STUDIES ON SOME ASPECTS OF BIOLOGY OF TWO ESTUARINE FISHES *MEGALOPS CYPRINOIDES* AND *SCATOPHAGUS ARGUS*

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By

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To my parents

CERTIFICATE

This is to certify that this thesis is an authentic record of the research work carried out by Miss Anney K.Mathew M.Sc., under my supervision and guidance at the School of Marine Sciences, Division of Marine Biology, Microbiology and Biochemistry, Cochin University of Science and Technology, in partial fulfilment of the requirements for the degree of Doctor of Philosophy and that no part thereof has been presented before for any other degree, diploma, associateship, fellowship, or other similar titles of any University.

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DECLARATION

I, Anney K.Mathew do hereby declare that the thesis entitled "Studies on some Aspects of Biology of two Estuarine Fishes Megalops cyprinoides and Scatophagus argus" is a genuine record of the research work done by me under the scientific supervision of Dr. A.Antony, Reader, School of Marine Sciences, Cochin University of Science and Technology, under the Faculty of Marine Sciences, and has not previously formed the basis for the award of any degree, diploma, associateship or other similar titles or recognition of any University.

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PREFACE

The protein requirement of growing population in India cannot be met fully through conventional sources such as agriculture, animal husbandry or fisheries. It is absolutely necessary to find alternate areas for generating additional food resources. This is where coastal aquaculture comes into prominence. Coastal aquaculture can be looked upon as a part of the great effort to improve the food resources of India. Fish is relished by about 60% of Indians and as such an adequate supply of fish could substantially improve the consumption of animal protein.

Tropical brackish water fishes are of immense importance in providing protein food for man and the demand for such protein is rising exponentially with the rapidly accelerating increase in human population. The ever increasing demand for fish at the present day has made it imperative to exploit fully the resources of the estuaries and backwaters. Thus brackish water fish farming has drawn the attention of the fishery scientist during recent years.

Fishery resources can be classified under two major categories, viz., capture fisheries and culture fisheries. Capture and culture fisheries have now developed into a systematic branch of fishery science. Many years of research and development have helped to tide over the serious problems encountered in rearing the fishes in captivity, induced breeding and nurturing the larval forms. These achievements have gained aquaculture a world wide popularity.

Many countries have made advancements in pisciculture. Norwegians have mastered salmon production. Hungary also stands equal in her aquaculture production. Salmon culture has become a success in Chilean and Icelandic aquaculture programmes. This being the background of European Aquaculture Society (EAS), India along with other Asian countries, is only stepping up her efforts for developing aquaculture, liberally backed by FAO and other

international organisations, both financially and technically. Brackish water aquaculture is the youngest among the programmes of fisheries development in India. It is estimated that we have about 2.6 million hectares of estuaries and brackish water lakes (Swaminathan, 1980). But the traditional system exploits only about 35,000 hectares of this vast available area. Our species resources are varied and rich with a number of commercially important fishes suited for culture. In future the gap between production and demand for fishery will have to be filled through aquaculture.

The exploitable fishery potential of Indian Exclusive Economic Zone (EEZ) is about 4.5 million tones of which only a minor portion is being exploited. The maximum exploitation of the natural resources has become a necessity in a highly populated country like India where fish is one of man's main source of animal proteins. The development of fishery in India is indispensible not only to meet the food requirement, but also to improve the socio-economic conditions of fisherman community. So the research programmes undertaken in this field should be so oriented to provide worthy information regarding the exploited and unexploited wealth of the sea and inland waters, and methods to tap it.

Based on the adaption of fishes to their habitat, they are divided into three ecological groups - marine, fresh water and estuarine or brackish water forms. Estuarine fishes inhabit the less saline region of the sea, estuaries and other inland waters. These fishes are more subjected to pollution than fresh water fishes or marine fishes as they encounter pollutants present in the outgoing river water and the incoming sea water during low and high tides respectively. So, the study of the biology of the estuarine fishes has become unavoidable to assess their suitability in aquaculture.

The development of both capture and culture fisheries related to any brackish water system is dependent on the availability of scientific data on the various biological factors in respect of the different species. Such a study on fishes will be helpful in formulating suitable schemes for the management of brackish water for capture and culture fisheries. It was therefore felt that

a study of the biological and biochemical aspects of two estuarine fishes - Megalops cyprinoides Broussonet and Scatophagus argus Bloch which are not fully exploited in aquaculture programmes, was worth undertaking. The present study is expected to advance our knowledge on the biology of the two fishes which are very desirable for brackish water fish farming.

INTRODUCTION

INTRODUCTION

Teleost fishes are the most numerous and varied group of vertebrates which constitute about 40 percent of the living vertebrate species. About 21,000 species of bony fishes have been reported so far. They are present in all oceans and most land-locked waters. They find habitat in abyss to spray zone, in thick swamps to rushing torrents of Andes and Himalayas and in warm spring to freezing ponds and marine waters so cold that antifreeze is required in their blood. They range from 1 cm gobies to giant tunas, marlins and sword fishes. They afford food for millions all over the world, sport for anglers and many other commercial uses such as animal food and raw materials for industries.

Man is interested in fishes for diverse reasons. Fish has been an item in the diet of mankind since time immemorial. Traditionally fishing has been the principal avocation for the livelihood of the people living in the coastal regions. Even before man settled in agricultural communities, he depended on fishing and hunting for food. Fish is perhaps the cheapest, but the best animal protein available to man. So, the use of fish for food has appreciably increased in recent years. The declaration of Exclusive Economic Zone has provided a great opportunity to the coastal nations, especially to India for economic, social and industrial benefits. Indian fisheries is now in a transitional phase from the traditional to modern ways of exploitation. Modern fishery science has merged as a result of the blending of many basic sciences such as ecology, biology, hydrography, biochemistry, microbiology, economics, commerce, etc. Fishery resources, once thought to be infinite is now realized to be finite. So scientific research to upgrade culture practice has become a necessity of the time to uplift fishery and to satisfy world's demand for fish.

Fish food is an important source of proteins and vitamins. Basic nutrients such as proteins, sugars, fats, vitamins and amino acids are readily available from fish in a balanced form. According to the FAO classification based on the per capita consumption of fish, India comes under the category

'low'. An average Indian is still undernourished and his per capita fish consumption rate is only 4.13 kg/annum, against 15 kg, 29 kg and 41 kg in USA, U.K. and Japan respectively (CMFRI, 1977). Kerala being one of the maritime states in India, stands in frontline of fisheries with per capita fish consumption rate of 10.1 kg. This shows the necessity to accelerate studies on fishes and improvement of fishery in India. Regarding India, estuarine fishery is less developed than marine and fresh water fishery. To achieve a full potential for aquaculture production, a vast knowledge of estuarine systems which serve as extensive nursery grounds for many fishes is needed.

The brackish water biotic niche is highly productive and is always characterised by rich and varied fish fauna which can tolerate the extremely fluctuating physico-chemical parameters of this environment. Thus a characteristic type of fish population has developed in each brackish water lake on which the commercial fishes are based. The distribution and abundance of these fishes are predominantly controled by the master environmental factor viz., salinity.

The major sources of brackish water fishes and fisheries resources in India are the estuaries and brackish water lakes located in the east and west coasts of the country. Among the brackish water lakes in the west coast, Vambanad lake is the largest, with an area of about 250 sq.km. The estimated average fish production in the Vembanad lake is more than 10,000 tonnes (Kurup 1982). This is the second largest shrimp farming area in the country. The lake serves as an extensive nursery ground for many marine organisms. It also serves as a major source of lime shell to the state. The majority of the fishermen in Ernakulam district depend on the fisheries of the backwaters for their livelihood. This is especially so during the monsoon months, when rough sea prevails along the Kerala coast making sea fishing difficult. The progress of brackish water fishery can improve the socio-economic condition of the poeple in the area, which is essential for the progress of the country as a whole.

Vembanad lake and its tributaries support a very wide variety of fishes. This lake is a lucrative fishing ground and also provides ample scope for undertaking large scale brackish water fish farming. When compared to marine fishes, our knowledge on the biology of estuarine fishes in meagre. In recent years, brackish water fishes have been a subject of considerable research owing to their importance in capture and culture fisheries developmental programmes. A thorough knowledge on the biological and biochemical characteristics of the brackish water fishes is essential to have a clear idea on the nutritive value and dietary importance of these organisms. Considering this fact, two species of estuarine fishes, Megalops cyprinoides Broussonet and Scatophagus argus Bloch, inhabiting the Vembanad lake have been selected for the study. The present work comprises the following aspects:

- Food and feeding habits;
- Biochemical constituents of the different tissues;
- Haematological changes accompanying exposure to copper;
- Effects of copper on different enzymes like Acid phosphatase, Alkaline phosphatase, Glutamic oxaloacetic transaminase and Glutamic pyruvic transaminase in different tissues, and
- Glycogen and lactic acid levels in different tissues of fishes exposed to different concentrations of copper.

Feeding is one of the important activities of an organism since energy for the various activities is obtained from food. A knowledge of the feeding behaviour is also a necessity for the successful operation of different gears. It is also imperative to understand how the food influences growth, abundance, migration, reproduction etc. Studies on the day and night feeding can help to reveal the relationship between illumination and fish congregation in the euphotic zone. The present investigation has been undertaken to study in detail the food and feeding habits of the two fishes mentioned above. The results are presented and discussed in the first part of the thesis.

Fishes are consumed either in fresh condition or in preserved form. They are important sources of protein, vitamins and oils of medicinal and industrial importance. The wastes from fishery industry are used for animal feed and manure. A clear idea about the biochemical constituents of the food fishes is essential for the standardisation of proper processing methods and to estimate their food value. In the bioenergetic studies, calorific content of different tissues of organisms is an important parameter and it is used for converting the biomass value to energy units. Much emphasis is being given in recent years on the study of energy values of organisms and calorific measurements as units of energy transfer. Factors such as developmental stages, reproductive condition and food intake are known to have effects on the proximate composition and calorific values. The literature regarding the proximate composition and calorific values of the Indian fishes are meagre when compared to that of other countries and as such a study on this aspect is important. The present study attempts to analyse the proximate composition and calorific values and their seasonal variations in different tissues of the two fishes. The observations are presented and discussed in the second chapter of the thesis.

Copper is essential for normal metabolism in most organisms, but is toxic above threshold levels. The biological importance of copper is great. Like other heavy metals it is an essential trace element for life. The presence of copper is known in the structure of ascorbic acid, several enzymes like oxidases, tyrosinases and in other important molecules such as cytochrome oxidases. Species specific requirements have been understood with reference to copper. Copper is known to become toxic to aquatic organisms, when the concentration exceeds tolerable limits. Functional responses could be altered deleteriously when copper level in water exceeds threshold or tolerance limits. Copper is a principal ingredient in many antifouling compounds. Copper sulphate is commonly used in aquaculture for the treatment of ectoparasites and to eradicate certain diseases. But little is known about the effects of this chemical used for therapeutic purpose on the physiological systems of the exposed fish. So copper was selected in the present study to examine its

effects on the functional responses of the exposed fishes. In highly organised animals, especially vertebrates, blood is a very efficient connective tissue assigned with numerous functions of vital importance. This tissue is present throughout the body from organ to cellular level and provides access to each and every cell of the body. So it is subjected to the stress and strain the animal experiences even in the minute level. As blood reflects the physical, chemical and biological variations even at negligible levels, a study of it has become a necessity. The normal concentration of the different entities of the blood changes during pathological conditions. So the application of the haematological studies for the investigation of pathological conditions is at present a routine procedure. But such information is fragmentary in fishes. Elaborate and systematic details still lack in this branch of ichthyology. So a study of the variations of different haematological parameters induced by exposure to varying sublethal concentrations of copper was undertaken. The results of the study are presented and discussed in the third chapter.

Fishery industry like all other developing sectors is facing natural and man-made hazards. Pollution due to the illegal dumping of industrial wastes and pesticides is a major problem. Consequently this brings in not only mass mortality of fishes but also lasting and genetically transferable changes in their physiology and enzyme systems. Tissues of all species of animals contain enzymes. Alterations in these enzyme systems due to any stress are immediately reflected in the functional responses of the organism. Phosphatases and transaminases are two important enzyme systems which have been subjected to considerable investigations. Alkaline phosphatase and acid phosphatase are two phosphomonoesterases that catalyse transphosphorylation and hydrolysis of numerous orthophosphate esters. Transaminases are a group of enzymes that catalyse the process of biological transamination. Of the many transaminases, the most important and widely investigated ones are Glutamic pyruvic transaminase (GPT) and Glutamic oxaloacetic transaminase (GOT). In fishes, study of phosphatases and transaminases has been mostly restricted to serum. The role of these enzymes on other tissues is still to be properly understood. So an experiment was designed to observe the variations in the specific activity of these enzymes in different tissues when exposed to different concentrations of copper. The observations are presented and discussed in chapters four and five.

Glycogen has a significant role in the accumulation and dissipation of energy in animals, especially in fishes. Lactic acid is the end product of glycolysis in the muscle of vertebrates under anaerobic conditions. This lactic acid is immediately transported to liver where it is converted to liver glycogen. The accumulation of lactic acid in muscle or liver can be considered as a failure in the transport system, the continuation or the intensity of anaerobic condition. The estimation of glycogen and lactic acid levels in liver and muscle tissue is necessary to have an idea of the "stress" imposed on fish while handling, transportation, exposure to toxicants or other pathological conditions. But the breakdown of glycogen and accumulation of lactic acid in different tissues of fish under stress is a least investigated aspect. In the present study glycogen and lactic acid levels in various tissues of fish exposed to different concentrations of copper were analysed. The results are presented and discussed in chapter six.

Detailed methods pertaining to each aspect of the investigation are given in the respective chapters.

A summary of the present study is given at the end followed by a list of the references.

The study area

Vembanad lake is situated between latitudes 9^{o} 28' and 10^{o} 10' N and longitudes 76^{o} 13' and 76^{o} 31' E. The lake and its tributaries extend into Alleppey, Ernakulam, Kottayam and Trichur Districts of Kerala State. It has a permanent connection with the Arabian sea by a canal about 500 m wide, which forms the main entrance into Cochin harbour. The depth of the lake varies from 8 to 10 m in the lower reaches close to the sea. The upper reaches of the lake is relatively shallower with depth ranging from 2 to 5 m. The bottom of the lake is generally muddy. The average tidal range of the lake is about 1 m in the lower part of the lake and diminishes progressively towards the upper region.

Fig.1 Megalops cyprinoides Broussonet 1782

Fig.2 Scatophagus argus Bloch 1788

The tidal current from the sea into the lake on one hand and the discharge of fresh water from the rivers and their tributaries on the other, mix salt and fresh water and make the lake a typical estuary. The salinity in the Vembanad lake fluctuates from about 1‰-33‰ during the course of the year. The distribution and abundance of fishes in the area is predominantly controlled by saline conditions, with sea water dominating the system in summer and fresh water in monsoon. Vembanad lake offers a rich and diversified fishery which contributes to the economy of the State and also provides ample scope for undertaking large scale brackishwater fish farming. Both the fishes under study are adapted to waters of varying salinities.

Description of the species

Megalops cyprinoides Broussonet 1782 (Fig.1)

The family Megalopidae is represented by a single genus Megalops and Megalops cyprinoides is the only Indian representative of the family. The species is generally known as 'tarpon' and is widely distributed in the backwaters of Kerala. This species is recorded from Indian and Pacific Ocean, estuaries and rivers of India and Ceylon. The young ones of these fishes get land locked and are usually found in rivers with accessibility to sea. This fish resembles herring fishes in colour and appearance and posses large eyes. These characters have secured it the name 'Ox-eyed herring' among the fishermen.

The body is compressed. The cleft of the mouth is oblique and lower jaw prominant. Scales are absent on the head. The dorsal fin commences opposite to ventral. It's upper edge is concave. The caudal fin is deeply lobed. Pectoral, ventral and anal fins are diaphanous. The last ray of the dorsal fin is prolonged into a filament.

Scatophagus argus Bloch 1788 (Fig.2)

The genus Scatophagus is the only known representative of the family Scatophagidae and Scatophagus argus is the only species recorded

from the waters of India and Sri Lanka. It is more often found in estuaries and backwaters and prefers waters of higher salinity adjacent to the sea throughout the whole life cycle. The family name Scatophagidae means 'dung - eater'. The euryhaline nature and beautifully spotted rhombic body ranks it as a fascinating aquarium fish. This species is generally known as 'Scats'. It has been recorded from Indian Ocean along sourthern Africa, Sri Lanka and east and west coasts of India including the Andamans.

Body is rhombic and well compressed. It is greenish brown in colour with dull white belly and black blotches of varying sizes all over the body. Mouth is small, terminal and horizontal. Minute scales are present on the body and head. The first dorsal fin is spinous and the second dorsal fin soft. Pectoral fin is small and rounded. Caudal fin is truncate.

Collection of materials

The materials for the study were collected using different types of fishing gears operated in the Vembanad lake. Fortnightly collections were made for a period of two years during April 1984 to March 1986. A total of 653 specimens of Megalops cyprinoides (188 juveniles and 465 adults) ranging in size from 6.3 cm to 26.5 cm and weighing 15 gm to 242 gm were collected from the Vembanad lake. Four hundred and forty seven specimens of this species (133 juveniles and 314 adults) ranging in size from 6.8 cm to 25.7 cm and weighing 10.8 gm to 246 gm were also collected from the fish culture farms of Cochin area.

Specimens of Scatophagus argus were collected from the lower part of the lake using cast nets and chinese dip nets. Six hundred and seventy two specimens (207 juveniles and 465 adults) were collected for the study.

Five percent formalin (3-5 ml) was injected into the abdominal cavity of the specimens to make the gastric muscle stiff to avoid regurgitation. Regurgitated stomachs if any, were not included in the present study. The preserved specimens were brought to the laboratory for further analysis of the

stomach contents. Besides this, required number of live specimens of both the species were brought to the laboratory in large plastic troughs and kept in aquarium tanks for other studies.

CHAPTER I

FOOD AND FEEDING HABITS

The basic functions of an organism - growth, development, reproduction - all take place at the expense of the energy which enters the organism in the form of its food. The first stage in the life cycle of a fish is completed at the cost of the reserved food, which it receives from the maternal supply (the yolk of the egg). However, fish can live on its yolk only for a comparatively short time and then goes over completely to the consumption of external food. Hence, a correct knowledge of the relationship between the fishes and their food items is essential for the prediction and exploitation of the fish stocks.

From the beginning of fishery research, the problem of food and feeding have drawn the attention of fishery biologists. Generally, stomach sampling is employed for gaining knowledge on the feeding habits of fishes. It gives general information of the food items in view of competition for food, helps in evaluating the predator-prey relationship, indicates seasonal, geographical and diurnal variations in food composition and reckoning the feeding rate. The knowledge on the rate of consumption of food and its availability to fishes, especially to plankton feeders, can be related to the eutrophication of its habitat which may lead to the flourishing or diminishing of the existing species and its replacement by other species, a phenomenon which is of great significance to fishery industry.

Grove et al. (1978) suggested that low energy food items are emptied from the stomachs more rapidly than those of high energy content. In the light of their experiments, they have given an idea about the overall energy content of the different feeds of fishes. The study of the food and feeding habits can lead to an awareness of even minor differences in the structure and position of mouth (Keast et al. 1966). As Johnson (1975) stated, the size, appearance and taste of the essential organisms most likely determine their vulnerability to fishes. Based on this theory an attempt can be made to relate food organisms and fishes feeding on them. The above factors emphasises the importance of the study of food and feeding habits of fishes for the

management and exploitation of a fishery stock.

The problem of food of fishes first attracted the attention of a few ichthyologists towards the end of the last centuary. The beginning of such a type of study was first made by Day (1882), who investigated the food of herring. Since then a number of papers dealing with the food and feeding habits of fishes have been published. But the credit of developing the problem and providing it the important status it occupies today in fishery research goes to Lebour (1918-1934) who published a series of papers on the food and feeding habits of larval and post-larval fishes. She described the change of food habits of different stages in the life history of fishes. An ecological study of the feeding habits of fishes was conducted by Hertly (1953) and Whitefield and Blaber (1978). Studies on the predator-prey relationships in some fishes by Grossman (1962), Paloheim (1979) and Anderson (1982) are worth mentioning.

The feed of larval stages and juveniles of fishes was initiated by many workers. The works done by Sparrow (1968) on salmon fry of British Columbia, Kjelson et al. (1975) on feeding ecology of post-larval fishes in the New Port estuary of United States, Zisman et al. (1975) on the feeding habits of early stages of mullets in the Haifa Bay region of Israel, Keast (1977) on the feeding relationships between the year classes of yellow perch in Canada, are sources of valuable information in this field. In their study on omnivorous and planktivorous fishes, Confer and Blades (1975) formulated a linear relationship of the prey size and distance of which fish reacts to zooplankton. Feeding modes and prey size selection and measuring the preferences in selective predation has been the topic of study by Janssen (1976) and Chesson (1978).

Food selectivity in fishes has also attracted attention of Allen (1960) and Starostka and Applegate (1970). Jenkins et al. (1970) recorded the feed of rainbow trout in relation to the abundance of invertebrates drifting in the mountain stream. Noteworthy among other workers who have made significant contributions to our knowledge on this subject are Corbet (1961), Jean (1970), Cadwallader (1975), Arthur (1976), Frost (1977), Papova and Sylina (1977), Cadwallader and Eden (1979), Bell (1979), Cyrus and Blaber (1983) and

Clark (1985).

The daily feeding periodicities and food uptake in different hours of the day were analysed by Keast and Linda (1968). Variations in feeding in relation to natural parameters like light (Hunter, 1968) and temperature (Keast, 1968) have extended the horizon of the investigation in this field. Seasonal variation in food intake was examined by Northcote and Lorz (1966), Manzer (1968), Chaston (1969) and Elliot and Jenkins (1972).

Interspecific and intraspecific competetion for food in fishes cohabiting the same location was studied by Thomas (1962), Mc Comish (1966), Clifford (1968) and Scott and Tibbo (1968). The energetics of food intake, assimilation and growth in different fishes were analysed by Johnson (1966), Kohler (1966), Tyler (1972) and, Wolfer and Miller (1978). Keast (1966) studied mouth and body form relative to feeding ecology of fishes and Koinka and Windell (1972), the differential movement of digestible and undigestible food fractions in rainbow trout, Salmo gairdneri. Goldschmid et al. (1984) extended their study to the analysis of food and gut length of fourteen blennid fishes.

In India, considerable attention has been paid to this aspect of study from very early time. Hamidkhan (1934) scanned the habits and habitats of food fishes of Punjab and Job (1940) studied the nutrition of perches off Madras coast. Mukherjee (1944) has given an account of the food of fresh water fishes and Chacko and Venkataraman (1944) recorded the food of mullets. Rahimullah and Das (1945) prepared a descriptive record on the physiology of the pyloric caeca in carnivorous and herbivorous fishes, Mukherjee and Das (1945) studied the gut contents of carnivorous and herbivorous fishes connected to their feeding in different stages. Studies on this subject on some fishes of Bengal was made by Mukherjee et al. (1946) and Sarojini (1948, 1954). The food of some common fishes of Utter Pradesh was studied by Das and Moitra (1955). Among others, particular mention should be made of the works of Kuthalingam (1956), Kamasastri (1960), Venkataraman (1960), Rajan (1964), Bennet (1967), Patnaik (1970), Rajendran (1970), Sekharan (1971), Qazim (1972), Devaraj (1973), Bhusari (1976), Jayaseelan and Krishnamurthy (1980), Jacob and

Balakrishnan (1982), Kurup (1982), Pati (1982) and Srinivasan (1984).

The food of Indian mackeral Rastrelliger kanagurta was studied by Noble (1962). Studies on the food habits of Indian oil sardine, Sardinella longiceps are confined to the works of Dhulkhed (1962), Kagwade (1964) and Noble (1965), and that of Polynemus indicus by Karekar and Bal (1958) and Polynemus heptadactylus by Kagwade (1967).

Investigations on the feeding habits of some fishes off Calicut and Madras coasts were made by Venkataraman (1960), those of Gulf of Mannar and Palk Bay by Talwar (1962) and those of Bombay coast by Suseelan and Somasekharan (1967). George et al. (1969) published a report on the investigation on the food of some demersal fishes from the trawl grounds off Cochin.

Attention had been drawn on the adaptation of the alimentary canal in relation to food of milk fish by Chacko (1945), of grey mullet, Mugil tade by Pillay (1953) and of Indian major carp Cirrhina mrigala by Yusuf (1967). Paloheimo and Dicke (1966) emphasized the nature of food, rate of intake, their conversion efficiency and growth under different parameters like salinity and temperature.

The feeding of post-larval fishes off Trivandrum was studied by Gopinath (1942) and of juvenile fishes of the coastal waters of Madras by Basheerudin and Nayar (1961). Differences in food and feeding adaptations of juveniles and adults of Indian oil sardine studied by Benzam (1964) is an authentic work of its kind. Devdas and Mahadevan (1973) noticed differences in feeding habits of juveniles of Psettodes erumii of different size groups. Jayaprakash (1974) reported the food and feeding habits of the juveniles of Otolitholdes brunneus in Bombay waters. Joseph (1976) carried out a field study on the feeding rate and growth of mullet fingerlings of Kayamkulam lake.

Studies on the anatomy and histology of the alimentary canal of Megalops cyprinoides were carried out by Pasha (1964). But he has paid little attention to study the food items and his work was confined to the study of

the adaptations of the alimentary canal with regard to its food. Noble (1973) observed the food items of the post-larvae and juveniles of Megalops cyprinoides by rearing them in the laboratory. But no study has been conducted by him on food and feeding habits of the adult fish. As his work was based on post-larvae reared in the laboratory and fed with zooplankton, it was not possible to find out whether they were omnivorous or carnivorous.

A preliminary study on the food and feeding habits of Scatophagus argus was conducted by Saramma (1967). This work mainly dealt with the adaptation of the mouth of the fish in relation to its feeding habits, and no detailed studies have been made by her on the food items of this fish. A perusal of the literature reveals that only limited information is available on the food items of these fishes. So, the present investigation was undertaken with a view of study in detail the food items of the two fishes of different size groups and from different habitats. Seasonal variations in feeding and day and night feeding rhythms are also studied.

MATERIALS AND METHODS

Details of materials collected for the study are given in the first part of the thesis.

It has been observed by Philips (1929) and Job (1940) that when a fish is captured or put into formalin for preserving, very often it vomits the remains of the last meal, due to the shock sustained. So the stomach may be empty while the gut may show the presence of partially digested food matter. An examination of the rectal contents may reveal the presence of items that are excreted without digestion which can not be considered as the food item of the fish. Because of this, Kjelson et al. (1975) followed the method of examining the entire alimentary canal of fishes. In the present study also the contents of the entire alimentary canal were taken for examination.

The intensity of feeding can be judged by the degree of the distension of the stomach, or by the quantity of food contained in it. The

distension of the stomach was judged visualy and classified as 'gorged' or 'distended', 'full', '3/4 full', '1/2 full', 'little' or 'trace' and 'empty' with due regard to the distension of its walls and the quantity of the food in it, and points were allotted. Stomachs were considered 'gorged' when it was expanded fully with packed food and the walls thin and transparent, 'full' when it was filled with food normally and the walls thick and intact and '3/4 full' when it was partly collapsed and walls thick. Stomachs were designated '1/2 full' and 'trace' based on the food present and nature of the walls. For the sake of convenience, fishes with 'gorged' and 'full' stomachs were considered to have actively fed, '3/4 full' stomachs as moderately fed and 'trace' as poorly fed. The percentage frequency of 'empty', 'trace', '1/2 full', '3/4 full' and 'gorged' stomachs were calculated from the total number of fishes examined each month.

The gastro-somatic index (GSI) was calculated for each fish to determine the feeding intensity by applying the formula,

$$GSI = \frac{\text{weight of the gut}}{\text{weight of the fish}} \times 100$$

and the average was calculated for each month.

Reviews of the literature on the methods of gut content analysis have been made by several workers (Hynes, 1950; Pillay, 1952; Rounsefell and Everhart, 1953; Lagler, 1956 and Hyslop, 1980). The most common methods employed for stomach content analysis are volumetric (Job, 1940), points (Swynnerton Worthington, 1940) and index of preponderance (Natarajan and Jhingran, 1962). Pillay (1952) suggested that the method adopted must depend entirely on the particular diet of the fish. In the present study points method of Swynnerton and Worthington (1940) is followed. This method is especially useful when the food consists of many small organisms (Frost, 1977). As stated in the report of FAO (1974), this method is rapid, easy and requires no special apparatus, and with experience the method could be very accurate.

The contents of the alimentary canal was emptied and examined

Fig.3a Monthly percentage occurrence of stomachs in different degrees of fullness in M.cyprinoides collected from Vembanad lake.

Fig.3b Monthly percentage occurrence of stomachs in different degrees of fullness in M.cyprinoides collected from culture farms.

Fig. 3a

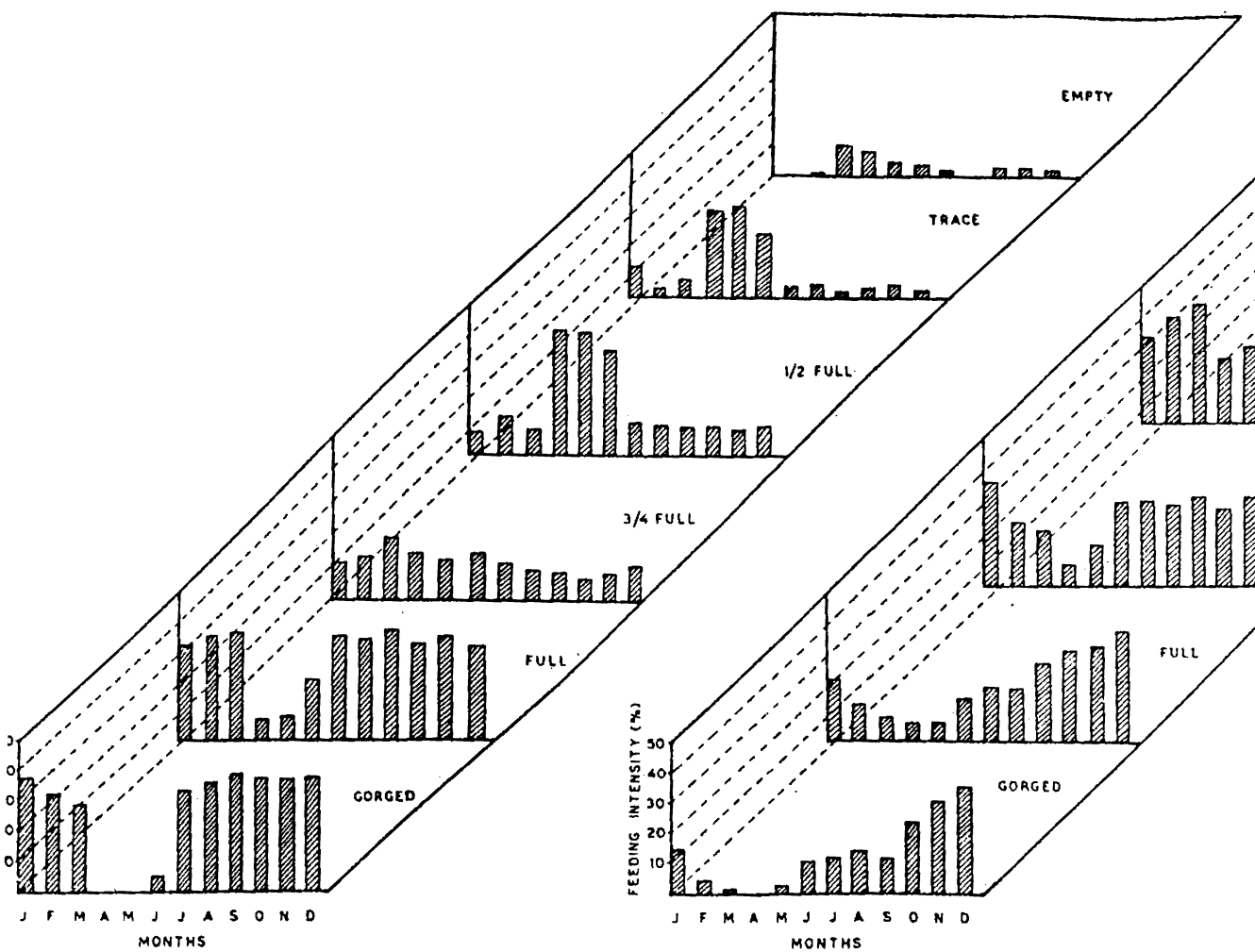

EMPTY
TRACE
1/2 FULL
3/4 FULL
FULL
GORGED
J F M A M J J A S O N D
MONTHS

Fig. 3b

EMPTY
TRACE
1/2 FULL
3/4 FULL
FULL
GORGED
FEEDING INTENSITY (%)
50
40
30
20
10
J F M A M J J A S O N D
MONTHS

under a binocular microscope. The food items were identified upto generic level or groups depending on their state of digestion. For evaluating the preference of food consumed, different food items were assessed by a general eye estimation and listed under the categories plenty, common, few, little and rare. Due consideration was given to the size of the food organism as well as its abundance and points were allotted to stomachs, depending on their degree of fullness and distension. The points gained by each food item were either increased or decreased proportionately to the total allotted to the stomach and then summed up. The summations of the points obtained by each food item for each month were then scaled down to percentages to show the composition of the food items in different months.

RESULTS

Megalops cyprinoides

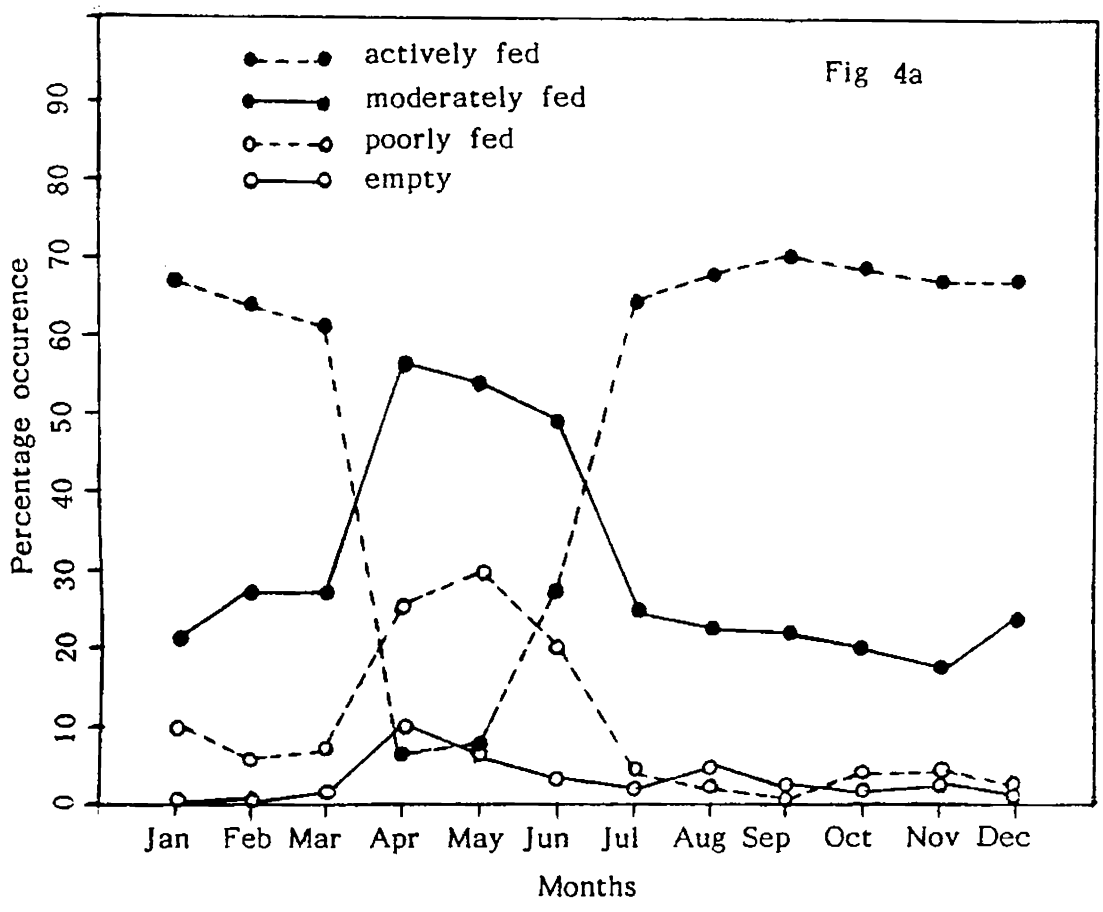
The data given here are the monthly mean values of the results obtained for the specimens.

a. Feeding intensity

The percentage occurrence of feeding intensity of Megalops cyprinoides collected from Vembanad lake is given in Table 1 and Fig. 3a. In general the percentage of 'gorged' and 'full' stomachs were high when compared to other categories. Fishes with 'gorged' stomachs were present in all the months except April and May. The highest percentage of 'gorged' stomachs was observed in September (39.63) and the lowest in June (5.72). More or less the same trend was followed for the 'full' stomachs, showing a maximum value of 36.76% in September and a minimum value of 7.16% in April. '3/4 full' and '1/2 full' stomachs were present throughout the year. The percentage of '3/4 full' stomachs ranged between 20.64 in March and 8.88 in October. 'Half-full' stomachs were observed in all the months. The highest percentage of such guts was present in April (40.83) and the lowest in March (8.25). Stomachs with 'little' or 'trace' amounts of food items were low compared to

Fig.4a Monthly percentage frequency distribution of different feeding intensities (actively fed, moderately fed, poorly fed and empty stomachs) in M.cyrpinoides collected from Vembanad lake.

Fig.4b Gastro-somatic index of M.cyprinoides collected from Vembanad lake.


actively fed
moderately fed
poorly fed
empty
Fig 4a
Percentage occurence
0
10
20
30
40
50
60
70
80
90
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Months

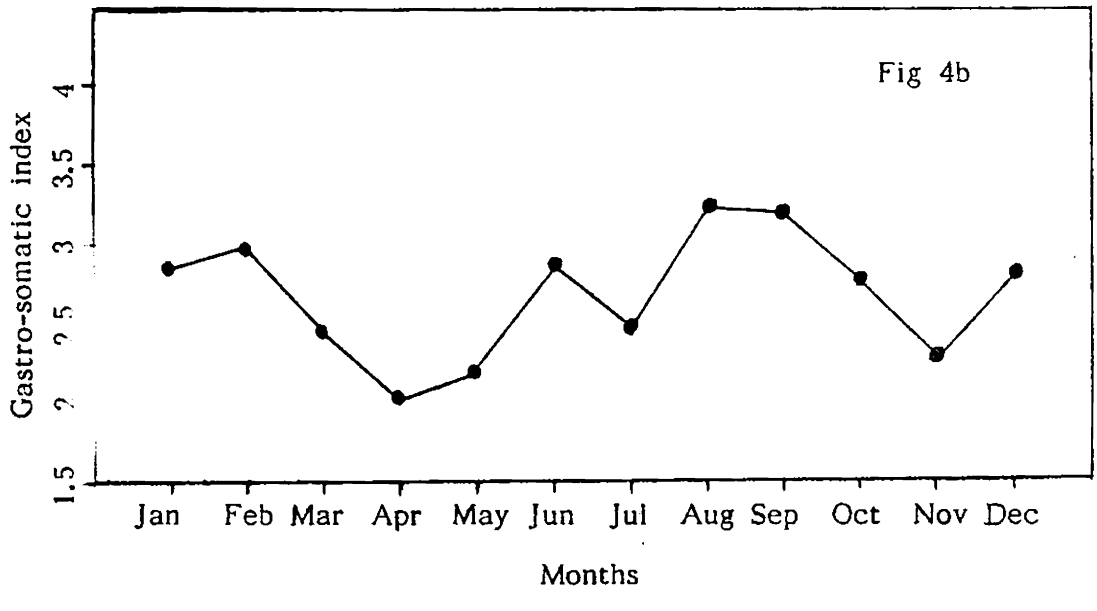


Fig 4b
Gastro-somatic index
1.5
3
3.5
4
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Months

the other groups, except in April, May and June, showing percentages of 28.82, 30.24 and 21.95, respectively. Empty stomachs were absent in January and September. Their frequency was very low in February (0.89%). The highest value of empty stomachs (9.13%) was observed in April.

The details of the fullness of stomachs of M. cyprinoides collected from culture farms during the period of investigation are shown in Table 2 and Fig. 3b. In fishes collected from culture farms 'gorged' stomachs were abundant during December (35.76%), November (30.47%) and October (23.91%). They were not present in April, and the occurrrence was very low in March (1.14%) and May (2.22%). Though 'full' stomachs were noticed in all months, higher values were always noticed from September to December (26.37% to 36.45%). Such stomachs were rare in April (7.77%) and May (7.80%). '3/4 full' stomachs were observed throughout the year, their frequency of occurence being 6.65% in April to 32.16% in January. The occurrence of '1/2 full' stomachs varied from a minimum of 6.42% in December to a maximum of 38.79% in August. Though the guts having 'trace' amounts of food were abundant in April (44.88%) and May (38.42%). they were very low in October (0.83%) and December (0.66%). This category was absent in November. Empty stomachs were also absent in November and December. Maximum number of fishes with empty stomachs were found in April (21.39%).

The results of the monthly variations in stomach fullness under various categories ('actively fed', 'moderately fed' and 'poorly fed') are shown in Fig. 4a. From the figure it is seen that the percentage occurrence of 'actively fed' specimens collected from the open waters of Vembanad lake was high from June to January. From February onwards there was a gradual decline in feeding intensity followed by a deep decline during April and May. This was followed by a sudden increase in values during June and July. The percentage occurence of 'moderately fed' stomachs was found to be high during April, May and June and then decreasing from July onwards upto November.

Fig.5a Percentage composition of different food items in juveniles of M.cyprinoides collected from Vembanad lake.

Fig.5b Percentage composition of different food items in adults of M.cyprinoides collected from Vembanad lake.

Fig 5a

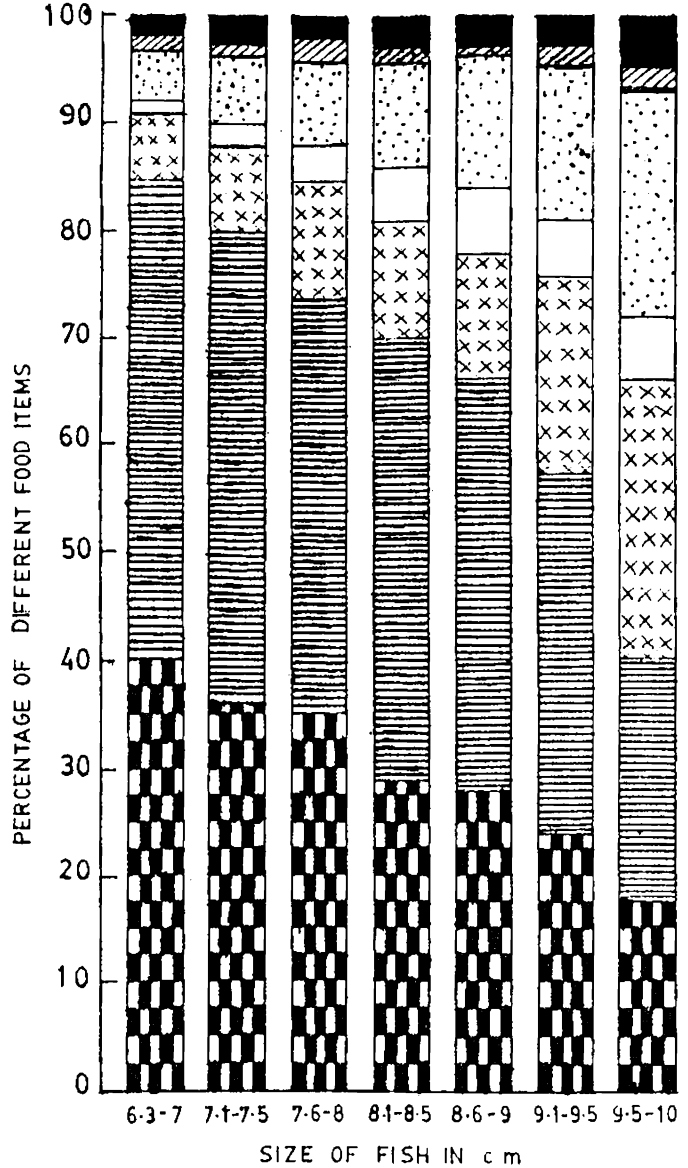
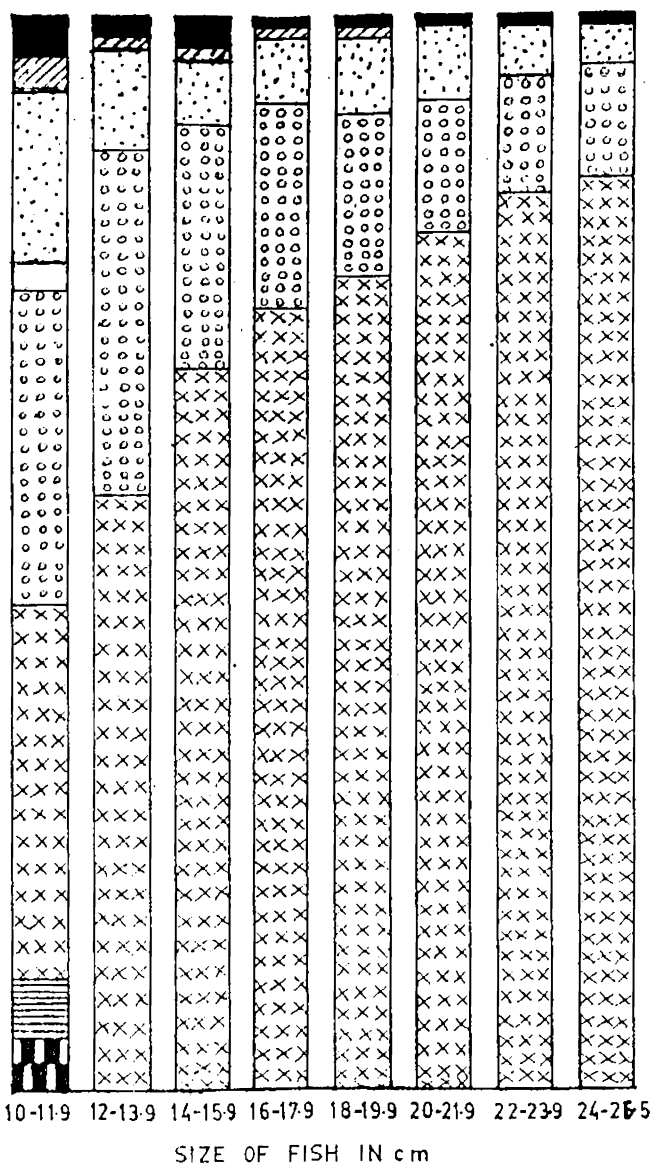
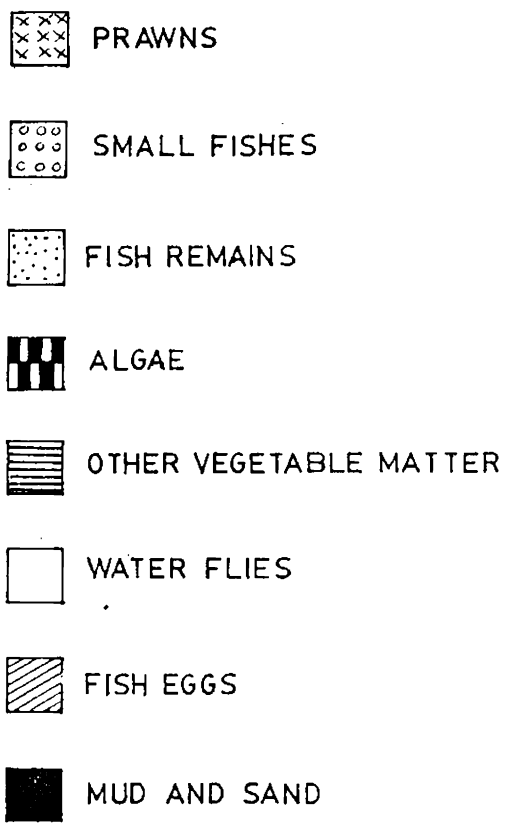

100
90
80
70
60
50
40
30
20
10
0
PERCENTAGE OF DIFFERENT FOOD ITEMS
6·3-7
7·1-7·5
7·6-8
8·1-8·5
8·6-9
9·1-9·5
9·5-10
SIZE OF FISH IN cm

Fig 5b


10-11·9
12-13·9
14-15·9
16-17·9
18-19·9
20-21·9
22-23·9
24-25·5
SIZE OF FISH IN cm


PRAWNS
SMALL FISHES
FISH REMAINS
ALGAE
OTHER VEGETABLE MATTER
WATER FLIES
FISH EGGS
MUD AND SAND

b. Food composition in relation to size of fishes

The percentage composition of different food items of the juveniles and adults of M.cyprinoides is given in Figs. 5a and 5b. The analysis of the gut contents of the juveniles revealed that algal filaments and other vegetable matter formed the major portion of the food. About 68% of the food was either algae or fragments of aquatic pteridophytes. They included Volvox sp. and Spirogyra sp. of Chlorophyceae; Oscillatoria spp., Synechococcus sp. and Synechocystis sp. of Cyanophyceae; Skeletonema spp., Coscinodiscus spp., Nitzchia spp., Bidulphia spp., Thalassiosira spp. and Pleurosigma spp. of Bacillariophyceae and fragments of Salvinia auriculata. Animal matter like water flies, insects and small prawns (28.85%), in addition to mud and sand (3%) formed the rest of the diet which is only a supplementary feed. This shows that the juvenile fish accepted both plant and animal matter. But plant matter increased as the size of the fish decreased and animal matter followed the reverse pattern. In the present study, it has been observed that vegetable matter had an inverse relation to the size of the fish.

Adult fishes did not seem to select a wide range of food items. They preferred prawns and fishes, with prawns as the major food item (70.8%). Small fishes, fragments of fishes and fish eggs ranked second and they formed about 26.5% of the diet (Fig. 5b). Mud and sand formed a small category (1.75%). No vegetable matter was observed in the gut contents of the adult fishes. This showed that the adult fishes were strictly carnivorous. To be more specific they were piscivorous in feeding habits and preferred to feed on prawns to fishes.

c. Food composition in relation to habitat

In the guts of adult fishes collected from natural habitat (Vembanad lake), prawns formed the common and major food item. They formed about 70% of the food items and were found intact without any damage. Small fishes ranked next. Their share was about 20% of the food.

Fragments of fishes, fish scales and fish eggs followed (about 8%). No vegetable matter was noticed in these stomachs. Mud and sand formed about 2% of the diet.

In the gut contents of fish collected from fish culture farms, animal matter other than prawns formed the main food consumed by them. Fishes and fish remains predominated. Prawns were only second in importance. Here also, no vegetable matter was observed. Another factor observed in the study was that fishes with empty stomachs were noticed more in these collections, particularly in collections made from culture fields left after harvesting.

d. Feeding intensity in relation to day and night hours

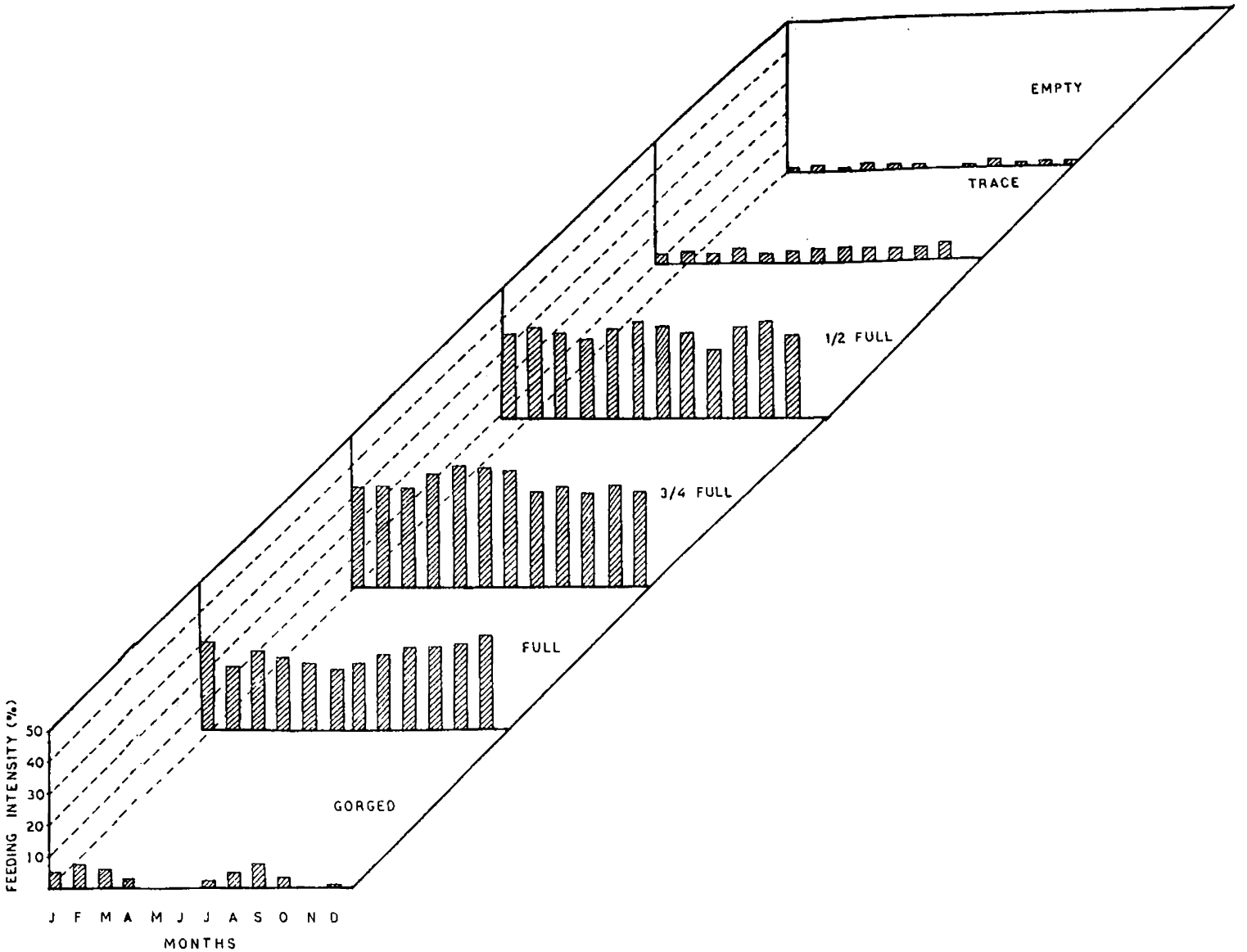
'Gorged' and 'full' stomachs were present in day and night collections. Their frequency of occurrence did not vary in light and dark hours of the day. Empty stomachs were also represented equally at all times of the day. But they were few in numbers. Same was the case with fishes having 'trace' amounts of food in their guts. '3/4 full' and '1/2 full' stomachs were also encountered unbiased by light. This proves that light is not a factor that influences food intake in M.cyprinoides. Light and darkness did not seem to control the persuite and capture of prey by this predator.

e. Feeding intenstiy in relation to spawning

M.cyprinoides is reported to migrate to deep waters of the sea for spawning. Since the present collections were made from the shallow areas only, no specimen with mature gonads was obtained. Studies by Gopinath (1946) have shown that larvae and post-larvae of M.cyprinoides appeared in abundance along Trivandrum coast during December. Noble (1973) reported the larvae of this species from Karwar coast. But there is no previous records of the collections of M.cyprinoides with ripe gonads from coastal waters. This may be due to the fact that gonads mature only in high saline waters of the sea. Since the present study was based on specimens collected from backwaters and

Fig.6 Monthly percentage occurrence of stomachs in different degrees of fullness in S.argus.

Fig. 6


EMPTY
TRACE
1/2 FULL
3/4 FULL
FULL
GORGED
FEEDING INTENSITY (%)
50
40
30
20
10
J F M A M J J A S O N D
MONTHS

ponds, the influence of maturation and spawning on feeding could not be revealed.

The gastro-somatic index of adult fishes collected from the natural habitat showed irregular increase and decrease during different months of the year (Fig. 4b). During August and September the values of GSI were high which may be due to active feeding. The lower values of GSI during April and May, may be due to decrease in feeding intensity of the fishes. But no seasonal variations in the intensity of feeding was evident in this rise and fall of GSI values.

Scatophagus argus

a. Feeding intensity

The percentage occurrence of stomach in different degrees of fullness in adult of Scatophagus argus is given in Table 3 and Fig. 6. The percentage of 'gorged' and 'empty' stomachs were lesser than the other categories. 'Gorged' stomachs were absent in May, June and November. The highest percentage of 'gorged' stomachs was noticed in February (8.65) and September (8.48) and lowest value in December (1.23). 'Full' stomachs were present in all the months and the feeding intensity was observed to be high throughout the year. Their percentage of frequency was highest in December (29.59) followed by January (28.68) and November (27.24). The lowest value was recorded in June (20.97). '3/4 full' and '1/2 full' guts were abundant than any other groups throughout the year. These categories did not exhibit wide variations in their monthly appearence. The highest percentage of '3/4 full' stomachs was 41.68 (May) and the lowest 32.72 (December). The maximum and minimum values of '1/2 full' stomachs were 32.98% in November and 22.39% in September respectively. Guts with 'trace' or 'little' amounts were observed in all the twelve months, though in lesser numbers. Empty stomachs were absent in July. They were represented in all other months in very low percentages (0.72 to 2.93).

Fig.7a Monthly percentage frequency distribution of different feeding intensities (actively fed, moderately fed, poorly fed and empty stomachs) in S.argus.

Fig.7b Gastro-somatic index of S.argus

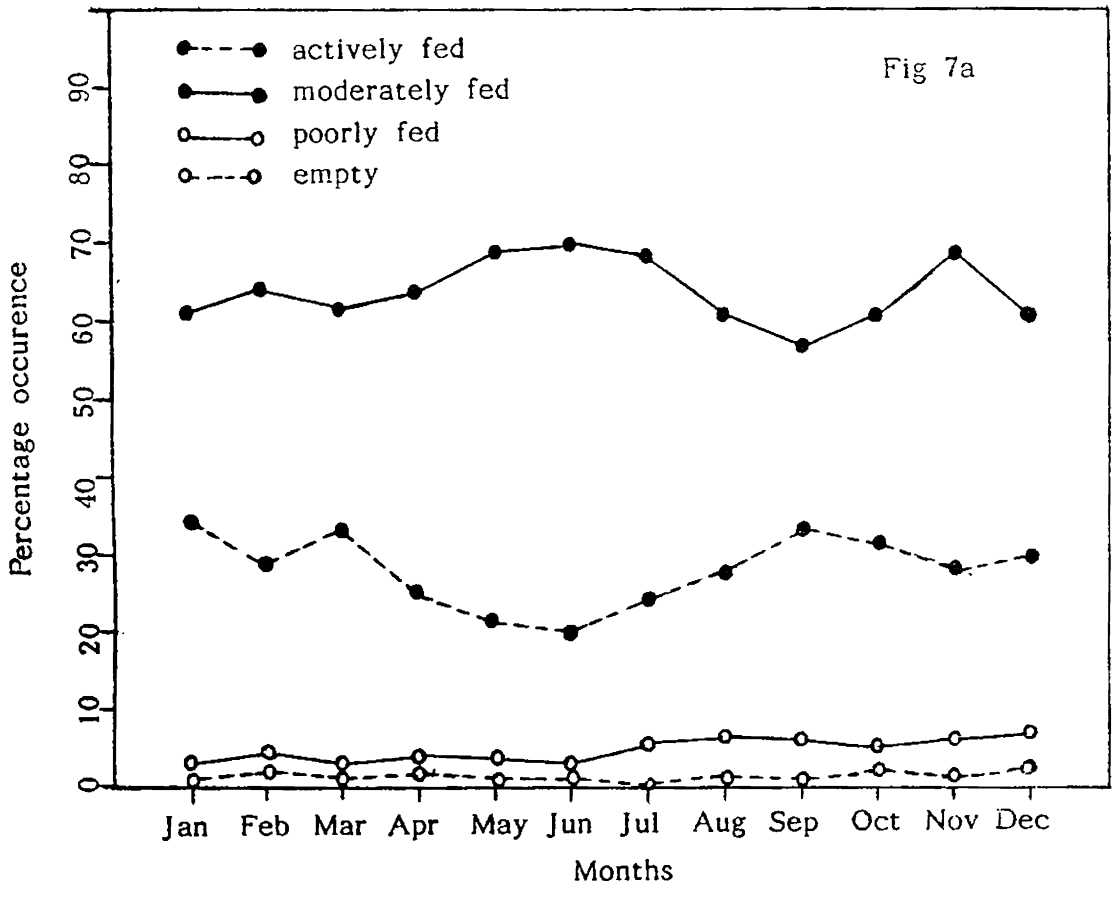

actively fed
moderately fed
poorly fed
empty
Percentage occurence
0
10
20
30
40
50
60
70
80
90
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Months

Fig 7a

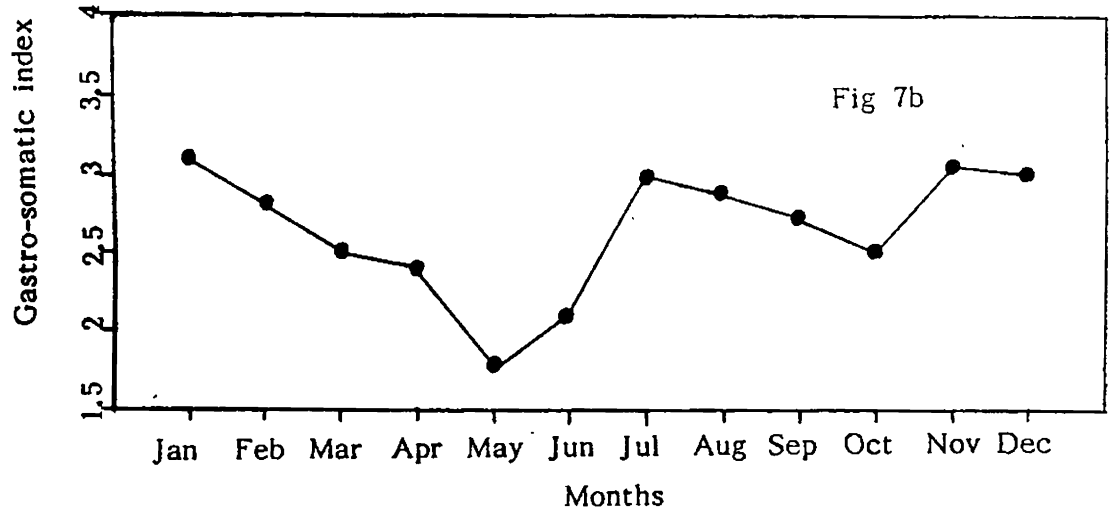

Gastro-somatic index
1,5
2
2,5
3
3,5
4
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Months

Fig 7b

The monthly percentage of various categories of stomach fullness in S.argus are shown in Fig. 7a. The percentage occurrence of 'actively fed' stomachs was found to be lower than 'moderately fed' stomachs in the year around. Fishes with 'actively fed' stomachs showed the minimum value (24%) in July and maximum value (35%) in September. The occurence of 'moderately fed' stomachs varied from a lower value of 57% in September to a higher value of 72% in June. The percentage of 'poorly fed' stomach was low in all the months.

b. Food composition in relation to size

Juvenile fishes were found to feed on smaller food items. In the early stages they consumed mainly diatoms and smaller algae. Diatoms like Skeletonema spp., Coscinodiscus spp., Nitzchia spp., Bidulphia spp., Thalassiosira spp., Pleurosigma spp. and Navicula spp. were found to be present in abundance and those like Rhizosolenia spp., Thalassiothrix sp. and Chaetoceros sp. were found less in abundance. Trichodesmium sp. (Rhodophycae), Synechococcus sp., Synechocystis sp., Merismopedia sp. (Cyanophycae) etc., were the common algal forms.

The percentage composition of different food items in S.argus is given in Fig.8. The results of the study reveal that adult of S.argus preferred algae, diatoms, hydroids and crustaceans in that order. Algae formed the major item (about 30%) of the food. Maximum occurrence of algae in the stomach contents were found during July to December period. Rhodophycae was represented by Polysiphonia sp. and Ectocarpus sp., and Chlorophyceae represented by Spirogyra sp., Volvox sp., Cladophora sp. and Mougeotia sp. The most abundant group was Cyanophycae. They included Phormidium sp., Schizothrix sp., Lyngbya sp., Spirulina sp., Nostoc sp. and Oscillatoria spp. Among them Oscillatoria spp. were most abundant. Diatoms which occurred in abundance were Skeletonema spp., Thalassiosira spp., Coscinodiscus spp., Pleurosigma spp., Nitzchia spp., Rhizosolenia spp. and Navicula spp. and diatoms observed commonly were Bidulphia spp., Melosira sp. and Hemidiscus sp. Forms like Licmophora sp., Corethron sp. and Surirella sp. were

Fig.8 Percentage composition of different food items in S.argus.

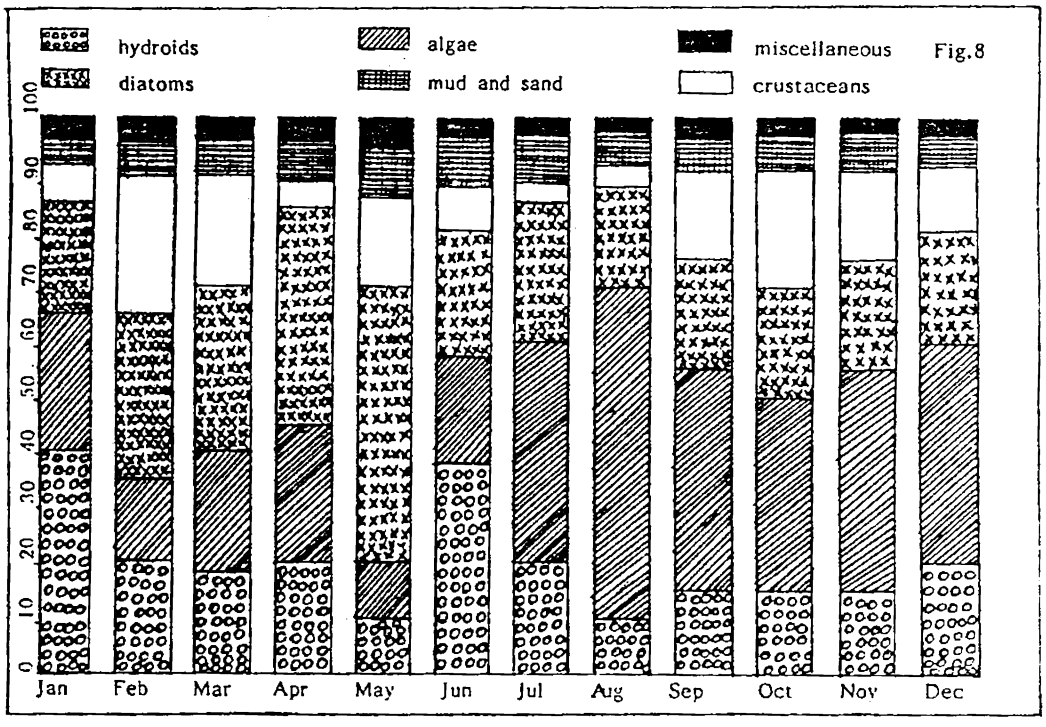

hydroids
diatoms
algae
mud and sand
miscellaneous
crustaceans
0
10
20
30
40
50
60
70
80
90
100
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec

Fig.8

found only in smaller numbers. Hydroids constituted a significant portion of the food of this fish (about 20%), their peak occurrence being in January. Very often the perisarc of these hydroids were undamaged and seen in the rectum. Crustaceans (about 13%) were present in the stomach in all the months eventhough their percentages were low in June, July and August. Crustaceans belonged to the groups copepods, amphipods, isopods and mysids. The copepods included Paracalanus sp., Acrocalanus sp., Canthocalanus sp., Eucalanus sp., Acartia sp., Centrophages sp., Temora sp., Labidocera sp. and Oithona sp. Amphipods such as Eriopisca sp., Corophium sp. and Ampelisca sp. and isopods like Cirolana sp. and Synidotea sp. were found to be present. Among crustacean larvae, zoea was common and they were found in maximum in January and March, and a second peak during September and October. Prawn larvae were present in the stomach contents with dominance during March, April and May. Fish eggs were also found with a maximum occurrence in November and December months. The miscellaneous groups (about 7%) including fish scales, crustacean remains, unidentifiable matter, molluscan shell fragments, mud and sand were the other inclusions of the stomach contents, but in lesser quantity.

c. Feeding in relation to habitat

Fishes were collected from chinese dip nets, operating in the bar mouth of Cochin and cast nets, operating in the backwaters. The specimens collected from these two areas showed significant variations in their gut contents. The most striking difference was the reduced appearance of hydroids in the stomach of fishes collected from backwaters. Hydroids had only nominal representation in these fishes while they were abundant in the gut contents of fishes collected from the bar mouth area. In few collections from backwaters (April and May) hydroids were totally absent. The quantity of diatoms were also reduced in these fishes. The absence of some species of diatoms like Halopteris sp., Licmophora sp., Bacillaria sp., Prasiola sp. and Skujaella sp. was noticeable. Not much difference was noticed in the presence of algal filaments in the gut contents of fishes from two localities. But Spirogyra sp. was the most common form present in the gut contents of fishes from backwaters.

Crustaceans were present equally in fishes collected from both the areas. Comparatively more vegetable matter was observed in the stomach contents of fishes from backwaters.

d. Feeding intensity in relation to day and night hours

Unlike in M.cyprinoides, difference in day and night feeding was noticed in S.argus. Decreased rate of feeding was noticed in dark hours of the day. Fishes caught in the night hours and early in the morning, never had 'gorged' or 'full' stomachs. Only semifilled stomach with almost digested matter was present. This digested or semidigested matter in the stomach can be accounted as the remnants of a good feed of late light hours. It should be considered that the fish did not engage in active feeding during darkness. The food items that entered the mouth cavity along with the water engulfed, formed the feed in the dark hours of the day.

e. Feeding in relation to spawning

No distinct difference was noticed in the rate of feeding depending on spawning. But the guts were less gorged during May and June. More than a reduction in feeding, the animal exhibited a selection in feeding during this period. A preference in feeding on diatoms was observed during April and May. Hydroids were represented in very little quantity during these months (about 20% and 10% respectively). But it increased and formed about 40% of the gut contents in June.

Monthly variation in the diet was observed. Algae was rich in the gut contents during the postmonsoon months. There were two peaks of occurrence for crustaceans throughout the year, first during February and March and the second during September to November. There was a decrease of crustaceans during April and December months.

Gastro-Somatic index varied monthly (Fig.7b). A sharp fall in the GSI value was seen in May and June, followed by an increase in July.

Succeeding this peak, a gradual decrease in the value was noted till October. The ebbs and falls in the GSI values coincides with the spawning seasons of the fish.

DISCUSSION

In M.cyprinoides collected from Vembanad lake, the percentage occurence of 'gorged' and 'full' stomachs were high when compared to other categories. This shows the abundant supply of food in this habitat. But the absence of 'gorged' stomachs were observed in April and May. More than availability of food, this can be due to the restriction of the intake of food. The interval of food intake may be shorter and the daily intake of food by icthyophagus species is discontinuous than other feeders. The reason for it being that the calorific value of fish is higher than other items (Longhurst, 1987). Regarding fishes collected from culture farms, the intensity of feeding is purely a function of the availability of food items. The percentages of 'actively fed' animals were low and 'poorly fed' ones were high in farms left after harvesting during April and May. The reverse situation occured when the farms were nearing harvest, during October, November and December.

The value of gastro-somatic index was found to differ every month. In specimens of M.cyprinoides collected from open waters of Vembanad estuary, it is noted that from August onwards there was a gradual decline in the gastro-somatic index till November and another decline from February to April (Fig.4b). This may be due to the decline in feeding intensity of the organism, perhaps due to the spawning period of this species. During August and February the gastro-somatic index was high which can be due to active feeding.

In S.argus, gorged stomachs were absent during May, June and November. These months are reported to be the breeding period of the fish (Saramma, 1967). The increased demand of the maturing gonads for space may be the probable reason to avoid gorged stomach. But active feeding is

observed since full stomachs were present. Moderate feeding was also high during this period. Empty stomach was absent in July, the time when spent fishes recover after spawning. Since food scarcity is very rare regarding an omnivore, only physiological parameter like breeding could be considered to influence feeding.

In S.argus, the gastro-somatic index differed every month (Fig.7b). It showed the lowest value in May, coinciding with the peak spawning period. Greater numbers of mature specimens could also be collected during May and early June. A subsequent increase in the value of gastro-somatic index in July may be due to active feeding after peak spawning during May and June.

From the present analysis of the gut contents of M. cyprinoides and S.argus, it could be observed that the juveniles of both fishes fed on smaller food items while adult fishes preferred larger ones. This change in the size of food materials may be due to the increase in the gape of the mouth, as it dictates the size of the prey that can be swallowed by the fish. Nilson (1978) pointed out that as fish increase in size, energy requirement also increases. So the energy expended in capturing small prey become uneconomical. According to Hynes (1961) and Sheldon (1969) this alteration in feeding reduces intraspecific competetion and permit more efficient utilization of the available food. Such changes in feeding, depending on size has been reported by many other workers like Basheerudin and Nayar (1961) in some juvenile fishes off Madras, Bensam (1964) in Juveniles and adults of Indian oil sardine and Kohler and Fitzzerald (1969) in the adults of cod and haddock.

Adult of M.cyprinoides depends on animal feed completely, and adult of S.argus includes hydroids and crustaceans in its feed, in addition to the major vegetable items. This increase in animal matter in the food can be due to the increased energy requirement for augmented activities accompanying maturation. According to Nair and Sobhana (1980), feeding on other animals provides the double credit of a pre-packed food. The predator along with the prey, obtains the energy and nutrition of the food on which the prey had fed and which may be still present in the gut undigested.

The present study agrees with the reports of Pasha (1964) that adult M.cyprinoides is a carnivore and that of Rajan (1964) that it is a piscivore. According to Allen and Webb (1966), piscivorous fishes have a fusiform or tubular body, narrow caudal peduncle of short or medium length, forked tail and efficient propulsive surfaces. They are adapted to prolonged cruises and for making quick rushes. The mouth opening is wide and jaws strong. These charactors fit M.cyprinoides as a piscivorous fish and the results of the present study concretes the views of Allen and Webb (1966).

Analysis of the gut contents shows that S.argus can be considered as an omnivore, consuming a wide range of food materials. This study supports the views of Bapat and Bal (1952) and Saramma (1967). But a point of disagreement prevails with the studies of Saramma. According to her, the main food item of adult S.argus is hydroids. But in the present study, the major food item is algae. Next comes diatoms and hydroids which are only third in the order of preference. This difference in the food items may be due to the difference in the availability of these items.

Variation in the abundance of different food items of the gut contents observed in the fishes caught from different localities, supports the reports of many earlier workers. Studies on the prey-predator relationship in pikes by Crossman (1962) established that prey selection is principaly based on its relative abundance. The increased occurrence of animal matter, other than prawns in M.cyprinoides collected from culture farms and reduction of hydroids in S.argus from backwaters, has been supported by Qazim (1972). He pointed out that food association of fishes is generally governed by its availability in the locality and feeding intensity appears to depend on the abundance and fluctuation of food organisms readily available in the environment. The experiments of Drenner et al. (1978) show that the feeding selectivity is influenced by the capture probability which in turn is directly proportional to its abundance. The suggestions of Jacob and Balakrishnan (1982) and, Cyrus and Blaber (1983) that the composition of the diet is probably a reflection of the food in the habitat, sustains validity in this case.

There are reports on the pursuit and capture of prey by fishes even after sun set, as observed in the present investigation in M.cyprinoides. Active feeding at night is reported in Thunnus obsesus by Talbot and Penrith (1963) and in Magalaspsis cordyla by Sreenivasan (1974). They have indicated that in carnivorous or piscivorous fishes, the prey being larger in size, its visibility is not affected in diminished light and the search for food can be accomplished even in low illumination.

But many workers have cited difference in feeding based on diurnal variation, as noticed in the present study in S.argus. Karekar and Bal (1958) and Rao (1964) reported that feeding takes place only to a limited extent in darkness and that too probably by contact. Maitland (1965) explained that passive feeding depending on natural habit may occur at night. Northcote and Lorz (1966) stated that intensity of light influences prey selection and variation in feeding can occur according to day-night changes. Allen and Welsh (1968) also emphasised on such ecological changes in daily feeding periodicities in some lake fishes. The reduced feeding observed in S.argus during night time was also noticed in brown trout and rainbow trout by Jenkin (1969).

Seasonal variation in feeding has been recorded by several workers. Such variation in feeding was observed in S.argus during spawning season. The fish exhibited a preference to smaller items of feed like diatoms and algae, and avoided larger inclusions like hydroids. But such a difference has not been noticed by Saramma (1967) in S.argus. Works of Karekar and Bal (1958) in Polynemus indicus, Kagwade (1967) in horse mackeral and Malhotra (1967) in fishes of Kashmir valley support the present observation. Mojumdar (1969) also noticed such a variation in food items in catfish Trachysurus thalassinus during spawning time.

The present study on food and feeding habits of the two fishes reveals that M.cyprinoides is an omnivore in the juvenile stage. But as it grows, the fish becomes more selective feeder and starts feeding on prawns and fishes. The availability of the food items in the habitat

determines their rank as chief and supplementary food. Feeding was not limited to the light hours of the day.

Scatophagus argus is an omnivore, consuming a wide range of food materials. The juvenile fishes fed mainly on diatoms, small algae and detritus. But the stomach contents of adult fishes included hydroids and crustaceans, in addition to algae and diatoms. The fishes avoided large food items like hydroids and preferred algae and diatoms during the spawning months. The feeding was reduced during night time.

Table 1 Percentage occurence of stomachs in different degrees of fullness in adults of M.cyprinoides collected from Vembanad lake.

Month	gorged	full	3/4 full	1/2 full	trace	empty
January	37.86	32.24	11.93	7.61	10.36	-
February	32.85	34.28	14.82	13.45	3.67	0.89
March	28.78	34.88	20.64	8.25	6.43	1.02
April	-	7.16	14.20	40.83	28.82	9.13
May	-	8.18	12.88	40.30	30.24	8.42
June	5.72	18.16	15.68	33.70	21.95	4.79
July	33.68	34.98	11.12	11.17	4.43	4.66
August	36.95	33.67	11.48	10.46	4.90	2.65
September	39.63	36.76	10.35	9.94	2.72	-
October	37.60	33.78	8.88	10.32	4.65	4.57
November	36.57	34.97	10.46	8.88	4.49	4.63
December	37.55	32.79	12.46	10.26	3.88	3.07

Table 2 Percentage occurence of stomachs in different degrees of fullness in adults of M.cyprinoides collected from culture farms.

Month	gorged	full	3/4 full	1/2 full	trace	empty
January	14.70	20.31	32.16	28.28	3.43	1.18
February	7.26	12.29	20.55	34.47	14.68	10.85
March	1.14	8.28	17.21	38.64	18.92	15.78
April	-	7.77	6.65	19.32	44.88	21.39
May	2.22	7.80	13.36	24.56	38.42	13.64
June	10.55	14.48	26.56	32.72	10.26	5.46
July	12.31	19.42	26.73	34.68	4.72	2.14
August	13.15	18.12	25.52	38.79	3.18	1.24
September	10.48	26.37	28.21	31.42	2.63	0.92
October	23.91	30.57	24.70	19.63	0.83	0.36
November	30.47	32.69	27.96	8.98	-	-
December	35.76	36.45	20.81	6.42	0.66	-

Table 3 Percentage occurence of stomachs in different degrees of fullness in adults of S.argus collected from Vembanad lake.

Month	gorged	full	3/4 full	1/2 full	trace	empty
January	5.47	28.68	33.35	28.25	2.97	1.28
February	8.65	20.68	33.42	30.55	4.54	2.26
March	6.28	26.66	32.94	29.61	3.31	1.20
April	3.56	24.69	37.82	26.91	4.39	2.66
May	-	22.71	41.68	30.14	3.85	1.70
June	-	20.97	40.92	32.88	3.66	1.48
July	2.61	22.78	38.83	30.75	5.11	-
August	5.33	24.45	32.93	28.68	6.75	1.82
September	8.48	26.76	33.86	22.39	5.57	2.93
October	3.87	27.76	32.88	30.24	4.53	0.72
November	-	27.24	33.62	32.98	4.74	1.83
December	1.23	29.59	32.72	27.19	6.83	2.45

CHAPTER II

BIOCHEMICAL CONSTITUENTS

Fish occupy the foremost position among food products of animal origin in nutritive value due to the presence of valuable proteins and easily assimilable oils rich in vitamins. Besides, fish products are also rich in other organic and mineral substances. Fish flesh consists of simple proteins - albumins, myogens, globulins, and myostromines as well as conjugated proteins. Fish fat contains saturated acids such as palmitic, myristic and stearic acids and unsaturated fatty acids such as linoleic, linolenic and arachidonic acid. The relative ease of oxidation and high iodine value of fish oils are due to the presence of fairly high quantity of unsaturated fatty acids. Minerals which are the necessary participants of water-salt metabolism, are supplied by fish products. They also provide phosphorous, an essential component of nucleo-proteins and tri-phosphates, and also iodine for the proper functioning of the thyroid. Kandoran (1976) explained that consumption of 100 gm of fish per day would meet about 25% of our daily requirement of protein, 10% of fat, half of vitamins and most minerals. Fish flesh contains very little glycogen. Fish liver contains large quantities of vitamins from which pharmaceutical preparations are made. It is also used for preparing high quality medicinal oil as well as high calorie canned products.

1. Nutritive value of fish protein

It is the content of proteins, fats, vitamins and minerals which makes fish the most valuable food. It was Drummond (1918) who first studied the nutritive quality of fish protein and found it as effective as beef protein and superior to casein in promoting growth. Kick and Mc Collum (1927) investigated the quality of haddock and herring protein. Beveridge (1947) carried out experiments by comparing fish protein with those of livestock origin. He concluded that fish protein was better than beef or egg protein. Fish proteins are most important as they provide amino acids for the synthesis of proteins during growth, for maintenance of cellular nitrogenous constituents

and for other metabolic needs. Stansby and Olcott (1963) stated that out of the 19 amino acids occuring in fish, 10 are supposed to be essential to man.

2. Nutritive significance of fish oil

Fish oils are the richest known source of vitamins A and D. The stores of those vitamins which accumulate in the livers of many fishes are much higher than those found in the livers of most other animals. This is due to the consumption of diatoms as the base link of food chain (Eithel, 1962). Liver is the important storage site of fat and vitamin A. This quality of liver varies in different species. There is also evidence that vitamins are not distributed uniformly throughout the liver tissue. In some species, potency of liver oil diminishes from upper hepatic portions to the tip of the lobe (Molteno et al., 1945). This uneven distribution of oil controls the distribution of fat-soluble vitamins. In teleosts, fat may be localized mainly in liver. These fishes yield liver oil of relatively high nutritive significance.

3. Fish as the source of minerals

The importance of fish as a source of minerals is well established. The mineral composition of cooked fish meat shows that fish is an important source of iodine, a significant source of phosphorus, potassium, iron, sulphur, calcium and magnesium, but poor in sodium and chlorine. The only rich source of iodine commonly included in the human diet is from marine teleost fishes. Fish can be used as a sodium restricted diet and it is prescribed to persons suffering from hypertension (Thurston, 1958). Dried fish which provides calcium and phosphorus is an important item of the diet of the people in villages (Holemans and Lambrechts, 1958).

Other than the nutritive significance, a clear idea about the proximate composition of fishes is essential for the standardisation of proper processing methods in fishery industry.

Though early works of Biot (1807), Morin (1822), Kuhne (1859) Haliburton (1888), Atwater (1892), Holmes (1918) and Van Furth (1918) are worth mentioning in the field of fishery science, systematic investigations of the nutritive value of fishes were initiated by Drummond (1918). He isolated the proteins of herring, cod and salmon by heat coagulation in acid medium followed by consecutive extractions with water, hot alcohol and ether.

Further elaborate work was carried out by Heart et al. (1940) regarding the proximate composition of herring in British Columbia. Reay et al. (1943) studied the chemical composition of some fishes and recommended their status as chief food item. Other notable works are that of Busson et al. (1953), Kendrew et al. (1954), Deuel (1955), Gilbert (1957), Leim (1958) and Dambergs (1959). Idler and Binters (1960) studied the biochemical composition of sockeye salmon during spawning migration while Mc Leod et al. (1960) investigated the biochemistry of coho salmon maturing sexually in aquarium. Water content of cod muscle was estimated by Love (1960). Thurston (1961) estimated the proximate composition of nine species of rock fishes and Slobodkin and Richman (1961) studied the calorific content of some fishes.

Balridge (1972) observed the accumulation and function of liver oil in Florida sharks. Goodyear and Boyd (1972) analysed the elemental composition of large mouth bass, and Love et al. (1973) the changes in proximate composition of land locked sea lamprey during the period of metamorphosis. Niimi (1974) investigated the relationship between the ash content and body weight in lamprey, trout and large mouth bass. Investigations on the caloric measurements of some estuarine organisms including fishes were made by Thayer et al. (1973). Studies on fat and moisture composition of some canadian fishes by Eaton (1975), seasonal changes in fat content of yellow perch by Newsome and Leduc (1975) and variations in fillet lipid content in Atlantic herring by Ackman and Eaton (1976) are some of the important works in this field.

The seasonal changes in protein, lipid and energy content of carcasses, ovaries and liver of adult plaice Pleuronectus platessa was reported by Dawson and Gremon (1980). Proximate composition of various stages of adult life of lamprey was studied by Bird and potter (1983), the effect of temperature on chemical composition of pink salmon by Kepshire et al. (1983) and changes in fat and protein levels in tropical anabantid by Hails (1983). Proximate composition of certain Red Sea fishes were estimated by Hanna (1984) and river lamprey by Heikkala et al. (1984). Investigations of Norman et al. (1984) on biochemical composition in red grouper and black sea bream during hypoxic exposure, and Tudor's (1984) report on the proximate composition of the white muscle of young grey mullet, Liza saliens are worth mentioning. The study made by Reinhardt and Van (1984) on lipid composition of Antartic midwater fishes is a single available report of that habitat.

In India, works on the nutritive value of fishes are comparatively scarce. Saha (1940) made investigations on the value of the proteins of fishes of Bengal. Other reports on the nutritive value of fishes are that of Setna et al. (1944) on some marine fishes off Bombay and Chari (1948) on some fishes off Madras coast. Chidambaram et al. (1952) studied the fat variations in mackeral, Venkataraman and Chari (1953) correlated the fat variations of mackeral and plankton on which they feed. Notable works on the chemical composition of freshwater fishes are those of Sreenivasan and Natarajan (1961) on certain fishes of Bhavanisagar reservoir, Natarajan and Sreenivasan (1961) on thirty five species of freshwater fishes, Jafri et al. (1964) and Khawaja (1966) on various species of freshwater fishes. Gopakumar (1968) published an account of the seasonal variations in lipid of oil sardine and chemical composition of pomfrets.

Some recent studies in this field are the works of Radhakrishnan et al. (1972), Gopakumar (1973), Jafri (1974), Jafri and Shremi (1974), Royan and Venkataramanujam (1975), Sumithra et al. (1975), Kutty et al. (1976), Mukundan and James (1978) and Mukundan et al. (1979). Some of the other recent reports of concern are those of Afser and Ali (1981), Mukundan et al. (1981), Afsar (1982), Bhagawathi and Ratha (1982), Mukundan et al. (1982), Keshava and Sen (1983) and Vijayakumar (1987).

MATERIALS AND METHODS

Details of materials collected for the study are given in the earlier part.

In the laboratory, fishes were cleaned and samples of muscle and liver were taken for the study. Care was taken to ensure that the muscle was free of bones. The wet samples were weighed and dried in an oven at 90 - 95^{o}C till a constant weight was attained. The difference in the initial and final weight (after drying) was noted to calculate the moisture content. The dried tissue was then ground well to make it into powder and stored in a dessicator for further analysis.

a. Estimation of carbohydrate

Total carbohydrate content was determined by the micromethod of Mendel et al. (1954). Glycogen in the tissue was first hydrolysed to glucose. The final determination including any glucose and fructose originally present as monosaccharides yielding those sugars, were made calorimetrically. To about 5-10 mg of dried and powdered tissue sample, added 5 ml of 5% TCA containing 0.2% silver nitrate. The mixture was boiled in a water bath for 10 minutes and then cooled. After centrifuging, 1 ml of the supernatant was pipetted out. To it 3 ml of conc.H_2SO_4 was added and warmed in a water bath to develop colour which was measured in a spectrophotometer at 530 nm. Calibration curve using glucose was prepared for the conversion of photometric readings and the results were expressed as percentage of wet weight of tissue.

b. Estimation of lipid

Sulphophosphovanillin method described by Barnes and Blackstock (1973) was adopted. The lipid content of the dried and powdered sample was extracted using a 2:1 solution of chloroform and methanol. The extract was centrifuged and 1 ml of the supernatant was pipetted into a test tube and the solvent removed under vacuum. 0.5 ml Conc.H_2SO_4 was added and the mixture

was well agitated. After stoppering with cotton wool, heated for 10 minutes in a boiling water bath. The solution was cooled and to 0.1 ml of it, 2.5 ml of phosphoric acid - vanillin reagent was added. The mixture was thoroughly mixed and left for 15-30 minutes at room temperature. The colour developed was measured in a spectrophotometer at 530 nm or 540 nm. Cholesterol was used for preparing the calibration curve. The fat content was calculated as the percentage of wet weight of tissue.

c. Estimation of protein

Protein was estimated by using Lowry's method (Lowry et al., 1951). This method is based on a combination of biuret reaction and the Folin-Ciocalteu reaction. The sample was warmed with alkali in a water bath. The mixture was centrifuged and to an aliquot of the supernatant, an alkaline solution of copper sulphate was added followed by Folin's reagent. The solution becomes blue in colour. The intensity of colour is proportional to the protein concentration of the sample. The colour is estimated spectrophotometrically and the concentration of protein was calculated from the calibration curve prepared using bovine serum albumin. The protein content was expressed as the percentage of wet weight of the tissue.

d. Determination of ash content

Ash content of the sample were determined gravimetrically as described by Weinberg (1971). Duplicate samples were weighed in previously weighed clean silica crucibles. They were placed in a muffle furnace and the temperature retained at 550°C for 12 hrs till the ashing was complete. The weight of the residual ash was noted and expressed as the percentage of the wet weight of the tissue.

e. Determination of the moisture content

Determination of the moisture content was carried out as explained by FAO publication (1981). Labelled clean watch glasses were weighed

accurately in an analytical balance. The samples to be analysed were taken in watch glasses and the weight noted. The samples were left overnight at 90-95^{o}C till a constant weight was attained. They were again weighed after cooling in a dessicator. From the difference in weight, the percentage of moisture was calculated.

f. Estimation of calorific value

The method developed by Karzinkin and Tarkovskaya (1964) was adopted for this estimation. 8-15 mg of dried and powdered sample was transferred to a clean round-bottom flask. Added 3 ml of 5% potassium iodate solution and 20 ml of Conc.H_2SO_4. Connected the flask to a reflux condenser and heated in a heating mantle for 1 hour. Oxidation of the organic matter commences immediately and lilac-coloured free iodine was liberated. The intensity of the colour is directly proportional to the amount of organic matter present in the sample. Cooled and diluted the solution with distilled water to 50 ml. Free iodine is liberated turning the mixture pink. Warmed till the colour and smell of iodine disappeared. Cooled and diluted to 250 ml with distilled water. Transferred to a conical flask of 500 ml capacity. Added 10 ml of 10% potassium iodide and kept in dark for about 10 minutes. Titrated against 0.1 N solution of sodium thiosulphate using starch indicator till the blue colour disappeared. The calorific value was calculated from the amount of thiosulphate used, weight of sample, oxygen consumed and oxycaloric-coefficient and expressed as K cals/gm tissue.

RESULTS

1. Carbohydrate level

a. Megalops cyprinoides : Muscle tissue

Carbohydrate level of the muscle tissue of M.cyprinoides was found to be very low. It varied from 1.28% to 3.37% wet weight of the tissue. The lowest value of 1.34% was recorded in the month of February and the

Fig.9 Biochemical constituents of muscle tissue of M.cyprinoides.

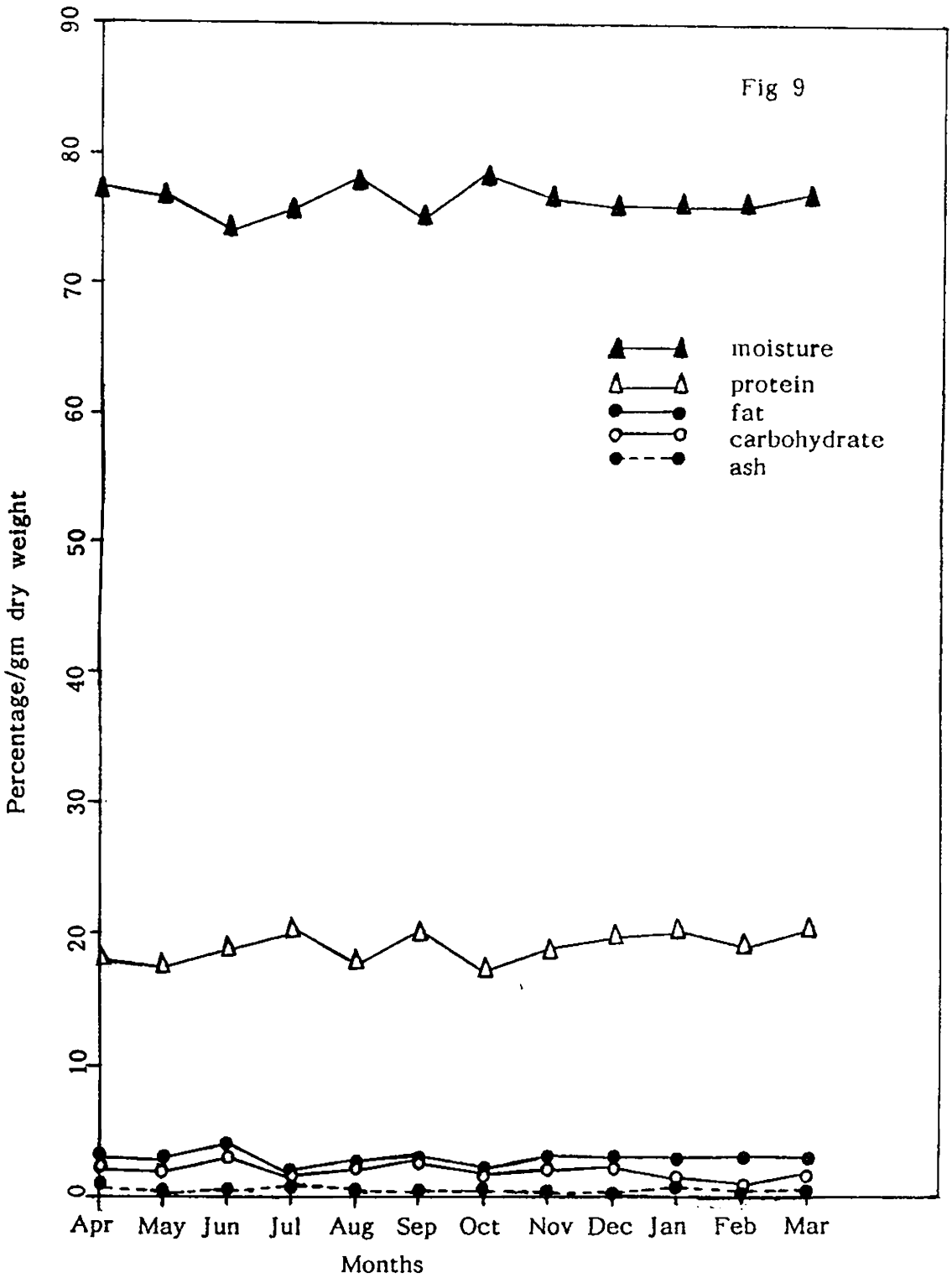


Fig 9
Percentage/gm dry weight
90
80
70
60
50
40
30
20
10
0
moisture
protein
fat
carbohydrate
ash
Apr
May
Jun
Jul
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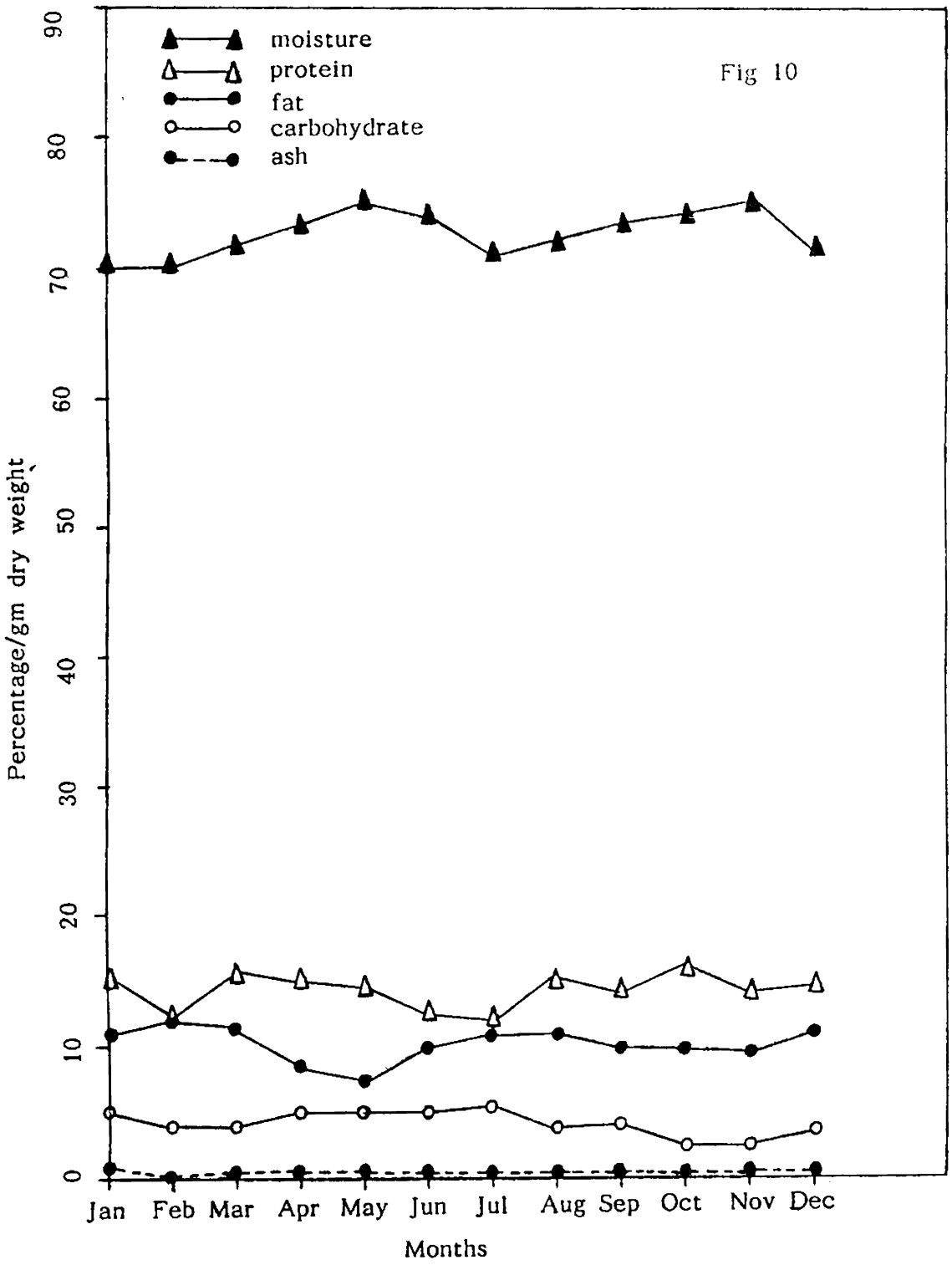
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Months

Fig.10 Biochemical constituents of liver tissue of M.cyprinoides.


moisture
protein
fat
carbohydrate
ash
Fig 10
Percentage/gm dry weight
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Jan
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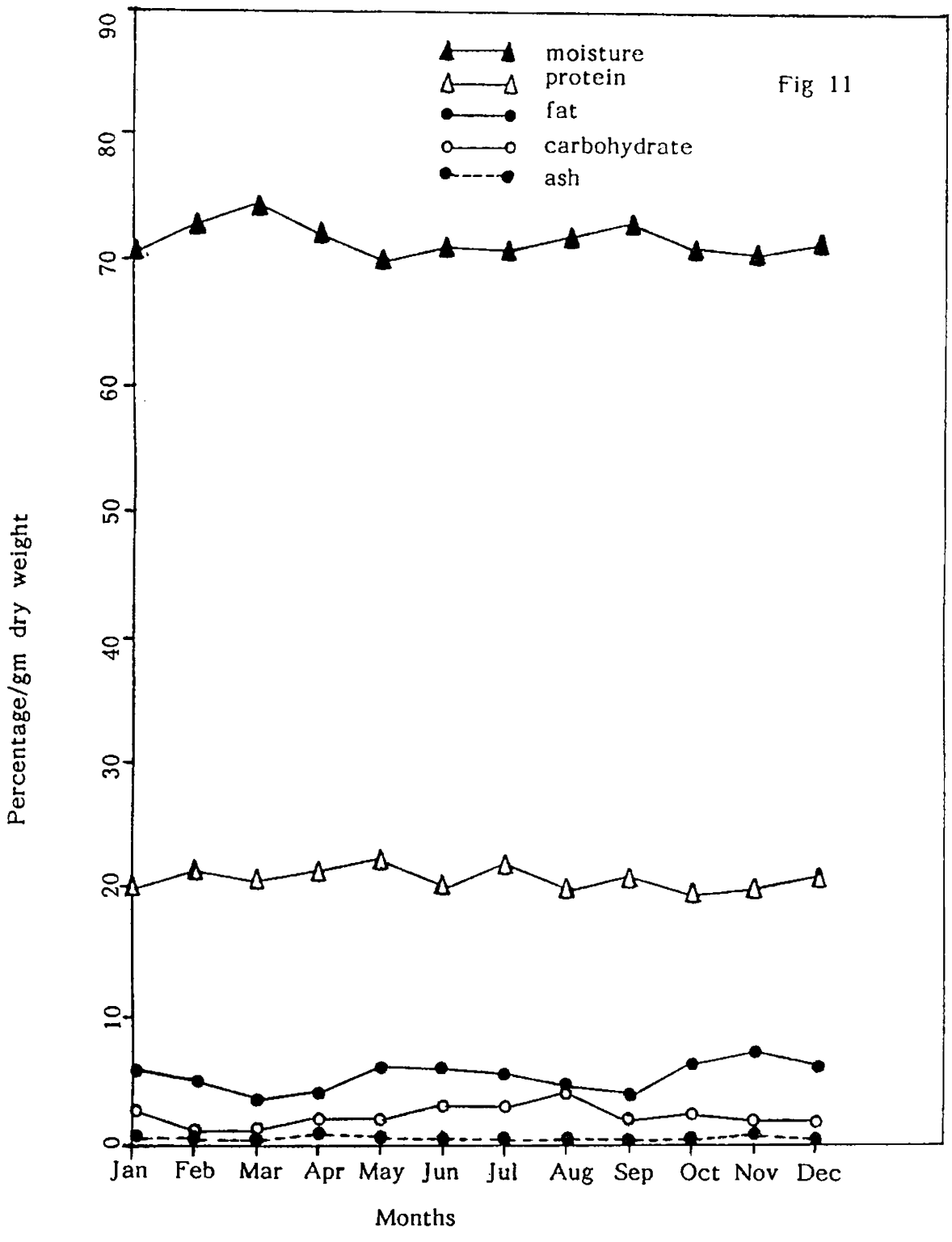
Dec
Months

Fig.11 Biochemical constituents of muscle tissue of S.argus.


moisture
protein
fat
carbohydrate
ash
Fig 11
Percentage/gm dry weight
0
10
20
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40
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Jan
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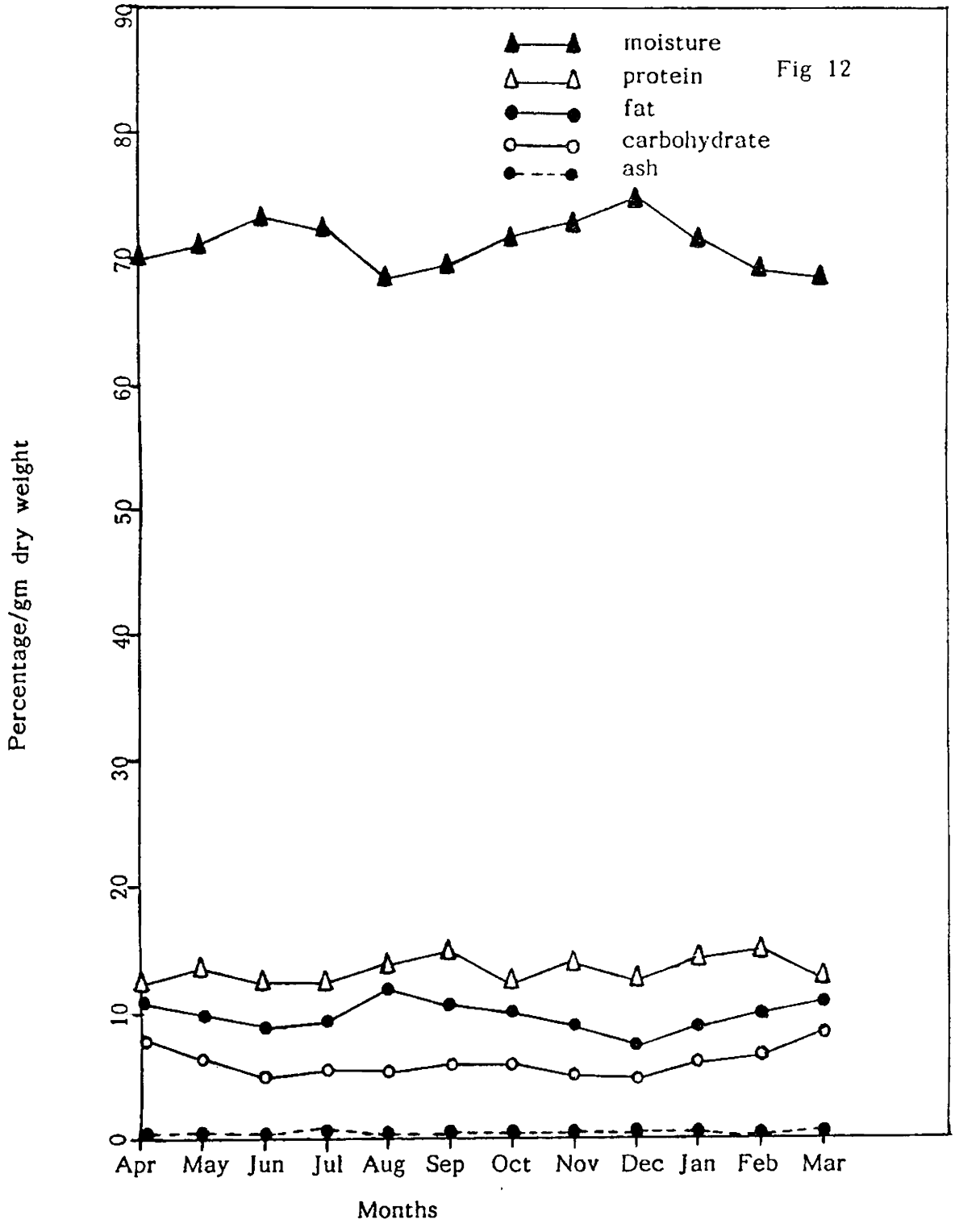
Months

Fig.12 Biochemical constituents of liver tissue of S.argus


moisture
protein
fat
carbohydrate
ash
Percentage/gm dry weight
0
10
20
30
40
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Apr
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Jan
Feb
Mar
Months

Fig 12

highest value of 3.37% in June. The carbohydrate levels did not vary considerably in other months except the slight decrease from January to March. The values of carbohydrate are shown in Table 4 and Fig.9.

b. M.cyprinoides : Liver tissue

The results of the analysis of carbohydrate for the liver tissue of M.cyprinoides are given in Table 5 and Fig.10. The liver tissue registered a higher level of carbohydrate than the muscle tissue. The level varied between 2.5% to 5.5% wet weight. The lowest value of carbohydrate in the liver was noticed in January and February and the highest in October. Monthly fluctuation of carbohydrate value in the other months were negligible.

c. Scatophagus argus : Muscle tissue

The level of carbohydrate present in the muscle tissue of S.argus was low. It showed a range of 1.05% to 3.89% wet weight of the tissue. The months of May-June and November recorded the lowest and highest levels respectively. After the peak level in November, carbohydrate value decreased and was steady till March, except a little increase in January. This was followed by an increase in April. The level again dropped in May-June after which gradual increase was noticed till it reached the maximum in November. The low levels of May-June and December coincide with the spawning months. The values obtained are presented in Table 6 and Fig.11.

d. S.argus : Liver tissue

The level of carbohydrate in the liver tissue of S.argus varied from 5.1% to 8.5% wet weight. This value was considerably higher than that recorded in the muscle tissue of the same fish. The lowest level was recorded in June and in November-December period. After June the increase in carbohydrate level was little. But after December, there was a rapid increase in carbohydrate value and reached the maximum value in March (Table 7 ; Fig.12).

2. Fat level

a. M.cyprinoides : Muscle tissue

The results of the fat content are shown in Table 4 and Fig.9. The fat values of muscle tissue of M.cyprinoides resembled very much to its carbohydrate value except in January, February and March, when fat level was steady. The highest and lowest values recorded were 3.75% in June and 2.4% in July. In the other months the fat level did not exhibit much fluctuations.

b. M.cyprinoides : Liver tissue

The fat level of the liver tissue of M.cyprinoides was high and it varied between 7.5% and 12%. Two peaks were noticed in the yearly analysis, one in May (12%) and the other in October - November (11%). Though a depletion of fat content was clear after October - November period, it was not a sharp fall as observed after the peak in May. After this peak, the fat level dropped to the lowest value of 7.5% in August, (Table 5 ; Fig.10).

c. S.argus : Muscle tissue

In the muscle tissue of S.argus the fat level was higher than the carbohydrate level and it varied between 3.5% in June and 7.5% in February. The low levels of 3.5% and 4% were recorded in June and December respectively. A gradual increase was observed after these months and attained a maximum in February, August and September. Fat level did not seem to be proportionate to carbohydrate level. Both followed independant trends. But unlike the carbohydrate levels, fat levels were more subjected to monthly variations (Table 6 ; Fig.11).

d. S.argus : Liver tissue

A high level of fat (between 7.5 and 11.68%) was observed in the liver tissue of S.argus. As in the case of carbohydrate in the liver tissue, fat values also diminished in June and December. After these depressions, the fish regained the fat level to the maximum of 11.68% in August and 10.68% in March and April (Table 7 ; Fig.12).

3. Protein level

a. M.cyprinoides : Muscle tissue

The values of protein in the muscle tissue of M.cyprinoides are given in Table 4 and Fig.9. The analysis reveals that muscle tissue is rich in protein. The protein values varied between 17.2% and 20%. Protein values did not exhibit a regular variation monthwise. The maximum value of 20% was registered in July, September, January and March and the minimum of 17.2% in October. The protein values were independent of carbohydrate or fat values.

b. M.cyprinoides : Liver tissue

The liver tissue of M.cyprinoides contained a lesser level of protein than the muscle tissue. The highest level of protein was recorded in January (16%) and the lowest value of 11.6% in October. The protein level expressed an irregular trend with considerable monthly fluctuations. The protein values were dependant neither on carbohydrate nor on fat (Table 5 ; Fig.10).

c. S.argus : Muscle tissue

Protein values were rather higher in the muscle tissue of S.argus. Though the protein values varied monthly, it never reached above 22.5% and

below 19.5%. The greatest amount of protein in the muscle tissue was present in August. The low value of 19.5% was found in January, February, April and September. In all other months, the fluctuation were negligible. Protein content had no significance on carbohydrate or fat content (Table 6 ; Fig.11).

d. S.argus : Liver tissue

As in the case of M.cyprinoides, the protein level in the liver tissue of S.argus was lower than the muscle tissue. February and September recorded the highest value of 15%. The lowest value of 12.5% was recorded in the months of June, July and December. The monthly fluctuations were irregular and did not show any similarity to fat or carbohydrate constituents of the same tissue (Table 7 ; Fig.12).

4. Ash level

a. M.cyprinoides

Ash content of muscle and liver tissues of M.cyprinoides was very low. It did not vary more than 0.5% in both cases except a record of 1% in April and July in the muscle, and April alone in the liver. Data obtained in ash content of the muscle and liver are presented in Tables 4 and 5, and Figs. 9 and 10.

b. S.argus

The ash content of muscle and liver tissues of S.argus was around 0.5% in almost all the months. But this reached upto 1% in July and February in muscle tissue, and July in liver (Tables 6 and 7; Figs.11 and 12).

5. Moisture level

a. M.cyprinoides : Muscle tissue

74% to 78% moisture was noticed in the muscle tissue of M.cyprinoides. These values were obtained in the months of June and October respectively. The moisture values showed an inverse relationship with fat. Protein or carbohydrate values had no such correlations to moisture level. The values are given in Table 4 and Fig.9.

b. M.cyprinoides : Liver tissue

The liver tissue had a lesser amount of moisture when compared to muscle. The range of moisture level was between 70% to 75%. A level of seventy percent occurred in April and May, and the peak in February and August. Moisture and fat registered an inverse proportion in this analysis. But no correlation of moisture could be noticed with carbohydrate or protein (Table 5 ; Fig.10).

c. S.argus : Muscle tissue

The moisture levels of the muscle tissue of S.argus varied from 70% in August to 74.5% in June. The moisture level increased when fat decreased and the increase in fat was accompanied by a decrease in the moisture content. Such a relation with the level of moisture was not recorded by carbohydrate or protein values (Table 6 ; Fig.11).

d. S.argus : Liver tissue

The liver tissue registered a lower level of moisture than the muscle tissue ranging from 68.5% to 74%. The lowest moisture level was noticed in August and the highest in December. The highest moisture values were observed when the fat values were lowest, and the lowest moisture level corresponded to the highest fat values. But carbohydrate and protein values

Fig.13 Calorific value of muscle tissue of M.cyprinoides.

Fig.14 Calorific value of liver tissue of M.cyprinoides.

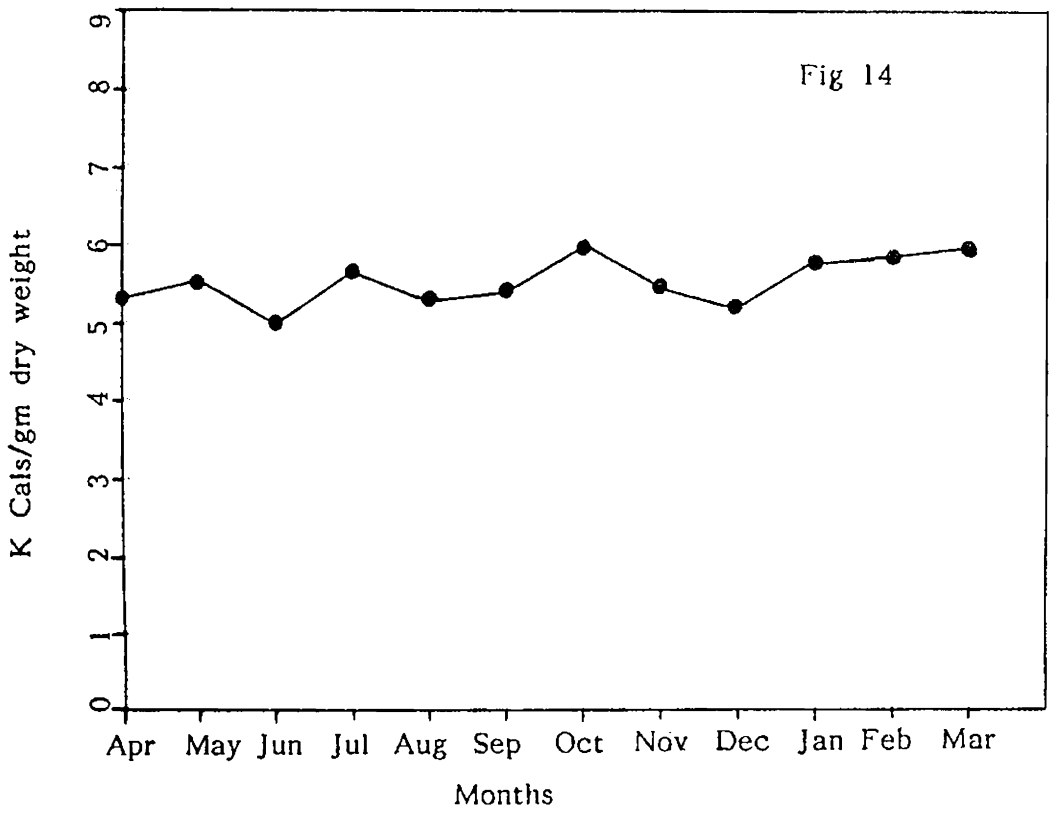
Fig 14
K Cals/gm dry weight
0
1
2
3
4
5
6
7
8
9
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Months

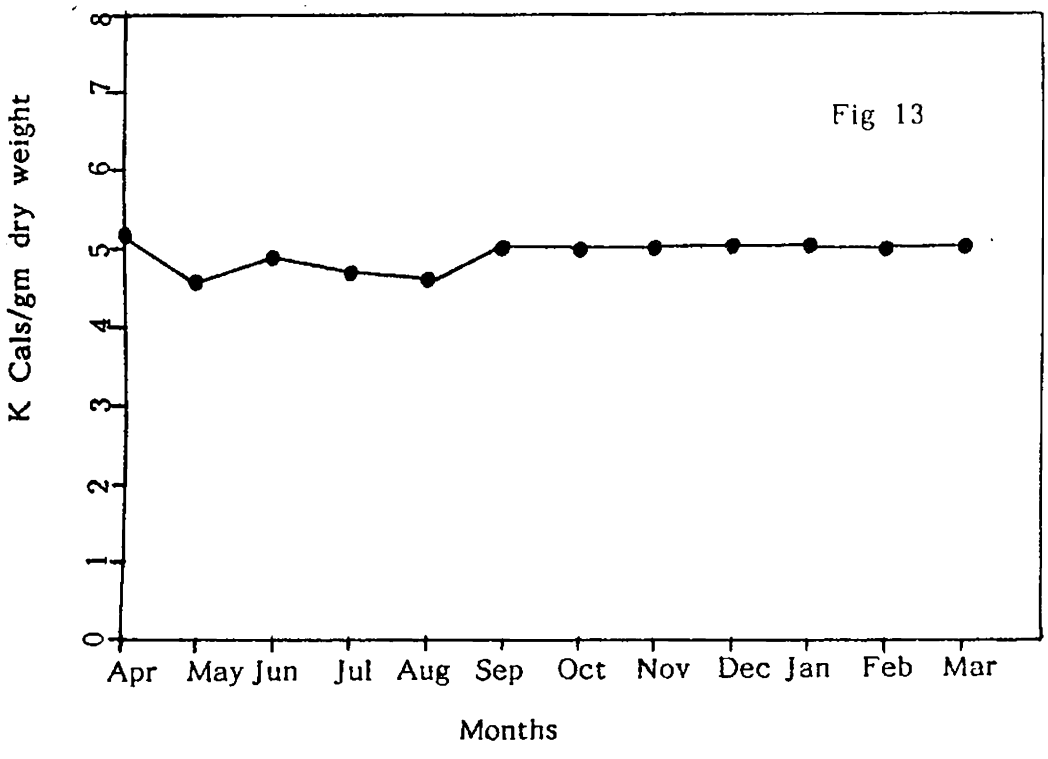
Fig 13
K Cals/gm dry weight
0
1
2
3
4
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8
Apr
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Jan
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Mar
Months

Fig.15 Calorific value of muscle tissue of S.argus.

Fig.16 Calorific value of liver tissue of S.argus.

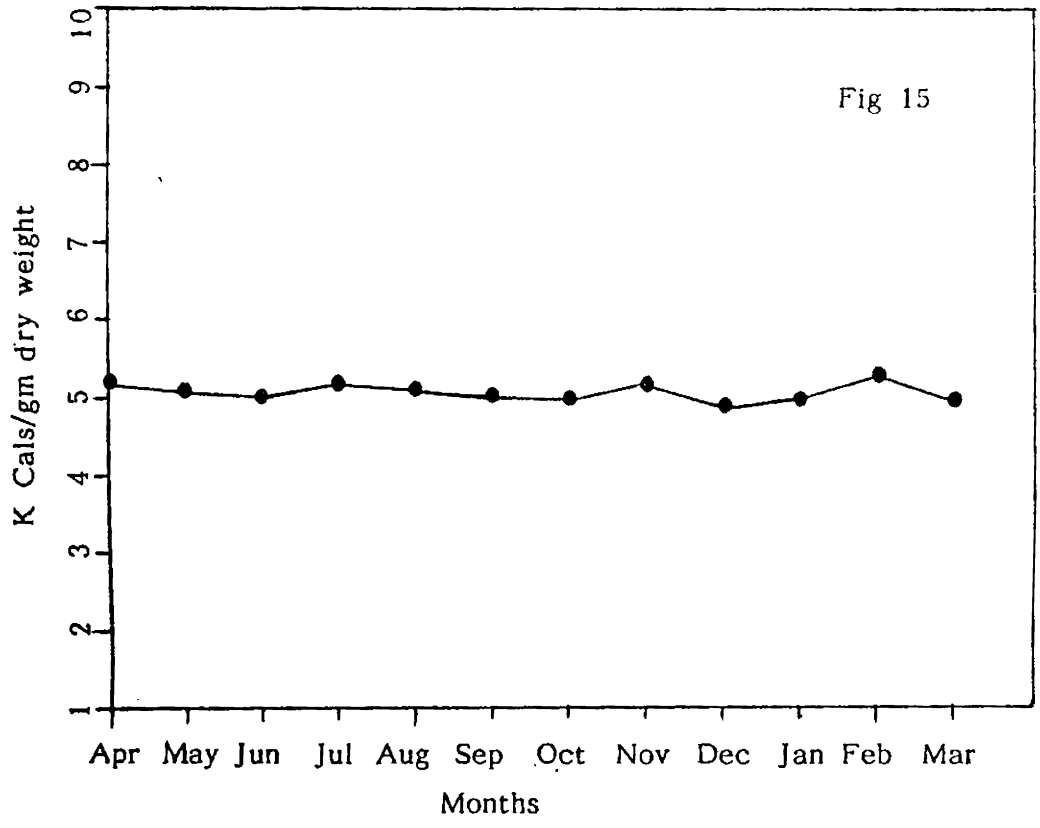
Fig 15
K Cals/gm dry weight
1
2
3
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5
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Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar
Months

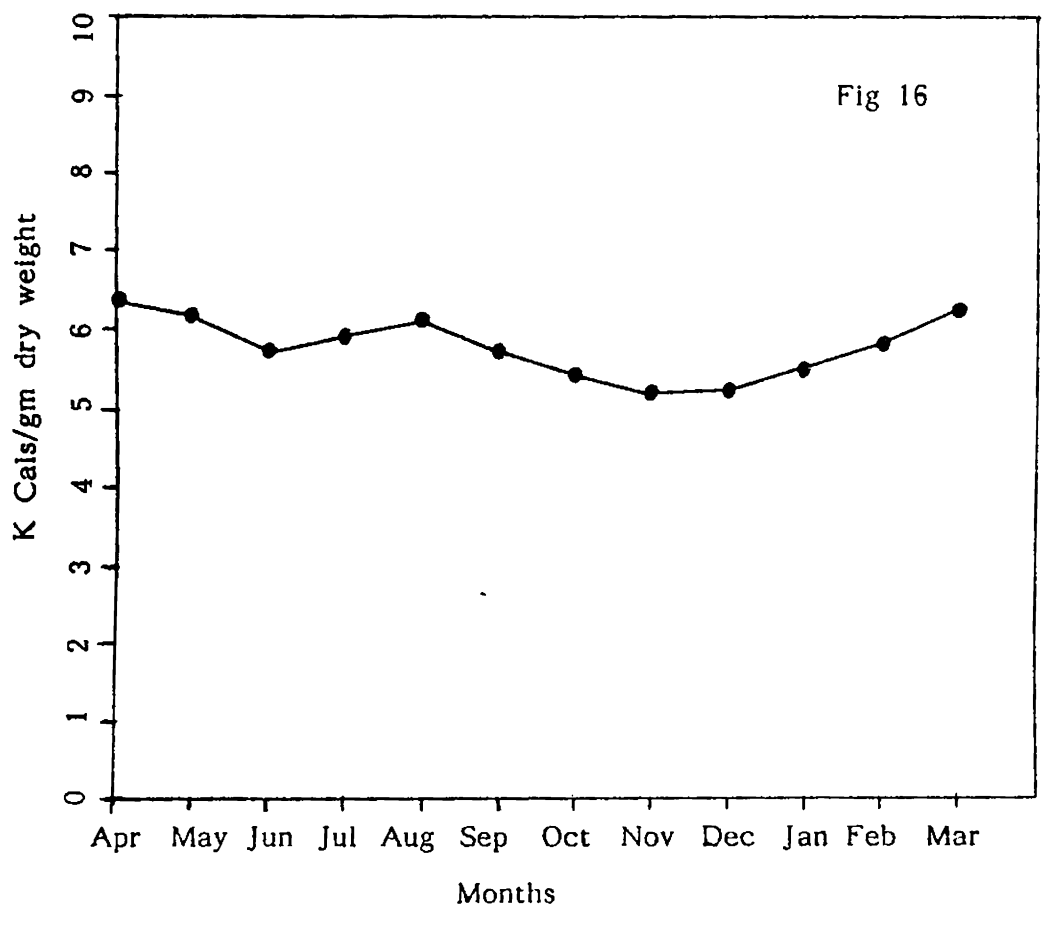
Fig 16
K Cals/gm dry weight
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2
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Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar
Months

were independent of moisture level (Table 7 ; Fig.12).

6. Calorific value

a. M.cyprinoides : Muscle tissue

5.2 K cals/ gm tissue was the maximum calorific value recorded in muscle tissue of M.cyprinoides in April. The lowest recorded value was 4.6 K cals/gm tissue during May to August. Calorific values were steady from March to September, registering a value of 5 K cals/gm tissue. Results are given in Table 4 and Fig.13.

b. M.cyprinoides : Liver tissue

The calorific value of the liver tissue of M.cyprinoides varied widely in different months from 5 K cals/gm tissue in June to 6 K cals/gm tissue in October and March. The fluctuations exhibited during the annual period were irregular. (Table 5 ; Fig.14).

c. S.argus : Muscle tissue

In the muscle tissue of S.argus the calorific value observed ranged between 4.9 K cals/gm tissue in December to 5.3 K cals/gm in February. Monthly fluctuations were narrow. A low value of 5 K cals was recorded in June, September, October, January and March (Table 6 ; Fig.15).

d. S.argus : Liver tissue

Liver tissue exhibited a higher calorific value, the highest being 6.4 K cals/gm tissue in April and the lowest of 5.2 K cals/gm tissue in November and December. The annual variation appeared in 2 cycles. After the 1st peak in April, there was gradual lowering till June. From June onwards the value increased and reached the maximum in August. The second cycle reached the maximum value in April (Table 7 ; Fig. 16).

DISCUSSION

Low carbohydrate values were recorded in the muscle tissue of both M.cyprinoides and S.argus. But higher values were present in the liver tissue of both the fishes. The variation in the values of carbohydrate in the muscle and liver tissues of some fishes has been established by some earlier workers. According to Caulton and Bursel (1977), liver is the storage site of glycogen and the role of muscle in the storage of glycogen is very little. Based on the studies on three spined sticklebacks, Wotton et al. (1978) showed that the glycogen content of the carcass was negligible when compared to liver. This variation in carbohydrates has been proved by Vijayakumar (1987) in Puntius filamentosis. The fishes under the present study also show that the glycogen content is only little in the body tissue with values ranging from 1.3% to 4.1%. This situation is explained by Shul'man (1972) that glycogen is the main source of energy only in animals with low levels of energy metabolism and which reside in anaerobic conditions. In very active animals like fishes, this role is taken up by some other factors with a higher energy content and less solubility.

The same trend was followed in the fat levels of both the fishes. The liver tissue of the fishes registered higher values (7.5% - 12%) than muscle tissue (2.4% to 3.75% in M.cyprinoides and 3.5% to 7.5% in S.argus) as reported by Vinogradov (1985) in silver hake. According to him since the fat content of the organism is linearly related to the weight of its liver, an indication of the fat content of the entire fish can be determined from the amount of fat in the liver which is the primary fat depot of the fish. Swift (1955) and Watanabe (1963) reported that fat content in the muscle of fishes is low throughout the year as noticed in the present study. The studies of Jafri (1974) on Cirrhina mrigala and Kepshire et al. (1983) in pink salmon showed the higher level of fat content in the liver tissue. As Shul'man (1972) stated, fat deposit of the liver is consumed only finally after the mesenterial, connective tissue and muscular fat is utilized. This also increases the chances for more fat content in the liver. But Lee et al. (1975) and Mc Leod et al. (1960) have stated that the lateral line muscle tissue of fish is very rich in

fat. In the present study, lateral muscle tissue was analysed for the fat content and it was found that the values were lower than the liver tissue.

In the present observation of the two fishes, it is seen that the fat content varied seasonally. Seasonal variation in the fat content of fishes has been recorded by some previous workers. Many of the teleost fishes are known to build up large quantities or fat in their tissues during feeding season to be used as readily available source of energy during period of food scarcity. This is accounted as the reason for the fluctuations of fat content in different months of the year by Jafri (1968). Jafri (1974) found that Cirrhina mrigala builds up considerable quanity of fat reserves in its liver during post-winter and post monsoon and it was in the latter period the fish is known to indulge in active feeding. He observed that seasonal fluctuations in fat content of liver is governed by maturation and depletion of gonads. According to Lovern (1934), fat is usually the largest biochemical fraction, second to protein and this lipid content varies with season related to sexual cycle. In the present study, a fall in lipid level is noticed in May-June and November-December which are the spawning months of the fish. Such a variation of fat was noticed by Rifaat (1984) in some species of Red Sea fishes, Leim (1957) in herring of Canadian Atlantic waters and Ackman and Eaton (1976) in Atlantic herring of south eastern Newfoundland. But the result of the present study disagrees with the studies of Newson and Leduc (1975). They are of opinion that significant differences in fat content is absent in yellow perch with and without gonads. But majority of the workers establish fat as the main energy source during maturation and spawning.

According to Shil'man (1972), fat is the main form of accumulation of reserve energy for metabolic processes in the different periods of annual cycle. He suggested that because of the particular mode of life, fish, unlike other vertebrates have very high reproductive capacity and fat supplies energy for the synthesis of genital products, a process which has very strict time limits and therefore calls for specially intense energy expenditures. This is due to its occurrence in most concentrate (most calorific), most convenient deposition and stability. Fat is more inert biochemically than glycogen and

protein. It is more fully utilized in the production of energy. All these make fat the most important energy source for the highly active and most energy demanding animals like fishes. Dawson and Greemm (1980) and Jafri (1974) suggest that lipid is by far the most important energy reserve, supplying 75% energy for metabolism.

The muscle tisue of S.argus contained more fat than the muscle tissue of M.cyprinoides. The difference in the fat content in two fishes is due to the difference in their diet. Being a carnivorous fish, M.cyprinoides contains lower values of lipid (with a range of 2.4% to 3.75%) than that of S.argus (with a range of 3.5% to 7.5%), which is an omnivorous feeder. The variation in fat content in fishes based on their feeding habits has been reported earlier by Venkataraman and Chari (1953). According to them, fishes feeding on plankton-rich feed contain more lipid. Sreenivasan and Natarajan (1961) while studying the chemical composition of some fishes of Bhavanisagar reservoir revealed that predatory and carnivorous fishes have a lower fat content than plankton feeding fishes. Venkataram and Solanki (1968) suggested that the quantity of fat in fish is dependant on the nature of their diet. The results of the present study also revealed that a carnivorous fish M.cyprinoides has a lower value of lipid than an omnivorous fish S.argus.

The protein content of the muscle tissue of M.cyprinoides and S.argus are given in figs.9 and 11. Khawaja (1966) found that carps contain an average of 18% protein and cat fishes and murrels an average of 17% protein. Kutty et al. (1976) estimated a protein content of 20.7% to 16.02% in some species of Indian fishes. Tudor (1984) observed an increased amount of protein in grey mullet, Liza saliens. Rifaat (1984) estimated protein contents in some Red Sea fishes. In the present study M.cyprinoides shows the value of proteins ranging from 17.2% to 20.4% and S.argus 19.5% to 22.5%. The monthly fluctuations of protein throughout the year in the muscle tissue of the two fishes were low as observed by the previous workers. Seasonal variations in protein content was also not prominent, as noticed by Kovalchuk (1954) in some cultivated fish species.

In both the fishes under present study, muscle tissue contained higher amount of protein (17.2% to 22.5%) than liver tissue (11.6% to 15.7%). Dambergs (1963) made a similar observation in his studies on cod. Bhagowati and Ratha (1982) arranged the tissues of some fishes in accordance with their abundance in protein content as muscle > liver > kidney > gill. Mukundan et al. (1982) registered a higher level of protein in white muscle of oil sardine. Rifaat (1984) estimated protein content in Red Sea fishes and classified muscle into five fat-protein content categories. In all these previous investigations, it is suggested that the relative amount of flesh is a decisive factor influencing the protein content of the fish. A plankton rich feed is reported to increase the protein content in fishes (Marimokovic and Zei, 1959). Of the two fishes investigated at present, S.argus, an omnivore, which includes a considerable amount of plankton in its feed, recorded a higher protein level (19.5% - 22.5%) than the carnivorous M. cyprinoides.

Kleiminov (1971) reported the protein values of highly demanded table fishes. According to him Barracuda (21.30%), Drenpane (18.2% - 18.4%), Caranx (19.8%), stripped mullet (19.6%), Flounder (18.20%), Butterfish (19.50%), Swordfish (19.50%), Halibut (18.2%), Threadfin (18.3%), Indian mackerel (20.5%), Lizardfish (19.4%) etc. are highly nutritive due to their protein value. The results of the present analysis suggest that the two fishes studied, can also be included in this group, since their protein levels (17.2% - 22.5%) are similar to the above mentioned fishes.

Workers like Natarajan and Sreenivasan (1961), Radhakrishnan et al. (1972) and Rifaat (1984) reported an ash content ranging from 1% to 1.5% in several species of fishes. But in the present study the ash content ranged between 0.4% to 1% which is slightly lower than the previous reports. Nimii (1974) remarked that a variation in ash content upto 1% of the body weight can occur based on feeding levels. There is no marked seasonal variation in the ash contents of muscle tissue and liver tissue of the two fishes. Though the results of the studies of Elliott (1976) in brown trout and Solanki et al. (1976) in pomfret agrees with the present observation, Love (1970) disagrees with it. According to him, the proper functioning of

the muscle depends on a correct balance of water, protein and ionic constituents. So it is logical to expect a readjustment of the inorganic substance when the proportions of water and protein alter.

The moisture content (68.5% - 75%) of the tissues of the two fishes studied can be compared with the previous reports of Eaton et al. (1975) in canadian capelin, Kutty et al. (1976) in some Indian fishes and Kleimenov (1971) in some species of fishes from different oceans. Kleimenov (1971) has also reported a higher content of moisture (80% and above) in some fishes like thread fin (80%), pike conger (81.5%), Rosy rockfish (80.5%) which can be considered as exceptions to the normal trend.

Based on the results obtained in the present studies, it is seen that a negative correlation exists in the moisture and fat contents of the two fishes. This inverse proportion was more prominent in liver tissue since the liver tissue contained more fat than the muscle tissue. A similar inverse relationship has been recorded earlier by some workers like Natarajan and Sreenivasan (1961), Rao (1967), Jafri (1968), Love (1970) and Vijayakumar (1987). Jafri (1974) reported that this fat-water inverse relationship was constant under different conditions associated with seasonal cycles. The present studies on M.cyprinoides and S.argus also indicate that a negative correlation exists between fat and water constituents of the tissues uninfluenced by seasonal changes.

The calorific values of the tissues of the fishes (5 to 6.4 in liver tissue and 4.5 to 5.3 K cals/gm in muscle tissue) are similar to those reported by Royan and Venkataramanujam (1975) in Ambassis commersoni and Sumitra et al. (1975) in some estuarine fishes. The calorific value of liver tissue (5 K cals to 6.4 K cals) was higher than the muscle tissue (4.6 K cals to 5.3 K cals). This could be due to the higher fat level. Increase in calorific value based on fat content was recorded by Mukundan and James (1978) in grey mullets and Jafri et al. (1964) in catfishes. Since the level of fat is inversely proportional to that of water, Craig (1977) opined that calorific value

is also inversely proportional to moisture content. Seasonal fluctuations seen in fat content are observed in the calorific value also.

While studying the calorific value of Ambassis commersoni Royan and Venkataramanujam (1975) noticed the highest calorific value in the eggs. This increased calorific value could be as a result of the lipid present in the yolk in plenty. In the present analysis, S.argus being more fatty than M.cyprinoides, recorded a higher value of calorific content in muscle and liver. The positive correlation between lipid and calorific content indicates that lipid is the principal determining factor of energy content in fish. Correlation between lipid and calorific content has been reported in a number of teleost fishes by Love (1970), Nimmi (1974) and Craig (1977). As stated earlier, fat is the main source of energy for animals like fishes with high muscular activity. The significance of fat and energy is emphasised by Mc Cance and Widdowson (1956) by the statement "fat depot is the great energy bank of the body which regulates the energy metabolism, accumulating surplus energy and supplying it when a dificiency is encountered".

Table 4 **Biochemical composition** of muscle tissue of M.cyprinoides.

Biochemical Composition	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nove	Dec
Carbohydrate %	1.53	1.28	1.52	1.95	2.36	3.37	2.30	2.10	2.70	1.95	2.20	2.50
Protein %	19.17	19.30	20.40	18.40	17.50	18.50	19.70	17.50	20.05	17.24	18.50	19.50
Fat %	2.70	2.90	3.14	3.12	3.20	3.70	2.40	2.50	2.80	2.50	3.20	2.90
Ash %	1.02	0.63	0.51	1.33	0.54	0.52	1.05	0.49	0.52	0.39	0.32	0.54
Moisture %	75.80	75.40	75.00	75.30	75.50	74.30	75.50	78.10	75.00	78.50	76.50	75.70
Calorific value in K cals/gm	5.00	5.30	5.00	5.20	5.10	5.00	5.20	5.10	5.00	5.00	5.20	4.90

Table 5 Biochemical composition of liver tissue of M.cyprinoides.

Biochemical Composition	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Carbohydrate %	2.51	2.48	3.50	5.12	4.30	3.90	5.05	5.10	5.30	5.50	3.70	3.60
Protein %	15.72	13.70	14.50	14.58	12.50	15.35	15.07	14.50	12.50	11.60	14.60	13.80
Fat %	9.80	9.45	10.65	11.04	11.95	11.40	8.35	7.50	9.70	10.80	10.70	10.10
Ash %	0.38	0.45	0.58	0.96	0.45	0.47	0.61	0.64	0.45	0.49	0.53	0.44
Moisture %	73.60	74.70	71.50	70.00	70.00	71.35	73.00	74.70	73.50	71.50	71.70	73.50
Calorific value in K cals/gm	5.90	5.95	6.00	5.30	5.00	5.00	5.70	5.30	5.40	6.00	5.50	5.20

Table 6 **Biochemical** composition of muscle tissue of S.argus.

Biochemical Composition	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Carbohydrate %	2.50	2.10	2.35	2.50	1.05	1.10	2.30	2.12	2.63	2.78	3.89	2.30
Protein %	19.50	20.20	20.90	20.08	21.50	20.50	21.50	22.50	20.30	21.60	20.09	21.10
Fat %	6.50	7.50	6.50	6.20	5.10	3.50	3.80	5.60	6.10	5.45	4.50	4.30
Ash %	0.52	0.87	0.55	0.51	0.56	0.35	0.90	0.62	0.59	0.51	0.50	0.48
Moisture %	71.20	70.50	71.50	71.30	72.50	74.50	72.40	70.10	69.60	70.65	72.10	72.80
Calorific value in K cals/gm	5.00	5.00	5.00	5.20	4.50	4.90	4.70	4.60	5.00	5.00	5.00	5.00

Table 7 Biochemical composition of liver tissue of S.argus

Biochemical Composition	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Carbohydrate %	5.60	6.50	8.50	7.80	6.50	5.40	5.50	5.50	5.90	6.10	5.30	5.10
Protein %	14.50	14.80	12.53	12.42	13.45	12.54	12.46	14.20	14.90	12.53	14.10	12.45
Fat %	8.70	9.55	10.68	10.64	9.60	8.80	9.51	11.68	10.46	9.68	8.72	7.52
Ash %	0.52	0.48	0.69	0.55	0.75	0.41	0.83	0.59	0.62	0.43	0.75	0.55
Moisture %	71.50	69.00	68.50	69.70	70.60	73.00	72.00	68.50	69.50	71.50	72.50	75.00
Calorific value K cals/gm	5.50	5.80	6.20	6.40	6.20	5.70	5.90	6.10	5.70	5.40	5.20	5.20

CHAPTER III

EFFECT OF COPPER ON BLOOD

In physiological studies of fish, blood sampling is often required as an index of the effect of xenobiotic compounds on these animals. The measurement of specific physiological and biochemical changes in the blood of fish exposed to sublethal concentrations of environmental stressors may provide a sensitive method for predicting the effects of chronic exposure on survival of the animal. Such analysis are widely used for clinical purposes in mammals. But in teleosts, such applications are only moderate. A broader knowledge of the pathological effects of water pollutants on the circulating blood elements can provide a frame work for simpler routine analysis of blood in teleostian toxicology.

Most of the transportation functions of the blood can be carried out in an aquous medium represented by plasma. But an adequate transportation of gases needs special adaptation. Capacity of the protein - haemoglobin, as an efficient carrier of gases raises it to the standard of an inevitable component of blood. The shortcoming due to low solubility of oxygen in the aquous medium is overcome by the efficiency of the haemoglobin to bind oxygen. In vertebrates, plasma contains only about 0.3 ml of dissolved O_2/100 ml of blood, but the presence of haemoglobin increases the amount to about 20 ml O_2/100 ml of blood (Nielson 1963). This proves the significance of haemoglobin in gas transportation. Estimation of the changes in haemoglobin values, following acute stress is useful as indicators of haemodilution or haemoconcentration. This application is reliable to assess the pathological condition of fish.

The fact that the carrier protein-haemoglobin is accommodated in the red corpuscels of the blood, shows the high ranking of these cells in oxygen transport system. The increase or decrease in their count, or variations in the packed cell volume of this component, or the variations in the size and diameter of these cells indirectly expresses the increase or decrease of the efficiency of blood in its gas transport function. These alterations can be due to physical factors like retention of metabolites, metabolic problems as the

diminished supply of various inevitable constituents or environmental stress. Haematocrit (packed cell volume expressed as the percentage of the whole blood volume) is a method proposed as a substitute for the tedious red blood cell counts for the rapid approximation of the volume of circulating erythrocytes. It is amenable to routine fish culture or field studies as an aid to haematological diagnosis of fish health.

White blood cells are mainly asociated with immunity of the animlas. Phagocytic property and efficiency to synthesise immunoglobulins, assist these cells in this function. Retention of the normal number of leucocytes is a must for the animal to be healthy. Since the number of leucocytes in the circulating blood reflects accurately the pathological condition of the animal, or the stress it is subjected to, the estimation of the white corpuscle count has become a regular practice in haematology. Measurement of leucocrit (packed cell volume of leucocytes expressed as the percentage of the whole blood volume) values is suggested as a simple test method for rapid assessment of changes in the number of leucocytes and thrombocytes in fish blood. It is analogous to haematocrit value. In estimating WBC-total counts this technique offers advantages similar to haematocrit method. Measurement of leucocrit values has proved helpful in early detection of incipient disease conditions and in the selection of healthy stock for breeding purpose. It is also an approximate screening test to provide information on the physiological effects on environmental stress on fish health.

Piscian haematology is a comparatively modern branch of fishery science in which publications are rather less. Works of Preston (1960), Black et al. (1966), Curtois (1975), Mc Carthy et al. (1975), Carl (1979), Meade and Perrone (1980), Breasel et al. (1982), Al-Mehdi (1984), Banerjee (1984) and Mavares and Perez (1984) are worth mentioning as baseline studies. The influence of different parameters and pollutants on blood cells and plasma has been carried out by various investigators. Weinreb (1958) studied the haematology of rainbow trout, Salmo gairdneri under normal and experimental conditions of inflammation. Haematological effects of stress on teleosts were observed by Soivio and Oikari (1976) and, Wedemeyer and Mc Leay (1981). Changes in haematological parameters associated with capture and captivity

were analysed by Hickey (1982) in Cunners, and Bourne (1986) in Pleuronectus platessa. The seasonal study of blood of some wild freshwater fishes was conducted by Van vuren and Hatting (1978). Effects of pulp mill effluents on the haematological parameters were reported by Mc Leay (1973, 1975) in Coho salmon exposed to different durations. Similar work was carried out in Heteropneustes fossilis and Sarotherodon mossambicus by Haniffa et al. (1986). Falkner et al. (1966) analysed the haematological responses of goldfish, Carassius auratus to sublethal thermal shock.

Variations in the blood constituents due to the exposure to metals were examined by some investigators. The effect of cadmium on the circulatory tissue of an estuarine teleost, Killifish was subjected to detailed study by Gardner and Yevich (1970). Mercury induced variations of blood of Coho slamon were studied by Storoshuk and Gulevan (1983). Toxic effects of copper, reflected in blood were reported by Hount and Stephan (1969) in minnow, Hickim et al. (1970) in brook trout, Weiwood (1980) in rainbow trout, Dixon and Dick (1985) in Salmo gairdneri and Lus Tort et al. (1987) in dogfish.

Some observations on the relationship of microhaematocrit values to haemoglobin concentration and erythrocyte count were done by Huston and De Wilde (1972) in carp. Raizada et al. (1983) studied the monthly variations in the haematocrit value in Cirrhina mrigala. Munkittrick and Leatherland (1983) estimated the haematocrit values in goldfish, as an indicator of health and Wedemeyer et al. (1983) examined the potentials and limits of leucocrit test as an assessment method of fish health.

MATERIALS AND METHODS

M.cyrprnoides of the size 11-16 cm (30-80 gm weight) and S.argus of the size 8-11 cm (30-70 gm weight) were selected for the study. They were collected from their habitat as described earlier, and maintained in aerated aquarium tanks for two weeks for acclimatization. They were fed regularly. When the experiment was set, the fishes were transferred to three experimental tanks of 100 litre capacity and kept at room temperature

($27^{o}C \pm 2^{o}C$) for a day. This assisted to relieve the specimen from disturbance due to transfer. Brackishwater (salinity 10‰) was maintained throughout the experiment. Feeding was stopped during the experimental period. Particular care was taken to ensure that in none of the tanks, oxygen or salinity acted as limiting factor, as physiological stress may affect the toxicity of metals. In each experimental tank, eight fishes were exposed to the test medium or undosed water. Water in the two tanks was dosed with 0.1 ppm and 0.15 ppm Cu^{2+}. The third tank, undosed, served as control. These three batches constituted the experimental group. For dosing with Cu^{2+}, the salt used was $CuSO_4 \cdot 5H_2O$ (Glaxo). Water was changed partially every day and the metal ion concentrations in the experimental tanks were maintained at their respective levels throughout the experimental time. Care was taken to siphon out the faecus regularly from all the tanks.

For the purpose of collecting blood, the fish was caught and stunned by a quick blow on the head. The animal was suddenly cleaned and a slanting cut was made just above the caudal peduncle. The blood that flowed out was collected into a petri dish rinsed with heparin. Direct addition of heparin was avoided to minimise dilution. Aliquotes of this blood was used for different estimations. The experiment was set up seven times for accuracy and the statistical significance was calculated using students' 't' test (Zar 1974).

Estimation of haemoglobin

Cyanomethemoglobin method described by Ortho Diagnostic systems (1986) was followed for estimating the haemoglobin content. To 0.02 ml of blood was added 5 ml of Aculute reagent (modified Drabkin reagent) and stirred well. The pot. ferricyanide present in the reagent converts the haemoglobin iron from ferrous to ferric state to form methaemoglobin. The resulting methaemoglobin combines with pot. cyanide of the Aculute reagent to produce a stable pigment cyanomethaemoglobin which represents the sum of oxyhaemoglobin, carboxyhaemoglobin and methaemoglobin. The cyanomethaemoglobin formed is estimated calorimetricaly at 540 nm.

Measurement of haematocrit and leucocrit values

Haematocrit and leucocrit values were measured by applying the method of Mc Leay and Gordon (1977). Blood was drawn into heparinised microhaematocrit tube (0.55 ± 0.05 mm D). One end of the tubes was sealed and centrifuged at 11500 rpm for 3-5 minutes. Haematocrit and leucocrit values were measured within 30 minutes of centrifugation. Leucocrit value was measured by determining the height of the buffy layer (greyish white layer of cells separating the erythrocytes and plasma) using ocular micrometer. The measurement was converted to percentage of the whole blood. Haematocrit being a longer column was directly measured as the percentage of whole blood using the scale provided along with the microhaematocrit centrifuge used for centrifuging.

RESULTS

Mecalops cyprinoides

a. Haemoglobin content

Haemoglobin content of the control fishes remained almost stationary throughout the experimental duration. The variations were insignificant. The range was between 7.8 - 8.1 gm/100 ml blood having a mean value of 7.96 gm/100 ml blood. But in fishes challenged with 0.1 ppm and 0.15 ppm of Cu^{2+} ions, the haemoglobin content varied significantly. Variations in these two groups of animals were similar.

Haemoglobin content of fishes exposed to 0.1 ppm of Cu^{++} was significantly higher ($p<0.05$) than the control (8.3 g/100 ml against the control value of 8.1 gm/100 ml). But in the following hours of exposure a reverse trend was observed. The Hb content showed a drop to 7.85 gm/100 ml in 72 hrs ($P<0.05$), 7.4 gm/100 ml in 120 hrs ($P<0.02$) and 7.05 gm/100 ml in 168 hrs ($P<0.005$).

Fig.17 Variations in the haemoglobin content (gm/100ml blood) of M.cyprinoides exposed to two concentrations of Cu^{++} at different time periods.

Fig.18 Variations in the haematocrit values (% of whole blood) of M.cyprinoides exposed to two concentrations of Cu^{++} at different time periods.

Fig.19 Variations in the leucocrit values (% of whole blood) of M.cyprinoides exposed to two concentrations of Cu^{++} at different time periods.

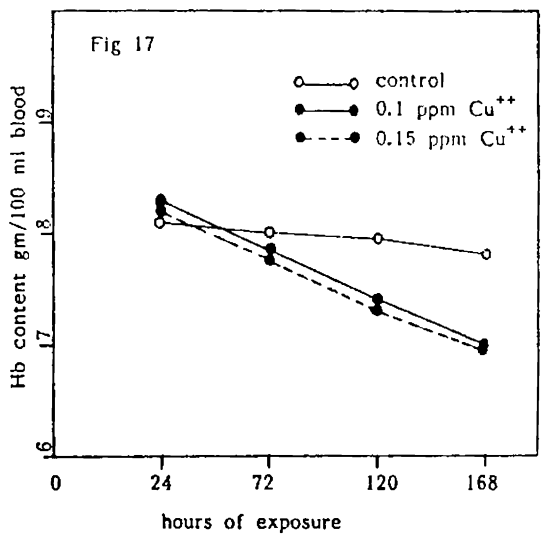
Fig 17
control
0.1 ppm Cu++
0.15 ppm Cu++
Hb content gm/100 ml blood
19
18
17
16
0
24
72
120
168
hours of exposure

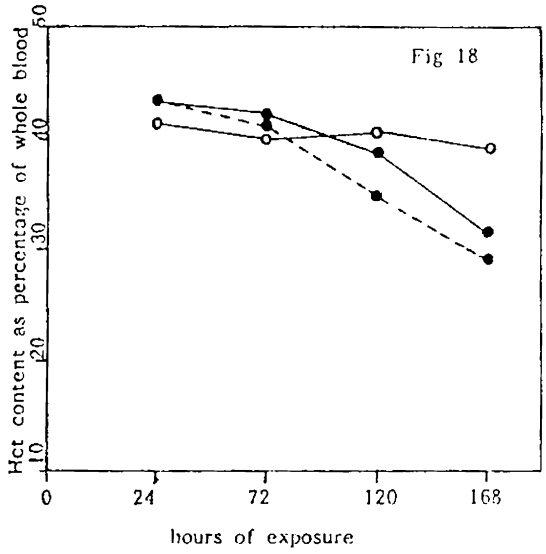
Fig 18
Hct content as percentage of whole blood
50
40
30
20
10
0
24
72
120
168
hours of exposure

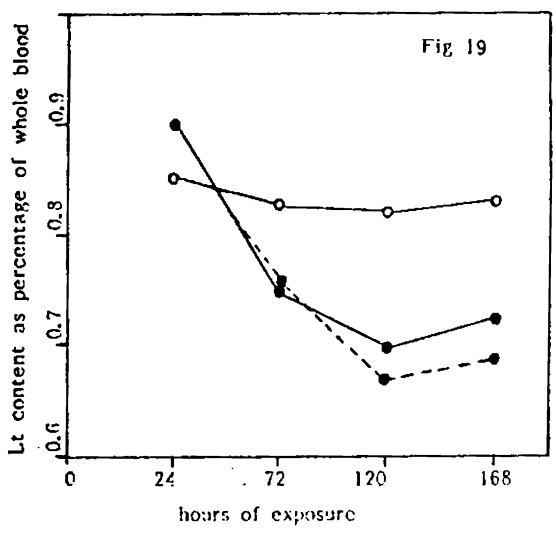
Fig 19
Lt content as percentage of whole blood
0.9
0.8
0.7
0.6
0
24
72
120
168
hours of exposure

The same pattern of response was noticed when fishes were dosed with 0.15 ppm of copper. But the increase after 24 hrs of exposure was lesser and the decrease in the following days was higher. These variations are given in Table 8 and Fig.17.

b. Haematocrit value

The variations in the haematocrit (Hct) values are given in Table 9 and Fig 18. It did not differ much from the Hb content. The Hct values of the control fish were within a narrow range of 39% to 41.5% having a mean value of 40%.

In 0.1 ppm Cu^{++} dosed fishes, an increase in Hct values was noticed after 24 hrs of exposure (43.5%; $P < 0.05$). This increase continued till 72 hrs of exposure when the Hct value was 42.2% ($P < 0.01$). A dose-dependent depression followed and the Hct values dropped to 38.8% after 120 hrs of exposure ($P < 0.05$) and 31.4% at 168 hrs of exposure ($P < 0.05$).

The variations in the Hct values of animals challenged with 0.15 ppm Cu^{2+}, resembled very much to that of fishes exposed to 0.1ppm Cu^{2+}, except in the magnitude of the increase after 72 hrs of exposure. After 24 hrs of exposure, the Hct values increased to 43.5% ($P < 0.01$) and after 72 hrs the increase was insignificant (40.57%). The Hct values reduced considerably after this duration. The recorded value after 120 hrs of exposure was 35% ($P < 0.05$) and 29% ($P < 0.05$) after 168 hrs of exposure.

c. Leucocrit value

The mean value of leucocrit observed in the control fishes was 0.825% and the range was 0.82% to 0.85%.

After 24 hrs of exposure an increase independent of the conc. of Cu^{++} occured (0.9% ; $P < 0.05$) in both groups of fishes. A significant depression followed to 0.75% ($P < 0.05$) and 0.76% ($P < 0.05$) in 0.1 ppm and 0.15 ppm Cu^{2+}

dosed fishes respectively. The difference between the values of two groups of fishes was insignificant. The depression of Lt values continued till 120 hrs of exposure (0.7% in 0.1 ppm Cu^{++} dosage; $P < 0.05$ and 0.67% in 0.15 ppm Cu^{++} dosage; $P < 0.05$). After this duration, a trend of regaining the Lt values was observed. The percentage of Lt registered was 0.72% ($P < 0.05$) in 0.1 ppm Cu^{++} dosed fishes and 0.69% ($P < 0.05$) in 0.15 ppm Cu^{++} dosed fishes after 168 hrs of exposure (Table 10; Fig.19).

Scatophagus argus

a. Haemoglobin content

In control fishes, haemoglobin content did not undergo much variation during the experimental period. Insignificant variations within the range of 7.6 - 8 gm/100 ml blood with a mean value of 7.91 gm/100 ml occurred. But in fishes exposed to 0.1 ppm and 0.15 ppm Cu^{++}, dose-dependent fluctuations were observed. The Hb content of control and dosed animals at different exposure periods are given in Table 11 and Fig.20.

A significant increase in haemoglobin content was recorded after 24 hrs of exposure to 0.1 ppm Cu^{2+} (8.5 gm/100ml; $P < 0.001$). In the following hrs of exposure, a depression in the haemoglobin content as 7.5 gm/100 ml after 72 hrs ($P < 0.2$), 7.3 gm/100 ml after 120 hrs ($P < 0.01$) and 7.2 gm/100 ml after 168 hrs ($P < 0.02$) was observed.

A similar trend was expressed when the fishes were exposed to 0.15 ppm of Cu^{2+}. Though the increase after 24 hrs was less prominent (8.2 gm/100 ml; $P < 0.05$), the decrease was more than that in the other group of fishes. After 72 hrs of exposure, haemoglobin content dropped to 7 gm/100 ml ($P < 0.01$) ; at 120 hrs it was 6.75 gm/100 ml ($P < 0.01$) and at 168 hrs haemoglobin still diminished to a level of 6.6 gm/100 ml ($P < 0.005$).

Fig.20 Variations in the haemoglobin content (gm/100 ml blood) of S.argus exposed to two concentrations of Cu^{++} at different time periods.

Fig.21 Variations in the haematocrit values (% of whole blood) of S.argus exposed to two concentrations of Cu^{++} at different time periods.

Fig.22 Variations in the leucocrit values (% of whole blood) of S.argus exposed to two concentrations of Cu^{++} at different time periods.

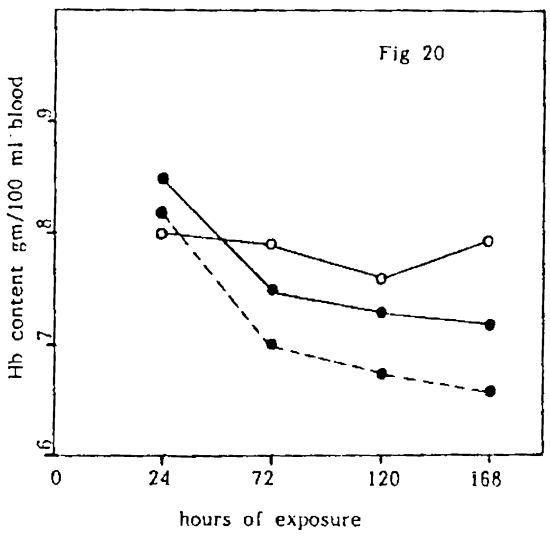
Fig 20
Hb content gm/100 ml blood
9
8
7
6
0
24
72
120
168
hours of exposure

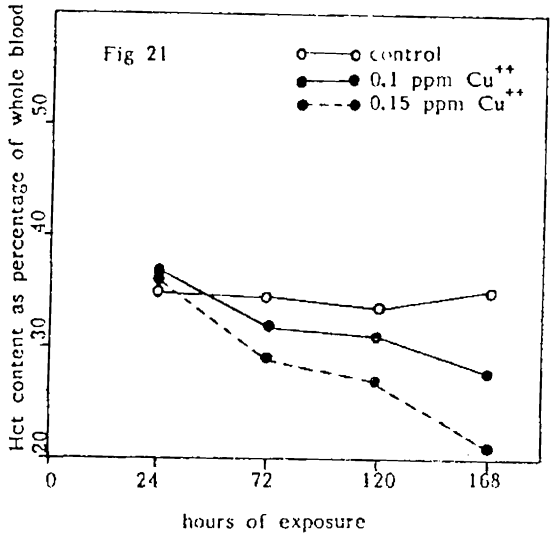
Fig 21
control
0.1 ppm Cu++
0.15 ppm Cu++
Hct content as percentage of whole blood
50
40
30
20
0
24
72
120
168
hours of exposure

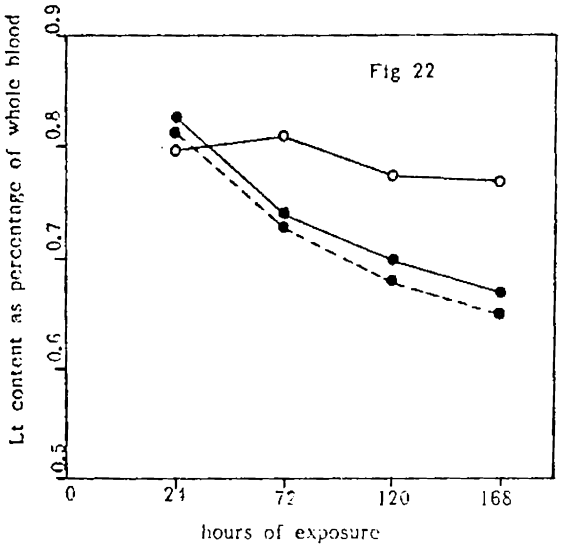
Fig 22
Lt content as percentage of whole blood
0.9
0.8
0.7
0.6
0.5
0
24
72
120
168
hours of exposure

b. Haematocrit value

The haematocrit value of the control animals and its deviations in the dosed ones are tabulated and plotted in Table 12 ; Fig.21. The haematocrit (Hct) values of the control fish registered only minor variations between 33.5 - 35% with an average of 34.5%. But the two groups of fishes challenged with 0.1 ppm Cu^{++} and 0.15 ppm Cu^{++} exhibited wide variations in their haematocrit values. The variations were directly proportional to the concentrations of Cu^{++} and duration of exposure.

After 24 hrs of exposure 0.1 ppm Cu^{++}, the Hct values increased slightly (37%; $P < 0.001$) followed by a sharp decrease. The Hct value was 32% ($P < 0.01$) after 72 hrs, 31% ($P < 0.01$) after 120 hrs, and 27.5% ($P < 0.02$) after 168 hrs of exposure.

A similar pattern of response was noticed when fishes were dosed with 0.15 ppm Cu^{2+}. A mild increase (36%; $P < 0.001$) in Hct values was observed after 24 hrs of exposure. But in the following hrs of exposure the fall in Hct values was manifested as 29% ($P < 0.001$) after 72 hrs, 27% ($P < 0.01$) after 120 hrs and 21% ($P < 0.001$) after 168 hrs.

c. Leucocrit value

The variations observed in the leucocrit (Lt) values were not different from those of Hb or Hct values. The control fishes did not exhibit much variation in their Lt values (between 0.77% to 0.81%). The mean value recorded was 0.7875%. A dose and duration dependant variation was evident in the other two groups of fishes dosed with 0.1 ppm Cu^{++} and 0.15 ppm Cu^{++}. The control values and the dosed values are tabulated and plotted in Table 13; Fig.22.

In fishes exposed to 0.1 ppm of Cu^{2+}, Lt values increased after 24 hrs of exposure by 0.025% ($P < 0.05$). But the Lt values decreased later to 0.74% ($P < 0.1$) after 72 hrs, 0.7% ($P < 0.02$) after 120 hrs and 0.67% ($P < 0.001$)

after 168 hrs of exposure.

Variations similar to the above trend were noticed in fishes challenged with 0.15 ppm Cu^{++}. The increase after 24 hrs of exposure was 0.01% ($P < 0.02$). The decrease in the Lt values observed after prolonged hours of exposure was 0.725% ($P < 0.001$) after 72 hrs, 0.675% ($P < 0.005$) after 120 hrs and 0.65% ($P < 0.001$) after 168 hrs of exposure.

DISCUSSION

Adsorption of xenobiotics from solution by most animals seems to involve passive diffusion of the compound, probably as a soluble complex, down gradients created by adsorption at the surface (through cuticle, mucus layer,etc.) and binding by the constituents of the surface cells, body fluids and internal organs. There is no evidence that any animal can prevent the entry of metals by changing the permeability rapidly, although organisms like bivalve molluscs can temporarily prevent absorption by closing the shell (Bryan 1976). In fish, chances of absorption are greater as the circulatory fluid is more exposed to these xenobiotic compounds through the gills. So this system is subjected to the exotic factors to a greater extent than any other system and the pathological effects are frequently evident in circulatory tissues.

In the present study, a marked increase in blood parameters like Hb, Hct and Lt was noticed at 24 hrs post-dosing. This reflects the pathological effects induced by the excessive intake of Cu^{2+} into the body systems, specially the haematopoietic tissues. A similar increase in Hb and Hct was recorded in rainbow trout after 24 hrs of exposure to 0.5 ppm $CuSO_4$ (Wotten and Williams, 1980). Such an increase can be the initial response to stresses, similar to the mechanisms in mammals. Exposure to stress results in the release of Adreno cortico tropic hormone (ACTH) from the pituitary gland which stimulates the release of cortisol from the interrenal gland, the homologue of the mammalian adrenal (Donaldbon and Dye, 1975). In fish, cortisol is mainly regarded as the major secreted corticosteroid. The prime function of this

corticosteroid is the enhancement of glucose metabolism to meet the energy for the increased demand of energy by other systems. The increase in glycolysis leads to the release of lactic acid and consequently decrease in the P^H. Barton et al. (1972) demonstrated that lactic acid, a product of anaerobic glycolysis, increased in zinc-poisoned fish. This is also proved in rainbow trout exposed to zinc (Hodson, 1976 a). As the normal permeability of gill membrane may be altered by the exposed Cu^{++}, gas exchange can be interrupted. Though the animal acclimatizes and gets over the crisis on continued exposure, alteration in the P^H of plasma results during the early period of exposure. As established in the animal body, erythrocyte is the major component involved in buffering the P^H of the circulatory tissue. This necessiates increased erythropoiesis which results in an augmented Hct and Hb content. A transient increase in Hct, Hb and number of erythrocyte is also observed in brook trout (Mc Kim et al., 1970) and in brown bullheads (Christenser et al., 1972). They are of opinion that increased Hct, Hb and erythrocytes might be offset by haemodilution due to reduced osmolarity. Such a rise in Hb and Hct content was noticed after 24 hrs when rainbow trout was challenged with different concentrations of $CuSO_4$. More over the release of adrenal cortisol, by itself increases circulation. The stress and the variation in the cellular components of blood in turn stimulate the immune system including lymphocytes which augment the Lt values. But anaemia was an early manifestation of acute or chronic lead intoxication in vertebrates (Leland and Kuwabara, 1985) which is a contradiction to the results of the present study in which anaemia appeared in the later period. This variation can be due to the difference in the binding sites of the trace metals.

After the initial increase, the Hct and Hb content declined. Though Hb and Hct values dropped after 24 hrs, the decrease in Hct value was lesser. The decrease in Hct and Hb values were not equal in magnitude. Hb content was lesser than the Hct value. Such a discrepancy suggests that either swelling of the RBC or an unproportionate erythropoiesis and haemopoiesis occur. The former can be due to the change in osmolarity of the erythrocyte membrane caused by the variation in the major plasma electrolytes due to Cu^{++}. The swelling of erythrocytes could also be a consequence of factors like high PCO_2,

high lactate concentration or low PO_2 in the blood leading to a low ATP concentration, which increases the oxygen affinity of the blood (Soivio and Nikiumaa, 1981). Since metals produce change in pressure of gases and lactate concentration, the swelling of red blood cells can be the response of the fish to heavy metal pollution.

The unproportionate erythropoiesis and haemopoiesis may be accounted as the inhibition of any enzyme system involved in haem synthesis. Leland and Kuwabara (1985) have established that impaired haeme synthesis occurred in vertebrates exposed to lead toxicity. The activity of α-aminolevulinic acid dehydratase (ALAD), an enzyme involved in the biosynthesis of haeme, appears to be specifically inhibited by trace metals like lead and this results in an accumulation of protoporphyrin. This may also occur in the case of copper toxicity too. But, when Haux et al. (1980) exposed rainbow trout to different concentrations of lead, despite the persistent and pronounced inhibition of some erythrocyte enzymes, no significant effects were detected in the Hct and Hb content.

The anaemia observed after 120 hrs and 168 hrs exposure to Cu^{++} can result from two basic defects, haemodilution due to the influx of body fluid into the plasma or due to the shortened life span of erythrocytes. Osmoregulatory failure is the more likely basis of acute copper toxicity (Lewis and Lewis, 1971). Studies designed by Leland and Kuwabara (1985) examined the mechanism of toxicity of copper and zinc in fish, and indicated that decreased osmoregulation is the consequence of copper toxicity in fish. Copper altered Na^+ and K^+ activated ATPase activity in coho salmon (Lorz and Mcpherson, 1976). Elevated levels of Na^+ Cl^-, the major plasma electrolytes were noticed in flounders exposed to 1 mg Cd^{++}/l (Larsson et al. 1976). A failure in osmoregulation as observed in copper toxicity can lead to decreased uptake of monovalent ions which are the major plasma electrolytes (Na^+, Cl^-). There is also chance for the loss of these electrolytes into the hypotonic external media. Both the situations result in the reduction of the levels of the major plasma electrolytes. The consequence is manifested as haemodilution by the influx of body fluid into the plasma. Decrease in osmolarity is reported

in brook trout after short-term and long-term exposure to Cu^{++} (Mc Kim et al. (1975).

A graded loss of osmoregulatory function with exposure to increasinig concentrations of copper was observed in Carcinus maenas and Cancer irroratus by Thurberg et al. (1973). In the same study, the animal registered a total absence of osmoregulatory function at a concentration of 20 ppm Cu^{++}. In phytoplankton, Cu^{++} is reported to inhibit photosynthesis by altering the cell membrane which induces the accumulation of photosynthetic products and this in turn inhibits photosynthesis (Steeman and Anderson, 1970).

In toxic concentrations, Cu^{++} may cause cytotoxicity and reduce the life span of erythrocytes, by its oxidant action and can affect the function of erythrocyte enzymes leading to an oxidation of Hb, a disulphide formation of membrane proteins, and a decrease in the intracellular concentration of reduced glutathione. Another possible reason for haemolysis is the inhibition of erythrocyte enzymes such as glutathione reductase, glucose-6-phosphate dehydrogenase and pyruvate kinase (Metz and Sagone, 1972). Cu^{++} can combine with sulphhydryl groups of membrane proteins (Salhany et al., 1978) leading to oxidation and disulphide bond formation. In mammals following copper poisoning, a reduction in blood glutathione concentration to nearly zero and an increase in methaemoglobin concentration were observed indicating that marked oxidative changes occur in erythrocyte. It is preceded by development of haemolytic anaemia (Bremmer, 1979).

The decrease in leucocrit as observed in the present experiment could be the consequence of haemodilution. According to Lewis and Lewis (1971) decrease in Lt due to haemodilution is caused by the changed osmolarity. Wedemeyer et al. (1983) related this decrease to the lowering of lymphocyte number and haemodilution. A decrease in leucocrit was noticed in dog fish exposed to 2 mg/l of copper by Lius Tort et al. (1987). According to Peters et al. (1980) leucocrit is inversely related to physiological stress. These observations agree with the results of the present study.

Table 8 Haemoglobin content (gm/100 ml of whole blood) of M.cyprinoides exposed to two concentrations of Cu^{2+} at different time periods.

	Period of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	8.10	8.00	7.95	7.80
	± SD	0.13	0.07	0.101	0.19
	Range	8 - 8.30	7.9 - 8.10	7.8 -8.10	7.6 - 8.10
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	8.30	7.85	7.38	7.05
	± SD	0.12	0.11	0.23	0.15
	Range	8.2-8.40	7.7 -7.90	7.1- 7.60	6.9- 7.30
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	8.20	7.82	7.32	7.00
	± SD	0.18	0.03	0.07	0.07
	Range	8.1 -8.30	7.6 - 8.00	7.2 - 7.40	6.9- 7.10

N = Number of animals; SD = Standard deviation.

Table 9 **Haematocrit values (% of whole blood) of** M.cyprinoides **exposed to two concentrations of** Cu^{2+} **at different time periods.**

	Period of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	41.50	40.20	41.20	39.00
	± SD	0.50	0.60	0.80	1.10
	Range	41-42	39.6 - 40.80	40.4 - 42.00	37.2 - 40.00
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	43.63	42.20	38.80	31.42
	± SD	1.10	0.39	1.01	0.58
	Range	41.9 - 45.00	41.8 - 42.80	37.5 - 40.00	30.81 - 32.00
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	43.70	40.57	35.11	29.40
	± SD	0.76	0.89	0.90	0.63
	Range	43.1 - 45.00	39.3 - 41.80	34.17 - 36.00	28.75 - 30.00

N = Number of animals; SD = Standard deviation.

Table 10 Leucocrit values (% of whole blood) of M.cyprinoides exposed to two concentrations of Cu^{2+} at different time periods.

	Period of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	0.85	0.83	0.82	0.83
	± SD	0.03	0.01	0.10	0.03
	Range	0.83 -0.88	0. 81 - 0.84	0.69 - 0.95	0.8 - 0.88
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.90	0.75	0.67	0.73
	± SD	0.0035	0.0280	0.0216	0.0300
	Range	0.90 - 0.91	0.729 - 0.80	0.618 - 0.69	0.7 - 0.73
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.91	0.76	0.68	0.69
	± SD	0.016	0.028	0.071	0.012
	Range	089 - 0.930	0.725 - 0.800	0.641 - 0.791	0.703 - 0.679

N = Number of animals; SD = Standard deviation.

Table 11 Haemoglobin content (gm/100 ml of whole blood) of S.argus exposed to two concentrations of Cu^{++} at different time periods.

	Period of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	8.08	7.98	7.69	7.94
	± SD	0.14	0.11	0.25	0.13
	Range	7.19 - 8.25	7.72 - 7.90	7.28 - 7.93	7.82 - 8.71
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	8.51	7.52	7.31	7.22
	± SD	0.21	0.48	0.25	0.83
	Range	8.16 - 8.73	6.85 - 8.19	7.06 - 7.71	6.74 - 7.66
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	8.17	6.99	6.75	6.62
	± SD	0.02	0.84	0.33	1.10
	Range	7.95 - 8.58	6.67 - 7.18	6.25 - 7.50	6.53 - 6.81

N = Number of animals; SD = Standard deviation

Table 12 Haematocrit values (% of whole blood) of S.argus exposed to two concentrations of Cu^{++} at different time periods

	Period of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	34.92	34.45	33.42	34.82
	± SD	0.49	0.24	1.11	1.46
	Range	34.4 - 35.50	33.5 - 36.59	32.7 - 35.18	32.6 - 36.36
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	37.18	32.73	31.98	28.28
	± SD	0.24	1.01	0.91	2.99
	Range	36.2 - 39.50	31.4 - 34.00	31.02 - 33.00	26.03 - 30.33
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	36.70	29.31	26.87	20.72
	± SD	0.71	0.10	2.77	2.89
	Range	35.7 - 37.70	28.4 - 29.88	23.7 - 29.82	19.2 - 23.13

N = Number of animals; SD = Standard deviation

Table 13 Leucocrit values (% of whole blood) of S.argus exposed to two concentrations of Cu^{++} at different time periods.

	Period of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	0.80	0.81	0.78	0.77
	± SD	0.01	0.01	0.07	0.01
	Range	0.78 - 0.82	0.80 - 0.83	0.69 - 0.87	0.76 - 0.79
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.84	0.82	0.71	0.68
	± SD	0.02	0.14	0.01	0.02
	Range	0.79 - 0.85	0.80 - 0.84	0.68 - 9.71	0.65 - 0.71
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.82	0.73	0.67	0.65
	± SD	0.01	0.02	0.01	0.05
	Range	0.80 - 0.84	0.71 - 0.74	0.65 - 0.68	0.58 - 0.71

N = Number of animals; SD = Standard deviation

CHAPTER IV

EFFECT OF COPPER ON THE ACTIVITY PATTERN OF ACID AND ALKALINE PHOSPHATASES IN THE RENAL AND HEPATIC TISSUES

The impact of pollutants on an organism is initiated as the disturbances at the subcellular and cellular levels of the organism. Since lysosomes are the subcellular units involved in the concentration, disintegration and elimination of toxicants, a knowledge of the concentration of important lysosomal marker enzymes is inevitable to monitor the extent of pollution caused by biotic and abiotic factors. Cell membrane which surrounds the cell and the continuous endoplasmic reliculum are the first to confront pollutants. They are susceptible to the effect of pollutants as they bind to the lipoprotein layer of the membrane and induce variation in the permeability which upset the whole cellular systems. So a study of the activity of the membrane bound enzymes becomes a useful index of the extent of pollution imposed. In the present chapter, investigation on the activity of two phosphomonoesterases; acid phosphatase, a lysosomal marker enzyme (Kendall and Hawkins, 1975) and alkaline phosphatase, an enzyme bound to the cell membrane and endoplasmic reticulum (Ciro et al., 1975) is designed.

Acid and alkaline phosphatases are groups of enzymes that hydrolyse phosphomonoesters in a relatively non-specific manner with optimal activity in the acidic and alkaline P^H respectively (Chin-yin and Hiroyuki, 1987). These phosphomonoesterases are involved in carbohydrate metabolism and glycolysis (Miller and Crane, 1961), in energy transfer through ATP (Schaefler, 1967), in the metabolism of nucleotides and phospholipids (Goodman and Rothstein, 1957), in tissue growth and differentiation (Barker and Alexander, 1958) and active transport of materials through phosphorylated intermediates (Goodman and Rothstein, 1957). Acid phosphatase is associated with lysosomes and is designated as one of marker enzymes (Edward and James, 1987). It is a hydrolytic enzyme which takes part in the dissolution of dead cells and as such is a good indicator of stress conditions in biological system (Gupta et al. 1975; Verma et al. 1980).

The site of copper accumulation was investigated in carp exposed to 0.1 ppm copper for 2 weeks by Yamamoto et al. (1977). They concluded that, by copper loading, the statistically highest increase in copper content was observed in the hepatopancreas. More than 70% of the copper was present in the supernatant fraction of the hepatopancreas extract. They also noticed that in carp hepatopancreas, copper induces the synthesis of a copper binding protein which can be identified as a metallothionein-like protein. Kidney is accepted as the major organ system designated for the physiological elimination of endobiotic and xenobiotic factors. So these two tissues, liver and kidney were considered for the study of phosphatases, a major group of hydrolytic enzymes, when the animal encountered a xenobiotic stress.

The mechanism of di-nitrophenol activity and its toxicity was first reported by Simon (1953). Phosphomonoesters are involved in the active transport of materials through phosphorylated intermediates. Such an involvement of phosphomonoesters was established in yeast cells by Goodman and Rothstein (1957). The acid and alkaline phosphatases in house-flies of different ages was studied by Barker and Alexander (1958). The distribution of alkaline phosphatase in the arteries of several organisms was analysed by Manilow et al. (1959). Miller and Crane (1961) while studying the digestive function of the epithelium of small intestine, revealed that these enzymes mediate carbohydrate metabolism. The coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism was investigated by Mitchell (1961). Localized areas of high alkaline phosphatase activity in the endothelium of arteries was studied by Romanul and Baninister (1962). This major group of enzymes participate in energy transfer through ATP. This property was studied in E.coli by Schaefler (1967). An investigation of lysosomal enzymes (which includes phosphatases also) was carried out by Bird et al. (1969). The mechanism of the action of reagents that uncouple oxidative phosphorylation was explained by Weinbach and Garbus (1969). Brunnel and Cathala (1973) worked on the activation and inhibition process of alkaline phosphatase from bovine brain by metal ions.

Phosphomonoesterase of the digestive tract in porcellio laevis Latrailla was elaborated by Saleem and Alikhan (1973 a, 1973 b, 1973 c and 1974). The purification and properties of rat liver alkaline phosphatase was studied by Ohkubo et al. (1974). Cathala et al. (1975 a) undertook the purification, analysis of the submit structure and metalloenzyme properties of bovine kidney alkaline phosphatase. The same workers (1975 b) examined the catalytic properties, subunit interaction in the catalytic process and Mg^{2+} stimulation in bovine kidney alkaline phosphatase. Gaudet et al. (1975) while analysing the enzyme activities of plasma and selected tissues in rainbow trout Salmo gairdneri, analysed the alkaline phosphatase activity in serum and various tissues of the same fish. Gupta et al. (1975) while investigating the effect of malathion and radiation upon rat enzymes in vivo, concluded that acid phosphatase is a good indicator of stress conditions in biological system. Racicot et al. (1975) established the diagnostic use of the enzymes by inducing CCl_4 toxicity and Aeromonas infection in rainbow trout. The effect of pentachlorophenol on oxidative phosphorylation by uncoupling was revealed by Desiah (1978). Ikehara et al. (1978) purified and characterized alkaline phosphatase from plasma membrane of rat. Rao (1979) studied the alkaline phosphatase activity in the ovaries of some clupeoids. Dalela et al. (1980) applied alkaline phosphatase as an index of the extent of physiological stress induced by sublethal concentrations of phenol and pentachlorophenol in Notopterus notopterus. Verma et al. (1980) made a similar investigation of recordinig the effects of phenol and di-nitrophenol on acid and alkaline phosphatases in the tissues of Notopterus notopterus. The role of serum alkaline phosphatase in relation to inorganic phosphorus with respect to mercury poisoning in teleost of Red Sea was studied by Himly et al. (1981). Dalela et al. (1982) chronically exposed Notopterus notopterus to phenolic compounds and assayed acid phosphatase activity in different tissues of the fish.

The effect of seasonal changes on the activity of intestinal alkaline phosphatase of pike perch and bream was examined by Gelman et al. (1984). Shinichi et al. (1985) partially purified alkaline phosphatase from bovine polymorphonuclear neutrophils. The subunit structure and carbohydrate

composition of acid phosphatase were studied by Hanna and Aleksandra (1985). Hashimoto et al. (1985) estimated alkaline phosphatase activity present in the waters of Bay of Tokyo by utilizing the function that alkaline phosphatase participates in the release of orthophosphate from organic phosphorus. Kubiiz (1985) isolated and characterized a homogenous acid phosphatase from catfish liver. Chiu-yin-kwan and Hiroyuki Ito (1987) examined the acid and alkaline phosphatase activities in the vascular smooth muscle of rat and dog. In the same study they investigated species differences and subcellular distribution of the two enzymes. Another elaborate report of acid phosphatase was made in culex mosquito by Edward and James (1987). A recent report of alkaline phosphatase activity is in rat mammary tissue under different physiological states such as normal, lactating and neoplastic conditions by Colston et al. (1988).

MATERIALS AND METHODS

Methods of collection of specimens, acclimatisation and dosing with copper were done as described in chapter III. For the collection of tissue samples for analysis, the fishes were caught and stunned by a hard blow on the head. They were soon dissected, liver and kidney were removed and homogenised in de-ionised water below $0^{o}C$. The homogenate was centrifuged in a refrigerated centrifuge at 12,000 rpm. The supernatant was collected and used as enzyme extract for analysis. Care was taken to maintain the tissue and extract cool till incubation.

Acid phosphatase activity was determined by following the methodology described in sigma technical bulletin (No.104) with slight modifications. To 1 ml of 0.1M fhozen citrate buffer of p^{H} 5.3, 0.05 ml of the enzyme extract was added. This buffer-enzyme mixture was kept in a water bath at 37 $\pm$ $0.1^{o}C$. When the temperature reached $37^{o}C$, 0.1 ml of substrate (2 mg of p-nitrophenyl phosphate sodium salt (Merck) in 0.1 ml distilled water) was added to initiate the reaction. After incubating for 1 hour, the reaction was stopped by adding 2 ml of 0.25 N NaOH. P-nitrophenyl phosphate is hydrolysed to p-nitrophenol by the enzyme during the incubation

period. The yellow colour of the p-nitrophenol in the alkaline medium was read at 410 nm. The concentration of the p-nitrophenol formed was found out from a standard graph of p-nitrophenol. Simultaneously, the protein content of 0.1 ml of tissue extract was estimated. From this μmol of p-nitrophenol liberated per mg protein per hour was calculated and enzyme activity is expressed as μ mol of p-nitrophenol liberated/mg protein/hr.

The same procedure was followed to estimate alkaline phosphatase activity. The buffer used was 0.05 M Glycine-sodium hydroxide buffer of p^H 9. 0.1 mg of $MgCl_2$ was added. The volume of enzyme extract added was 0.1 ml. The calculations were the same as described above. Statistical significance was calculated in both cases.

RESULTS

Megalops cyprinoides

I. Acid phosphatase activity

1. Kidney tissue

The acid phosphatase activity in kidney tissue of M.cyprinoides exposed to 0.1 ppm Cu^{++} and 0.15 ppm Cu^{++} for different durations are given in Table 14 and Fig.23.

A dose-dependent decrease in the acid phosphatase activity was observed in the kidney tissue. A significant decrease occurred in the enzyme activity of fishes exposed to both concentrations of Cu^{++}. In 0.1 ppm Cu^{2+} dosed animals, the enzyme activity was reduced to 0.087 μmol/mg protein/hr against the control value of 0.099 μ mol ($P < 0.005$) after 24 hrs of exposure. After 72 hrs of exposure, it was 0.084 μmol while the control value was 0.094 μmol ($P < 0.02$). In 120 hrs of exposure, the enzyme activity recorded was 0.083 μ mol against the control value of 0.09 μ mol ($P < 0.02$) and after 168 hrs of exposure, 0.09 μ mol while the control value was 0.1 μ mol ($P < 0.05$).

In animals exposed to 0.15 ppm Cu^{++}, after 24 hrs of exposure the enzyme activity was 0.079 μ mol ($P< 0.02$), 0.075 μ mol ($P< 0.02$) after 72 hrs of exposure, 0.072 μ mol ($P< 0.001$) after 120 hrs of exposure and 0.081 μmol ($P< 0.01$) after 168 hrs of exposure against their respective control values stated above.

2. Liver tissue

The acid phosphatase activity in the liver tissue of M.cyprinoides exposed to two concentrations of Cu^{++} for different durations are tabulated and graphed in Table 15 and Fig.24.

In the liver tissue, the enzyme activity decreased sharply throughout the exposed period in 0.1 ppm Cu^{++} dosed fishes. The activity decreased still in the liver tissue of fishes exposed to 0.15 ppm Cu^{++}. In fishes exposed to 0.1 ppm Cu^{2+}, the enzyme activity recorded was 0.076 μmol (control value - 0.083 μ mol; $P< 0.005$) after 24 hrs exposure, 0.073 μ mol (control value 0.08 μ mol; $P< 0.02$) after 72 hrs of exposure, 0.071 μ mol (control value - 0.075 μ mol; $P< 0.05$) after 120 hrs of exposure and 0.07 μ mol (control value - 0.075 μ mol $P< 0.05$) after 168 hrs of exposure.

In fishes exposed to 0.15 ppm Cu^{++} the enzyme activity recorded were 0.071 μ mol ($P< 0.05$) after 24 hrs exposure, 0.07 μ mol ($P< 0.01$) after 72 hrs exposure, 0.069 μ mol ($P< 0.05$) after 120 hrs of exposure and 0.067 μ mol ($P< 0.05$) after 168 hrs of exposure against their respective control values noted above.

II. Alkaline phosphatase

1. Kidney tissue

Table 16 and Fig.25 illustrate the alkaline phosphatase activity in the kidney tissue of M.cyprinoides exposed to two concentrations of Cu^{++} for various durations.

Fig.23 Acid phosphatase activity (μ mol/mg protein/hr) in kidney tissue of M.cyprinoides exposed to two concentrations of Cu^{++} at different time periods.

Fig.24 Acid phosphatase activity (μ mol/mg protein/hr) in liver tissue of M.cyprinoides exposed to two concentrations of Cu^{++} at different time periods.

Fig.25 Alkaline phosphatase activity (μ mol/mg protein/ hr) in kidney tissue of M.cyprinoides exposed to two concentrations of Cu^{++} at different time periods.

Fig.26 Alkaline phosphatase activity (μ mol/mg protein/ hr) in liver tissue of M.cyprinoides exposed to two concentrations of Cu^{++} at different time periods.

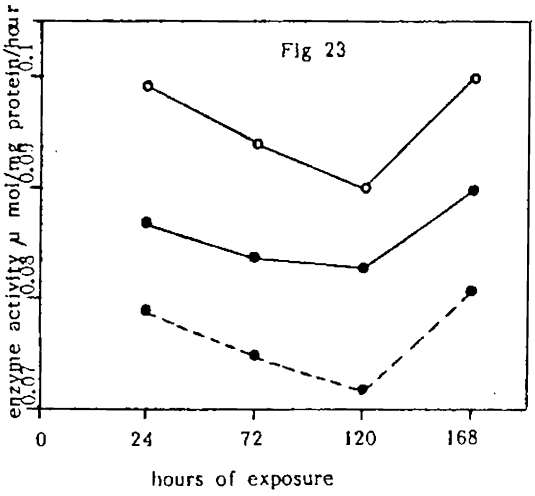
Fig 23
enzyme activity μ mol/mg protein/hour
0.07
0.08
0.09
0.1
0
24
72
120
168
hours of exposure

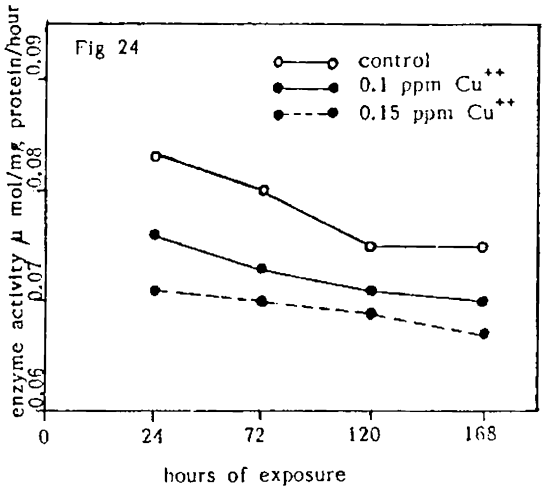
Fig 24
control
0.1 ppm Cu++
0.15 ppm Cu++
enzyme activity μ mol/mg protein/hour
0.06
0.07
0.08
0.09
0
24
72
120
168
hours of exposure

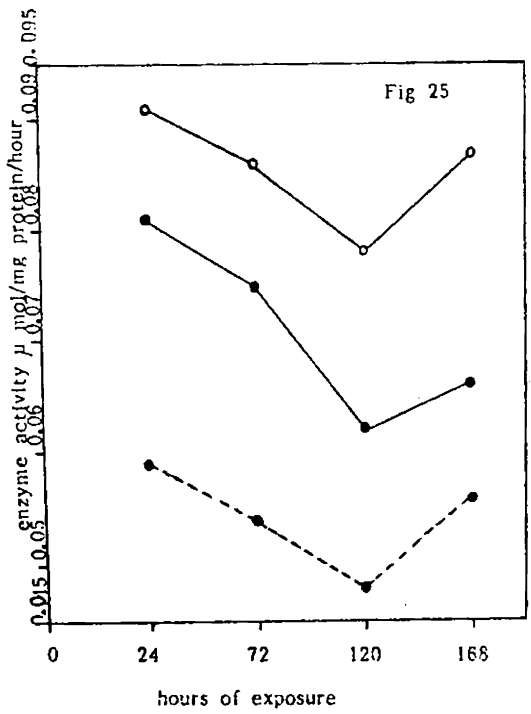
Fig 25
enzyme activity μ mol/mg protein/hour
0.05
0.06
0.07
0.08
0.09
0.095
0
24
72
120
168
hours of exposure

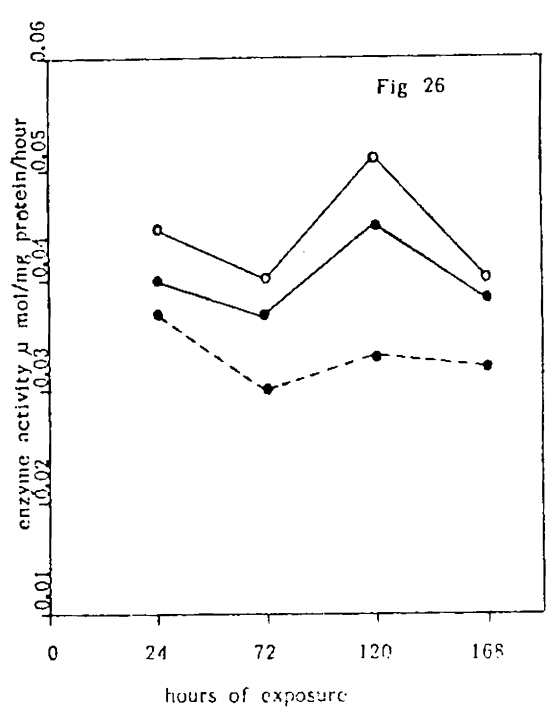
Fig 26
enzyme activity μ mol/mg protein/hour
0.01
0.02
0.03
0.04
0.05
0.06
0
24
72
120
168
hours of exposure

A dose-dependent decrease in the enzyme activity was observed. The enzyme activity levels observed in fishes exposed to 0.1 ppm Cu^{++} were 0.081 μ mol (control value - 0.091 μ mol; $P < 0.001$), 0.075 μ mol (control value - 0.86 μ mol;$P < 0.001$); 0.062 μ mol (control value - 0.078 μ mol; $P < 0.001$) and 0.06 μ mol (control value - 0.082 μ mol; $P < 0.005$) after 24, 72, 120 and 168 hrs of exposure, respectively.

The enzyme activity levels observed in the kidney tissue of fishes exposed to 0.15 ppm Cu^{++} were 0.059 μ mol ($P < 0.001$), 0.054 μ mol ($P < 0.001$), 0.048 μ mol ($P < 0.001$) and 0.056 μ mol ($P < 0.001$) after 24, 72, 120 and 168 hrs exposure against their respective control values listed previously.

2. Liver tissue

The values of the alkaline phosphatase activity in the liver tissue of 0.1 ppm and 0.15 ppm Cu^{++} dosed fishes are listed and plotted in Table 17 and Fig.26.

A decrease in the enzyme activity was observed in the liver tissue of the fishes exposed to both the concentrations. The enzyme activity of 0.1 ppm Cu^{2+} exposed fishes and their appropriate control values in μ mol mg protein/hr were as 0.04; 0.045 ($P < 0.002$) after 24 hrs, 0.037; 0.04 ($P < 0.05$) after 72 hrs, 0.045; 0.051 ($P < 0.005$) after 120 hrs and 0.038; 0.04 ($P < 0.005$) after 168 hrs of exposure.

In animals exposed to 0.15 ppm Cu^{++}, the enzyme activity levels observed were 0.037 μ mol ($P < 0.001$), 0.03 μ mol ($P < 0.01$) 0.033 μ mol ($P < 0.001$) and 0.032 μ mol ($P < 0.01$) during 24, 72, 120 and 168 hrs of exposure. The control values were the same as in the group above.

Fig.27 Acid phosphatase activity (μ mol/mg protein/hr) in kidney tissue of S.argus exposed to two concentrations of Cu^{++} at different time periods.

Fig.28 Acid phosphatase activity (μ mol/mg protein/hr) in liver tissue of S.argus exposed to two concentrations of Cu^{++} at different time periods.

Fig.29 Alkaline phosphatase activity (μ mol/mg protein/ hr) in kidney tissue of S.argus exposed to two concentrations of Cu^{++} at different time periods.

Fig.30 Alkaline phosphatase activity (μ mol/mg protein/ hr) in kidney tissue of S.argus exposed to two concentrations of Cu^{++} at different time periods.

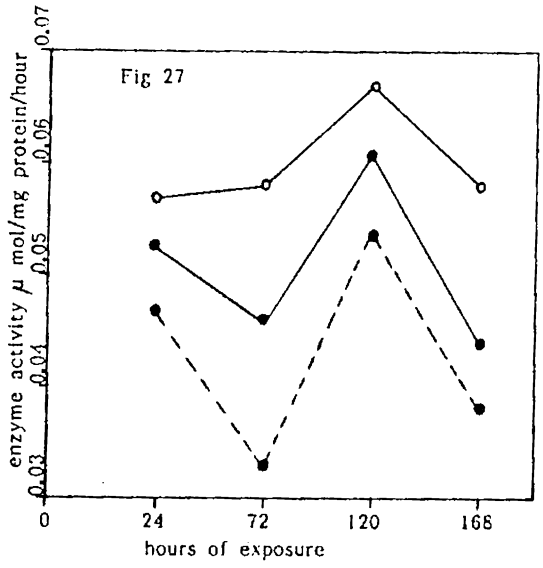
Fig 27
enzyme activity μ mol/mg protein/hour
0.03
0.04
0.05
0.06
0.07
0
24
72
120
168
hours of exposure

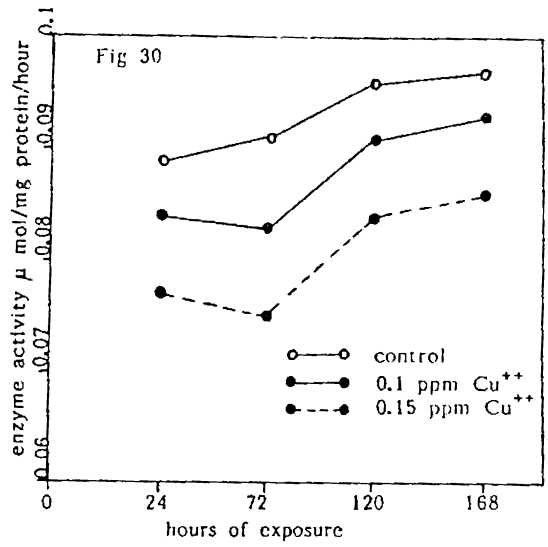
Fig 30
enzyme activity μ mol/mg protein/hour
0.06
0.07
0.08
0.09
0.1
control
0.1 ppm Cu++
0.15 ppm Cu++
0
24
72
120
168
hours of exposure

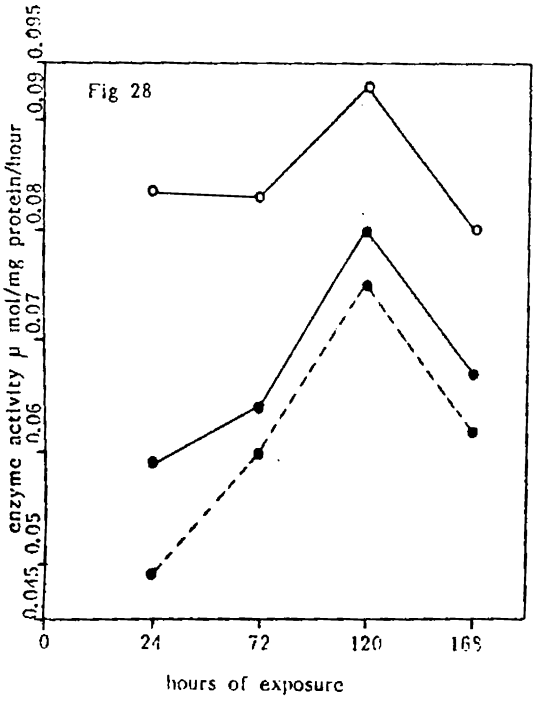
Fig 28
enzyme activity μ mol/mg protein/hour
0.045
0.05
0.06
0.07
0.08
0.09
0.095
0
24
72
120
168
hours of exposure

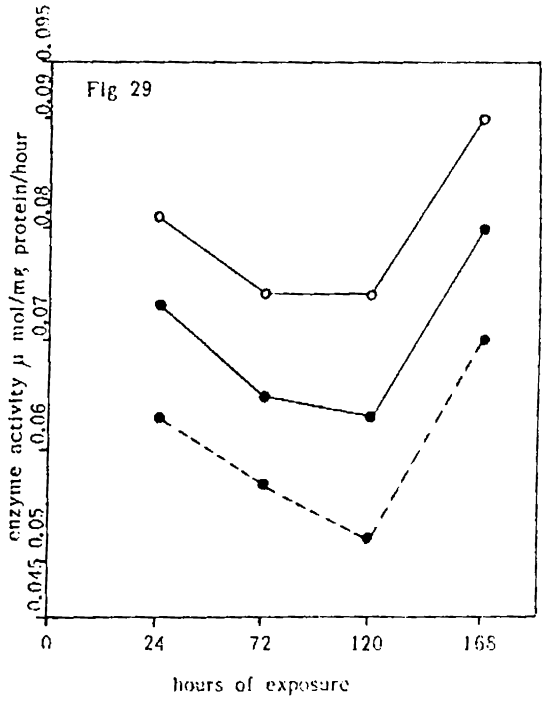
Fig 29
enzyme activity μ mol/mg protein/hour
0.045
0.05
0.06
0.07
0.08
0.09
0.095
0
24
72
120
168
hours of exposure

Scatophagus argus

I. Acid phosphatase

1. Kidney tissue

The data of the enzyme activity in the kidney tissue of S.argus exposed to two concentrations for the experimental period are given in Table .18 and Fig.27.

The enzyme activity was significantly low in both groups of fishes exposed to the two concentrations of copper. In fishes exposed to 0.01 ppm Cu^{++}, the enzyme activity levels for different periods of exposure were observed as 0.053 μ mol ($P < 0.001$), 0.001), 0.046 μ mol ($P < 0.005$), 0.061 μ mol ($P < 0.02$) and 0.044 μ mol ($P < 0.001$) after 24, 72, 120 and 168 hrs of exposure against the respective control values of 0.057 μ mol, 0.058 μ mol, 0.067 μ mol and 0.058 μ mol.

The kidney tissue of fishes exposed to 0.15 ppm of Cu^{++} registered enzyme activity of 0.047 μ mol ($P < 0.001$), 0.033 μ mol ($P < 0.001$), 0.054 μ mol ($P < 0.001$) and 0.038 μ mol ($P < 0.001$) during 24, 72, 120 and 168 hrs of exposure. The control values were common for both concentrations of Cu^{++}.

2. Liver tissue

The details of acid phosphatase activity in the liver tissue of fishes exposed to two concentrations of Cu^{++} for different durations are given in Table 19 and Fig.28.

When compared to the control values of 0.0835 μ mol, 0.093 μ mol, 0.093 μ mol and 0.08 μ mol for different durations of exposure as 24, 72, 120 and 168 hrs, the enzyme activity in dosed fishes were very low. In fishes dosed with 0.1 ppm Cu^{2+}, the enzyme activity levels recorded were

0.059 μ mol ($P < 0.001$) 0.064 μ mol ($P < 0.001$), 0.08 μ mol, ($P < 0.001$) and 0.067 μ mol ($P < 0.001$) and in fishes exposed to 0.15 ppm Cu^{2+} the activity levels observed were 0.049 μ mol ($P < 0.001$), 6.06 μ mol ($P < 0.001$), 0.075 μ mol ($P < 0.001$) and 0.062 μ mol ($P < 0.001$), after the respective periods of 24, 72, 120 and 168 hrs of exposure.

III. Alkaline phosphatase

1. Kidney tissue

Table 20 and Fig.29 provide the details of the alkaline phosphatase activity in the kidney tissue of fishes challenged with 0.1 ppm Cu^{2+} and 0.15 ppm Cu^{2+} for different durations.

The dose-dependent decrease in the enzyme activity of animals exposed to the two concentrations of Cu^{++} was very prominant. The enzyme activity levels noticed in 0.1 ppm Cu^{++} dosed fishes were 0.073 μ mol ($P < 0.002$), 0.065 μ mol ($P < 0.02$), 0.063 μ mol ($P < 0.005$) and 0.08 μ mol ($P < 0.001$) and in fishes exposed to 0.15 ppm Cu^{++} they were 0.063 μ mol ($P < 0.001$), 0.057 μ mol ($P < 0.002$), 0.052 μ mol ($P < 0.002$) and 0.07 μ mol ($P < 0.001$) against the respective control values of 0.081 μ mol, 0.074 μ mol 0.074 μ mol and 0.09 μ mol after 24, 72, 120 and 168 hrs of exposure.

2. Liver tissue

Table 21 and Fig.30 explain the variations in the alkaline phosphatase activity in the liver tissue of fishes exposed to two concentrations of Cu^{2+} for the experimental durations.

Though the alkaline phosphatase activity levels in the liver tissue of control fishes were 0.089 μ mol, 0.091 μ mol, 0.096 μ mol and 0.097 μ mol respectively after 24, 72, 120 and 168 hrs of exposure, the activity recorded in the liver tissue of fishes exposed to 0.1 ppm Cu^{++} were 0.084 μ mol ($P < 0.02$), 0.083 μ mol ($P < 0.005$), 0.091 μ mol ($P < 0.05$) and 0.093 μ mol

($P < 0.05$) and in the liver tissue of fishes exposed to 0.15 ppm Cu^{++} were 0.077 μ mol ($P < 0.001$), 0.075 μ mol ($P < 0.005$) 0.084 μ mol ($P < 0.01$) and 0.086 μ mol ($P < 0.002$) after the respective durations of exposure of 24, 72, 120 and 168 hrs.

DISCUSSION

As mentioned in the introduction, lysosomes and cell membranes are the first targets of pollutants because the lysosomes are concerned with the disintegration of unwanted elements and the cell membrane is the first barrier to a xenobiotic encounter. So, a variation in their functional systems is unavoidable. In the present study such a variation is manifested as a reduction in the activity of their enzyme system either by reduced synthesis or by inhibition or inactivation of the released enzymes. In any case, it is expressed as the decrease in enzyme activity. Such a lowering in the enzyme activity has been reported by previous workers. Hilmy et al. (1981) noticed a decline in the activity of serum alkaline phosphatase when a teleost Aphanius dispar was subjected to mercury poisoning. According to them this can be due to the replacement of the cofactor Mg^{2+} by mercury (Hg^{2+}) and thus the release of the former as a toxic free ion into the serum. Fouad (1973) also recorded a decreased level of alkaline phosphatase activity in the serum of rats subjected to nitrofuran furadoxyle injection. Harper (1968) reasons that a reduction in enzyme activity can occur due to pathological changes in liver. Nelson (1955) on the other hand has recorded an increase in the activity of the enzyme alkaline phosphatase in serum of rats exposed to endrin.

In the present analysis, a reduced activity of both phosphatases was observed in liver and kidney depending on the concentration of Cu^{++}. This diminution of the enzyme activity is probably due to the inhibition of the enzyme by the metal ions. The binding affinity of heavy metal cation and protein is generally intense (Hilmy et al. 1981). Since enzymes are protein, they can not be exceptions to this general affinity. Desiah (1978) has put forward another inhibitory activity of pollutants on phosphomonoesterases. He used pentachlorophenol in his studies as the polluting chemical. He

suggested that uncoupling (inhibition) of oxidative phosphorylation was the main cause of the inhibition of phosphatases. Uncoupling of oxidative phosphorylation was also pointed out by Dalela et al. (1980) and Verma et al. (1980) for the inhibition of acid and alkaline phosphatases. Simon (1953) stated that concentration of toxic substances higher than those needed to prevent oxidative phosphorylation injured the mitochondrial system so greatly as to block the action of enzymes concerned with oxidative metabolisms.

Action of uncouplers on oxidative phosphorylation has been pointed out on a chemical basis by Pressman (1963) and by chemi-osmotic interactions by Mitchell (1961). According to Mitchel (1961) uncouplers promote the conductivity of protons within mitochondrial membranes and subsequently prevent the gradient formation across the membrane. This points to the trapping of the enzyme in the area of formation as the new altered gradient does not permit its passage. According to Pressman (1963), uncouplers promote the splitting of an energy-rich intermediate compound prior to ATP formation. This hinders oxidative phosphorylation and the enzyme system catalysing the reaction. Weinbach and Garbus (1969) suggested that uncouplers traverse through lipo-protien layer of mitochondrial membrane and interact with the protein groups which then undergo structural changes. This directly affects the enzyme bound to the lipo-protein layer, namely alkaline phosphatase. It is generally assumed that major changes in mitochondrial function are reflected in morphological alterations and that mitochondrial profiles are dependent on the continuing supply of energy-rich intermediates produced by oxidative phosphorylation. Weinbach and Garbus (1969) indicated that these uncouplers bind tightly with mitochondrial proteins which are involved in amino acid metabolism. All these interactions and processes occur simultaneously when fishes are exposed to toxicants.

In the experiments of Ikehara et al. (1978) alkaline phosphatase was inactivated by EDTA (0.15 mM). He explained that since alkaline phosphatase is known to be a Zn^{2+} containing enzyme, the inhibition of EDTA seems to reflect the removal of Zn^{2+} from the enzyme protein. This inactivation was overcome by the addition of excess Mg^{2+} (1 mM). There is evidence that alkaline phosphatase has a Mg^{2+} binding site (Ohkubo et al. 1974). When this

site is occupied by Mg^{2+}, the conformation of the enzyme is altered in such a way that the binding between Zn^{2+} and the enzyme increases, and Zn^{++} can not diffuse readily away from the enzyme, resulting in the stabilization of the enzyme. Brunel and Cathala (1973) and Cathala et al. (1975, a,b) has reported that Mn^{2+} and Co^{2+} bind to alkaline phosphatase in the same site of Mg^{2+}. Cu^{++} also may combine with the enzyme in the same site of Zn^{++} and bring in competitive inhibition causing a reduction in the enzyme activity.

In the present study the inhibition of alkaline phosphatase can probably be due to the occupation of the binding site of Mg^{2+} by Cu^{2+} which alters the conformation of the enzyme. Since a dose dependent decline in the enzyme activity was observed, it should be assumed that the inhibitation occurred according to the concentration of the available Cu^{++}. As the Cu^{++} was not suficient enough to remove Mg^{++} completely from the enzyme, a total inactivation of the enzyme did not occur. The reduction of the acid phosphatase activity also may have resulted from various reasons explained above. It can be due to the uncoupling of oxidative phosphorylation or the prevention of gradient formation across the mitochondrial membrane or the interaction with the lipoprotein layer of the mitochondrial membrane and altering the protein structure, all due to copper ions. A very active subcellular unit like lysosomes have increased demand for energy which is supplied by mitochondria. So, a destruction of the mitochondrial component of the cell will be reflected in the general functional status of the lysosome which is manifested as the reduced enzyme activity in the present case. Such an inhibitory action of $CuSO_4$ on acid and alkaline phosphatase was reported in Porcellio laevis by Saleem and Alikhan (1973 a). In the present observation a time-dependent decrease was not noticed which confirms that the inactivation of the enzymes was not continuous or reactivation of the inactivated enzymes did not take place. Once an alteration occurred in the enzyme structure or mitochondrial structure, that state remained as such for the remaining period with out any increase or decrease in the activity of the enzyme.

Table 14 Acid phosphatase activity (μ mol of p-nitrophenol liberated/mg protein/hr) in the kidney tissue of M.cyprinoides exposed to two concentrations of Cu^{++} at different time periods.

	Hours of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	0.09896	0.09438	0.08534	0.10792
	± SD	0.12000	0.00646	0.00269	0.00597
	Range	0.0865 -0.1136	0.0818 -0.9950	0.0846 0.9130	0.0985-o.1124
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.08768	0.08486	0.08318	0.09088
	± SD	0.00576	0.00176	0.00357	0.00713
	Range	0.0806 - 0.0882	0.0829 - 0.0881	0.0794 - 0.0898	0.0903- 0.0986
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.07918	0.07566	0.07201	0.08134
	± SD	0.01833	0.00696	0.00340	0.00268
	Range	0.0561 - 0.1088	0.0697 - 0.0759	0.0641 - 0.0735	0.0805- 0.0862

N = Number of animals; SD = Standard deviation

Table 15 Acid phosphatase activity (μ mol of p-nitrophenol liberated/mg protein/hr) in the liver tissue of M.cyprinoides exposed to two concentrations of Cu^{++} at different time periods.

	Hours of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	0.08354	0.08054	0.07550	0.07544
	$\pm$ SD	0.00723	0.00931	0.00365	0.00847
	Range	0.0837 - 0.0878	0.0683 - 0.0962	0.0689 - 0.0792	0.0649 - 0.0869
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.07648	0.07378	0.07134	0.07088
	$\pm$ SD	0.00347	0.00268	0.00133	0.00182
	Range	0.0712 - 0.0795	0.0745 - 0.0814	0.0695 - 0.0736	0.0684 - 0.0735
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.07180	0.07036	0.06972	0.06717
	$\pm$ SD	0.00260	0.00513	0.00152	0.00244
	Range	0.0676 - 0.0755	0.0711 - 0.0861	0.0678 - 0.0717	0.0641 - 0.0712

N = Number of animals; SD = Standard deviation

Table 16 Alkaline phosphatase activity (μ mol of p-nitrophenol liberated/mg protein/hr) in the kidney tissue of M.cyprinoides exposed to two concentrations of Cu^{++} at different time periods.

	Hours of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	0.0914	0.0865	0.0788	0.0870
	$\pm$ SD	0.0023	0.0117	0.0033	0.0028
	Range	0.0879 - 0.0950	0.0707- 0.1072	0.0715-0.0797	0.0824-0.0906
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.08116	0.07122	0.06208	0.06686
	$\pm$ SD	0.00249	0.00136	0.00305	0.00892
	Range	0.0785 - 0.0854	0.0725 - 0.0781	0.0573 - 0.0665	0.0518 - 0.0784
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.05948	0.05401	0.04832	0.05606
	$\pm$ SD	0.00280	0.00271	0.00073	0.00759
	Range	0.0548 - 0.0627	0.0508 - 0.0584	0.0472 - 0.0491	0.0491-0.0664

N = Number of animals; SD = Standard deviation

Table 17 Alkaline phosphatase activity (μ mol of p-nitrophenol liberated/mg protein/hr) in the liver tissue of M.cyprinoides exposed to two concentrations of Cu^{++} at different time periods.

a

	Hours of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	0.04458	0.04012	0.05176	0.04000
	± SD	0.00269	0.00487	0.00162	0.00528
	Range	0.0417 - 0.0495	0.0311- 0.0447	0.0505- 0.0540	0.0307- 0.0455
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.04029	0.03762	0.04596	0.03800
	± SD	0.00227	0.00311	0.00228	0.00097
	Range	0.0375 - 0.0432	0.0330- 0.0412	0.0426- 0.0488	0.0388- 0.0401
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.03724	0.03018	0.03368	0.03218
	± SD	0.00293	0.00382	0.00428	0.00084
	Range	0.0347 - 0.0428	0.0278 - 0.0360	0.0276- 0.03491	0.0307- 0.0330

N = Number of animals; SD = Standard deviation

Table 18 Acid phosphatase activity (μ mol of p-nitrophenol liberated/mg protein/hr) in the kidney tissue of S.argus exposed to two concentrations of Cu^{++} at different time periods.

	Hours of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	0.057826	0.05844	0.06770	0.5876
	± SD	0.00054	0.00423	0.00288	0.000372
	Range	0.0573 - 0.0587	0.0556- 0.0618	0.0617- 0.0694	0.0582- 0.0592
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.0532	0.04970	0.06180	0.04420
	± SD	0.00116	0.00136	0.00267	0.00204
	Range	0.0515 - 0.0546	0.0479- 0.05168	0.0584- 0.0655	0.0416- 0.0468
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.0477	0.0330	0.0541	0.03852
	± SD	0.001233	0.00117	0.001906	0.00088
	Range	0.0462 - 0.0494	0.0313- 0.0348	0.0517- 0.0566	0.0372- 0.0395

N = Number of animals; SD = Standard deviation

Table 19 Acid phosphatase activity (μ mol of p-nitrophenol liberated/mg protein/hr) in the liver tissue of S.argus exposed to two concentrations of Cu^{++} at different time periods.

	Hours of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	0.08352	0.08325	0.09328	0.07966
	± SD	0.00173	0.00144	0.00457	0.00062
	Range	0.0811 - 0.0856	0.08109- 0.0849	0.0886- 0.991	0.0790- 0.0805
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.0590	0.06455	0.08028	0.06792
	± SD	0.00173	0.00318	0.00289	0.00109
	Range	0.0529 - 0.0612	0.0602- 0.06829	0.0762- 0.08504	0.0661- 0.0694
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.04902	0.06052	0.07512	0.06223
	± SD	0.004480	0.001193	0.002334	0.001409
	Range	0.0410 - 0.0528	0.0589 - 0.0621	0.0735- 0.0788	0.0528- 0.0648

N = Number of animals; SD = Standard deviation

Table 20 Alkaline phosphatase activity (μ mol of p-nitrophenol liberated/mg protein/hr) in the kidney tissue of S.argus exposed to two concentrations of Cu^{++} at different time periods.

	Hours of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	0.08152	0.07386	0.07402	0.0905
	± SD	0.002299	0.00572	0.00653	0.001613
	Range	0.0782 - 0.0832	0.0656 - 0.0793	0.0663- 0.0826	0.08850.0930
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.07366	0.06569	0.062285	0.07991
	± SD	0.0020	0.00209	0.00169	0.00268
	Range	0.0715-0.0763	0.04186-0.0692	0.0595-0.0645	0.0768- 0.0845
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.06352	0.05531	0.05218	0.0711
	± SD	0.006417	0.003185	0.00522	0.00214
	Range	0.0508-0.0730	0.05135-0.0591	0.0440-0.0586	0.0672- 0.0735

N = Number of animals; SD = Standard deviation

Table 21 Alkaline phosphatase activity (μ mol of p-nitrophenol liberated/mg protein/hr) in liver tissue of S.argus exposed to two concentrations of Cu^{++} at different time periods.

	Hours of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	0.08958	0.09164	0.09624	0.09704
	± SD	0.00869	0.00492	0.00798	0.00627
	Range	0.08009 - 0.0897	0.09271- 0.09962	0.0816 - 0.1041	0.08671- 0.1059
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.08446	0.08300	0.09174	0.09716
	± SD	0.00379	0.00096	0.00074	0.00210
	Range	0.0818 - 0.08959	0.0818 - 0.0843	0.09108- 0.09296	0.09116- 0.09716
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.07760	0.07518	0.08440	0.08569
	± SD	0.00107	0.00312	0.00120	0.00247
	Range	0.0761 -0.0792	0.0719- 0.0788	0.0824-0.0857	0.0822- 0.0895

N = Number of animals; SD = Standard deviation

CHAPTER V

EFFECT OF COPPER ON THE ACTIVITY PATTERN OF TRANSAMINASES IN THE RENAL AND HEPATIC TISSUES

Transaminases are a group of enzymes that catalyze the process of biological transamination. Transamination reactions involve the transfer of an amino group of an amino acid to keto acid with the formation of an amino acid from the latter, and the generation of a new keto acid. These reactions present a prime mechanism for the synthesis and deamination of various amino acids. As stated by Meister (1955) "Transamination is a chemical reaction in which an amino group is transfered from one molecule to another without the intermediate participation of ammonia". Transamination represents one of the principal metabolic pathways for the synthesis and deamination of amino acids. It is a reversible reaction of broad scope involving various tissues. Many transamination reactions are linked to a carrier pair glutamate- α - ketoglutarate. Ammonia can be incorporated indirectly into a variety of amino acids by the action of glutamic dehydrogenase on α-keto-glutaric acid to form glutamate. Transamination, therefore allows an interplay between carbohydrate, fat and protein metabolism, an activity which can serve the changing demands of the organism (Cohen and Sallach, 1961).

In fish, transamination reactions probably play significant role at some stage in the autolytic degradation of muscle protein (Siebert and Schmitt, 1965). It is probable that the pool of free amino acids present in the fish muscle is utilized by transamination to produce a variety of keto acids. Some of these keto acids could be volatile and some might be decarboxylated and contribute a variety of aldehydes to the pool of carbonyl compounds which probably determine the overall flavour of the fish (Murray and Burt, 1974). Differences in the activity of amino transferases among fish and fish cells are helpful in tracing the evolutionary differences in fishes (Mounib and Eisan, 1969). They are also significant from aspects of post-mortem handling and preservation of fish since freezing and thawing result in the release of certain transaminases (Chhatbar and Velankar, 1977).

Of the many transaminases the most important and widely investigated are Glutamic pyruvate transaminase (GPT) and Glutamic oxaloacetate transaminase (GOT). Their stability and relative easiness made them subject of analysis in a variety of animals. In fishes, their investigation has gained only limited popularity though tissue enzyme analysis is gaining increasing importance in the field of environmental toxicology for the detection of toxic effects of chemical pollutants. Of the other transaminases, these two enzymes are used by fishery biologists to diagnose sublethal insult of pollutants to animal as a whole or organs like liver. These two transaminases play an important role in the detoxification of ammonia in teleosts.

Due to the significance mentioned in the previous chapter, the tissues - liver and kidney were selected for the analysis of enzyme activity. More over, since transaminases are both cytoplasmic and mitochondrial in location, liver parenchyma is the richest source of GOT and GPT (Hilmy et al. 1981).

The scope of transamination reactions in animals was reported by Cammerata and Cohen (1950). Meister and Tice (1950) explained the transamination from glutamine to α-keto acids. Serum glutamic oxalacetic transaminase activity in man during acute transmural myocardial infraction was studied by La Due et al. (1954). Meister (1955) elaborated the role of transamination in amino acid metabolism. Rowsell (1956 a) published his investigation on transamination with L-glutamate and α-oxoglutarate in fresh extracts of animal tissue. Hug and Weskman (1957) carried out experiments on transamination in Rhodospirillum rubrum. Reitman and Frankel (1957) developed the popularly accepted spectrophotometric method for the estimation of GOT and GPT. Transamination permits an interplay among carbohydrate, fat and protein. It was established by Cohen and Sallach (1961). The function of Vitamin B_6 in transamination reactions was studied by Guirard and Snell (1964). Transamination probably plays a significant part in the autolytic degradation of muscle proteins in fish as reported by Siebert and Schmitt (1965). The requirement of PLP or PMP as cofactors of transaminases was analysed by Fasella (1967). Bell (1968) investigated the diagnostic use of GOT and the

distribution of transaminases in tissue of pacific salmon. The serum level and tisue level of two transaminases in six vertebrate species was correlated by Zimmerman et al. (1968). The activity of amino transferases is useful in tracing evolutionary differences in fishes as reported by Mounib and Eisan (1969).

Lane and Scura (1970) examined the effects of dieldrin on glutamic oxaloacetic transaminase in Poecilia latipinna. Wilson (1973) studied the tissue distribution of two transaminases in channel catfish, Ictalurus punctatus. The role of transaminases in fat and protein metabolism was also reported by Malevski and Montgomeri (1974). Mehrle and Bloomfield (1974) analysed ammonia detoxifying mechanism in rainbow trout and the use of plasma enzyme level as an index of liver damage. According to Murray and Burt (1974) transamination contributes to the overall flavour of fish. Enzyme activities of plasma and selected tissues in rainbow trout were studied by Gaudet et al. (1975). Chhatbar and Velankar (1977) developed the estimation of transaminases as a biochemical test for the distinction of fresh fish from frozen and thawed ones using the property that some transaminases were released while freezing and thawing.

Effect of chemicals on hepatopancreatic enzymes was examined in blue crab Callinectes sapidus by Fox and Rao (1978). Chhatbar and Velankar (1980) studied aminotransferase enzyme in fish and shell fish. Apollonia and Anderson (1980) investigated the optimal assay conditions for serum and liver GOT and GPT in Salmo gairdneri. The role of serum transaminases with respect to mercury poisoning was analysed in Aphanius dispar by Hilmey et al. (1981). Verma et al. (1982) has reported the effect of phenol on in vivo activity of tissue transaminases in Notopterus notopterus. This study was elaborated by Gupta and Dalela (1985).

MATERIALS AND METHODS

Collection of specimen and their acclimatization in aquarium was done as described in chapter III. The setting of experiment and dosing the animals with copper ions is already mentioned in the same chapter. The

procedure followed in the previous chapter for preparation of enzyme extract was carried out in the present analysis also. For the estimation of GOT and GPT the calorimetric method of Reitman and Frankel (1957) was adopted.

For estimating GPT, phosphate buffer of P^H 7.5 was used. To 1 ml of 0.1 M frozen phospate buffer 0.1 ml of enzyme extract was added followed by 0.5 ml of substrate (0.2 M L - alanine and 2 m M 2-oxoglutarate in 0.5 ml distilled water), incubated for 1 hour at 37^oC, added 1 ml of chromogen (1 m M 2,4-dinitrophenyl hydrazine), mixed well, and kept for 20 minutes at room temperature. The medium was made alkaline by adding 0.4 N NaoH. The colour developed by 2.4-dinitrophenyl hydrazone of the reaction product - pyruvate was determined spectrophotometrically at 546 nm.

GOT was estimated in the same way. The reagents required were also the same except that the P^H of phosphate buffer was 8 and the substrates used were 0.1 M aspartate and 2 m M 2-oxoglutarate. Oxaloacetate is formed which combines with the chromogen to form 2,4-dinitrophenyl hydrazone of oxaloacetate which was read at 546 nm. Sodium pyruvate was used to prepare calibration curve for both.

RESULTS

I. M.cyprinoides

1. Glutamic pyruvate transaminase

a. Kidney tissue

An increase in the activity of the enzyme was noticed in the kidney tissue when the fishes were exposed to Cu^{++}. Table 22 and Fig.31 give the details of the results of GPT activity in the kidney tissue of M.cyprinoides.

The increase was relative to the concentrations of Cu^{++}. In the kidney tissue of fishes exposed to 0.1 ppm Cu^{++}, the enzyme activity was 1.583 ($P < 0.001$), 1.4596 ($P < 0.001$), 1.6340 ($P < 0.002$) and 1.5735 ($P < 0.001$)

Fig.31 GPT activity (µ mol/mg protein/hr) in kidney tissue of M.cyprinoides exposed to two concentrations of Cu^{++} at different time periods.

Fig.32 GPT activity (µ mol/mg protein/hr) in liver tissue of M.cyprinoides exposed to two concentrations of Cu^{++} at different time periods.

Fig.33 GOT activity (µ mol/mg protein/hr) in kidney tissue of M.cyprinoides exposed to two concentrations of Cu^{++} at different time periods.

Fig.34 GOT activity (µ mol/mg protein/hr) in liver tissue of M.cyprinoides exposed to two concentrations of Cu^{++} at different time periods.

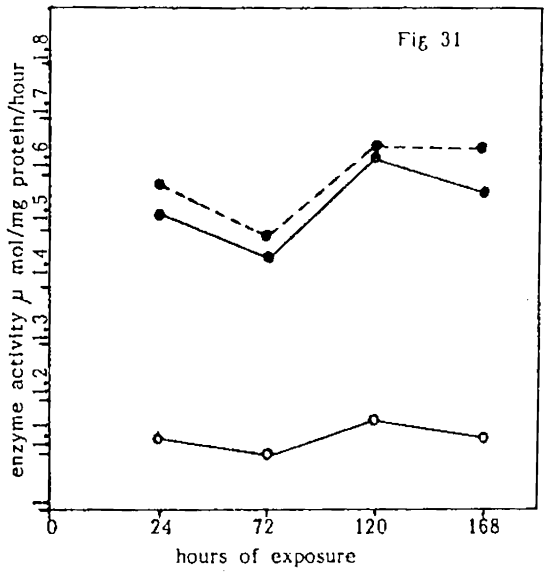
Fig 31
enzyme activity μ mol/mg protein/hour
hours of exposure
0
24
72
120
168

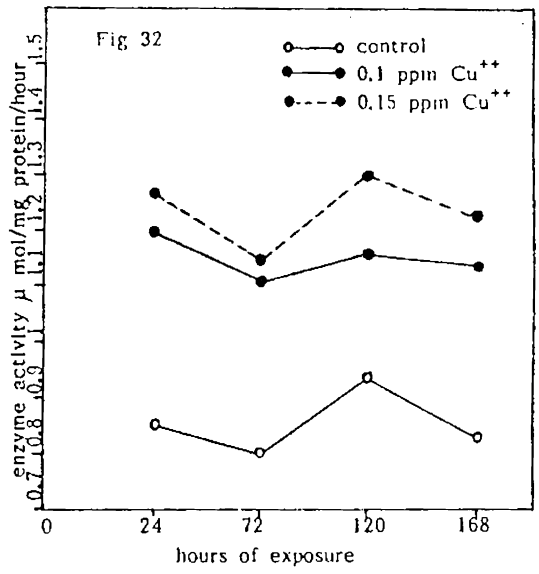
Fig 32
control
0.1 ppm Cu++
0.15 ppm Cu++
enzyme activity μ mol/mg protein/hour
hours of exposure
0
24
72
120
168

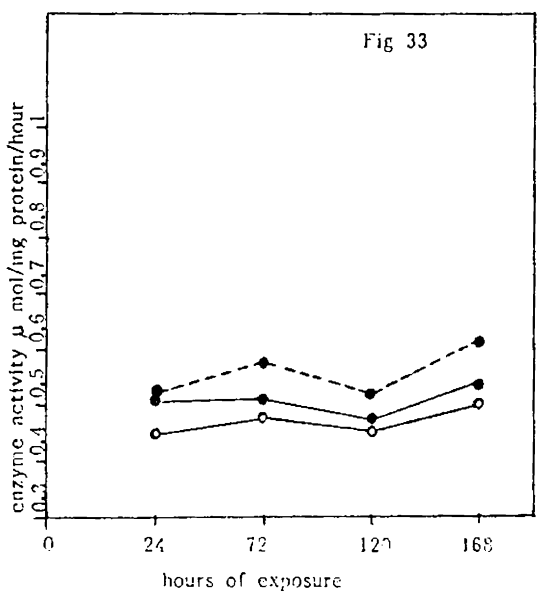
Fig 33
enzyme activity μ mol/mg protein/hour
hours of exposure
0
24
72
120
168

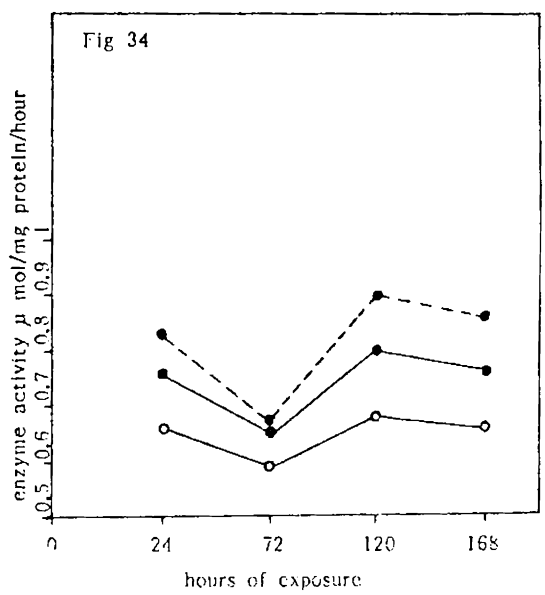
Fig 34
enzyme activity μ mol/mg protein/hour
hours of exposure
0
24
72
120
168

μ mol/mg protein/hr, and in fishes exposed to 0.15 ppm Cu^{++} it was 1.5832 ($P < 0.001$), 1.4988 ($P < 0.001$), 1.6486 ($P < 0.005$) and 1.7105 ($P < 0.001$) μ mol/mg protein/hr at 24, 72, 120 and 168 hrs time periods of exposure against the respective control values of 1.135, 1.1047, 1.175 and 1.3460/ μ mol/mg protein/hr.

b. Liver tissue

The details of GPT activity in the liver tissue of M.cyprinoides when dosed with two conc. of Cu^{++} for various durations are given in Table 24, and Fig.32.

The values of the enzyme activity in the liver tissues of M.cyprinoides exposed to 0.1 ppm Cu^{++} were 1.20601 ($P < 0.001$), 1.1014 ($P < 0.001$), 1.1663 ($P < 0.001$) and 1.14012 ($P < 0.001$) μ mol/mg protein/hr and in fishes exposed to 0.15 ppm Cu^{++} they were 1.2742 ($P < 0.01$), 1.1556 ($P < 0.001$), 1.3062 ($P < 0.001$) and 1.23668 ($P < 0.001$) μ mol/mg protein/hr after 24, 72, 120 and 168 hrs of exposure, respectively. Their respective control values were 0.8585, 0.8008, 0.948, and 0.8335 μ mol/mg protein/hr.

2. Glutamic oxaloacetic transaminase

a. Kidney tissue

Table 24 and Fig.33 provide the details of the GOT activity in the kidney tissue of M.cyprinoides when exposed to the two conc. of Cu^{++} for durations of 24, 72, 120 and 168 hrs. The enzyme activity increased to levels of 0.5156 ($P < 0.001$), 0.5133 ($P < 0.001$), 0.4741 ($P < 0.002$) and 0.5403 ($P < 0.001$) μ mol/mg protein/hr in fishes exposed to 0.1 ppm Cu^{++} and in those exposed to 0.15 ppm Cu^{++} they were 0.52202 ($P < 0.002$), 0.58008 ($P < 0.001$), 0.5262 ($P < 0.001$) and 0.61376 ($P < 0.001$) mg/protein/hr. The corresponding control values were 0.4538, 0.4817, 0.45506 and 0.5005 μ mol/mg protein/hr.

b. Liver tissue

Table 25 and Fig.34 explain the GOT activity of the liver tissue of fishes exposed to 0.1 ppm and 0.15 ppm Cu^{++} at different exposure periods.

The increased enzyme activity recorded was 0.767 ($P < 0.001$), 0.6559 ($P < 0.001$), 0.8007 ($P < 0.001$) and 0.7642 ($P < 0.001$) μ mol/mg protein/hr in liver tissue of 0.1 ppm Cu^{++} dosed fishes and 0.831 ($P < 0.001$), 0.6757 ($P < 0.001$), 0.9048 ($P < 0.002$) and 0.8608 ($P < 0.001$) μ mol/mg protein/hr in liver tissue of 0.15 ppm Cu^{++} dosed fishes during the respective exposure periods of 24, 72, 120 and 168 hrs. Their corresponding control values were 0.6668, 0.5986, 0.6840 and 0.6613 μ mol/mg protein/hr.

II. S.argus

1. Glutamic pyruvate transaminase

a. Kidney tissue

The data of the GPT activity in the kidney tissue of S.argus when exposed to 0.1 ppm and 0.15 ppm Cu^{++} are tabulated and plotted in Table 26 and Fig.35.

Significant increase in the enzyme activity was observed in the kidney tissue of the two groups of fishes. In fishes exposed to 0.1 ppm Cu^{++}, the enzyme activity was elevated to 0.51734 ($P < 0.001$), 0.4414 ($P < 0.001$), 0.4703 ($P < 0.002$) and 0.4042 ($P < 0.001$) μ mol/mg protein/hr and in fishes exposed to 0.15 ppm Cu^{++} the elevated values were 0.5497 ($P < 0.001$), 0.4655 ($P < 0.001$), 0.5242 ($P < 0.001$) and 0.4301 ($P < 0.001$) μ mol/mg protein/hr during the respective periods of exposure of 24, 72, 120 and 168 hrs. The control values during these periods were 0.3879, 0.3464, 0.42834 and 0.3433 μ mol/mg protein/hr respectively.

Fig.35 GPT activity (μ mol/mg protein/hr) in kidney tissue of S.argus exposed to two concentrations of Cu^{++} at different time periods.

Fig.36 GPT activity (μ mol/mg protein/hr) in liver tissue of S.argus exposed to two concentrations of Cu^{++} at different time periods.

Fig.37 GOT activity (μ mol/mg protein/hr) in kidney tissue of S.argus exposed to two concentrations of Cu^{++} at different time periods.

Fig.38 GOT activity (μ mol/mg protein/hr) in liver tissue of S.argus exposed to two concentrations of Cu^{++} at different time periods.

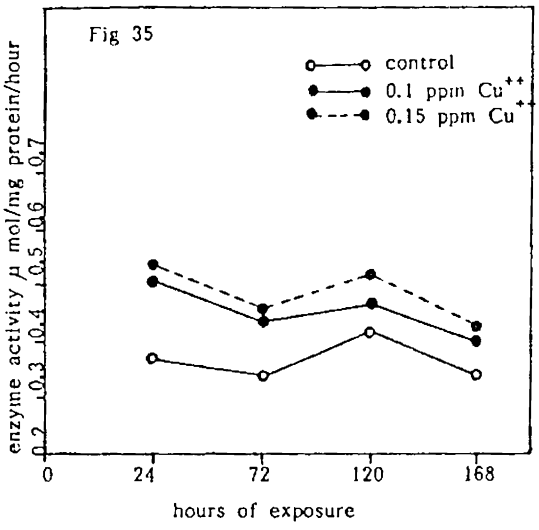
Fig 35
control
0.1 ppm Cu++
0.15 ppm Cu++
enzyme activity μ mol/mg protein/hour
0.2 0.3 0.4 0.5 0.6 0.7
0 24 72 120 168
hours of exposure

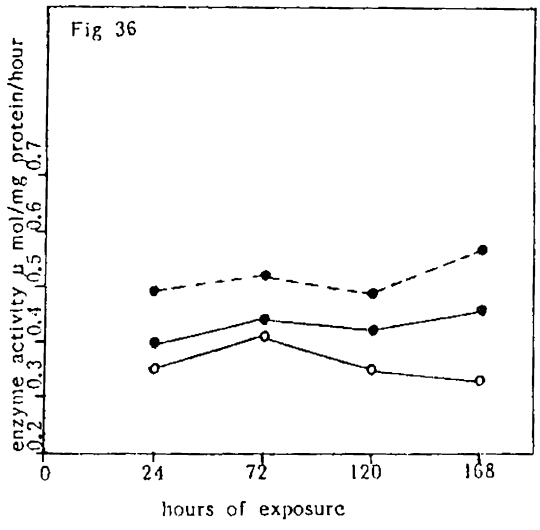
Fig 36
enzyme activity μ mol/mg protein/hour
0.2 0.3 0.4 0.5 0.6 0.7
0 24 72 120 168
hours of exposure

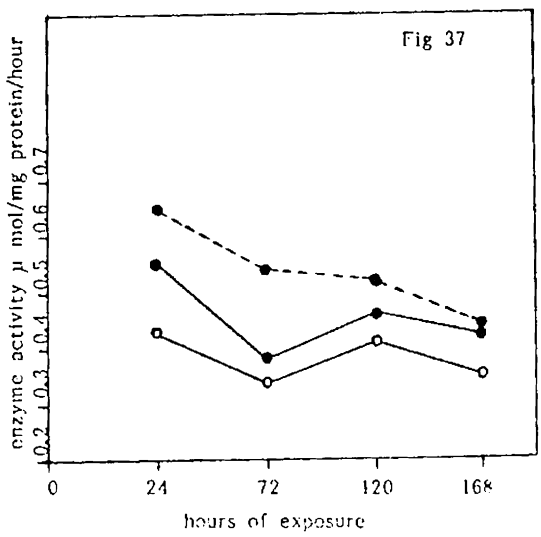
Fig 37
enzyme activity μ mol/mg protein/hour
0.2 0.3 0.4 0.5 0.6 0.7
0 24 72 120 168
hours of exposure

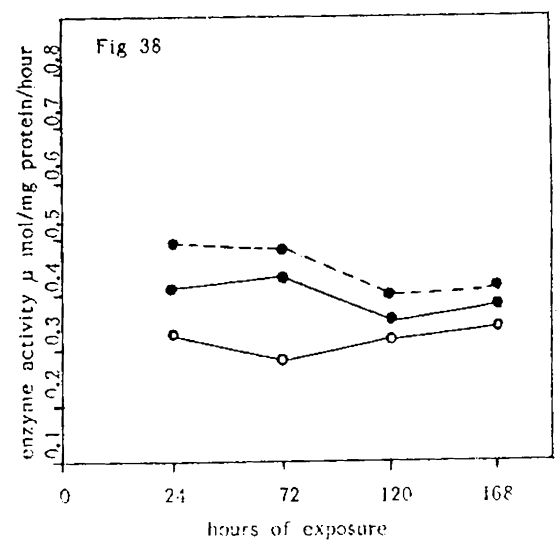
Fig 38
enzyme activity μ mol/mg protein/hour
0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8
0 24 72 120 168
hours of exposure

b. Liver tissue

The values of GPT activity in the liver tissue of S.argus exposed to 0.1 ppm and 0.15 ppm of Cu^{++} are given in Table 27 and Fig.36.

The enzyme activity was found to be higher in the exposed ones. In control fishes the enzyme activity was 0.3575, 0.4154, 0.35717 and 0.3313 μ mol/mg protein/hr for the durations of 24, 72, 120 and 168 hrs, respectively while in fishes exposed to 0.1 ppm Cu^{++} the values were as 0.4018 ($P< 0.05$), 0.44316 ($P< 0.001$), 0.4278 ($P < 0.001$) and 0.33183 ($P< 0.001$) μ mol/mg protein/hr and in 0.15 ppm dosed fishes the values were 0.4922 ($P < 0.001$), 0.52324 ($P < 0.001$), 0.4906 ($P< 0.001$) and 0.5716 ($P< 0.001$) μ mol/mg protein/hr.

2. Glutamic oxaloacetic transaminase

a. Kidney tissue

Table 28 and Fig.37 explain the enzyme activity in kidney tissue of S.argus exposed to 0.1 ppm and 0.15 ppm Cu^{++} for different durations.

The enzyme activities in kidney tissue of the dosed animals were above those of the control animals. The control values of the enzyme activity were 0.4369, 0.349, 0.414 and 0.3539 μ mol/mg protein/hr after 24, 72, 120 and 168 hrs, respectively. But in fishes dosed with 0.1 ppm Cu^{++} the enzyme activity in the kidney tissue was 0.5551 ($P < 0.001$), 0.3860 ($P< 0.001$), 0.4656 ($P< 0.001$) and 0.4218 ($P< 0.001$) μ mol/mg protein/hr and in fishes dosed with 0.15 ppm Cu^{++}, the enzyme activity registered was 0.6516 ($P< 0.001$), 0.5474 ($P< 0.001$), 0.5296 ($P< 0.001$) and 0.4396 ($P< 0.001$) μ mol/mg protein/hr after 24, 72, 120 and 168 hrs of exposure respectively.

b. Liver tissue

The values of the enzyme activity in the liver tissue of S.argus dosed with 0.1 ppm and 0.15 ppm Cu^{++} are given in Table 29 and Fig.38.

An increasing trend in the enzyme activity was observed in the two groups of fishes when compared to the control values of 0.3378, 0.2889, 0.3209 and 0.34152 μ mol/mg protein/hr after 24, 72, 120 and 168 hrs, resepectively. During these exposure periods the respective enzyme activity in fishes exposed to 0.1 ppm Cu^{++} was 0.4113 ($P < 0.001$), 0.4368 ($P < 0.001$), 0.3523 ($P < 0.001$) and 0.3877 ($P < 0.001$) μ mol/mg protein/hr and 0.4897 ($P < 0.001$), 0.4855 ($P < 0.001$), 0.4008 ($P < 0.001$) and 0.4087 ($P < 0.001$) in fishes exposed to 0.15 ppm Cu^{++}.

DISCUSSION

A large portion of the absorbed copper is excreted by way of bile in animals and kidney is engaged in the excretion of metals transferred to the circulatory tissue. So it is logical to assume that these two organs are subjected to the effect of the pollutant. In the present study an increase in the activity of both enzymes was observed in both the tissues. Such an elevated enzyme activity in serum was noticed in Aphanius dispar by Hilmy et al. (1981) after acute and long-term exposure to mercury. The activities of both GPT and GOT were elevated. The increase of enzyme activity in serum is the direct reflection of the augmented enzyme production in liver, since hepatic cells are the richest source of these enzymes. Sherlock (1968) considers GPT and GOT as specific enzymes for liver damage. Hilmy et al. (1981) suggested that the damage to liver tissue leads to increased levels of both transaminases in the serum due to the increased permeability of the liver cell membrane. This damage to liver cell membrane due to Hg^{++} poisoning may also occur due to Cu^{++} which increases the enzyme level and in turn enzyme activity in the liver tissue. The increased enzyme can be carried to the kidney via blood which may be manifested as an increased enzyme activity in the kidney.

Cheng (1965) showed that stimulation in the activity of both GOT and GPT occurred when fish was exposed to 0.1 mg/litre of pentachlorophenol. Fox and Rao (1978) reported a stimulation of GOT and GPT in tissues of blue crab exposed to low concentrations of sodium pentachlorophenate.

Verma et al. (1981) also observed a significant increase in GOT and GPT in the blood serum of Notopterus notopterus when exposed to different concentrations of phenol, dinitrophenol and pentachlorophenol. But these workers do not give a definite explanation about the mode of action of these pollutants on the transaminases. This stimulation in activity of GOT and GPT would alter the amino acid metabolism which in turn may disturb all the metabolic processes inside the body of the organism.

All transaminases appear to require specifically either pyridoxal-5-phosphate (PLP) or pyridoxamine-5-phosphate (PMP). These coenzymes are tightly bound to the apoenzyme (Guirard and Snell, 1964 and Fasella, 1967). An activation of the coenzymes by Cu^{++} may in turn elevate the activity of the transaminases in the tissues.

Another probable mechanism for the enhancement of GOT and GPT activity is the activation of the carrier pair by Cu^{++}. Many transamination reactions are linked to the carrier pair, glutamate-α-ketoglutarate (Cohen and Sallach, 1961). A temparory binding of Cu ions could activate the carrier function of glutamate-α-ketoglutarate which may enhance the transamination reaction. According to Cammarata and Cohen (1950) 2-oxoglutaric acid has a wider scope as an amino group acceptor. If Cu^{++} can alter the acceptor sites of the 2-oxoglutaric acid in such a way as to receive more amino groups, the transamination reaction may be increased which can be considered to have occurred in the present study also.

Table 22 GPT in kidney tissue of M.cyprinoides exposed to two concentrations of Cu^{++} at different time periods.

	Hours of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	1.1350	1.1047	1.1750	1.3460
	± SD	0.0934	0.1406	0.1224	0.1043
	Range	1.020 - 1.290	0.925- 1.282	1.012- 1.375	1.217 - 1.474
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	1.5330	1.4596	1.6340	1.5735
	± SD	0.0309	0.0887	0.1487	0.0943
	Range	1.495- 1.573	1.408 - 1.551	1.416- 1.839	1.462- 1.728
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	1.5832	1.4985	1.6486	1.7105
	± SD	0.0458	0.0839	0.2863	0.0750
	Range	1.514 - 1.644	1.396- 1.599	1.103 - 1.925	1.613- 1.819

N = Number of animals; SD = Standard deviation

Table 23 GPT activity in the liver tissue of M.cyprinoides exposed to two concentrations of Cu^{++} at different time periods.

	Hours of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	0.08585	0.8008	0.9480	0.8335
	± SD	0.0540	0.0333	0.2167	0.0717
	Range	0.813- 0.953	0.753 - 0.844	0.921 - 0.980	0.764 - 0.970
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	1.2061	1.1074	1.1663	1.1401
	± SD	0.0896	0.0162	0.0209	0.0297
	Range	1.076 - 1.313	1.087- 1.134	1.129- 1.841	1.093 - 1.179
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	1.2742	1.1556	1.3062	1.2367
	± SD	0.0979	0.0388	0.0465	0.0617
	Range	1.120- 1.398	1.109 - 1.207	1.282- 1.409	1.143- 1.332

N = Number of animlas; SD = Standard deviation

Table 24 GOT activity in kidney tissue of M.cyprinoides exposed to two concentrations of Cu^{++} at different time periods.

	Hours of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	0.4538	0.4817	0.4551	0.5005
	± SD	0.0815	0.0478	0.2642	0.2540
	Range	0.441 - 0.465	0.410- 0.539	0.418-0.491	0.466 - 0.538
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.5156	0.5133	0.4741	0.5403
	± SD	0.02664	0.02209	0.02568	0.04883
	Range	0.484 - 0.553	0.488 - 0.546	0.435 - 0.507	0.451- 0.591
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.5220	0.5801	0.5262	0.6139
	± SD	0.0428	0.0382	0.0929	0.0141
	Range	0.458- 0.576	0.509 - 0.617	0.518 - 0.540	0.595- 0.635

N = Number of animals; SD = Standard deviation

Table 25 GOT activity in the liver tissue of M.cyprinoides exposed to two concentrations of Cu^{++} at different time periods.

	Hours of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	0.0667	0.5981	0.6840	0.6613
	± SD	0.0652	0.0385	0.0826	0.0198
	Range	0.565 - 0.747	0.552- 0.646	0.555 - 0.788	0.637- 0.696
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.7670	0.6559	0.8007	0.7644
	± SD	0.0322	0.0364	0.0344	0.0439
	Range	0.718- 0.810	0.616 - 0.713	0.761 - 0.8582	0.684 - 0.809
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.831	0.675	0.905	0.861
	± SD	0.0186	0.0335	0.0209	0.0172
	Range	0.809- 0.858	0.619 - 0.710	0.875- 0.932	0.833- 0.881

N = Number of animals; SD = Standard deviation

Table 26 GPT activity in kidney tissue of S.argus exposed to two concentrations of Cu^{++} at different time periods.

	Hours of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	0.3879	0.3463	0.4283	0.3433
	± SD	0.0304	0.0359	0.0386	0.0147
	Range	0.352 - 0.4318	0.299- 0.4062	0.351 - 0.4501	0.322- 0.3635
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.5173	0.4414	0.4703	0.4042
	± SD	0.0728	0.9326	0.0419	0.0152
	Range	0.5064- 0.525	0.4207 - 0.456	0.4663- 0.474	0.3818- 0.423
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.5497	0.4655	0.5242	0.4301
	± SD	0.0823	0.0977	0.0122	0.0279
	Range	0.540 - 0.564	0.451- 0.476	0.508 - 0.542	0.381- 0.461

N = Number of animals; SD = Standard deviation

Table 27 GPT activity in the liver tissue of S.argus exposed to two concentrations of Cu^{++} at different time periods

	Hours of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	0.3579	0.4154	0.3572	0.3313
	± SD	0.03223	0.0341	0.0114	0.0293
	Range	0.3004- 0.3943	0.3850 - 0.4810	0.3437- 0.3743	0.2919- 0.3721
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.4018	0.4432	0.4278	0.4620
	± SD	0.0259	0.0142	0.0225	0.0188
	Range	0.371- 0.4415	0.425 - 0.4651	0.401- 0.4655	0.4112- 0.5742
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.4922	0.5232	0.4906	0.5716
	± SD	0.1117	0.0359	0.0611	0.0545
	Range	0.506- 0.523	0.459 - 0.563	0.481- 0.495	0.528- 0.693

N = Number of animals; SD = Standard deviation

Table 28 GOT activity in the liver tissue of S.argus exposed to two concentrations of Cu^{++} at different time periods.

	Hours of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	0.3378	0.2889	0.3209	0.3415
	± SD	0.0381	0.0219	0.0125	0.0104
	Range	0.2991- 0.3017	0.2516- 0.3138	0.3075- 0.3374	0.3281- 0.3535
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.4113	0.4368	0.3523	0.3877
	± SD	0.0355	0.0180	0.0206	0.0551
	Range	0.4070- 0.4163	0.417 - 0.4690	0.3166- 0.3775	0.3825- 0.3964
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.4897	0.4855	0.4008	0.4087
	± SD	0.03228	0.07159	0.02860	0.01680
	Range	0.4529- 0.5332	0.4795 - 0.4968	0.3643 - 0.4316	0.3889- 0.4321

N = Number of animals; SD = Standard deviation

Table 29 GOT activity in kidney tissue of S.argus exposed to two concentrations of Cu^{++} at different time periods.

	Hours of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	0.4369	0.3490	0.4140	0.3539
	± SD	0.0353	0.02020	0.0406	0.0257
	Range	0.3702- 0.4628	0.3225 - 0.3737	0.3638- 0.4507	0.3501- 0.3569
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.5551	0.3864	0.4656	0.4218
	± SD	0.0988	0.0169	0.0141	0.0116
	Range	0.05428- 0.5696	0.3610 - 0.4041	0.4494- 0.4823	0.4374- 0.4374
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	0.6516	0.5467	0.5296	0.4396
	± SD	0.0139	0.0322	0.0401	0.0170
	Range	0.6308- 0.6702	0.4961- 0.5308	0.5237- 5361	0.4117- 4613

N = Number of animals; SD = Standard deviation

CHAPTER VI

EFFECT OF COPPER ON THE GLYCOGEN AND LACTIC ACID CONTENT IN DIFFERENT TISSUES

When heavy metal ions exceed a limited concentration in the aquatic ecosystems, they turn to be pollutant and bring in anoxic or hypoxic conditions to the aquatic organisms. Environmental pollution is reported as one of the major factors causing hypoxemia to animals (Black et al., 1962). When tissues of the animal do not receive sufficient oxygen, they must either reduce the overall energy demand or respire anaerobically. Since glycogen is the ready source of energy even under anaerobic conditions, the accumulation of lactic acid and other end products of glycogen metabolism is expected to be the immediate manifestation of hypoxia.

To assess the interruption of metabolic activities due to anoxic conditions, the conventional method followed in pollution bioassay studies is the estimation of oxygen content in the environment. But in teleosts which are known to withstand anaerobic conditions for a longer duration (Blazka, 1958) this procedure may not porduce satisfactory results. As proved by the extensive investigations, it is recognised that different tissues of fish can sustain varying levels of anaerobic metabolism. In most teleosts fermentation of glucose or glycogen to lactate provides the main source of energy production under hypoxic conditions (Heath and Pritchard, 1965; Burton and Sephar, 1971). Estimation of this end product may provide a better picture of the hypoxic stress to which the fish is subjected. So evaluation of this end product of glycolysis was adopted here as a method of analysing the hypoxic effect of copper ions. As glycogen in the liver and muscle is the immediate anaerobic substrate (Jorgensen and Mustafa, 1965), these two tissues were selected as the assay materials.

The depletion of glycogen and accumulation of lactic acid have been a yard-stick of fatigue in animals following exercise. It is only a recent trend to relate it to the anaerobic conditions which the animal experiences due to environmental pollutants. A preliminary investigation was made by Jones and Erichsen (1952) by studying the reactions of fish to water of low oxygen

concentration. Black made an extensive study of blood lactic acid level following muscular exercise in fishes and published the data in a series of papers (Black, 1955, 1957 a, b and c). His further studies were on hyperactivity as a lethal factor. According to him hyperactivity causes the accumulation of lactic acid in the muscle which diffuses into the blood stream causing severe disturbance to the acid-base balance leading to the death of the animal (Black, 1958; Edgar et al., 1959 and 1960). Anaerobic metabolism of fish was studied elaborately by Blazka (1958). Miller et al. (1959) fed rainbow trout with feed of varying glycogen content to examine the effect of diet and glycogen reserve on fatigue-resistance.

Accumulation of lactic acid can also occur due to the stress caused by the gears used for fishing which in turn brings in fatigue and mortality. Such a chain of reactions were investigated in troll-caught pacific salmon (Oncorhynchus) by Parker et al. (1959) and Beamish (1966) in fishes caught by otter trawls. Setten and Setten (1960) undertook an elaborate study on glycogen metabolism in fishes. Black et al. (1961) have made a detailed study on glycolysis in fish. Evidence has been presented by Tomlinson et al. (1961) who established that duration of full rigor after death in fish is related to the continued production of lactic acid in the muscle tissue of fishes. The pattern of immediate changes in carbohydrate metabolism in fish, following muscular activity is similar to that in other vertebrates including mammals. This similarity was established by Black et al. (1962) while studying the changes in glycogen and lactate in rainbow trout (Salmo gairdneri). Increase in lactic acid also causes changes in metabolic rate (Heath and Pritchard, 1962). Hochachka (1962) and Richard (1962) analysed the influence of diet on glycogen stores in trout tissues. Migration is a major factor influencing glycogen and lactic acid levels as explained by Anne et al. (1964). According to Edgar and Anne (1964) anaesthetics also effect glycogen metabolism. The effects of severe hypoxia on carbohydrate energy stores and metabolism were studied by Heath and Pritchard (1965) in freshwater fishes.

There is a view that metabolism during effort varies in different tissues of the same animal (Wittenberges and Diaciac 1965). Physical conditions are also considered as controlling factors altering carbohydrate metabolism

(Hammond and Hickman, 1966). According to Stevens and Edgar (1966) other than continued exercise, intermittent exercise also induces some variations in carbohydrate metabolism. Metabolic change in mud-skipper during asphyxia or exercise was the field of analysis by Bandurski et al. (1968). A similar work was carried out in Atlantic cod (Gadus morhua) by Beamish (1968). Charles (1968) in one of his reports suggested that lactic acidosis beyond a threshold limit causes death by reducing the P^H of blood. This view was supported by Gronlund et al. (1968) in their work on sockeye and chinook salmon. A study of the details of lactate metabolism in fish was carried out by Dando (1969). Burton (1970 a) induced gradual hypoxia at two acclimation temperatures to study its effect on the anaerobic responses of brown bullhead catfish (Ictalurus nebulosus). The postmortem glycolytic changes were investigated in white sucker (Catostomus commersoni) by Manohar (1970).

Burton and Sephar (1971) made a re-evaluation of the anaerobic end products of freshwater fishes exposed to environmental hypoxia. Johnston and Goldspink (1973) carried out a comparative analysis of glycogen and lactate in the muscle and liver tissue of Gadus virens during sustained swimming. The same workers (1973) made quantitative studies of glycogen utilization during continuous swimming in crucian carp. The muscle metabolism of the swimming musculature during severe hypoxia in rainbow trout was examined by Johnston (1975). Hochachka et al. (1975) concluded that glycolysis plays a central role in the anoxic adaptation of diving vertebrates. Glycolysis is considered to vary in red and white muscles due to the difference in their vascular supply. A comparative study of glycolysis in red and white muscle of rainbow trout was undertaken by Johnston (1977). In animals, accumulation of lactic acid in the anaerobic muscle is an accepted stress reaction. The release or non-release of lactic acid from these anaerobic tissues is a function of stress hormones. The role of such hormones in the release of lactic acid in the swimming muscle of plaice Pleuronectes platessa was investigated by Wardle (1978).

The restoration of glycogen from lactic acid in the swimming muscle of plaice was studied by Batty and Wardle (1979). Burgen and Comeron (1980)

examined the anaerobic metabolism gas exchange and acid-base balance during hypoxic exposure in channel catfish (Ictalurus punctatus) and Jorgensen and Mustafa (1980) the utilization of glycogen and accumulation of glycolytic end products in various tissues of flounder. Conventional and modified metabolic pathways in red and white muscle during acute anoxia and prolonged hypoxia in rainbow trout were studied by Smith and Heath (1980). Narasimhan and Sundararay (1981) analysed the effects of stress on carbohydrate metabolism in Notopterus notopterus. Turner et al. (1983 a) studied the proton dynamics involved in lactate difusion from muscle tissue to blood.

MATERIALS AND METHODS

The collection, acclimatization and setting of the experiment were done as described in chapter III. For preparing tissue extracts, fishes were caught and stunned by a quick hard blow on the head. They were dissected and samples of liver and muscle were collected with minimum delay. The tissues were deproteinised in 10% TCA (Trichloroacetic acid). The tissue was then homogenised in TCA and centrifuged. The clear supernatant was decanted and used as tissue extract for the estimations.

Estimation of lactic acid

The quantitative determination of lactic acid was done following the method of Barker (1957). To 1.5 ml of the tissue extract 1.0 ml of 20% $CuSO_4$ solution and 3.0 ml of distilled water were added and shaken well. Approximately 1 gm of powdered $Ca(OH)_2$ was added and mixed thoroughly. The mixture was allowed to stand at room temperature for 30 minutes with occassional shaking and then centrifuged. Duplicate aliquotes of 1.0 ml of the supernatant solution were carefully withdrawn from beneath any surface particles and transferred to clean test tubes. 0.5 ml of 4% $CuSO_4$ solution was added and the mixture chilled in a cold water bath (below $4^{o}C$). 6.0 ml of concentrated H_2SO_4 was added slowly through the side of the test tube and the contents mixed well. The mixture was heated in a boiling water bath for 5 minutes, removed and cooled below $20^{o}C$. Subsequently, 0.1 ml of

p-hydroxy diphenyl solution was added. The precipitated reagent was dispensed throughout the acid as quickly and uniformly as possible. The reaction mixture was kept at room temperature for 30 minutes and then the reaction was stopped by warming the mixture in a boiling water bath for 90 seconds and then cooled in cold water. The colour developed was read spectrophotometrically against the reagent blank at 560 nm. Lactate content was calculated by referring to a calibration curve prepared by using lithium lactate.

Estimation of Glycogen

Glycogen content in the tissue was estimated by the method of Montgomery (1957). 0.75 ml of muscle tissue extract and 0.5 ml of liver tissue extract were used for the estimation. To this 1.0 ml of 95% ethyl alcohol was added and kept overnight in a refrigerator. The mixture was centrifuged at 2500 rpm for 15 minutes. The supernatant was carefully decanted. The precipitate was dessolved in 2.0 ml of distilled water and 0.1 ml of 80% phenol was added. To this 5.0 ml of concentrated sulphuric acid was added forcefully to aid mixing. It was then left at room temperature for 30 minutes. After cooling, the optical density was read at 490 nm. The concentration of glycogen in the samples was calculated from the standard graph prepared using Oyster glycogen (Sigma chemical company USA).

RESULTS

Megalops cyprinoides

1. Muscle tissue

A dose-dependent decrease in the glycogen content and increase in lactic acid content were observed in the muscle tissue. The variations in glycogen content are given in Table 30; Fig.39 and those of lactic acid in Table 31 and Fig.40.

The glycogen contents (μ gm/mg tissue) estimated in muscle tissue of control fishes were 450, 413, 341 and 254. The respective glycogen contents

Fig.39 Glycogen content (μ gm/mg tissue) in the muscle tissue of M.cyprinoides exposed to two concentrations of Cu^{++} at different time periods.

Fig.40 Lactic acid content (μ gm/mg tissue) in the muscle tissue of M.cyprinoides exposed to two concentrations of Cu^{++} at different time periods.

Fig.41 Glycogen content (μ gm/mg tissue) in the liver tissue of M.cyprinoides exposed to two concentrations of Cu^{++} at different time periods.

Fig.42 Lactic acid content (μ gm/mg tissue) in the liver tissue of M.cyprinoides exposed to two concentrations of Cu^{++} at different time periods.

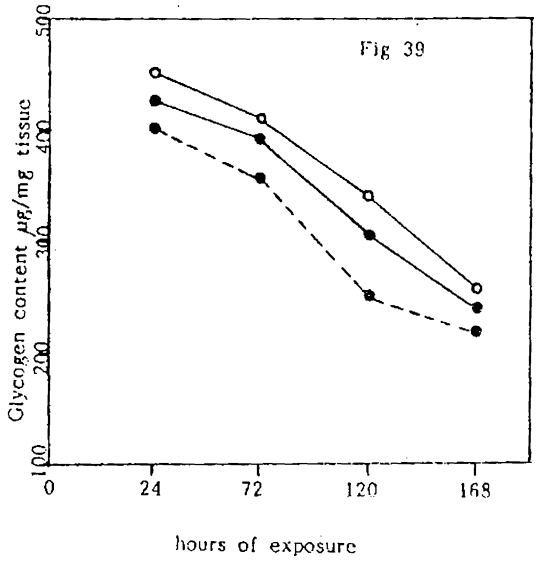
Fig 39
Glycogen content µg/mg tissue
100
200
300
400
500
0
24
72
120
168
hours of exposure

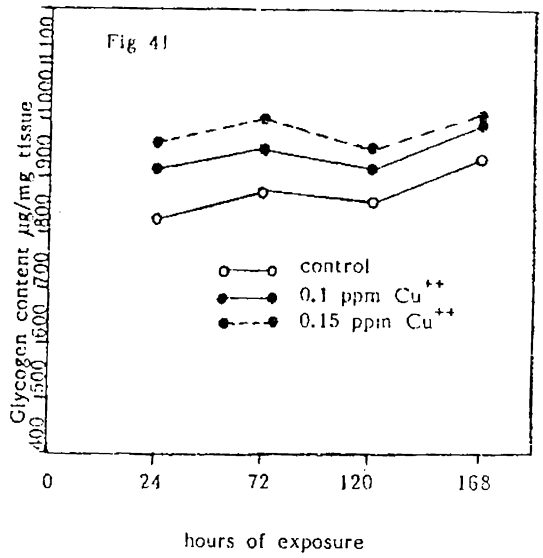
Fig 41
Glycogen content µg/mg tissue
1400
1500
1600
1700
1800
1900
2000
2100
0
24
72
120
168
hours of exposure
control
0.1 ppm Cu++
0.15 ppm Cu++

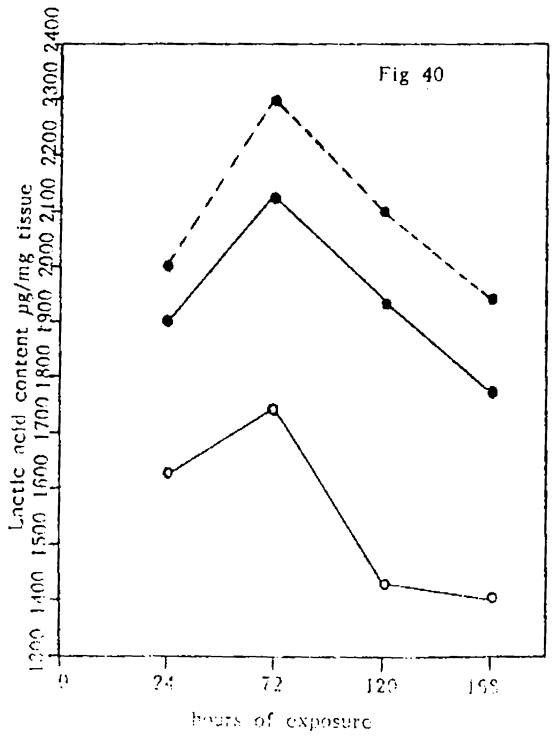
Fig 40
Lactic acid content µg/mg tissue
1300
1400
1500
1600
1700
1800
1900
2000
2100
2200
2300
2400
0
24
72
120
168
hours of exposure

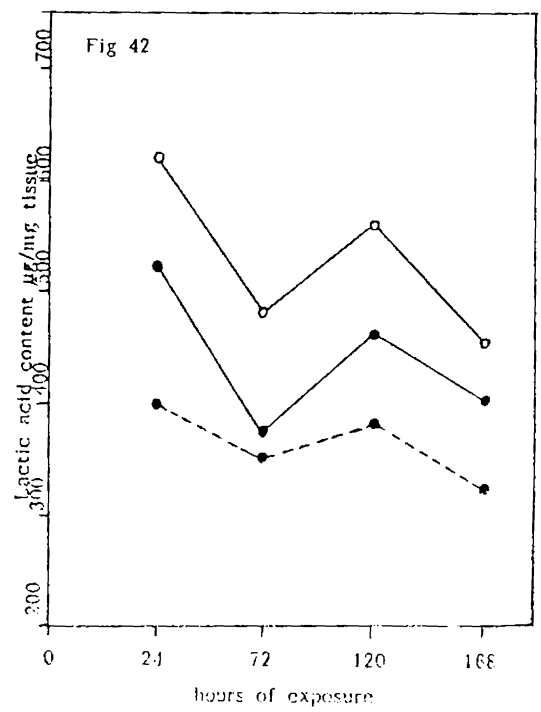
Fig 42
Lactic acid content µg/mg tissue
300
1400
1500
1600
1700
0
24
72
120
168
hours of exposure

in the muscle tissue of fishes exposed to 0.1 ppm Cu^{++} were 430 ($P< 0.05$), 401 ($P < 0.05$), 273 ($P < 0.001$) and 241 ($P < 0.05$) and in fishes exposed to 0.15 ppm Cu^{++} were 419 ($P < 0.05$), 360 ($P < 0.05$), 248 ($P < 0.001$) and 220 ($P < 0.01$) in units of μ gm/mg tissue after the corresponding exposure periods of 24, 72, 120 and 168 hours.

The lactic acid contents (μ gm/mg tissue) in the muscle tissue of dosed and control fishes after the respective hours of exposure of 24, 72, 120 and 168 were as follows. In fishes dosed with 0.1 ppm Cu^{++}, the values obtained were 1916 ($P < 0.001$) 2120 ($P < 0.001$), 1936 ($P < 0.001$) and 1773 ($P < 0.001$), and in fishes dosed with 0.15 ppm Cu^{++} they were 2093 ($P < 0.001$), 2308 ($P < 0.001$), 2306 ($P < 0.001$) and 1940 ($P < 0.001$), and their corresponding control values were 1628, 1747, 1424 and 1407.

2. Liver tissue

The reverse pattern of glycogen response was observed in the liver tissue. A decrease in lactic acid and an increase in glycogen content were registered in this tissue. Both the decrease and increase in lactic acid and glycogen content were dose-dependent and significant.

Table 32 and Fig.41 explain the details of the variations in glycogen content of the liver tissue when dosed with Cu^{++}. Glycogen contents in the liver tissue of fishes exposed to 0.1 ppm Cu^{++} were 908 ($P < 0.001$), 946 ($P < 0.001$) 909 ($P < 0.05$) and 989 ($P < 0.05$) and in fishes exposed to 0.15 ppm Cu^{++} 961 ($P < 0.001$), 1043 ($P < 0.001$), 949 ($P < 0.002$) and 1107 ($P < 0.05$) after 24, 72, 120 and 168 hours of exposure respectively. Their corresponding control values were 827, 877, 852 and 929. The glycogen content of all three groups are expressed in μ gm/mg tissue.

The variations in lactic acid content (μ gm/mg tissue) recorded in the liver tissue of dosed and undosed fishes are given in Table 33 and Fig.42. Fishes exposed to 0.1 ppm Cu^{++} recorded lactic acid content of 526 ($P< 0.001$), 370 ($P < 0.001$), 467 ($P< 0.001$) and 400 ($P < 0.001$), and in those dosed with

Fig.43 Glycogen content (μ gm/mg tissue) in the muscle tissue of S.argus exposed to two concentrations of Cu^{++} at different time periods.

Fig.44 Lactic acid content (μ gm/mg tissue) in the muscle tissue of S.argus exposed to two concentrations of Cu^{++} at different time periods.

Fig.45 Glycogen content (μ gm/mg tissue) in the liver tissue of S.argus exposed to two concentrations of Cu^{++} at different time periods.

Fig.46 Lactic acid content (μ gm/mg tissue) in the liver tissue of S.argus exposed to two concentrations of Cu^{++} at different time periods.

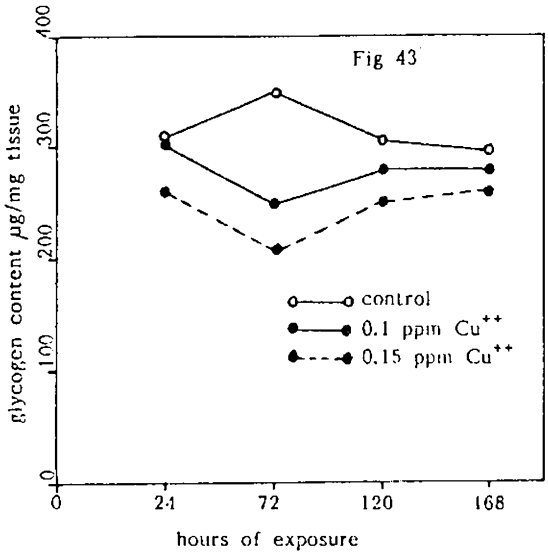
Fig 43
glycogen content µg/mg tissue
0
100
200
300
400
0
24
72
120
168
hours of exposure
control
0.1 ppm Cu++
0.15 ppm Cu++

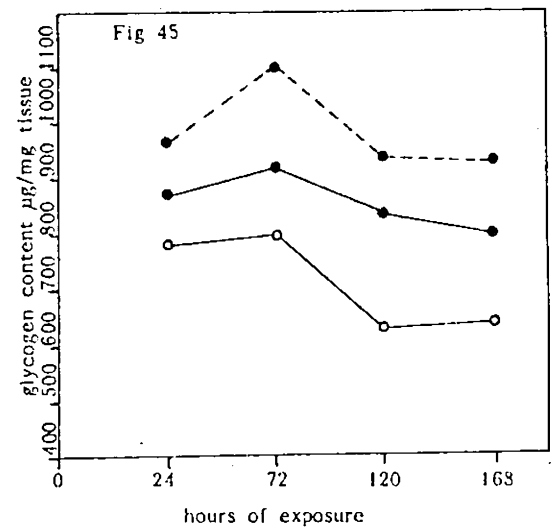
Fig 45
glycogen content µg/mg tissue
400
500
600
700
800
900
1000
1100
0
24
72
120
168
hours of exposure

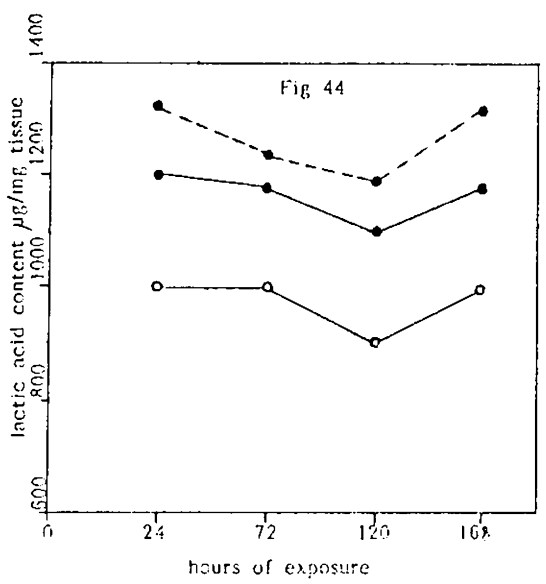
Fig 44
lactic acid content µg/mg tissue
600
800
1000
1200
1400
0
24
72
120
168
hours of exposure

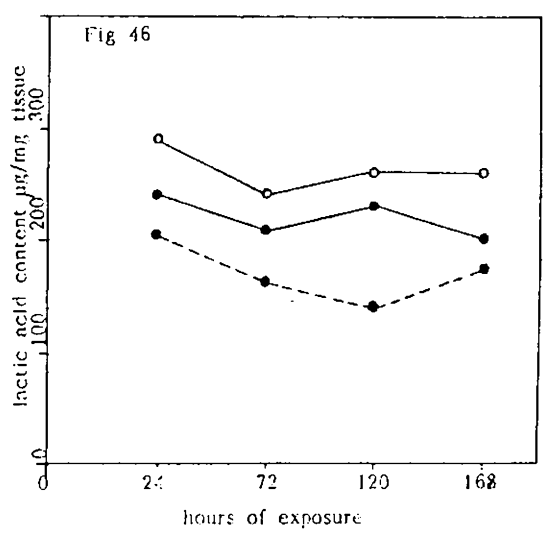
Fig 46
lactic acid content µg/mg tissue
0
100
200
300
0
24
72
120
168
hours of exposure

0.15 ppm Cu^{++} lactic acid contents were 403 ($P < 0.001$) 357 ($P < 0.001$), 381 ($P < 0.001$) and 323 ($P < 0.001$) after the respective exposure periods of 24, 72, 120 and 168 hours. Their corresponding values in the control fishes were 619, 485, 565 and 455.

Scatophagus argus

1. Muscle tissue

The same trend of glycolysis as seen in M.cyprinoides is evident in S.argus too. There was a depletion in the muscle glycogen and increase in muscle lactic acid. As in M.cyprinoides both glycogen and lactic acid is expressed as μ gm/mg tissue.

Table 34 and Fig.43 explain the concentration pattern of glycogen, and Fig.44 and Table 35 that of lactic acid.

The significant and dose-dependent decrease in glycogen content was observed. In fishes dosed with 0.1 ppm Cu^{++} the values were 306 ($P < 0.05$), 249 ($P < 0.001$), 279 ($P < 0.02$) and 281 ($P < 0.05$) and in fishes dosed with 0.15 ppm Cu^{++} the values were 268 ($P < 0.001$), 205 ($P < 0.002$), 251 ($P < 0.001$) and 262 ($P < 0.001$) after the respective durations of exposures of 24, 72, 120 and 168 hours against their corresponding control values of 313, 351, 308 and 293.

The increase in lactic acid content was also dose-dependent and significant. The control values obtained were 1174, 1082, 927 and 1054 and the corresponding values in 0.1 ppm Cu^{++} dosed fishes were 1218 ($P < 0.002$), 1183 ($P < 0.05$), 1104 ($P < 0.001$) and 1182 ($P < 0.001$) and in the muscle tissue of fishes dosed with 0.15 ppm Cu^{++}, they were 1327 ($P < 0.001$), 1246 ($P < 0.01$), 1194 ($P < 0.001$) and 1394 ($P < 0.001$) after the respective durations of exposure of 24, 72, 120 and 168 hours.

2. Liver tissue

The pattern of glycogen break down and lactic acid accumulation was similar to that found in the liver tissue of M.cyprinoides. Table 36, Fig.45; and Table 37 and Fig.46 give respectively the details of the alterations in glycogen and lactic acid content in the liver tissue of fishes dosed with Cu^{++}.

The increase in the glycogen content of the liver tissue of fishes exposed to 0.1 ppm Cu^{++} was as 870 ($P < 0.001$), 920 ($P < 0.001$), 839 ($P < 0.001$) and 803 ($P < 0.001$), and in fishes exposed to 0.15 ppm Cu^{++} the glycogen content of the liver tissue was 969 ($P < 0.001$), 1103 ($P < 0.001$), 935 ($P < 0.001$) and 930 ($P < 0.001$) after 24, 72, 120 and 168 hours of exposure respectively against the corresponding control values of 785, 802, 619 and 638.

The lactic acid content in the liver tissue of the fishes dosed with 0.1 ppm Cu^{++} was 243 ($P < 0.001$), 210 ($P < 0.001$), 238 ($P < 0.001$) and 203 ($P < 0.001$), and in fishes dosed with 0.15 ppm Cu^{++} the lactic acid content was 205 ($P < 0.001$), 166 ($P < 0.001$), 139 ($P < 0.001$) and 176 ($P < 0.001$) after the exposure periods of 24, 72, 120 and 168 hours. The corresponding control values were 287, 242, 262 and 264.

DISCUSSION

When an organism is subjected to stress, the functional systems of the body demand more energy. Since glycogen is easily catabolised and dispensed to the cellular level, it becomes the main fuel to be utilized. Under such circumstances of added energy requirement the first stage of energy release-glycolysis-is accelerated especially when the stress inducing factor is hypoxic since glycolysis is the anaerobic phase of energy production. Hochachka et al. (1975) consider that glycolysis certainly plays a central role in anoxic adaptations of diving animals. Liver and muscle glycogen become the immediate substrates of glycolysis. Variations in glycogen content of these tissues and the accumulation of glycolytic end products occur under anoxic conditions in animals.

In the present study decrease in the muscle glycogen is observed while the liver glycogen increased and a reverse pattern was observed for lactic acid. Such an increase in the muscle lactate level was also noticed by Turner et al. (1983 a) in the white muscle of rainbow trout after muscular exercise, and Smith and Heath (1980) in rainbow trout and mirror carp. Anaerobic glycolysis in white muscle is also reported in crucian carp by Johnston and Goldspink (1973 b). They are of opinion that the white muscle contains more of enzyme involved in anaerobic glycolysis. The same workers in another report (1973 a) have suggested an increase in muscle lactate in Gadus virens. But they noticed no depletion in muscle glycogen which is not in agreement with the result of the present study. According to them glycogen is replaced from other sites like liver so that muscle tissue does not encounter a reduced glycogen content. Another point of disagreement with the above authors is that they observed a fall in the liver glycogen. But in the present investigation liver glycogen increased while liver lactate fell consistently. Beamish (1968) noticed a depletion of muscle glycogen and an increase in muscle lactate following muscular exercise in Atlantic cod. Black et al. (1962) had similar results in his experiments in rainbow trout.

Though some workers have reported the utilization of muscle glycogen anaerobically and synthesis of lactic acid in the muscle, they opined that the lactic acid diffuses to the blood and is carried to the liver to be converted to glycogen by the Cori cycle (Black et al. 1960). In the present study this possibility can not be taken into consideration, since an increase in the liver lactic acid is not observed in fishes during the exposure to Cu^{++}. The liver lactate is found to be reduced in the present analysis and liver glycogen increased. Some workers have suggested that liver glycogen is not anaerobically used up and so a depletion in liver glycogen is not observed (Black et al., 1962). Anne et al. (1964) reported that muscular exercise had no effect on liver glycogen of fishes. This indicates that liver glycogen is not utilized for anaerobic glycolysis. So a reduction in liver glycogen cannot be expected. In the present study, liver glycogen increases while liver lactic acid decreases. The normal lactate content of the liver tissue may be used for the

synthesis of glycogen to keep up the blood glucose level resulting in a reduction in liver lactate and increase in liver glycogen. Hepatic glycogen thus formed is degraded to glucose which difuses to the blood stream to be transported to the metabolicaly active muscle tissue while maintaining the blood glucose level. The utilization of liver glycogen to maintain blood glucose level has been suggested by Black et al. (1960).

Since the level of lactate is found to increase considerably in the muscle and such an increase is not noticed in liver tissue, it should be considered that the transportation of muscle lactic acid to liver does not occur as reported by other workers mentioned above. But there are reports (Black, 1958; Charles, 1968) that lactic acid produced in the muscle diffuses to the blood which upsets the acid-base system by reducing the P^H and death is caused. This might have occurred when the level of accumulated lactic acid exceeded the threshold capacity of the muscle tissue due to lethal hypoxic activity and the lactic acid in excess may have diffused to the blood stream reducing the P^H to lethal level. In the experiment under discussion only sublethal concentration of Cu^{++} was used. So a lethal quantity of lactic acid production was not observed. In the present experiments, it seems that the lactic acid produced in the muscle tissue is retained in the site of production for further oxidation or glycogen synthesis. Though this opposes the conventional view that lactate is exported to liver to be reprocessed into glycogen via the Cori cycle, it is in accordance with the interpretation of Black et al. (1966), Wardle (1978), and Batty and Wardle (1979). A similar argument for lactic acid retention and metabolism in situ has been presented by Turner et al. (1983 a) in trout. The slow release or non-release of lactic acid from the exhausted anaerobic muscle may be under the control of catecholamine hormones involved in fright and fight reaction. Following appropriate stimulus, catecholamine is released into the blood which stimulates the lactate-loaded muscle cells to retain lactic acid against a concentration gradient and incorporated to glycogen by the muscle cells. At the same time the blood flow through the muscle is increased allowing greater dissolved gas and metabolic exchange Wardle (1978). Branchial or renal excretion of lactic acid seems to be of little importance (Turner et al., 1983 a). So chances of elimination of lactic acid to the exterior is negligible. The resynthesis of

muscle glycogen is a slow process in fish (Stevens et al., 1966). All these factors together assist the active retention of lactic acid in the myotome to be converted back to glycogen in situ.

Table 30 Glycogen content (µgm/mg tissue) in the muscle tissue of M.cyprinoides exposed to two concentrations of Cu^{++} at different time periods.

	Hours of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	450.4	413	341	254.4
	± SD	0.08436	0.05327	0.0551	0.0128
	Range	304 - 526	336 - 488	272 - 408	220 - 248
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	430.2	401	273.2	241.4
	± SD	0.02134	0.0520	0.0422	0.0399
	Range	402 - 459	350 - 509	212 - 328	89 - 357
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	419.8	360.8	248.4	237.8
	± SD	0.0641	0.0684	0.0767	0.06574
	Range	332 - 507	281 - 453	130 - 325	136 - 325

N = Number of animals; SD = Standard deviation

Table 31 Lactic acid content (µgm/mg tissue) in the muscle tissue of M.cyprinoides exposed to two concentrations of Cu^{++} at different time periods.

	Hours of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	1628	1747	1424	1407.4
	± SD	0.03538	0.1533	0.26672	0.210386
	Range	1589 - 1675	1505 - 1947	1063 - 1826	1020 - 1621
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	1916	2129	1936	1773.4
	± SD	0.0493	0.1559	0.02737	0.008529
	Range	1859 - 1980	2110 - 2339	1903 - 1971	1692 - 1714
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	2093.6	2308.6	2306	1940
	± SD	0.0331	0.15242	0.0496	0.04492
	Range	2034 - 2130	2349 - 2623	2049 - 2443	1843 - 1946

N = Number of animals; SD = Standard deviation

Table 32 Glycogen content (µgm/mg tissue) in the liver tissue of M.cyrpinoides exposed to two concentrations of Cu^{++} at different time periods.

	Hours of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	827.6	877.6	852.2	939.0
	± SD	0.004758	0.06058	0.08124	0.052642
	Range	826 - 834	809 - 968	740 - 951	904 - 1044
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	908.6	946.4	909	989.8
	± SD	0.03656	0.03088	0.20231	0.19926
	Range	871 - 968	912 - 997	629 - 1196	730 - 1275
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	961.2	1093.6	949	1071.2
	± SD	0.02618	0.0292	0.1054	0.26155
	Range	917 - 991	1052.9-1142.8	773 - 1147	753 - 1441

N = Number of animals; SD = Standard deviation

Table 33 Lactic acid content (µgm/mg tissue) in the liver tissue of M.cyprinoides exposed to two concentrations of Cu^{++} at different time periods.

	Hours of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	619.2	485.4	565.4	455.2
	± SD	0.0644	0.0305	0.06377	0.02711
	Range	534 - 713	438 - 525	444 - 621	427 - 491
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	526.8	370.8	467.6	400.8
	± SD	0.06771	0.04116	0.02008	0.0230
	Range	420 - 615	306 - 412	434 - 492	370 - 435
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	403.8	357.6	381.2	323.8
	± SD	0.05021	0.0285	0.0720	0.01057
	Range	386 - 513	325 - 461	287 - 476	309 - 339

N = Number of animals; SD = Standard deviation

Table 34 Glycogen content (µgm/mg tissue) in the muscle tissue of S.argus exposed to two concentrations of Cu^{++} at different time periods.

	Hours of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	313.2	351.4	308	293.8
	± SD	0.00762	0.0265	0.0546	0.02211
	Range	311 - 332	227 - 408	215 - 366	264 - 326
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	306.2	249	279.8	281.8
	± SD	0.0408	0.0796	0.03648	0.03289
	Range	271 - 368	178 - 382	213 - 315	226 - 320
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	268.2	205	251	262
	± SD	0.03955	0.05009	0.01863	0.02605
	Range	236 - 331	178 - 221	233 - 284	248 - 329

N = Number of animals; SD = Standard deviation

Table 35 Lactic acid content (µgm/mg tissue) in the muscle tissue of S.argus exposed to two concentrations of Cu^{++} at different time periods.

	Hours of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	1174.2	1082.2	927.4	1054
	± SD	0.11642	0.2693	0.01764	0.05963
	Range	1010 - 1326	990 - 1179	902 - 947	1077 - 1265
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	1218.14	1183.5	1104.8	1182.4
	± SD	0.08075	0.12365	0.018312	0.01028
	Range	1143.1 - 1338	903 - 1188.3	1076 - 1182	1167 - 1195
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	1327.8	1246.6	1194.8	1395.4
	± SD	0.07039	0.02229	0.0183	0.24419
	Range	1186 - 1325	1207 - 1273	1123 - 1178	1258 - 1883

N = Number of animals; SD = Standard deviation

Table 36 Glycogen content (µgm/mg tissue) in the liver tissue of S.argus exposed to two concentrations of Cu^{++} at different time periods.

	Hours of exposure	24 hours	72 hours	120 hours	168 hours
Control	N	10	10	10	10
	Mean value	785.8	802.4	619	638
	± SD	0.0328	0.0134	0.0471	0.12479
	Range	725 - 821	783 - 819	545 - 684	495 - 821
Dosed with 0.1 ppm Cu^{++}	N	10	10	10	10
	Mean value	870	920	839.8	803.8
	± SD	0.03536	0.01755	0.02708	0.03551
	Range	826 - 923	909 - 953	807 - 875	770 - 865
Dosed with 0.15 ppm Cu^{++}	N	10	10	10	10
	Mean value	969.16	1103.6	935	930.1
	± SD	0.00723	0.02672	0.02842	0.008221
	Range	960.1 - 979.7	1105 - 1181.4	902 - 986	930.3-952

N = Number of animals; SD = Standard deviation

Table 37 Lactic acid content (µgm/mg tissue) in the liver tissue of S.argus exposed to two concentrations of Cu^{++} at different time periods.

	Hours of exposure	24 hours	72 hours	120 hours	168 hours
	N	10	10	10	10
	Mean value	287.6	242.2	262.4	264.8
	± SD	0.01512	0.006829	0.03061	0.01312
	Range	269 - 310	234 - 250	219 - 303	249 - 281
	N	10	10	10	10
Dosed with	Mean value	243.4	210.4	238.4	203.7
0.1 ppm Cu^{++}	± SD	0.013836	0.01796	0.029152	0.04232
	Range	221 - 263	204 - 238	200 - 278	174 - 269
	N	10	10	10	10
Dosed with	Mean value	205.4	166	139.6	176.6
0.15 ppm Cu^{++}	± SD	0.006829	0.0321	0.036003	0.02758
	Range	197 - 214	136 - 213	87 - 137	146 - 227

N = Number of animals; SD = Standard deviation

SUMMARY

SUMMARY

CHAPTER I

An analysis of the food and feeding habits of two fishes, Megalops cyprinoides and Scatophagus argus are presented in the first chapter. The adults of M.cyprinoides are found to be strictly carnivorous, and preferred to feed on prawns and fishes. They did not seem to select a wide range of food items. S.argus can be considered as an omnivore, consuming a wide range of food materials.

In M.cyprinoides, significant variations could be seen between the juveniles and the adults, in the feeding habits. Juveniles feed on both plant and animal matter, with algae and diatoms forming the major portion of the food. The plant matter decreased in the diet, as the size of the fish increased. In S.argus, no significant variations could be seen in the food of juveniles and adults. The juveniles feed on diatoms and algae in the order of preference, whereas the adults feed on algae, diatoms, hydroids and crustaceans in that order. In both juveniles and adults, vegetable matter formed the major item of food.

Difference in feeding could be seen in relation to the habitat. In adults of M.cyprinoides collected from the Vembanad lake, prawns formed the major item of food, while in specimens collected from culture farms, animal matter, other than prawns formed the main food. In S.argus collected from bar mouth area of Vembanad lake, hydroids constituted a significant portion of the food. But hydroids showed only nominal representation in fishes collected from the inner parts of the lake. This indicates that the availability of food items in the habitat determines their rank as chief and supplementary food.

In both the fishes, breeding could be considered to influence feeding. In S.argus the GSI showed the lowest value in May, coinciding with the peak spawning period. A subsequent increase in the values of GSI in July, may be due to active feeding after peak spawning during May and June. The

ebbs and falls of GSI values in S.argus coincide with spawning periods of the fish.

The frequency of occurrence of food in M.cyprinoides did not vary in day and night collections, indicating that this fish feeds during night hours also. Unlike M.cyprinoides difference in day and night feeding was observed in S.argus. Decreased rate of feeding was noticed in this fish during night. All the above aspects were discussed in detail.

CHAPTER II

Aspects on the biochemical constitutents, including total carbohydrate, protein, fat content and calorific value of the muscle and liver tissues are included in this chapter. The muscle tissue of the two fishes showed very low carbohydrate values. But the liver tissue was richer in carbohydrate level when compared to muscle tissue. The carbohydrate level decreased during the spawning season.

Though the level of fat in the muscle tissue was higher than the carbohydrate level, liver was found to be the store house of fat. Of the two fishes, S.argus had increased level of fat in their tissues. This difference was attributed to the difference in the diet. The level of fat in the muscle and liver tissues of S.argus was low during the spawning period.

Both the fishes were rich in protein. Muscle tissue registered a higher level of protein than liver. The high protein content makes them highly nutritive food fishes.

Of the two fishes studied, S.argus, an omnivore, recorded a higher protein level than the carnivorous M.cyprinoides.

Ash content of the tissues was low in both the fishes. It was almost steady throughout the year, without much fluctuations.

Moisture content of the muscle and liver in both the fishes was inversely proportional to the fat level. This relationship was more prominent in liver tissue. Of the two fishes, S.argus had comparatively lesser content of moisture in the muscle and liver tissue, since they had higher level of fat.

The calorific values of the liver tissue of both the fishes were higher than the muscle tissue, due to its higher fat values. The muscle tissue of S.argus had a greater calorific value than the muscle tissue of M.cyprinoides. This is due to its higher fat content. This was discussed on the basis of similar studies on the proximate composition of some other fishes.

Based on the analysis of the biochemical constituents of the different tissues of the two fishes, it is evident that the high protein values of the flesh make them esteemed food fishes. The lipid rich liver also adds to their nutritive significance.

CHAPTER III

Though copper is an essential micronutrient, it is highly toxic at high concentrations. This chapter deals with the haematological changes such as total haemoglobin content, haematocrit and leucocrit values, when fishes are exposed to different concentrations of copper.

In the initial hours of exposure, an increase in Hb, Hct and Lt contents occurred in both the fishes, as a result of the stress due to the increased intake of copper into the circulatory tissues. This continued till 24 hrs. The stress-encounter activates the release of adrenocortico tropic hormone (ACTH) from the pituitary gland which stimulates the release of cortisol from the interrenal gland. Copper may alter the permeability of the gill membrane, and so the gas exchange can be interrupted. The stress stimulates the immune system including lymphocytes which elevates the Lt values.

The initial increase in the cellular components of the blood was followed by their decline in the later hours of exposure. This reduction can be due to the reduced life span of erythrocytes and haemodilution. The inhibition of erythrocyte enzymes and oxidation of erythrocyte membrane proteins enhance the destruction of erythrocytes. The alteration in plasma electrolytes causes impaired osmoregulation which leads to the influx of body fluid into the circulatory fluid. The decrease in the Hb and Hct content was different in magnitude, indicating unproportionate erythropoeises and haemosynthesis. This may either be due to the swelling of erythrocytes or inhibition of any enzyme involved in haemosynthesis. The haemodilution also caused a reduction in leucocyte numbers. The results of this study were discussed in detail.

CHAPTER IV

The variation in the specific activity of acid phosphatase and alkaline phosphatase in liver and kidney when the fishes were exposed to two conc. of copper is discussed in this chapter. Since lysosomes, cell membranes and endoplasmic reticulam are the major subcellular units to encounter xenobiotics, a variation in the activity of the enzymes bound to these cellular compounds is inevitable, while the animal is under stress. When the fishes were dosed with copper, its pathological effect was expressed as a decrease in the activity of the two main phosphomonoesterases, alkaline phosphatase and acid phosphatase, in both kidney and liver tissues. The copper ions combine with certain components of the enzyme unit causing a diminution in the enzyme activity. The decrease in the enzyme activity was dose-dependent which emphasises the influence of the heavy metal on the enzyme.

The inactivation of the lysosomal enzyme may be due to the deformed gradient across the mitochondrial membrane, or splitting of energy-rich intermediates prior to ATP fromation, or by the malfunctioning of the mitochondrial membrane.

Since alkaline phosphatase is a Zn^{++} containing enzyme it can be inactivated by the displacement of Zn^{++} by copper. Alkaline phosphatase has Mg^{2+} binding site which when occupied by Mg^{2+}, helps the enzyme to be tightly bound to the cofactor Zn^{2+}. When Mg^{++} binding site is occupied by Cu^{++}, it may reduce the binding capacity of the enzyme and Zn^{++}, and thus decrease the activity of the enzyme.

In the study, a time-dependent decrease in the enzyme activity was not registered. This confirms that the inactivation of the enzyme was not continuous or reactivation of inactivated enzyme did not occur. The alteration that took place in the enzyme structure or mitochondrial membrane, remained as such for the remaining duration without any increase or decrease in the enzyme activity. This aspect was discussed in this chapter.

CHAPTER V

In animals, glutamic pyruvic transaminase (GPT), and glutamic oxaloacetic transaminase (GOT) are two important enzymes that catalyse the process of biologial transamination. Since these two transaminases, selected for the present study, are specific enzymes of liver damage, variation in the enzyme activity in the liver tissue is an indicator of the pathological influence of pollutants on liver.

When fishes were exposed to 0.1 ppm and 0.15 ppm copper, an elevated activity of both GOT and GPT was observed in kidney and liver tissues. As liver is a rich source of GOT and GPT, the primary increase in the activity of the enzymes might have occurred in the liver tissue which in turn is due to the increased permeability of the hepatic cell membrane caused by copper. This increased release of the two transaminases was reflected in the kidney tissue, most probably via the circulatory system which may carry the additional enzymes to the kidney tubules to be eleminated.

The acceleration in the enzyme activity may also be due to the activitation of the coenyzmes PLP or PMP which are specific for the transaminases, by the copper ions.

Though the concentration of the copper was positively related to the enzyme activity, the duration of exposure did not seem to influence the activity.

CHAPTER VI

An attempt was made to study the glycogen and lactic acid levels in the muscle and liver tissues, when the fishes were exposed to two concentrations of copper. It was observed that under exposure conditions the muscle glycogen was depleted and lactic acid accumulated in the myotom. The increase in the lactic acid content in the liver was not proportionate to that of the muscle tissue. Moreover, a reduction in the liver lactic acid was recorded. This indicates that the lactic acid formed in the muscle is not transported to the hepatic tissue to be synthesized into liver glycogen.

The glycogen content of the liver tissue was observed to increase, while the lactic acid content decreased during the duration of exposure. Liver glycogen did not seem to undergo anaerobic glycolysis. Active glyconeogenesis occurred in the liver tissue resulting in a decrease in the lactic acid content and an increase in the glycogen content of the liver tissue. In fishes, chances of elimination of lactic acid to the exterior through branchial or renal excretion are negligible, and the resynthesis of muscle glycogen is a slow process. All these factors together induce the retention of lactic acid in the myotom against the concentration gradient and the consumption of liver lactic acid for glycogen synthesis to maintain the blood glucose level. This leads to the conclusion that the augmented glycogen content in the liver tissue is not due to the resynthesis of glycogen from the muscle lactate, but to the utilization of the normal content of liver lactic acid for maintaining the blood glucose level. All these aspects were discussed in this chapter.

It is hoped that the results of the present study will be helpful in formulating suitable schemes for the successful exploitation of these fishes.

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