was completely inhibited. In toadfish hepatocytes, a decrease in extracellular bicarbonates reduces the rate of urea synthesis, Walsh *et al.*, (1989). Nitrogenous waste in Sangana tilapia were predominantly ammonia, but during alkaline exposure, urea excretion increased three fold Randall *et al.*,

(1989); Wood *et al.*, (1989) with no change in plasma bicarbonate but an increase in plasma ammonia.

Increasing salinity increases the total nitrogen excretion in *Mystus gulio*. When exposed to sodium chloride stress this fish switches over to some adjustment in nitrogen excretion, which remains to be studied (Raveendran, 2000). However, high pH exposure leads to inhibited ammonia excretion Wilkie and Wood, (1994); (1995); (1996), and the disruption of internal acid base and electrolyte balance. Wilkie and Wood, (1995) reported an inhibition of ammonia excretion in rainbow trout by raising water pH from 8 to 9.5, air breathing fishes are opportunists. They can modulate excretion to the changing environmental conditions. Fresh water tilapia *Oreochromis alcalicus grahami* Wood *et al.*, (1994) shifts its nitrogen synthesis in tune with the environmental stress, pH, salinity and water temperature. Similar altered nitrogen excretion was observed in sturgeon (Gershanovich and Potatskiji, 1995). The amphibious air breathing fish, *Peripthalamus modestus* excretes more urea than ammonia (Iwata and Deguchi, 1996).

Teleosts are primary amminotelic, excreting ammonia as the major nitrogen excretory product by diffusion predominantly through the gills (Forster and Goldstein, 1969). However, an air breathing fish with limited gill ventilation is expected to restrict excretion of ammonia, since ammonia influences internal processes, such as blood pH and therefore elimination would be preferable (Campbell, 1973). Air breathing fishes can tolerate ammonia accumulation (Morii *et al.*, 1978) or they can shift to urea production and accumulation (Gordan *et al.*, 1969; 1978). Although majority of teleost fish

are amminotelic, urea also constitutes about 10 to 30% of the total nitrogenous wastes in most species (Wood, 1993; Saha and Ratha, 1989).

The above findings are in conformation with the present study, where *Oreochromis mossambicus*, a well known exotic teleost, excretes more ammonia and lesser urea in control. But the ammonia excretory rate decreased in the treated groups, which could be due to less mobilization of nitrogenous fuel, required to maintain homeostasis or conversion of highly toxic ammonia which requires plenty of freshwater for its elimination, into urea, a lesser toxic nitrogen end product, through ornitine urea cycle. Also, at 10ppt (LTT), 20ppt (MTT) and 20ppt (LTT), urea excretion increased with respect to control, and at 20ppt (LTT), it was roughly double the control values, which indicates increased proteolysis, further conversion of ammonia to urea.

An amphibious style causes difficulties for nitrogen metabolism. Many air breathing teleost are facultatively ureotelic when exposed to apparently stressful environmental conditions such as air exposure, Ramaswamy and Reddy, (1983); Natarajan, (1987); Rani, (1994); Vijayalaxmi, (1996) and high ambient ammonia exposures Saha *et al.*, (1997); Saha *et al.*, (1999) and infusing with different concentrations of ammonium chloride (Saha *et al.*, 1999). Recently excretion pattern involving urea that are biochemical adaptations to extreme environments or related to specific behaviour of the fish concerned (Randall *et al.*, 1989; Wood *et al.*, 1989). In toad fish, *Opsamus beta*, the extent to which urea and ammonia are excreted varies greatly intraspecifically and with experimental conditions. The most unusual chichlid, *Oreochromis alcalicus grahami* from alkaline lake Makadi, Kenya is

completely ureotelic which was interpreted as a specific strategy to survive in water with a very high pH (Randall *et al.*, 1989). The anadromous cyprinid *Chalcalbunnus tarichi,* Danulat and Kempe, (1992) resort to ureogenesis for living in alkaline environment such as soda lakes. The source of urea in teleost fish such as the alkaline lake adapted tilapia, *Oreochromis alcalicus grahami* Randall *et al.*, (1989), the marine toad fish, *Opsamus beta* and *Opsamus tau,* Read, (1971); Mommsen and Walsh, (1989) and Indian air breathing teleost *Heptoneustus fossilis, Clarias batrachus, Anguilla cuchia, Anguilla testudineus* Saha and Ratha, (1989) have ornithine urea cycle enzymes in the liver.

Aquatic teleosts normally excrete most nitrogen in the form of ammonia which is lost across gills (Smith, 1929). Teleost subjected to periods of emersion may be expected to restrict ammonia elimination which demands considerable water loss to prevent desiccation. A shift in nitrogen excretion from amminotelism to ureotelesim has been reported previously in a variety of air breathing Indian teleost subjected to prolonged emersion (Gordon *et al.*, 1969; 1970; 1978; Morri *et al.*, 1978; Morri, 1979; Davenport and Sayer, 1986; Natarajan, 1987; Sayer and Davenport, 1987; Rani, 1994; Vijayalaxmi, 1996).

In water, *Clarias mossambicus*, had a total nitrogen output of 0.3mm/kg/hr, predominantly ammonia with 13% urea. Returned to water after 24hr in air, the nitrogen output increased to 1.14mm/kg/hr containing 36% urea (Eddy *et al.*, 1980). *Anabas scandens* and *Channa gachua* when exposed to air and returned to water, showed marked increase in the rate of excretion of urea-N (Ramaswamy and Reddy, 1983). When in water, *Blennius pholis* excretes 53% of its ammonia and 67% of its urea from the head. In air, the

corresponding values are only 26 and 46% (Sayer and Davenport, 1987). The lake Megadi tilapia, *Oreochromis alcalicus grahami*, thrives in highly alkaline springs (pH=9.9, carbon dioxide=173m/mol) excretes all nitrogenous waste as urea-N at variable 80% rates (Randall *et al.*, 1989). These fishes make urea in large amounts disposing of both endogenous and exogenous bicarbonate and thus maintaining an internal pH compatible with life. A closely related species from a nearby river does not make urea and dies within an hour when placed in water from the alkaline lake. This example shows that synthesis of urea is not specifically an adaptation to life out of water (Atkinson, 1992).

Similarly in the anadromous cyprinid *Chalcalburnis tarichi* when lives in alkaline lake (pH=9.8), the nitrogen excretion is mainly ammonia and not urea (Danulat and Kempe, 1992). The fact that *Chalcalburnis tarichi* excretes ammonia at a higher rate is uprising in view of the report published on other teleost adapted to extremely alkaline adapted water. *Oreochromis alcalicus grahami* excretes urea predominantly (Randall *et al.*, 1989). Ammonia and urea excretion rates are affected by air exposure stress Rani, (1994); Vijayalaxmi, (1996), temperature and acclimation to salt water (Gershanovich and Pototskiji, 1995). Increasing reliance on urea as vehicle for nitrogen excretion was shown for the developing larvae of *Clarias gariepinus*. The high degree of ureotelism in this larvae may constitute an adaptive mechanism to a habit of water shortage (Terjesen *et al.*,1997; Peng *et al.*, 1998).

Bradley and Rourke, (1985) found a correlation between elevated NH_4^+ and reduced Na^+ plasma levels, and mortality, in juvenile steelhead trout *Salmo gairdneri* cultured in low ionic strength fresh water. They suggested that low environmental Na^+ likely was inhibiting NH_4^+ excretion occurring

normally NH_4^+ /Na^+ exchange mechanisms. Before the gills are functional, the blockage of transfer of NH_4^+ to the exterior via H^+/Na^+ or NH_4^+/Na^+ ion exchange mechanisms Heisler, (1982) presumably could lead to acidosis, ammonia toxicity and loss of regulatory capacity.

The tilapia fish *Oreochromis alcalicus grahami* from Kenya, has adapted to living in waters at pH 10.5 by excreting the end product of nitrogen metabolism as urea rather than as ammonia directly across the gills. The level of activity in liver of the first enzyme in the urea cycle pathway, carbamoyl-phosphate synthetase III (CPAse III), is too low to account for the observed high rates of urea excretion. Suprising findings that CPAse III and all other urea cycle enzymes activities are present in muscle of this species at levels more than sufficient to account for the rate of urea excretion, in addition, the basic kinetic properties of the CPAse III appears to be different from those of other known type CPAse III. This unusual form of adaptation may have occurred because of the apparent impossibility of packing the needed amount of urea cycle enzyme in liver (Lindley *et al.*, 1999).

In the present investigation, it is noted that *Oreochromis mossambicus* is amminotelic and to a lesser degree ureotelic in its natural freshwater environment. Excretion is directly proportional to seasonal temperature and ammonia excretion is inversely with salinity concentration. This is mainly due to maintain homeostasis. Greater excretion in summer is due to high metabolic activity for sustenance resulting in high ammonia and urea excretion, while in winter, low metabolic expenditure generating less excretory products. Hence after 24 hours, maximum ammonia excretory rate was noted to be 212.67±2.42µg gm. body wt. $^{-1}h^{-1}$ in normoxic water, while urea was

noted to be 76.27±1.20µg gm. body wt. $^{-1}h^{-1}$ during summer. While minimum ammonia excretion was recorded as 55.38±0.43µg gm. body wt. $^{-1}h^{-1}$ in normoxic water and urea was 40.13±0.49µg gm. body wt. $^{-1}h^{-1}$.

4.6 NITROGEN BIOMOLECULES

The augmentation of the steady state level of total proteins and free amino acids in the tissues of a variety of poikilotherms during cold acclimation and the reverse process during warm acclimation, under laboratory conditions have been well documented (Hochachka and Somero, 1973; Precht *et al.*, 1973; Hazel and Prosser, 1974; Das, 1984). The cold acclimation of the poikilothermic organisms like a fish, either in laboratory conditions or in nature during seasonal adaptation is characterized by a "biosynthetically directed metabolic reorganization" (Somero and Hochachka, 1976). This becomes possible plausibly because at a low temperature of acclimation, a poikilotherm can afford to channelize a larger proportion of its "free additional energy" into anabolic processes like net synthesis and accumulation of proteins, lipids, glycogen etc. in its tissues, due to a reduction of its maintenance metabolism Somero and Hochachka, (1976), and also due to a decrease in catabolic rate of the formed macromolecule (Das and Prosser, 1967; Somero and Doyle, 1973). This enhanced biosynthetic capacity during cold adaptation may be directed towards the synthesis of increased amount of enzyme proteins for compensation of the metabolic rate. Instead this may be phrased in terms of "season specific changes in metabolism" Somero and Hochachka, (1976), which is undoubtedly triggered by environmental thermal variation, but not directed primarily towards an adaptive compensation of a rate function against thermal effects.

The adaptive reorganization of the biochemical tissue compositions of the tropical air breathing teleost, *Channa punctatus*, during seasonal variation is induced by alteration of environmental temperature. Augmentation of the tissue levels of proteins due to cold acclimation of summer adapted *Channa* and diminution of these values due to warm acclimation of winter adapted fish reveal the role of environmental thermal fluctuation as the principal environmental triggering factor ("Zeitgeber") (Roy *et al.*, 1991).

The faster metabolic rate of fish living in a warm environment has been given as the probable cause of their shorter lives compared with the same species from colder water (Alm, 1959). *Cynolebias adloffi*, the "annual fish", which lives for about a year at its usual temperature of 22^0C, but which can be made to live much longer if kept at 16^0C (Liu and Walford, 1966). *Gadus morhua* rarely lives for more than 8yr in the North Sea, while the Barents Sea and Svalbard regions, it is known to live for considerably more than 20yrs. Metabolism of food (digestion) in *Perca flavescens* is three times as rapid in summers as in winters Pearse and Achtenberg, (1917), while the gastric secretion itself has been shown to increase at higher temperatures (Smith, 1967) in *Ictalurus nebulosus.*

Similarly, present investigation reveals that higher metabolic rate during summers and lesser during winters, which results in less tissue nitrogenous components during summer and high in winters. This could be due to low mobilization of various nitrogen fuels during winter and high catabolic activity during summers to maintain homeostasis, which is supported by lower excretion rates of ammonia-N and urea-N during winter and higher excretion rates in summer.

Total body protein decreased by 10% between February and April in large (>14cm) juvenile rainbow trout, but not in small fish under same conditions (Fessler and Wagner, 1969). In contrast, Woo *et al.*, (1978), found no change in liver and muscle protein content of coho salmon parr and smolts. Serum protein content of coho salmon smolts was 15% lower than in parr or postsmolts (Woo *et al.*, 1978). Cowery and Parry, (1963) found a 30% increase in muscle content of nonprotein nitrogenous constituents of smolts over than parr, due almost entirely to increased creatine content. The increased creatine content may be due to greater availability of N-phosphoryl creatine for endergonic reactions or to increased metabolism of several aminoacids for which creatine is an end product.

Phillips *et al.*, (1957) observed in *Salvelinus fontinalis*, that a capsule of food containing a red dye moved more rapidly along the intestine in warmer water, so peristaltic movement appears to be affected as well. Increased metabolic rate is seen in the activity of the enzymes of the liver. Vellas, (1965) found that uricase, allantoicase and allantoinase activities all increased in the livers of *Cyprinus carpio* kept at 20^0C compared to those kept at 8^0C increased metabolic and blood flow at higher temperatures appear to account for the smaller concentration of lactic acid in the blood of *Ictalurus melas, Lepomis macrochirus, Pomoxis anularis* and *Micropterus salmoides* after exercise of 20^0C as compared with 5^0C (Dean, 1962). Presumably the same amount is formed for the same energy output, but it is more quickly swept away and oxidised at the higher temperature.

The decrease in protein in response to NH_3-N, NO_2-N and NO_3-N toxicity could be due to proteolysis as is evident by the increased protease activity.

However, the steady state equilibrium of proteins in the body of an individual is governed not only by the rate of degradation but also by rate of synthesis. The fall in protein levels in all tissues is due to protein breakdown Tilak *et al.*, (2002), resulting in cellular depletion of amino acids resulting in inhibition of protein synthesis (Roger, 1980).

The progressive decrease in the protein content of the tissue with duration of exposure indicates a gradual accumulation of chemical resulting in gradual increase in toxicity.

Decrease in the protein content significantly, in response to fenvalerate treatment in gills, and other tissues of catfish, *Clarias batrachus* is mainly due to decrease in ribosomal activity. A reduced rate of translation of mRNA without a decrease in concentration of ribosomes (Tripathi *et al.*, 2002).

Protein content of liver, gill, brain, muscle, decreased during arsenic toxicity, which may be due to reduced protein synthesis and enhanced proteolysis (Shobha *et al.*, 2001).

Decrease in protein content in gill and liver tissue, during lindane intoxication, suggests an acceleration of protein catabolism as one of the possible physiological compensatory mechanism under lindane toxicity. The decrease in protein content, also suggests the possibility of impairment of protein synthesis (Zayapragassarazan and Anandan, 1996).

Gradual decrease in the levels of protein in brain and liver, after exposure to nickel salt of 10ppm, 20ppm, 30ppm and 40ppm may be due to proteolysis, or due to lack of protein biosynthesis, or inhibition of translation. It may also be due to reduced rate of glycine through intestine or nephrosis or low rate of protein synthesis (Desai *et al.*, 2002). Nagabhushanam and Talikhedkar,

(1997), reported that, in clam, *Donax cuneatus*, protein content was at a relatively high level throughout the year, being minimum in Dec. (56.59%) and maximum in May (68.31%). During monsoon period (July to Sept.), when clams are fully matured, was at moderate level (av. 59.58%). Then it declined slightly during breeding period from Oct. to Jan. (av. 57.51%). From March to June when clams are under maturation process, protein content increased (av. 63.33%).

The protein content in catfish, *Mystus vittatus*, in both acute and chronic exposures was found to be decreased than the control which could be attributed depressed feeding of the test animal at stress imparted by the pollutant or increased utilization of proteins to meet energy demand during stress (Sivakami *et al.*, 1994).

Protein content in gill, muscle, liver, intestine of fish *Channa striatus* exposed to 0.0004, 0.0006, 0.0008, and 0.001ppm of fenvalerate, decreased with increasing sublethal concentration of pesticide as compared to control fish. Decrease in protein concentration, might be due to mobilization of body proteins to meet the extra energy demand at chemical stress for maintenance. Such mobilized body proteins was metabolised to produce glucose through the process of gluconeogenesis (Sheela *et al.*, 1992).

4.7 LIPID BIOMOLECULES.

Warm adaptation is generally characterized by the increase in the relative proportion of 'cylindrical' shaped lipids, having large hydrophobic surface volumes. In contrast, increase in the concentration of 'inverted cone' or 'wedge shaped' lipid molecules with small hydrophobic surface volume is preferred during cold adaptation. The geometry of lipid components

determines the stability and phase composition which in turn helps in understanding and interpreting the consequence of temperature induced alteration in membrane lipid composition (Wieslander *et al.*, 1981; Rilfors *et al.*, 1984). Conically shaped molecules easily form non-bilayer (H^{II}) phases and it has been proposed that for normal membrane function a precise balance between bilayer and non-bilayer forming lipids are required (Hazel, 1995).

The physiological function of a cell and membrane associated cellular activities depend upon the membrane microviscosity /fluidity, which is very sensitive to physical environment. Any change in the environment can cause a drastic effect on the membrane bounded activities. Most of the cells of the poikilotherms have the capacity to maintain similar membrane viscosity or fluidity. Maintenance of similar membrane physical state due to any stress is known as homeoviscous adaptation, first reported for *E. coli* cells grown on 15^0C and 35^0C (Sinensky, 1974).

Singh and Das, 1980; Roy *et al.*, (1985) have reported the 'biosynthetically directed metabolic reorganization'. Somero and Hochachka, (1976), has shown in cockroach, *Periplanata americana*, either during winter acclimatization or after cold acclimation under lab conditions as revealed by an accumulation of total proteins, free amino acids, glycogen, RNA and total lipid sin fat bodies. The warm adaptation is marked by 'catabolic reorganization of the biomolecules'. Insects also possesses similar capacity of thermal compensation as exhibited by fish and other poikilotherms which shows reduced metabolism during cold with simultaneous accumulation of

lipid biomolecule in various tissues and the reversal with increasing temperature (Precht *et al.*, 1973; Hazel and Prosser, 1974; Das, 1984).

Fat content in *Donax* was minimum in Jan. (4.56%) and maximum in Aug. (7.15%). From Oct. to Feb., fat content remained low (5.15%). This could be related to spent condition of gonads during Dec. and Jan.,fat content was at moderate level from March to May (av. 5.48%). From June onwards, fat content began to increase reaching its peak in Aug. at the full mature condition of clam (Nagabhushanam and Talikhedkar, 1997).

Total gill filament, large intestine and liver lipids (Cholesterol (CH), Triglyceride (TG) and Phospholipid (PL)), decreases markedly in summers in *Oreochromis mossambicus*, in the present study which coincides with the changes in temperature to maintain homeostasis by "catabolic reorganization of the biomolecules" while in winters, there is accumulation of these lipids in various tissues, indicating "biosynthetically directed metabolic reorganization." Thus overall decrease in lipids is associated with increased utilization, for extra energy production during stress. Similar findings has been reported by Sheela *et al.*, (1992), in *Channa striatus* where the lipid content in gill, muscle, liver, intestine of fish exposed to 0.0004, 0.0006, 0.0008, and 0.001ppm of fenvalerate, decreased with increasing sublethal concentration of pesticide as compared to control fish. Decrease in lipid is associated with its increased utilisation for extra energy production during stress.

Total body and muscle lipid decreases markedly in spring in juvenile Atlantic coho and masu salmon and in rainbow trout coincide with other parr-smolt changes and is not dependent on changes in activity or temperature,

and return to prespring levels by late summer when fishes are retained in freshwater (Farmer, 1978; Saunders and Henderson, 1978; Woo, 1978).

Total lipid content increased in winter and spring with increased moisture content, and at higher temperatures, inverse condition as seen (Johnston and Saunders, 1981). Supression of smoltification results in the switch to hypoosmoregulatory mechanisms such as decreased kidney glomerular filteration rate, decreased drinking rate and changes in the gill epithelium associated with salt secretion, (Brit *et al.*, 1991).

The lipid of the fish on a hatchery diet is more saturated than that of wild fish, and therefore, by analogy with Hoar and Cottle, (1952), results on *Carassius auratus*, it is likely that the hatchery fish would be less resistant to temperature changes. Lovern, (1938) showed that lower temperature results in greater unsaturation (more double bonds) in the body lipids of *Anguilla anguilla*, and vice versa. Lovern, (1950) pointed out that at higher temperatures the bodily activity increases and requires a correspondingly greater expenditure of energy, so that less dietary lipid will be available for storage. The fatty acid composition of blue green algae was investigated by (Holton *et al.*, 1964), and found to change with environmental temperature. Farkas and Herodek, (1964) showed that the unsaturation of the lipids of crustacean plankton in lake Balaton increases as the temperature drops, and decreases again in the summer time.

Depletion of membrane lipid components (PL, CH, DG, TG) per unit of membrane protein in *Clarias batrachus* is a characteristic of summer adaptation. Decrease in the ratio of individual lipid component in summer adapted *Clarias* might be due to the utilization of lipid molecules to meet the

demand for energy maintaining the normal physiological processes (Roy *et al.*, 1997).

Although the phospholipid molecules are the main constituents of membrane lipid bilayer, other lipid molecules, like cholesterol and to some extent the triglycerol and diacylglycerol, may also contribute in maintaining the membrane physical state. The diacylglyceerol is known as an intermediate product of biosynthesis of phospholipid molecules (Longmuir, 1987). The first step in the synthesis of amino phospholipid is the hydrolysis of phosphatidic acid to diacylglycerol. The tropical air breathing teleost is more capable of adapting to higher 25 to 45^{0}C than a lower 5 to 25^{0}C thermal regime. This indicates an 'inverse compensation' of microviscosity during the winter season. It is noteworthy in this connection that this fish cannot survive at temperatures below 14^{0}C although it is capable of adapting to a higher temperature such as 38^{0}C (Precht *et al.*, 1973).

Although the apparent concentration of cholesterol is higher in winter, the ratio of cholesterol to phospholipid shows an inverse relation to the temperature. The cholesterol is known as 'packing' material in the phospholipid matrix and contributes to the ordering of the membrane. The cholesterol to phospholipid ratio increases at higher growth temperatures (Wodtke, 1978). The changes in the tissue cholesterol accompanying sexual maturation and spawning, has been investigated in several fish species (Idler and Bitner, 1958; 1960; Robertson *et al.*, 1961a; 1961b). Seasonal variation in cholesterol content in tissues has been well documented in fish (Mc Cartney, 1966; Siddiqi, 1966). Liver is the chief organ where active cholesterol synthesis occur. The rise and fall in CH level in *Heteropneustes fossilis* is

correlated with feeding, maturation and spawning (Shreni and Jafri, 1977). *Oreochromis mossambicus* are highly tolerant to saline conditions, growing well in full strength seawater (Ron *et al.*, 1995). Fish cultured in saline water need more energy for maintenance as shown by Orschot and Boon, (1993), in *Onchrorhynchus mykiss*. Decrease in the level of phosphotidylethanolamine PE, is elevated in livers of cold adapted fresh water and marine fish and shrimps, evolutionary adapted to low temperatures (Farkas *et al.*, 1994). Cold adaptation of membranes is generally associated with an increase in the relative concentration of phosphotidylethanolamine and less frequently, with a reduction in the level of phosphatidylcholine, Hazel, (1988), and warm adaptation resulted in an increase in the relative concentration of phosphatidylcholine with no change in the level of phosphatidylethanolamine.

Reduction in cholesterol (CH) in *Clarias batrachus*, exposed to carbaryl and phorate, may be because of more utilization of CH during coticosteroidgenesis, as it is precursor for steroid hormones (Jyothi and Narayan, 2001). The decrease CH might also be due to increased breakdown of CH into free fatty acids as observed by (Verma *et al.*, 1979). Hypocholestermic condition also frequently associated with anaemia (Goel *et al.*, 1984).

Leela *et al.*, (2000), showed a decrease in TG content, observed in liver and gill of two different size groups of *Tilapia mossambica* exposed to phosalone (long term and short term) suggests an increase in the breakdown of lipids possibly to meet extra energy demand. Srinivas *et al.*, (1991) observed that lipase activity was found to be more, at chronic exposure periods, suggesting higher requirements of TG to meet the extra energy

demands. The lipase acts on TG to form free fatty acids and glycerol. Free fatty acids contribute energy through beta oxidation by activating acetyl CoA in fishes. The increased free fatty acids are possibly utilized for lipogenesis with glycerol moiety through esterification to meet energy demands under stress condition because of shortage of carbohydrates. It is found that the changes are more pronounced in liver compared to gills, since liver is considered to be a major metabolic centre where synthesis, transport, storage and metabolism of TG occurs. Capuzzo *et al.*, (1984) also reported decreased TG due to increased catabolism of endogenous energy reserves in the American lobster, hydrocarbon stress.

Hunter, (1952), reported an average change in iodine value (a measure of unsaturation) of 0.5 unit per 1^0 change in acclimatization temperature in the body lipids of *Carassius auratus*. Lewis, (1962); Privol'nev and Brizinova, (1964) fish in general; and Knipprath and Mead, (1965), showed that in *Salmo gairdnerii* and *Carassius auratus* a relative increased unsaturation or decreased melting point of the body lipids to lower water temperatures.

Hoar and Cottle, (1952) could discern no relationship between the tolerance of *Carassius auratus* to cold and the degree of unsaturation of the body lipids, but found that the resistance to both low and high temperatures can be modified by feeding diets containing high concentrations of certain fatty acids. At higher temperatures the protein-lipid-calcium complex, which forms the various cellular membranes will be more stable. Hoar and Cottle, (1952); Johnston and Roots, (1964). Lewis, (1962) view is that many of the life processes can continue only if the cell lipids have a suitable viscosity, which is changed, of course, by a change of temperature. The selectivity of

the fish in utilizing its lipid stores is more interesting, although information is scanty. Lovern, (1934) noticed that there is a drop in the proportion of the remaining C-14 and C-16 acids as the lipid stores of *Salmo salar* are drained off. This selectivity was considerably more marked in the males than in females. *Clupea harengus* select the more highly unsaturated lipids when stores are being utilized Lovern, (1934) and the same would appear to be true of *Gadus morhua* in which the iodine value of the liver oil falls at the time of maximum depletion. The distribution of saturated fatty acids, however, changes little during the annual cycle (DeWitt, 1963). Wilkins, (1967) showed that a reduction in the proportion of phospholipid in *Clupea harengus* during starvation coincides with a reduction in certain protein fractions, indicating that a breakdown in body tissues is in fact occurring. It may be that the greater facility of fish for utilizing proteins during starvation enables them to utilize phospholipid at the same time. Accumulation of PUFAs in response to decreased temperature accompanied by increase fluidity of membranous structures has been reported in several cases (Cossins, 1977; Cossins *et al.*, 1980; Lee and Cossins, 1990; Kitajka *et al.*, 1996). On the basis of these results, phospholipid fatty acid composition differences, reflects the fluidity of phospholipid from liver tissue of *Hypophthalmichtys molitrix* and *Hypophthalmichtys nobilis* displaying greater fluidities than those from *Ctenopharingodon idella* and *Cyprinus carpio*. *Hypophthalmichtys molitrix* and *Hypophthalmichtys nobilis* contained twice as much 22:6 in their phospholipid than *Ctenopharingodon idella* and *Cyprinus carpio*. But when temperature was considered, *Hypophthalmichtys molitrix* and *Ctenopharingodon idella* showed greater PUFA than observed intraspecifically.

Differential response of lipid metabolism of various crustaceans to changes in environmental temperature showed *Cyclops vicinus* active in winter and summer, and Daphnia magna active only in summer, is that only the former could increase the level of long chain PUFA in PL when exposed to cold also a rapid temperature induced deacylation followed by chain elongation, desaturation and deacylation can be postulated to take place on exposure to cold. *Cyclops vicinus* uses the PUFA present in PE whereas *Daphnia magna* uses the molecular architecture of PC head groups to assure a comparable physical state of their membranes in warm temperatures. Failure to regulate PL physical state in *Daphnia magna* might be one of the reason why this species spends the cold season in the form of resting eggs (Farkas *et al.*, 1984).

Cold water fish brains were rich in 18:1 /22:6 diacylphosphatidylethanolamine (and to a lesser degree in diacylphosphatidylcholine), and its level decreased with increasing environmental body temperature. The ratio of 18:0 /22:6 to 16:0 /22:6 phosphatidylcholine and phosphatidylethanolamine was inversely related to body temperature (Farkas *et al.*, 2000). Accumulation of 18:1 /22:6 or, in general, sn-1 monoenic, sn-2 polyenic phosphatidylethanolamine molecular species in cold is demonstrated for the nematod *Caenorhabditis elegans* for the shrimp *Pandulus borealis* from the North Atlantic, and for marine crustacean from the Baltic sea, *Monoporeira* sp., *Gammarus* sp. and *Mysis* sp. A low level of 18:1 /22:6 was found in the brain of rhesus monkeys (Lin, 2000). A diminishing level of 18:1 /22:6 with increasing body temperature can be the result of reduced formation and thus incorporation of 18:1 into

phospholipids or reduced in desaturation of 18:1 /22:6 phosphatidylethanolamine. The sum of 18:1 /22:6 and 18:0 /22:6 is almost identical in winter and summer acclimated carp *C. carpio*, 38.6% vs 39.6% respectively, and in summer and winter acclimated silver carp *H. nobilis*, 32.8% v/s 34.5% respectively (Farkas *et al.*,2000). 18:1 excess in cold adapted /acclimated fish, is due to fish do not feed in winter. A plausible explanation is that phospholipid deacylation /reacylation in brain in cold adapted fish is more intensive than warm blooded fish.

Accumulation of phospholipids (PL), sterols and FFA declined where as the triglycerides and sterol esters and hydrocarbon (TG + SEH) fractions increased by increasing concentration of (0.5, 1, 2.5, and 5.0mM) lead, barium and zinc acetate, indicating, PL in the membranes are practically replaced by TG and SHE, without any adverse effect on the membrane viability (Sushma and Ameja, 1996). Bahuguna and Rawat, (1994), has shown in *Barilius bendelisis*, during 90 days of starvation, protein content varied from 15.63% to 11.30% while lipid content decreased from 3.95% to 0.857%. On the other hand, when fed with artificial food, little variation was observed. Protein content varied from 15.65% to 15.54% while lipid from 3.95% to 3.93%. During starvation, mobilization of biochemicals viz., lipid > protein is 78.30% and 27.79% respectively.

4.8 HISTOLOGY

Gills are the first organ, suspected to be affected by any kind of pollutant as compared to other organs. The gill tissue of *Oreochromis mossambicus* is of the typical teleostean type. The gill exposed to monocrotophos exhibited marked histopathological changes and the severity increased with the length

of exposure. The histopathological changes were characterized first by bulging tips of primary gill filament, lost their original shape and their curling occurred. Later their thickness increased. Hyperplasia and hypertrophy in epithelial cells and their nuclei pyknotic. Pools of congested blood were also seen within the sub epithelial space (Santhakumar *et al.*, 2001).

Proliferative thickening of gill epithelium was produced by most kinds of environmental toxicants as a general safety measure against its irritation (Skidmore and Tovel, 1972; Narain *et al.*, 1990). With increased exposure time, the secondary gill lamellae showed lamellar fusion and oedomatus separation of epithelial cells from pillar cells, which usually occur when the lymphoid space between the epithelium and its supporting elements gets enlarged by accumulation of fluid on account of factors like increased capillary permeability or lowered efficiency of the cells in maintaining normal water balance (Skidmore and Tovel, 1972). In fish, the respiratory epithelium is the barrier between the blood and the surrounding water through which respiratory exchanges takes place. Any damage to this epithelium affects not only ventilatory process but also other vital process like ion exchange, during the secretory and excretory functions of the gills (Narain *et al.*, 1990).

Perturbant stress induced in the gill epithelium leads to events like increased influx of hydrogen ions, which reduces the pH of the blood and thus decreases the oxygen carrying capacity of the haemoglobin (Haines and Schofield, 1980). On the other hand, the ionregulatory and excretory functions were hampered. Epithelial damage disturbs the exchange of ammonium and bicarbonate ions of the blood with sodium and chloride ions of the medium, which normally occurs across the gill epithelium of fish (Love, 1980).

Hyperplasia and fusion of gill epithelium due to the separation of epithelium, necrosis of gill epithelium, degeneration of pilaster cells and development of vacuoles in the epithelium are pathological changes observed in fishes exposed to pesticides (Roy and Munshi, 1991; Sunitha and Sahai, 1993). Hypersecretion of the mucous, impairs the fishes olfactory discrimination ability, which probably modifies the cell ionic environment which could affect the transduction process, and further this excessive mucous causes lesions during long term treatment (Getchell *et al.*, 1988).

In the acid and aluminium treatments, respiratory lamellae were frequently obliterated by hyperplasia of the inter lamellar epithelium, suggesting markedly compressed respiratory ability. In addition, chloride cell numbers were usually depressed, and those cells that were present were often vacuolated or covered by layers of pavement epithelium. Other histopathology was estimated to be hyperplasia of mucous cells, abundance of interlamellar mucous strands, edema, necrosis or sloughing of the epithelium, interlamellar plasma and red blood cells exudate, reduction in the number of red blood cells in capillaries of respiratory lamellae and fusion of the primary or respiratory lamellae. Also the thickened respiratory epithelium seen at 4^0C, pH 7 to 7.8 could be part of the normal adaptation response to cold temperatures. A thickened respiratory epithelium would decrease oxygen uptake but presumably would also resent a great barrier to ion loss (in fresh water fish most ions are lost through the respiratory epithelium). Thus in the cold induced torpor, this fish would not require as much oxygen, but would still have to maintain their electrolyte balance. The phenomena of the respiration

associate with the development of the gills and the supporting cells, which helps in regulating the osmosis mechanism (Rombough, 1988).

In the present study the loss of respiratory epithelium and formation of haematomas within the secondary lamellae might have led to a great reduction of the respiratory surface resulting in the impairment of oxygen uptake rate and will eventually create physiological imbalance in the organism. Present histopathological data could be correlated with those of our behavioural physiological and biochemical study ie. increased mucous secretion can be correlated with increased surfacing behaviour, decreased aquatic physiological respiration.

The common histopathological alteration of gill exposed to chemical induced shrinkage and disturbance of uniform, parallel distribution of gill lamellae on gill filament, with reduced interlamellar pore and decrease in blood cells, in the present study. Similar results were reported for various fishes exposed to different chemicals. In *Anabas testudineus* exposed to monocrotophos, Santhakumar and Balaji, (2000), in *Channa punctatus* exposed to aquatic pollutants, (Anitha and Ramkumar, 1997). In *Boleophthalmus dussumieri* (Cuv.) exposed to cadmium Manoj and Ragothaman, (1999). In *Channa punctatus* exposed to Cauvery water, (Dhanapakiam *et al.*, 1998). In the gill of loricariid fish, *Hypostomus plecostomus* (Fernandes and Perna, 1995). In rainbow trout, *Oncorhynchus mykiss* exposed to Chloramine-T, (Powell *et al.*, 1995). In *Oncorhynchus mykiss* exposed to copper, (Saucier *et al.*, 1991). In *Puntius stigma*, exposed to water variables (Khillare and Davane, 1998).

However, majority of the histopathological studies on different fish species are based on the induced histopathological changes occurred due to heavy metal intoxication at various doses is known, very little or no information is available on sodium chloride pollutant on fresh water fish. Although it is known that the metal ions get their entry into different biological systems through the surface of the intestinal and other biological membranes, Stein, (1967); Skoyma and Waldron, (1971). The reports on he quantitative accumulation of the same in the different tissues and organs particularly in the alimentary canal of teleost are rare (Anandkumar, 1989; Kaviraj, 1989; Sinha *et al.*, 1992). Tilapia is a freshwater fish, native of south east Africa. It populates reservoirs with a temporary oxygen deficiency. Based on the observations of the greatest oxygen deficiency occurs during night and specimens that live in low oxygen water and periodically swallow air, Carter and Beadle, (1931) suggested that the alimentary canal of such fishes acts as an additional respiratory organ. Similarly the stomach of *Ancistrus* species is adapted as an additional respiratory organ and breathes using only gills in well oxygenated waters but when oxygen concentration diminishes, it frequently comes to the surface and swallows air (Satora, 1998). The presence of lamellar bodies in the epithelial cells of the digestive canal suggests that these structures may be connected with the synthesis of a compound that plays a role similar to that of the surfactant in the lungs of Dipnoi, certain Amphibia and Mammalia, Goniakowska - Witalinska, (1995), and these could represent a morphological adaptation for respiratory function (Satora, 1998).

The normal structure of parallel gill lamellae on gill filament, presence of large interlamellar pore and large number of blood cells was disturbed leading

to shrinkage of gill filament, reduced interlamellar pore and decrease in blood cells, followed by obliterated gill lamellae, hyperplasia of interlamellar epithelium, inflammatory epithelium and vacuolated chloride cells marks interlamellar bridge and compressed respiratory ability.

The stomach and intestine in the present study animal are spirally bent in the body cavity and the liver gland is situated centrally. The walls of digestive tract are delicate and transparent. Permanent or seasonal dissolved oxygen deficiencies have a major influence on the evolution of additional respiratory organs in fish (Randall *et al.*, 1981). The ability to breathe atmospheric air in some fish species allows survival under unfavourable oxygen conditions, along with an increase in metabolic rate and activity that exceeds the limits imposed by gill respiration only. Such a relationship was described in the catfish *Hoplosternum thoracatum* (Gee and Graham, 1978). Examples of fish in which the digestive tract is adapted as an additional respiratory organ include *Misgurnus fossilis,* Jasinski, (1973); *Corydoras paleatus,* Kramer and McClure, (1980); *Hoplosternum thoracatum,* Gee and Graham, (1978); Huebner and Chee, (1978) and *Trichogenes longipinnis,* Pinheiro *et al.*, (1993). The intestine of fish, as in mammals, are longer in herbivorous than in carnivorous Lagler *et al.*, (1962); Nagase, (1964) and their shapes and structures may be modified by the nature of the diet (Gohar *et al.*, 1961). Further, the intestines are bigger if the fish are well fed than if they have been starved as in *Cyprinus carpio* (Luhmann and Mann, 1957). Therefore in the present study, the damage in the intestine, being the principle organ for absorption, has been found to be severe compared to other tissues. Further the extent of damage softer organs is closely related with time of exposure of

heavy metals tested. The longer the exposure, relatively more is the damage in the tissues. Similar findings has been observed by Kesh *et al.*, (1993), in the intestine of *Heteropneustes fossilis* (Bloch).

Liver being the main organ of various key metabolic pathways, the effects of a chemical usually appears primarily in the liver where the hepatic cells are not arranged to form distinct lobules, and the degree of pathology gradually increases from 7 days of exposure to 90 days. Degenerative changes in the hepatic cells of *Channa punctatus* exposed to heavy metals and ammonia could be characterized by vacuolation of the hepatocytes, pyknosis in many of the necrotic cells, cytoplasmic granulations and vacuolations with nucleus extruded, disintegration of the sinusoids and although being ammonotelic a slight increase in the ambient ammonia caused severe histopathological damage in the liver (Banerjee and Bhattacharya, 1997).

Joshi, (1982) reported histopathological alterations such as necrosis and vacuolisation in the liver of fish *Tilapia mossambica* within five days of treatment with monocrotophos and it was opined that vacuolization and fatty degeneration are independent of each other. Patil *et al.*, (1992) observed that the liver of fish *Boleopthalmus dussumieri* exposed to monocrotophos showed ruptured cell membranes, nuclei pushed aside, fatty degeneration, cellular content no longer distinct, large vacuoles in the cytoplasm of the hepatic cells, necrotic changes followed by vacuolation, liver histopathological lesions appeared to be the chief change by 48 to 72hr.

Histopathological effects reported for various fishes exposed to different chemicals included degenerative changes at the initial stage marked by swollen hepatocytes, vacuolation of hepatic cells, large number of necrotic

region seen and pyknosis of necrotic cells, blood sinusoids infiltered with half damaged blood cells, hyperplasia and hypertrophy in *Punntius ticto,* Rashmi *et al.*, (1994) on exposure to herbicide atrazine, in *Boleopthalmus dussumieri* Patil *et al.*, (1992) on exposure to monocrotophos, in freshwater fish *Gambusia affinis*, Patwardhan *et al.*, (1991) on exposure to sumithion, in *Clarias lazera*, Moussa *et al.*, (1997) on exposure to melia azedarach derivative, and in *Channa punctatus,* Banerjee and Bhattacharya., (1997) exposed to elsan, mercury and ammonia. The above observations are in conformation with the present findings.

Konar, (1970); (1975); (1977); (1981) has published insecticide induced histopathological alterations in the liver of fish. Histopathological alterations in the liver of fresh water teleost, *Puntius ticto* after exposure to herbicide, atrozine at two sublethal doses, 1ppm and 10ppm for different exposure durations showed histopathological lesions including infilteration of blood cells, hypertrophy /swollen hepatocytes and vacuolar degeneration of hepatocytes (Jain and Mishra, 1994). Acute exposure of the fresh water fish *Gambusia affinis* to sumithion caused severe effect on liver as vacuolation of hepatic cells, necrosis, damage to blood cells inside the blood sinusoids, rupture of the endothelial wall of the blood sinusoids and signet ring stage in the hepatic cells with hyperplasia and hypertrophy (Patwardhan and Gaikwad, 1991). Exposure of rats liver to xylene, toluene and methyl alcohol elicted peculiar ultrastructural changes viz. involution of nuclear membrane and irregular arrangement of mitochondrial cristae and concluded, enormous functional reserve is maintained by liver parenchyma, morphological changes may not strictly correspond to functional changes (Rana and Kumar, 1997).

Effect of copper showed dissolution and disentegration of gamatogenetic processes and gonadial connective tissue. This resulted in vacuolized acini and reduction in the number of sex cells. Spermatids formed clumps after 2hr of exposure. Sertoli cells appeared deshaped and vacuolised, their nuclei disappeared, cells lost their individual identity and became fragmented. The normal process of sperm detachment from sertoli cells on maturation got disturbed and sperms remained on acini wall with degenerated sertoli cells. Nurse cells and nutritive cells were also found degenerated. Thus copper produced histopathological damage leading to reduced reproductive capabilities (Mathur, 1994). The nuclei and nucleoli of germinal epithelium got disintegrated, disappeared in previtellogenic stage and ultimately resorbed by nurse cells (Josse *et al.*, 1968).

5. SUMMARY

- As various physico-chemical parameters have direct bearing on existence, growth and development of living organisms, assessment and estimation of these physico-chemical parameters will help in forecasting the type, nature, distribution and ultimately evolutionary success.
- Different types of behaviour in the same population vortexed in response to various environmental factors are exhibited in a way yo adapt to the changing physiologicl demand for sustainence.
- It was observed that there was no fixed behaviour, but rather a flexible and dynamic one as a trade off between maximum protection, maximum energy input and effects of other environment.
- Behavioural studies seem to be approaching a unified concept, that with increasing salinity concentration and exposure period, there is increase in metabolic demand.
- The predominant perception in population dynamics has been that of homeostasis. The concept of 'balance of nature' is well entrenched in biological thought and a stable equilibrium world is said to be normal. The chaotic fluctuations observed in population behaviour, over a span of time have been generally considered to reflect stochastic factors such as, weather conditions.
- The present work proves simple mechanism of physiology in fishes where, freshwater forms gain water and loose ion in osmoregulatory mechanism. To avoid the above problem, they excrete plenty of dilute urine to conserve body salts and the chloride cells are secretory in

function absorbing more salts from the medium. Reverse process is observed when treated with sodium chloride. Also water content in tissues is inversely related to lipid content in tissues during different seasons.

- It is clear that a major portion of the oxygen requirement by *Oreochromis* was met by gills and the air bladder plays a minor role in gas exchange. Thus *Oreochromis mossambicus* appears to be a facultative air breather which shows maximum dependence on gills.
- The enhanced aquatic respiration and aerial respiration in summers is correlated to increased oxygen demand to metabolise food stuff to liberate energy to sustain homeostatic state due to increased temperature or might be depletion of dissolved oxygen tension at higher temperatures during summers resulting in increased oxygen consumption or may be due to accumulation of sodium chloride, stress perturber in respiratory tissue thereby disturbing normal physiological process of gills or due to breeding activity which enhances respiration.
- Decreased aquatic respiration and aerial respiration in winters, may be due to decreased energy demand for sustenance, or may be due to decrease in acid phosphatase enzymes causing decrease of oxygen consumption from freshwater to saline water transfer or disorientation of epithelium of chloride cells, degeneration of cell structure and lamellar fusion are evidences for impairment of respiratory mechanism or respiratory inefficiency and total respiratory breakdown due to the formation of mucous on respiratory organs.

- From the present investigation, it is clearly indicative that *Oreochromis mossambicus* is a hardy fish, and can survive in environmental perturbations with respect to temperature and salinity, and this fish could be successfully aquacultured in any temperature and salinity perturbed environment in our own state.
- Though the endogenous nature of bimodal respiratory rhythms of air breathing fishes were studied, the relationship between the various environmental variables, and their influence on the rhythmic adjustments is fully explored. *Oreochromis mossambicus* displays differential weigh specific rhythmic pattern of bimodal respiration. The factors that are responsible for the development of this rhythm in this species is mainly temperature of various seasons and salinity.
- In Goa state, which has plenty of semi aquatic khazan land, temporary or permanently filled with saline water, and pleasant seasonal temperature ranging from 20 to 33±2^{0}C throughout the year, there is dearth of information on the use of this khazan land for pisciculture of hardy and exotic fish like *Oreochromis mossambicus*.
- From the present studies, it is clear that *Oreochromis mossambicus* is extremely amminotelic, and to a lesser degree ureotelic in its natural freshwater. But when exposed to increasing saline waters it excretes more urea and little ammonia. Similarly, exposure to saline water with increasing number of days showed increase urea and very little of ammonia excretion. Thus urea which is a very important and expensive nitrogen fertilizer could make this semi aquatic khazan lands more fertile for the production of various crops.

- The present work re-confirms that, nitrogenous ammonia and urea excretion are affected by acclimatised temperature and salinity parameters in the present study.
- Increase in (CH), (TG), (PL), during winters in various tissues is an indicative of cold adaptation to maintain homeostasis due to low mobilization of fats to sustain life and thus enhances growth. Or it could be the proteins that are utilized through gluconeogenesis.
- Decrease in (CH), (TG), (PL), during summers in various tissues is an indicative of warm adaptation to maintain homeostasis due to high mobilization of fats along with proteins to sustain life.
- Histology not only helps to study healthy tissue but also can be made use to diagnose diseased tissue thus extending its helping hand in biology and medicine including histopathology.
- Histology helps to understand *Oreochromis* hardy nature and know maximum adaptation, thus making them to be ubiquitous in distribution ranging from freshwater to saline sea water.

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