METABOLIC ADAPTATIONS OF THE YOUNG ONES OF SEA BASS, LATES CALCARIFER (BLOCH) WITH SPECIAL REFERENCE TO ENERGY UTILIZATION

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- to my beloved parents -

DECLARATION

I hereby declare that the thesis entitled **"METABOLIC ADAPTATIONS OF THE YOUNG ONES OF SEA BASS, LATES CALCARIFER (BLOCH) WITH SPECIAL REFERENCE TO ENERGY UTILIZATION"** has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar title or recognition.

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CERTIFICATE

This is to certify that this thesis is an authentic record of the work carried out by **Mrs. S.L. SREELATHA,** under my supervision at Central Marine Fisheries Research Institute and no part thereof has been presented before for any other degree in any University.

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I. PREFACE

An understanding of biotic and abiotic factors which affect the metabolic rate, growth and survival of fishes can be applied to the practices of fishery management and this can also be used as a means of assessing the responses of the fishes to environmental conditions. In culture practices there is a possibility of reducing the energy costs to a minimum and achieving food conversion to an optimum level. This can be carried out through an understanding of environmental and biological factors which affect the metabolic rate, with a view to reduce the demands on the system thereby increasing the rate of production. The rate of metabolism is directly or indirectly influenced by biotic (activity, weight, sex, age, oxygen debt, condition, starvation and diet) and abiotic factors (temperature, salinity, oxygen, carbondioxide, ammonia, pH, photoperiod, season and pressure) and among them ambient oxygen, temperature and activity exert the greatest influence on metabolism.

Like other poikilotherms, fishes do not have a definite basal metabolic rate. But it is possible to calculate the energy consumption very accurately during the metabolic activity from the rate of oxygen consumption, independently of the size of the respiratory quotient. The metabolic rate or rate of expending energy is of major importance because it affects the speed of all metabolic processes and reflects the loss of energy of fish and consequently their food requirements.

Studies on active metabolism are of great scientific and practical interest and the active metabolic rate of fish is far more sensitive to the availability of environmental oxygen. The metabolic rates can be estimated

from the subsequent measurements of oxygen consumption, carbondioxide production and ammonia excretion. The oxygen consumption and the carbon dioxide output are necessary for estimating respiratory exchange ratio or respiratory quotient (R.Q.) which can be used to judge the anaerobic capacities of fishes. The magnitude of the R.Qs over unity under low oxygen conditions is indicative of the intensity of anaerobic metabolism. The anaerobic abilities of many fishes are not known, because most of them are not subjected to proper experimentation. Anaerobic metabolism and its link with protein utilization can be calculated from the measurements of R.Q. and ammonia quotient (A.Q.). Protein degradation is reflected in the changes in A.Q. values.

The end product of protein metabolism is taken as ammonia and its production is of use to fish under anaerobiosis. When the supply of oxygen is not sufficient to meet the minimal energy demands of essential functions, fishes suffocate in natural condition when their well oxygenated water body is confronted with oxygen deficient water. In such conditions, some fish seem to become more passive and some more active thereby establishing a dichotomy in behaviour of fishes. Thus the decrease in ambient oxygen from air saturation down to the asphyxial oxygen level can cause different behavioural responses which have a major role in the survival of the species.

Lates calcarifer (Bloch) commonly called as sea bass/bhetki/giant perch/barramundi is one of the commercially important marine food fishes which also thrives in brackish water and freshwater. It is widely distributed

in tropical and sub-tropical waters of the Indo-west Pacific (Katayama and Taki, 1984). This large centropomid species with a 'delicate-flavoured' flesh is a euryhaline, eurythermal, catadromous, highly predacious and protandrous hermaphrodite which grows to a comparatively large size. Young ones spend most of their growing period in freshwater such as rivers, lakes etc. which are connected to the sea but the adults migrate to the sea, where the salinity range is 30 to 32‰, for gonadal maturation. Subsequently they spawn according to the lunar cycle and the larvae migrate further upstream for growth. The distribution pattern of different life stages is diverse in various ecosystems such as coastal waters, estuaries, lagoons, brackish waters and even in freshwater (Ghosh, 1973).

The giant perch is a carnivorous species and judging by its commercial value, this may be cultured in brackish water impoundments by providing trash fishes and uneconomic varieties of prawns as food. However, it is very important to know the physiological peculiarities of a particular species in order to develop a scientific basis for fish culture and management. In regard to Lates calcarifer, the information available is scanty. So the present investigation was undertaken to determine the physiological peculiarities of this species, in relation to different environmental factors and to understand the manner in which they adapt themselves to different media.

Only the young ones of sea bass with total length ranging from 7 to 17 cm were used for the present study because this is a critical stage at which they will enter the estuary, and also because this is the optimum or ideal size for stocking in culture programmes. In this species, all fishes

would be immature below a total length of 46 cm (Moore, 1979) and the young ones would be more active and osmotically efficient than adults.

Though distributed throughout the coast, in India, sea bass does not contribute much to the total fish landings except in certain areas in the northeast like Chilka lake, Hooghly-Matlah estuary etc. Since the capture fishery resources are limited, the alternative is to develop culture technology for this species (Kuldeep, 1991). It is expected that the results of the present investigation will throw some light on the metabolic adaptability of the young ones of sea bass which has immense potential for application in culture operations. Because of the relatively high market value, this fish has become an attractive commodity of both large and small scale aquaculture enterprises.

The details of the present study is descriptively documented in different chapters beginning with the Preface and Introduction, moving through Material and Methods, Results and Discussion, and finally concluding in a Summary followed by a Bibliography.

II. INTRODUCTION

Bioenergetic studies are of fundamental importance to the advance of aquaculture. Most of the earlier studies were based on the measurements of oxygen consumption, on the assumption that all fishes are obligate aerobes and that a measure of oxygen consumption is always a measure of metabolic rate (Fry, 1957; Kutty, 1966). Although anaerobic habitats had been recognized for a very long time, the diverse nature of the metabolic adaptations concerned with life under these conditions had been emphasized only quite recently (Hochachka and Somero, 1973; Hochachka, 1980). Now it is clear that a spectrum of organisms exist ranging from, the stable aerobic forms with restricted capacities for oxygen debts to the facultative and obligate anaerobes. Investigations on the dissolved oxygen requirements of several fishes have shown that most of the fishes have the ability to sustain complete or partial lack of oxygen (Blazka, 1958; Peer Mohamed and Kutty, 1981).

A wealth of information has been contributed on the energy metabolism of several species such as gold fish, Carassius auratus (Fry and Hart, 1948; Beamish and Mookerjii, 1964; Smit, 1965; Kutty, 1968a,b; Smit et al., 1971; Kutty and Peer Mohamed, 1975; and Peer Mohamed and Kutty, 1980); sockeye salmon, Oncorhynchus nerka (Brett, 1962, 1964 and 1973); rainbow trout, Salmo gairdneri (Rao, 1968; Kutty, 1968a,b, and Webb, 1971); mullet Rhinomugil corsula (Peer Mohamed, 1974; Kutty and Peer Mohamed, 1975; Peer Mohamed and Kutty, 1980; Sukumaran and Kutty, 1987); cichlid, Tilapia mossambica (Karuppannan, 1972; Kutty and Peer Mohamed, 1975; Peer Mohamed and Kutty, 1980 and 1981; and Peer Mohamed, 1981b); minor carp, Puntius sarana (Peer Mohamed, 1974; Kutty and Peer Mohamed, 1975;

Peer Mohamed and Kutty, 1980); cray fish, Cherax destructor (Head and Baldwin, 1986); and pumpkin seed, Lepomis gibbosus (Brett and Sutherland, 1964). But the information available on sea bass is inadequate. Hence there is a need for documentation of such information on this eurythermal species especially in view of its importance as a cultivable warm water species.

For fish culture practices in ponds, pens and cages, sea bass is considered to be the most important species in view of its excellent quality as a table fish. This particular species was selected as the test animal for the present study in order to understand its metabolic adaptability in different environments - marine, brackish and freshwater. Though considerable information had been accumulated on different aspects of this species viz. distribution (Greenwood, 1976; Hiroshi Kohno et al., 1986; Kasim and James, 1987); biology and ecoloy (Jhingran and Natarajan, 1969; De, 1971; Patnaik and Jena, 1976; Nacario, 1985; Kungvankij and Suthemechaikal, 1986); age and growth (Ghosh, 1971; Ghosh and Roy, 1977; Saha et al., 1978; Mukhopadhyay and Karmarkar, 1982; Reynolds and Moore, 1982; Davis and Kirkwood, 1984; Kosutarak and Watanabe, 1984a); food and feeding (Patnaik and Jena, 1976; Maneewong and Ruangpanit, 1977; Mukhopadhyay and Karmarkar, 1981, 1982; Maneewongsa and Tattanon, 1982a; Chou, 1984; Kosutarak, 1984; Davis, 1985b); culture (Arsjad, 1982; Aure, 1982; Awang, 1982; Pietersz, 1983; Banno and Amar, 1984; Young, 1985; Kungvankij et al., 1986; James and Marichamy, 1987); production (Maneewong et al., 1977; Maneewong et al., 1981; Sakaras and Sukbanteang, 1984; Kungvankij and Suthenmechaikal, 1986); reproduction (Patnaik and Jena, 1976; Kowtal, 1977;

Moore, 1982; Davis, 1984, 1985a; Harvey and Nacario, 1985; and Kuldeep Kumar, 1991); larval development (Ghosh, 1973; Patnaik and Jena, 1976; Kowtal, 1977; Mukhopadhyay and Verghese, 1978; Ghosh and Pandit, 1979; Moore, 1980, 1982; Barlow, 1981; Chan, 1982; Maneewongsa and Tattanon, 1982b; Moore and Reynolds, 1982; Russell and Garrett, 1983, 1985; Kosutarak and Watanabe, 1984b; Kosutarak et al., 1984; Davis, 1985a; Hiroshi Kohno et al., 1986); availability of the seed (Mukhopadhyay and Verghese, 1978; Maneewong et al., 1984); acclimatization (Rajyalakshmi and Reddy, 1984); pigmentation (Mukhopadhyay et al., 1983); migration pattern (Moore and Reynolds, 1982); genetic variations (Khuda - Bukhsh, 1979; Shaklee and Salini, 1985); parasitic fauna (Ruangpan, 1982; Leong and Wong, 1986; Wong, 1986); diseases (Limsuwan et al., 1983; Chao, 1984; Danayadol and Buranapanidgit, 1984; Danayadol et al., 1984; Bagarino and Kungvankij, 1986); sex inversion (Moore, 1979); fecundity estimation (Davis, 1984); tag shedding (Davis and Reid, 1982) etc., the present work appears to be the first record on energy metabolism of this species.

Extensive studies were made on the dissolved oxygen requirements of fishes belonging to temperate and subtropical waters (Doudoroff and Shumway, 1970; Fry, 1971; Smit et al., 1971). However, very few attempts have been made on tropical fishes, especially the influence of ambient oxygen on their random activity (Hamsa and Kutty, 1972; Peer Mohamed, 1974 and 1981a,b; Peer Mohamed and Kutty, 1980 and 1981) despite the fact that in nature they are often subjected to hypoxic (low ambient oxygen) and anoxic (lack of oxygen) conditions at high temperatures. The influence

of low ambient oxygen on metabolism was described in goldfish and rainbow trout (Kutty, 1968 a,b); in Tilapia mossambica (Kutty et al., 1971; Kutty, 1972); in some freshwater teleosts (Peer Mohamed and Kutty, 1980) and in fry and fingerlings of Chanos chanos and Mugil cephalus (Usha Devi, 1987).

A glance through the literature reveals the difficulty and limitations in the accurate measurements of total carbondioxide. Few workers had used the gas extraction and measurement technique (Kutty, 1968a; Kutty et al., 1971) but of late some physiologists have followed the distillation and titration technique with the help of Maros-schulek's apparatus (Maros et al., 1961; Kutty et al., 1971; Peer Mohamed, 1974 and 1982; Peer Mohamed and Kutty, 1983). For the present study, a direct titrimetric method was employed as described by Stroganov in 1962 (Kutty, 1970, 1971; Usha Devi, 1987) and decarbonated water was used as the experimental medium as described by Kutty (1968a) and Peer Mohamed (1974).

Knowledge of intermediary metabolism in fishes is fragmentary (Gumbmann et al., 1958; Drummond and Black, 1960; Ekberg, 1962). Anaerobic metabolism and its relation to protein utilization were studied in detail from the simultaneous measurements of respiratory quotient and ammonia quotient in different species by Kutty (1972), Karuppannan (1972), and Peer Mohamed (1974). Detailed investigations have been carried out on the respiratory quotients of fishes (Provencal and Humboldt, 1809; Grehant, 1870; Jolyet and Regnard, 1877 a,b; Knauthe, 1898; Zuntz, 1901; Bounhiol, 1905; Gardner and Leetham, 1914; Gardner et al., 1922; Gardner and King, 1923;

Bosworth et al., 1936) but the influence of ambient oxygen on respiratory quotient and ammonia quotient was reported only by Kutty (1968a and 1972); Karuppannan (1972); Kutty et al. (1971); Kutty and Peer Mohamed (1975); Peer Mohamed and Kutty (1981). Kutty (1968a,b) studied the changes of respiratory quotient in relation to activity and duration of exercise in gold fish and rainbow trout. Under high ambient oxygen concentrations, respiratory quotient near unity is usually maintained by the fish indicating that it was utilizing energy aerobically. The ammonia produced may be of advantage to fish under anaerobic condition (Prosser and Brown, 1961; Garcia Romeu and Motais, 1966; Kutty, 1972; Karuppannan, 1972; and Peer Mohamed, 1987) in acid-base regulation and iono-osmotic regulation. From the measurement of ammonia excreted by the fish, the involvement of protein in metabolism was found out.

The water temperature has a determinant influence on the oxygen demand of fish. Because of the scarcity of information on fish energetics mainly under tropical conditions, high ambient temperatures of 30 and 35°C were considered.

The activity of the fish also has a tremendous influence on metabolism. Since the fishes are neither continuously resting nor persistently active, it is necessary to determine the metabolic cost for intermediate levels of some well defined activity (Brett, 1962). Behavioural changes, especially those indicated by random (spontaneous) activity of fish exposed to different ambient oxygen concentrations are important in studying energy utilization and survival of the species (Doudoroff and Shumway, 1970; Peer Mohamed, 1987).

Generally it is noted that although there are studies on respiratory metabolism, locomotory metabolism, certain aspects of substrate utilisation and end product accumulation and disposal, no detailed study has been carried out on the total metabolism of fish with special reference to energy utilization in relation to the activity of the fish except the contributions of Kutty and Peer Mohamed (1975) and Sukumaran and Kutty (1987). The main objective of this attempt is to obtain a comprehensive picture of energy utilization of Lates calcarifer from the measurements of oxygen consumption, carbondioxide output and ammonia excretion by exposing the fish to different levels of oxygen (from air saturation down to asphyxial level), varying temperatures (30 and 35°C) and salinities (30‰ , 15‰ and 0‰).

III. MATERIAL AND METHODS

a. **Experimental fish**

The sea bass, Lates calcarifer (Bloch) was chosen as the test fish and, the young ones were subjected to the present study. The fish were collected from brackish waters in and around Cochin - mainly from Puduvyppu an island about 45 kilometres north of Kochi by road. The young fish were captured during the periods extending from May to August and from November to December.

221 fishes ranging in total length from 7.0 to 17.0 cm and weighing from 5.00 to 80.7 g were used. Details of the fish used for all the experiments are given in Table 26.

b. **Maintenance of fish-stock**

Fish were initially maintained in 300 l capacity rectangular fibreglass stock tanks in the laboratory for 10-15 days. Fish were reared in the same salinity from which they were collected. The stock tanks were aerated continuously by compressed air through air diffusers to maintain the dissolved oxygen concentration near air saturation. The salinity of the water in the stock tanks were checked periodically and the water was changed twice a week.

Fish were acclimatized to sea water (30‰), brackish water (15‰) and freshwater (0‰) by 'Drip method' (Plate 1). Five to ten young fish were kept in a rectangular perspex holding tank (20 l capacity) having an outlet (3 mm) at the lower side. Polythene tube was fixed to the outlet and arranged in such a way (inverted 'U' shape) that whenever the water

level in the tank increased, the water above the tube bend flushed out through the tube. Desired experimental medium (sea water/brackish water/ freshwater) was kept in another 70 l capacity perspex rectangular tank which was nearly 2 feet above the holding tank. The water from the upper tank dripped to the holding tank through a rubber tube. The water drip was regulated by an adjustable pinch-cock. Compressed air was bubbled through air-diffusers in both the tanks to maintain the oxygen concentration near air saturation and to mix the experimental medium in the holding tank. When the water dripped into the holding tank it mixed vigorously and the excess water was flushed out as explained earlier. This method was found suitable because of the gradual increase/decrease in salinity; it could be easily adapted to by the fish since the change in salinity was within the 'zone of tolerance' (Perry et al., 1984).

c. Acclimation

Healthy fish of similar size were transferred into the acclimation tank (Plate 2 and 3) of 70 l capacity containing sea water/brackish water freshwater. Five to ten fish were kept in each tank for about a week prior to the experiment. The acclimation tanks were provided with continuous aeration through air diffusers in order to maintain the dissolved oxygen concentration near air saturation. Since the toxicity of excreted ammonia is well known (Black, 1957; Kawamoto, 1961; Burrows, 1964), a biological filtering unit (Saeki, 1958; Kawai et al., 1965; Hirayama, 1974; Bower and

Bidwell, 1978; Kutty et al., 1976; Karuppannan, 1972; Peer Mohamed, 1974; and Usha Devi, 1987) used here, removed the suspended dirt and ammonia excreted by the fish.

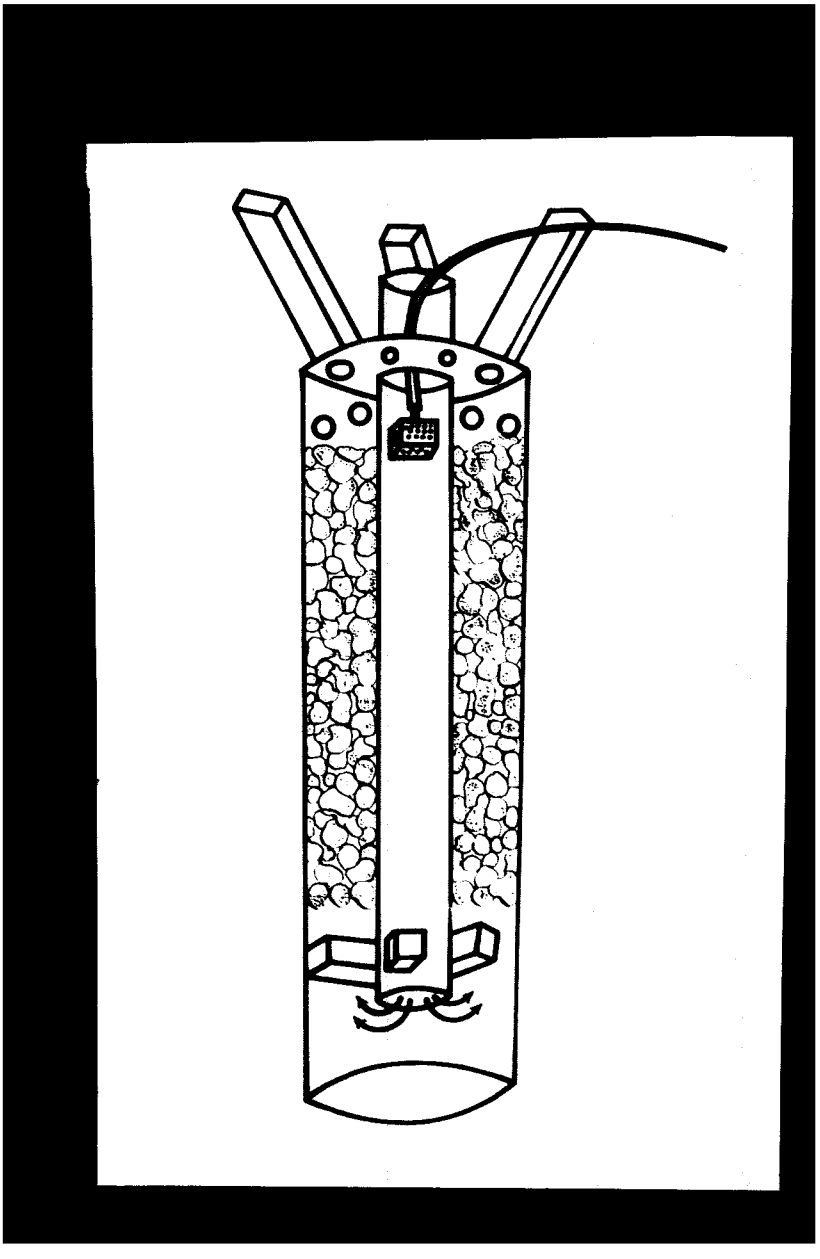
The temperature of the acclimation tank was controlled by thermostatic relays (Jumo thermometer relays). Temperature change in the acclimation tank was effected by a daily increase of 1°C, till the desired temperature was reached (Fry, 1957; Beamish, 1964; Mukhopadhyay and Karmarkar, 1981). All the experiments were done at the temperatures in which the fish were acclimated. The acclimation tank was partitioned by using an adjustable framed nylon netting so that fish to be experimented remained separated from others (Plate 2 and 3).

d. **Filtering unit**

The filtering unit (Plate 4) used in the acclimation tank was made up of two cylindrical perspex tubes of same length but of different diameters. The narrow inner tube (3.0 cm in diameter and 30 cm in length) was fixed inside the wider tube (9.0 cm in diameter and 30 cm in length) by three small perspex stuts so as to keep the inner tube exactly in the middle but 3.0 cm lower than that of the upper end of the outer tube. The inner tube was open on both the sides. The lower end of the annulus so formed between the inner and the outer tube was covered by a porous perspex sheet. Small holes (3 mm) were also made at the sides of the

PLATE 4

Diagrammatic representation of the biological filtering unit. Water in the inner tube got lifted by air bubbles and spilled out into the outer tube in which the water was filtered through the gravel bed. The filtered water came out through the holes present at the base of the unit.



lower end of the outer tube for easy filtration. The whole unit stood on three perspex legs. The space between the inner and outer tubes was partly (2/3) filled with gravel. This unit was kept in the middle of the acclimation tank and the water level was maintained at a level 1-2 cm below the upper end of the inner tube. An air diffuser was kept at the base of the inner tube, through which compressed air was passed. Water in the inner tube was lifted by air bubbles and spilled out into the outer tube in which water was filtered through the gravel bed. The filtered water was passed through the holes present at the base of the unit. In due course (days) the gravel bed developed ammonia fixing bacteria which could remove the ammonia present in the water of the acclimation tank (Peer Mohamed, 1974; Kutty et al., 1976; Usha Devi, 1987).

e. Feeding

Fish in the stock and acclimation tanks were fed ad libitum once daily with prawn (live and dead) and other fishes like mullets and tilapia. A preference for live prawn was noticed. This species is a highly predatory column feeder which can turn cannibalistic when food is scarce, hence the feeding schedule was carefully followed.

f. Experimental media and recirculation

For conducting the experiments three different media - sea water (30‰), brackish water (15‰) and freshwater (0‰) were used. Filtered sea

water of 30‰; sea water diluted to 15‰ by mixing with freshwater; and dechlorinated tap water, were used as the three experimental media.

The determination of total carbon dioxide produced by the fish in natural waters is very difficult as the amount of excreted carbondioxide added to the waters is comparatively very little (Kutty, 1968a). In order to get an accurate estimate of total carbondioxide, decarbonated experimental medium (sea water, brackish water and freshwater) of controlled pH (Bosworth et al., 1936; Kutty, 1968a; Peer Mohamed, 1974; Peer Mohamed and Kutty, 1980, 1981) was prepared as explained by Peer Mohamed (1974) and Peer Mohamed and Kutty (1983).

Owing to the limited amount of decarbonated experimental medium, the experimental system was so designed for circulation of the same water throughout the experiment (Plate 5). A 300 l capacity rectangular perspex tank provided with a thermostatic relay for temperature control and aeration, was used as overhead reservoir tank (10 feet high from ground level). The water from the overhead reservoir flushed through the respirometer to a 50 l capacity cylindrical fibre glass reservoir tank, which was kept at the ground level. Water from this reservoir was pumped up to the overhead reservoir and the pumping was regulated by a constant level device (Aqua-guard, Bombay) provided in the overhead reservoir. Compressed air was bubbled in both the reservoirs to maintain oxygen concentration of the water near air saturation.

g. **Respirometer**

The apparatus used for the present study (Plate 6 and 7) was a modification of the annular Fry's respirometer (Kutty et al., 1971) used by Peer

respirometer could be watched through the viewing windows. A red bulb was fitted inside, at the centre of the lid, which was kept "on" throughout the experiment to monitor the activity of the fish.

h. **Preparation of the fish for the experiment**

Prior to the experiments the fish were kept in the acclimation tank for about 7-10 days at the required temperature in the experimental medium (sea water/brackish water/freshwater). The fish to be experimented were kept within the partition (explained under 'Acclimation') and deprived of food for 24 hours (Brett, 1964; Beamish, 1964; Kutty, 1966; Peer Mohamed, 1974; Kutty and Peer Mohamed, 1975; Peer Mohamed and Kutty, 1980, 1982; Peer Mohamed et al., 1978; Sukumaran and Kutty, 1987) before the experiment, in order to maintain the physiological conditions of the fish almost uniform and to avoid contamination of the experimental medium by excretory wastes. Then the fish was weighed, the total length was measured and left in the respirometer overnight with continuous flushing and recirculation of the experimental medium to avoid handling effect (Peer Mohamed, 1981a).

i. **Experimental procedure**

Three series of experiments were carried out to investigate the following.

(i) Influence of random activity on the metabolic rates (oxygen consumption, carbondioxide output and ammonia excretion) and quotients (R.Q. and A.Q.) at high ambient oxygen (near air saturation).

(ii) Influence of hypoxia on metabolic rates, quotients and random activity.

(iii) Metabolic rates, quotients and random activity during hypoxia and recovery.

The experimental set-up (Plate 5) for the three series of experiments is same (already described under 'Respirometer'). The experimental procedure for these 3 series are similar in most respects, except during the later phase of the experiments.

(i) **Influence of random activity on metabolic rates and quotients at high ambient oxygen**

Each experiment consisted of 5-7 runs of 1 hour duration, when the respirometer remained closed.

At the start of the experiment, initial samples were collected and the circulation of decarbonated water through the respirometer was cut off. After an interval of one hour, final samples were collected. In each sampling time (initial and final of each run), three separate water samples were collected for analyses of dissolved oxygen, total carbondioxide and ammonia. The size of each sample was 30 ml for dissolved oxygen, 100 ml for carbondioxide and 60 ml for ammonia (25 ml of water collected first for rinsing the sample bottles was discarded). During sampling, care was taken to compensate the volume of sampling water by allowing water to flow into the respirometer. During the closure period, the random activity of the fish was recorded by visual counts (Peer Mohamed et al., 1978; Hamsa and Kutty, 1972; and Peer Mohamed and Gupta, 1983).

After collecting the final samples of the first run, the circulation of water through the respirometer was opened for 30 minutes to flush the

respired water from the respirometer as well as to bring the high ambient oxygen near air saturation. Initial samples for the second run were collected and followed by closure period of one hour, before the collection of the final samples. The random activity of the fish was also recorded during the closure period. Likewise the consecutive runs were made.

The concentration of oxygen, total carbondioxide and ammonia were determined in the samples collected (initial and final samples of each run). The activity of the fish observed by visual counts during the closure period was also multiplied to record the value in counts per distance travelled in one hour.

(ii) **Influence of hypoxia on metabolic rates, quotients and random activity**

The individual run of an experiment lasted for one hour in duration as was followed in series (i) except the last run in which the fish was allowed to reduce the ambient oxygen until it was asphyxiated.

The procedure followed was similar to that of the one described in series (i) till the completion of the first run. After collecting the final samples of the first run, the respirometer was not opened to the circulating water for complete flushing. But approximately 215 ml of decarbonated experimental medium was circulated once or twice through the respirometer with the help of a separating funnel for mixing it with the 'respired' water remaining unflushed in the respirometer. Then the initial samples of the next run (as described under series (i)) were collected. The overall time taken for sampling and adding water was about 3 minutes. To avoid using any correction factor for the initial oxygen content of second and consecutive

runs, this procedure (Peer' Mohamed, 1974; Kutty and Peer Mohamed, 1975; Peer Mohamed and Kutty, 1980, 1981) was followed by taking initial samples. During the last run, the final samples were collected only after the fish reached the asphyxial level, as indicated by the beginning of equilibrium loss of the fish. Then the respirometer was flushed by circulating the air saturated decarbonated water to revive the fish.

The collected samples were analysed and random activity was calculated as explained under series (i).

(iii) **Metabolic rates, quotients and random activity during hypoxia and recovery**

Until the fish was asphyxiated the experiment was similar to that of series (ii). Asphyxial oxygen concentration is the low lethal level of oxygen, below which fish cannot survive (Peer Mohamed and Kutty, 1981). Subsequent to the loss of equilibrium of the fish, they were revived by flushing the respirometer with air saturated, decarbonated water and the metabolic rates were determined for the next three to four hours as in series (i). During this recovery period the respirometer was completely flushed for 30 minutes between runs.

j) **Methods of water analysis**

(i) **Dissolved oxygen**

Oxygen was estimated by the modified Winkler's method as described by Strickland and Parsons (1968). For each determination 30 ml of sample was used.

(ii) **Total carbondioxide**

Fifty ml of the samle (in duplicate) was analysed by the method described by Stroganov (1962) and followed by Kutty (1970) and Usha Devi (1987). By following this method - direct titrimetric method to measure the free carbondioxide in the water; and addition of the conversion factor calculable from simultaneous measurement of alkalinity - the total carbon dioxide was estimated. The present method followed is quite adequate for measurements of carbondioxide production in fish, as proved by Kutty et al. (1971).

(iii) **Ammonia**

Immediately after collecting the samples, ammonia was determined by the phenol - hypochlorite spectrophotometric method (Solarzano, 1969). Methanol was used in place of ethyl alcohol to avoid high optical density. The reagents were added subsequently to 60 ml of sample and kept at room temperature for one hour. When a stable colour developed, optical density was measured in a Spectrophotometer (Electronic Corporation of India, GS 865 D) at 640 μ.

IV. RESULTS

The experimental animals were young ones of Lates calcarifer acclimated to and tested at two different temperatures - 30 and 35°C - and three different salinities - 30‰, 15‰ and 0‰.

The following experiments were carried out in each media at 30 and 35°C.

i) The influence of random activity on metabolic rates and quotients at high ambient oxygen (near air saturation).

ii) The influence of ambient oxygen (from air saturation down to asphyxial level) on metabolic rates, quotients and random activity.

iii) Metabolic rates, quotients and random activity during hypoxia and recovery.

a) **EXPERIMENTS IN SEA WATER**

(i) **Influence of random activity on metabolic rates and quotients at high ambient oxygen at 30 and 35°C**

Regression lines have been fitted (Fig.1 and 2) for pooled values to show the trends of routine metabolic rates - oxygen consumption, carbon dioxide production, ammonia excretion; and the two quotients - respiratory quotient and ammonia quotient - against random activity of all fishes acclimated to and tested at 30 and 35°C based on the regression equations (Fig. 1 and 2) at ambient oxygen concentration near air saturation. Mean values of routine metabolism and random activity at 30 and 35°C and standard metabolic rates and quotients (extrapolated values at 0 activity) are also presented in Table 1. At 30°C the regression coefficient (r value) for all parameters did not show any significant difference but at 35°C ammonia

Table 1. Mean routine and standard metabolic rates, quotients and random activity of sea bass acclimated to and tested at 30 and 35°C in sea water, brackish water and freshwater. Standard values are estimates obtained by extrapolation of the regression lines to zero activity through the plots shown in Figures 1-6.

Parameters	SEA WATER			
	30°C		35°C	
	Routine (Mean ± SD)	Standard	Routine (Mean ± SD)	Standard
O_2	84.55 ± 46.51	47.86	90.02 ± 48.71	120.23
CO_2	156.22 ± 103.49	288.40	158.86 ± 92.40	151.36
NH_3	15.58 ± 17.72	12.59	7.20 ± 10.62	79.43
R.Q.	2.26 ± 1.64	4.57	1.92 ± 1.15	0
A.Q.	0.31 ± 0.42	- 3.16	0.10 ± 0.16	- 1.58
Activity	35.98 ± 22.58	-	39.93 ± 22.71	-

Parameters	BRACKISH WATER			
	30°C		35°C	
	Routine (Mean ± SD)	Standard	Routine (Mean ± SD)	Standard
O_2	105.49 ± 71.98	69.18	111.70 ± 69.27	91.20
CO_2	195.84 ± 139.56	125.89	243.48 ± 143.07	181.97
NH_3	9.76 ± 14.32	3.16	15.24 ± 26.94	4.57
R.Q.	1.96 ± 1.21	2.04	2.21 ± 1.28	1.70
A.Q.	0.12 ± 0.16	- 1.40	0.25 ± 0.47	- 3.16
Activity	24.19 ± 20.71	-	24.27 ± 19.05	-

Parameters	FRESHWATER			
	30°C		35°C	
	Routine (Mean ± SD)	Standard	Routine (Mean ± SD)	Standard
O_2	74.84 ± 41.15	53.70	130.05 ± 28.24	107.15
CO_2	122.84 ± 54.82	45.71	261.36 ± 125.23	208.93
NH_3	8.31 ± 5.59	5.25	20.61 ± 12.02	30.20
R.Q	1.93 ± 0.99	1.66	1.96 ± 0.75	1.82
A.Q.	0.16 ± 0.17	- 2.14	0.17 ± 0.12	- 2.75
Activity	23.17 ± 13.93	-	53.00 ± 25.06	-

FIGURE 1

Regression lines fitted for oxygen consumption, carbon dioxide production, ammonia excretion, R.Q. and A.Q. in relation to random activity of Lates calcarifer acclimated to and tested in air saturated sea water at 30°C. Data obtained are mean values observed for 22 fishes. The lines showing the trends, are according to the regression equations given for the pooled data for all the fishes, in each case. The details of fishes used (Fish No.L_1 to L_{22}) are given in Table 26. Correlation coefficient or 'r' values are also given.

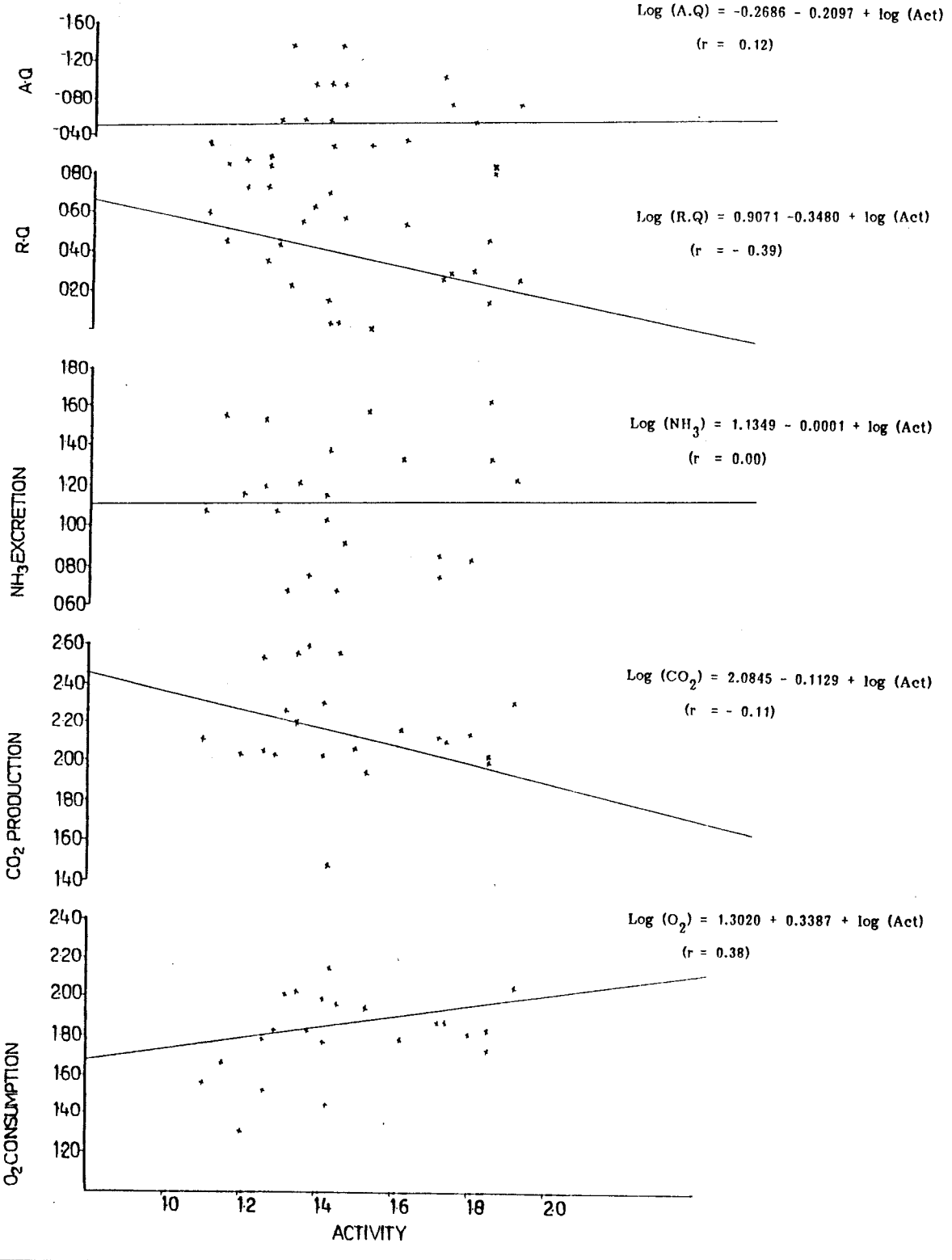
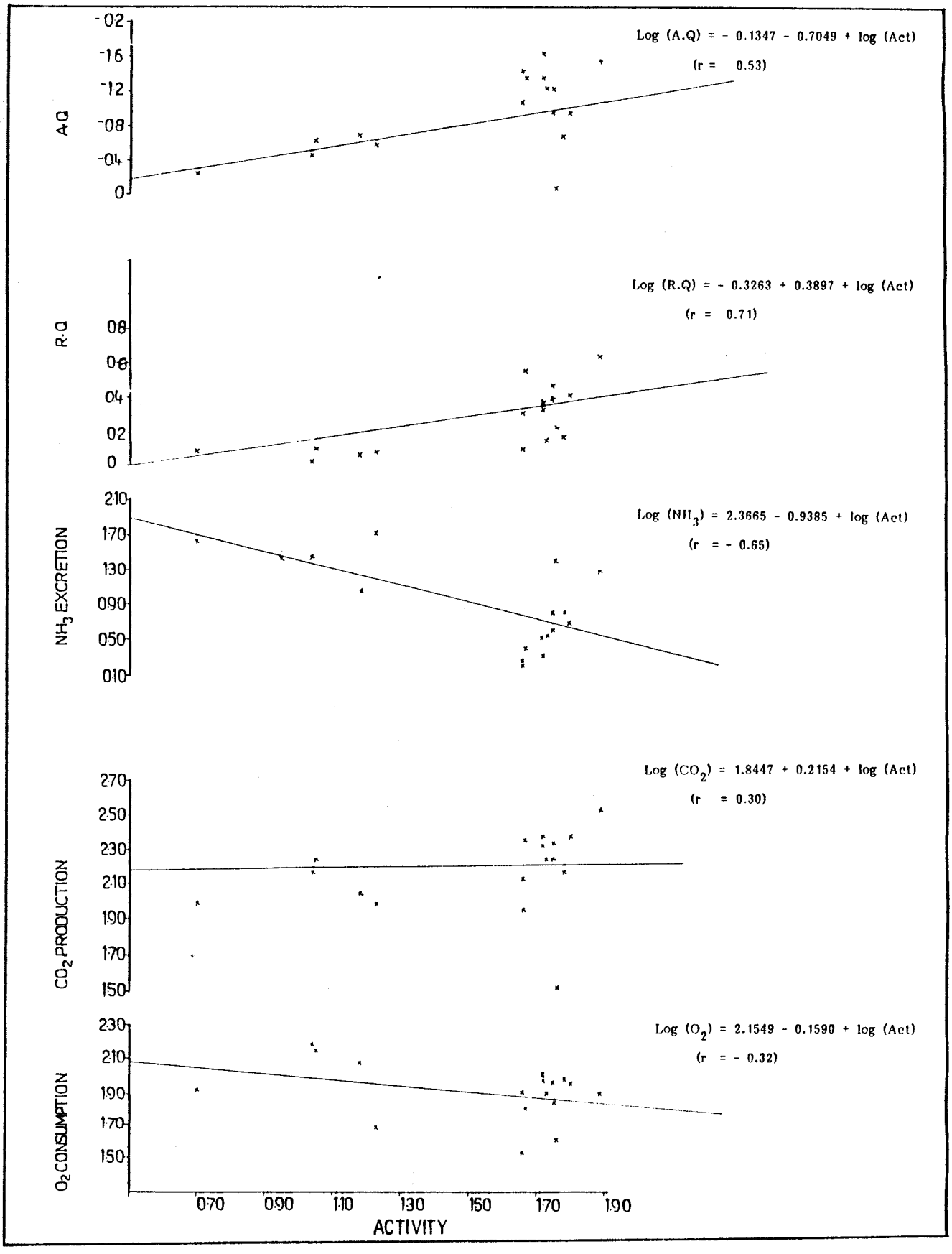

Log (A.Q) = -0.2686 - 0.2097 + log (Act)
(r = 0.12)
A·Q
160
120
080
040
Log (R.Q) = 0.9071 -0.3480 + log (Act)
(r = - 0.39)
R·Q
080
060
040
020
Log (NH_3) = 1.1349 - 0.0001 + log (Act)
(r = 0.00)
NH_3EXCRETION
180
160
140
120
100
080
060
Log (CO_2) = 2.0845 - 0.1129 + log (Act)
(r = - 0.11)
CO_2 PRODUCTION
260
240
220
200
180
160
140
Log (O_2) = 1.3020 + 0.3387 + log (Act)
(r = 0.38)
O_2CONSUMPTION
240
220
200
180
160
140
120
10
12
14
16
18
20
ACTIVITY

FIGURE 2

Regression lines fitted for metabolic rates and quotients in relation to random activity of sea bass acclimated to and tested in air saturated sea water at 35°C. The lines drawn, to show the trends, are based on the regression equations presented in the figure itself. The data obtained are mean values obtained for 17 fishes and the details of fishes used (Fish No.L_{45} to L_{61}) are given in Table 26. Correlation coefficient of each parameter is also presented.


A.Q
·02
·16
·12
·08
·04
0
Log (A.Q) = - 0.1347 - 0.7049 + log (Act)
(r = 0.53)
R.Q
08
06
04
02
0
Log (R.Q) = - 0.3263 + 0.3897 + log (Act)
(r = 0.71)
NH3 EXCRETION
210
170
130
090
050
010
Log (NH3) = 2.3665 - 0.9385 + log (Act)
(r = - 0.65)
CO2 PRODUCTION
270
250
230
210
190
170
150
Log (CO2) = 1.8447 + 0.2154 + log (Act)
(r = 0.30)
O2 CONSUMPTION
230
210
190
170
150
Log (O2) = 2.1549 - 0.1590 + log (Act)
(r = - 0.32)
070
090
110
130
150
170
190
ACTIVITY

excretion and R.Q. showed significant difference at 1% level and A.Q. at 5% level.

The high and low rates of ambient oxygen in sea water observed were found to be 7.10 ml/l and 1.67 ml/l at 30°C and 4.67 ml/l and 2.57 ml/l at 35°C. The respective high and low values for oxygen consumption, carbondioxide production, ammonia excretion, R.Q., A.Q. and random activity at 30°C and 35°C are also presented in Table 2.

(ii) **Influence of ambient oxygen (from air saturation down to asphyxial level) on metabolic rates, quotients and random activity at 30 and 35°C**

Oxygen consumption, carbondioxide production, ammonia excretion, their corresponding quotients (R.Q. and A.Q.) and random activity of Lates calcarifer (acclimation and test at 30 and 35°C) subjected to a hypoxic phase until the fish was asphyxiated are graphically shown in figures 7 and 8 based on the mean values presented in the Tables 3 and 4.

Metabolism

The change in metabolic rates refers to the corresponding changes in oxygen consumption, carbondioxide production and ammonia excretion. These changes at both temperatures resemble one another. Oxygen consumption was found to increase from 42.57 mg/kg/hr to 91.12 mg/kg/hr and further decline to 66.60 mg/kg/hr at 30°C. When the temperature was increased, there was a corresponding increase in oxygen consumption first from 46.34 mg/kg/hr to 93.20 mg/kg/hr and again to 118.65 mg/kg/hr with a drop in between to 72.93 mg/kg/hr. Carbondioxide production and ammonia

Table 2. Measurements of oxygen consumption, carbon dioxide production, ammonia excretion, respiratory quotient (R.Q), ammonia quotient (A.Q.) and random activity in sea bass at 'high' and 'low' ambient oxygen at 30 and 35°C in sea water. The 'high' oxygen refers to mean ambient oxygen concentration near air saturation and 'low' oxygen refers to lowest mean ambient oxygen concentration tested. Each value is the mean of 15 determinations. S.D is also given.

	30°C		35°C	
	High oxygen	Low oxygen	High oxygen	Low oxygen
Mean of mean ambient oxygen (ml/l)	7.10(15) ± 2.09	1.67(15)± 0.54	4.67(15) ± 0.71	2.57(15) ± 0.61
Mean rate of oxygen consumption (mg/kg/hr)	58.73(15) ± 39.84	72.69(15) ± 41.94	111.61(15) ± 43.82	92.15(15) ± 46.52
Mean rate of carbon dioxide production (mg/kg/hr)	218.19(15) ± 91.14	240.29(15) ±139.47	106.35(15)± 47.32	229.00(15) ± 112.46
Mean rate of ammonia excretion (mg/kg/hr)	436.59(15) ± 313.01	405.50(15) ±315.61	622.65(15) ± 865.81	319.15(15) ± 340.64
Mean respiratory quotient (R.Q.)	6.67(15) ± 5.94	3.96(15) ± 2.26	1.01(15) ± 0.51	2.92(15) ± 1.42
Mean ammonia quotient (A.Q.)	12.59(15) ± 13.95	6.78(15) ± 5.37	6.41(15) ± 9.17	4.55(15) ± 2.68
Mean random activity (c/hr)	27.6(15) ± 20.47	28(15) ± 21.27	9.14(15) ± 7.43	16.57(15) ± 12.40

Table 3. Oxygen consumption, carbon dioxide production, ammonia excretion, R.Q., A.Q. and random activity in relation to ambient oxygen below air saturation in sea bass acclimated to and tested at 30°C in sea water. Each value given is the average of groups of data pertaining to 10 determinations (except the last group, 22 determinations) after processing a total of 139 determinations in ascending order of mean ambient oxygen to facilitate the analysis. Mean ambient oxygen is the average of initial and final oxygen concentrations of an experimental run which was determined from samples of water withdrawn from the respirometer at the beginning and the end of the run. Each run in an experiment lasted for 60 minutes. S.D. is also given.

Mean ambient oxygen (ml/l)	Mean rate of oxygen consumption (mg/kg/hr)	Mean of carbon dioxide production (mg/kg/hr)	Mean rate of ammonia excretion (mg/kg/hr)	Mean respiratory quotient (R.Q)	Mean ammonia quotient (A.Q)	Mean random activity (counts/hour)
1.22 ± 0.18	42.57 ± 23.29	110.38 ± 50.39	9.03 ± 7.27	3.37 ± 2.02	0.33 ± 0.42	32.69 ± 17.77
1.72 ± 0.11	71.06 ± 35.04	149.11 ± 115.28	19.31 ± 31.08	2.04 ± 0.99	0.39 ± 0.60	50.25 ± 26.57
2.01 ± 0.09	67.88 ± 30.36	166.41 ± 137.80	16.96 ± 19.34	2.23 ± 1.67	0.31 ± 0.42	42.08 ± 24.42
2.28 ± 0.11	91.12 ± 30.45	247.26 ± 122.75	25.13 ± 16.47	3.09 ± 2.08	0.31 ± 0.23	44.00 ± 29.16
2.63 ± 0.09	83.43 ± 51.05	93.72 ± 71.82	11.65 ± 10.28	1.43 ± 1.07	0.17 ± 0.16	29.19 ± 14.35
3.03 ± 0.14	88.65 ± 46.81	118.98 ± 80.47	11.40 ± 12.18	1.46 ± 0.95	0.29 ± 0.46	35.50 ± 24.67
3.56 ± 0.13	135.39 ± 53.41	191.53 ± 138.88	10.29 ± 8.37	1.85 ± 1.71	0.11 ± 0.14	30.92 ± 14.48
3.87 ± 0.08	95.10 ± 47.07	144.88 ± 98.22	11.91 ± 15.18	1.90 ± 1.44	0.17 ± 0.20	29.39 ± 15.24
4.31 ± 0.22	86.89 ± 48.54	195.36 ± 111.11	18.96 ± 19.23	2.60 ± 1.28	0.39 ± 0.45	22.62 ± 17.82
6.38 ± 2.02	66.60 ± 36.40	164.81 ± 97.44	13.51 ± 9.84	2.91 ± 1.67	0.38 ± 0.45	28.46 ± 18.37

Table 4. Metabolic rates, quotients and random activity in relation to low ambient oxygen below air saturation in sea bass acclimated to and tested at 35°C in sea water. Each value is the mean of groups of data pertaining to 11 determinations (except the last group, 18 determinations) after arranging the total of 117 determinations in the ascending order of mean ambient oxygen to facilitate the analysis. Each run lasted for 60 minutes. S.D. is also given.

Mean ambient oxygen(ml/l)	Mean rate of oxygen consumption(mg/kg/hr)	Mean of carbon dioxide production(mg/kg/hr)	Mean rate of ammonia excretion. (mg/kg/hr)	Mean respiratory quotient (R.Q.)	Mean ammonia quotient (A.Q.)	Mean random activity. (counts/hour)
1.14 ± 0.18	46.34 ± 22.01	118.30 ± 114.59	3.88 ± 5.65	2.15 ± 1.81	0.07 ± 0.10	43.09 ± 14.95
1.69 ± 0.14	72.61 ± 19.26	209.31 ± 104.40	5.95 ± 6.60	2.78 ± 1.05	0.07 ± 0.07	48.18 ± 16.60
1.93 ± 0.06	91.69 ± 33.02	227.02 ± 126.00	8.50 ± 6.65	2.75 ± 1.23	0.10 ± 0.07	37.90 ± 22.60
2.10 ± 0.05	93.20 ± 40.03	235.94 ± 105.39	11.26 ± 17.63	2.54 ± 0.77	0.11 ± 0.15	45.18 ± 22.32
2.30 ± 0.08	86.31 ± 39.68	169.31 ± 83.72	8.03 ± 7.90	2.32 ± 2.07	0.14 ± 0.21	50.73 ± 23.75
2.52 ± 0.07	77.40 ± 37.34	160.42 ± 69.09	4.03 ± 3.24	2.60 ± 2.32	0.06 ± 0.20	32.73 ± 19.46
2.80 ± 0.14	72.93 ± 28.03	219.74 ± 51.74	3.81 ± 2.50	3.38 ± 1.35	0.06 ± 0.07	42.00 ± 16.13
3.36 ± 0.17	103.28 ± 46.01	161.60 ± 109.01	5.78 ± 8.04	1.62 ± 1.15	0.07 ± 0.10	24.00 ± 24.48
3.80 ± 0.10	135.56 ± 79.60	156.38 ± 83.26	11.79 ± 11.54	1.38 ± 0.90	0.18 ± 0.26	9.00 ± 2.71
4.56 ± 0.69	118.65 ± 37.82	119.88 ± 68.81	19.86 ± 22.79	1.08 ± 0.57	0.21 ± 0.25	8.41 ± 6.77

FIGURE 7

Trends pertaining to oxygen consumption, carbon dioxide production, ammonia excretion, R.Q., A.Q. and random activity in relation to ambient oxygen below air saturation in sea bass acclimated to and tested at 30°C in sea water based on Table 3. Ambient oxygen in the respirometer was reduced by the respiration of the fish itself. The experiment was terminated when the test fish lost its equilibrium. The number of fishes used are 15 and their details are given in Table 26 (Fish No.L_{30} to L_{44}).

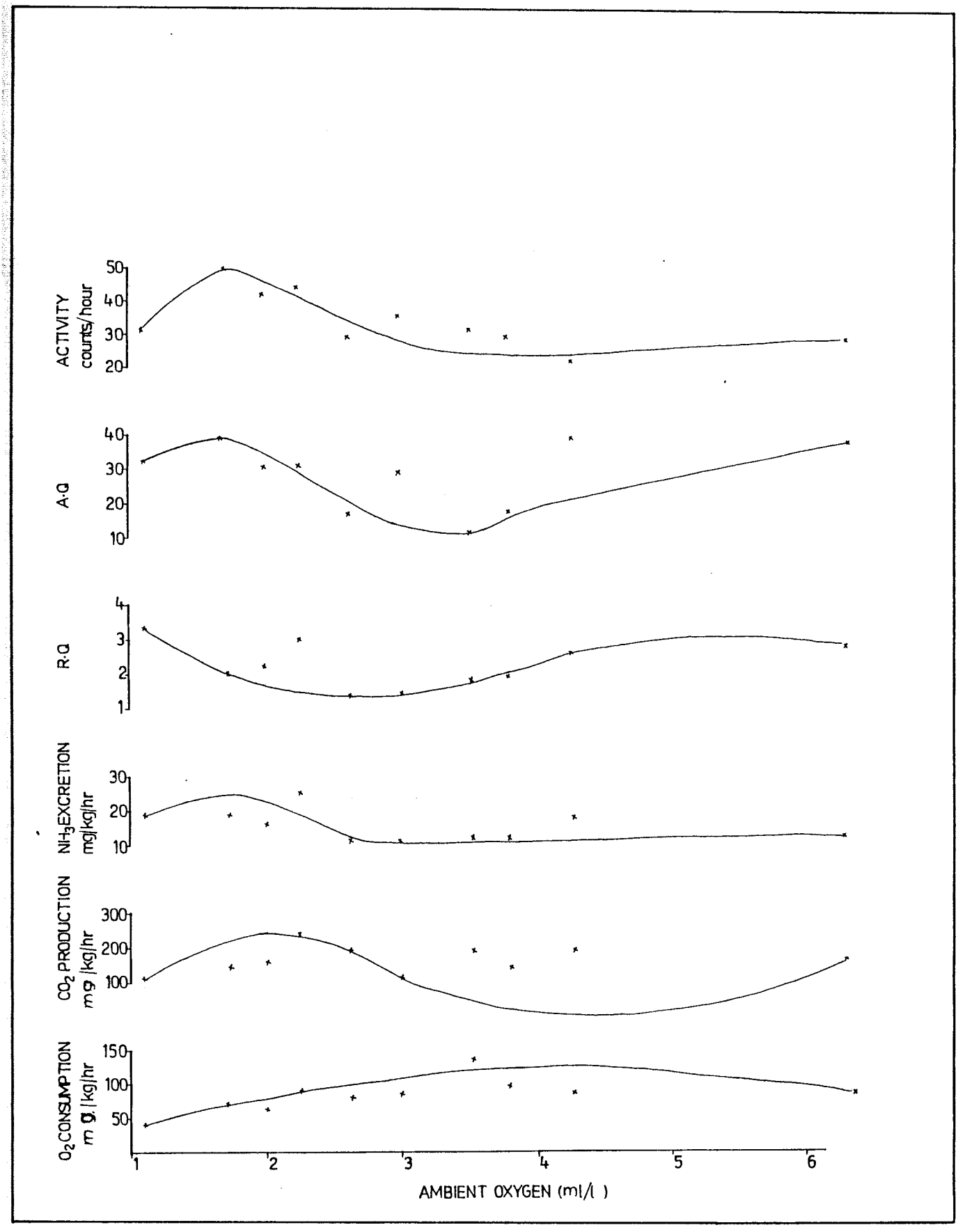
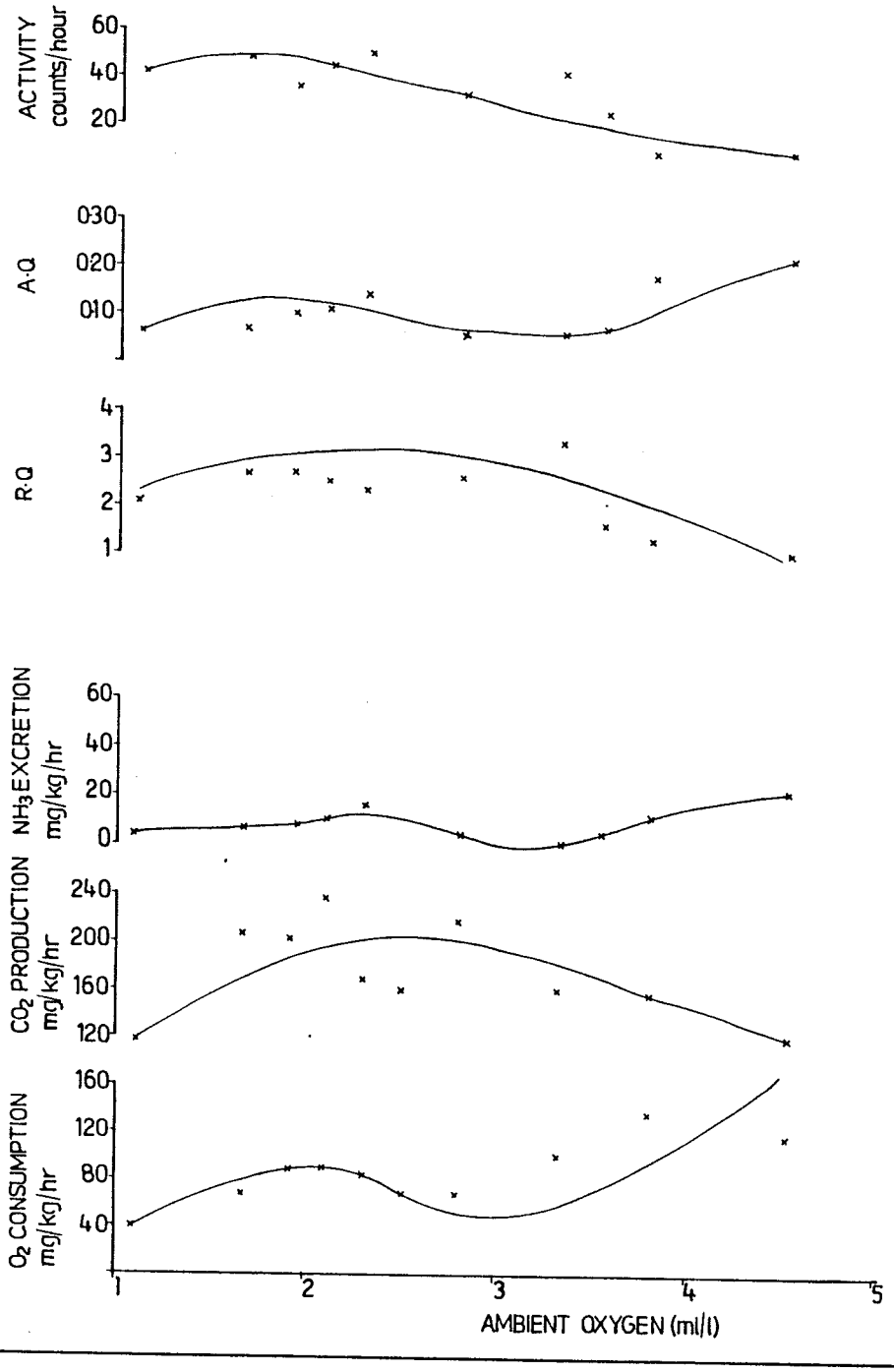

ACTIVITY
counts/hour
50
40
30
20
A·Q
40
30
20
10
R·Q
4
3
2
1
NH3EXCRETION
mg/kg/hr
30
20
10
CO2 PRODUCTION
mg /kg/hr
300
200
100
O2CONSUMPTION
m g. /kg/hr
150
100
50
1
2
3
4
5
6
AMBIENT OXYGEN (ml/l)

FIGURE 8

Trends pertaining to metabolic rates, quotients and random activity in relation to ambient oxygen below air saturation, in sea bass acclimated to and tested at 35°C in sea water based on the Table 4. The details of fishes used are (15 fishes - L_{62} to L_{76}) given in Table 26.


ACTIVITY counts/hour
60
40
20
A·Q
030
020
010
R·Q
4
3
2
1
NH3 EXCRETION mg/kg/hr
60
40
20
0
CO2 PRODUCTION mg/kg/hr
240
200
160
120
O2 CONSUMPTION mg/kg/hr
160
120
80
40
1
2
3
4
5
AMBIENT OXYGEN (ml/l)

excretion showed similar trends at both temperatures. Carbondioxide rose to 247.26 mg/kg/hr from 110.38 mg/kg/hr then decreased to 144.88 mg/kg/hr and again escalated to 164.81 mg/kg/hr at 30°C. Similarly when the temperature was increased to 35°C the carbondixode production also exhibited a rise from 118.30 mg/kg/hr to 235.94 mg/kg/hr and then decreased to about 119.88 mg/kg/hr. The trend in ammonia excretion showed an increase in the beginning from 9.03 mg/kg/hr to 19.31 mg/kg/hr and then declined to 11.91 mg/kg/hr and further rose to 13.51 mg/kg/hr at 30°C. Whereas at 35°C it first increased from 3.88 mg/kg/hr to 11.26 mg/kg/hr, then decreased to 3.81 mg/kg/hr and again rose to 19.86 mg/kg/hr.

Metabolic quotients

In 30‰, the quotients showed a marked difference at 30 and 35°C. At 30°C, R.Q. first decreased from 3.37 to about 1.43 and then increased to 2.91 but at 35°C, it initially increased from 2.15 to 3.38 and then decreased to about 1.08 showing a counter trend. All the R.Q. values at 30 and 35°C were above unity. R.Q. trend line resembles the trend given for Puntius sarana at 30°C by Peer Mohamed (1974). A.Q. values at 30°C showed a rise from 0.33 to 0.39 initially. The final value was 0.38 with an in between decrease of about 0.11. The trend resembles the curve in Rhinomugil corsula (Kutty and Peer Mohamed, 1975) at 30°C. At 35°C the A.Q. first increased from 0.07 to 0.14 and finally to 0.21 with an in between decrease of only about 0.06.

Activity

Trend lines for activity at both the temperatures were similar. With the increase in ambient oxygen, activity first increased from 32.69 counts/

hour to 50.25 counts/hour at 30°C and then declined to about 28.46 counts/hour whereas at 35°C it first increased from 43.09 counts/hour to 48.18 counts/hour. Activity, however, declined considerably to a low of 8.41 counts/hour towards the end of the experiment at 35°C.

Asphyxial oxygen

In sea water the mean asphyxial oxygen was found to be 1.17 ml/l at 30°C and 1.45 ml/l at 35°C (Table 5). The high and low asphyxial oxygen observed was 2.5 ml/l (Fish weight, 33.04 g) and 0.5 ml/l (Fish weight, 43.35 g) at 30°C and 2.4 ml/l (Fish weight, 38.10 g) and 0.9 ml/l (Fish weight, 34.33 g) at 35°C.

(iii) **Metabolic rates, quotients and random activity during hypoxia and recovery at 30 and 35°C**

Results from hypoxia and recovery experiments relating to oxygen consumption, carbondioxide production, ammonia excretion, R.Q., A.Q. and activity at 30 and 35°C are presented in Tables 6 and 7 and the graphical representations are shown in Figures 13 and 14.

Metabolism

The trends of ambient oxygen during hypoxia and recovery at 30 and 35°C are similar and this is in agreement with the trends of ambient oxygen in Rhinomugil corsula (Kutty and Peer Mohamed, 1975), Tilapia mossambica (Peer Mohamed and Kutty, 1981) and Mystus armatus (Sukumaran and Kutty, 1977). During hypoxia, ambient oxygen was found to decrease from 2.84 ml/l to 1.8[illegible] ml/l and from 4.06 ml/l to 2.62 ml/l at 30

Table 5. Asphyxial oxygen concentrations (the concentration at which the fish loses its equilibrium) and the time taken for asphyxia of sea bass acclimated to and tested at 30 and 35°C in sea water, brackish water and fresh-water. The values in brackets indicate the number of determinations. Details of fish are given in Table 26.

SEA WATER						BRACKISH WATER						FRESH-WATER					
30°C			35°C			30°C			35°C			30°C			35°c		
Fish No.	Asphyxial oxygen (ml/l)	Time taken for asphyxia (After closure for last run)Min.	Fish No.	Asphyxial oxygen (ml/l)	Time taken for asphyxia (after closure for last run)Min.	Fish No.	Asphyxial oxygen (ml/l)	Time taken for asphyxia (after closure for last run)Min.	Fish No.	Asphyxial oxygen (ml/l)	Time taken for asphyxia (after closure for last run) Min.	Fish No.	Asphyxial oxygen (ml/l)	Time taken for asphyxia (after closure for last run) Min.	Fish No.	Asphyxial oxygen (ml/l)	Time taken for asphyxia (after closure closure for last run) Min.
L30	0.8	27	L62	1.3	22	L127	1.3	40	L147	1.5	7	L177	3.0	25	L207	1.0	20
L31	0.9	52	L63	1.4	12	L128	2.6	50	L148	1.3	25	L178	3.0	20	L208	0.8	13
L32	2.1	25	L64	1.5	23	L116	2.2	28	L149	0.8	8	L179	3.1	21	L209	1.4	13
L33	1.4	10	L65	1.8	20	L117	1.2	20	L150	0.8	7	L180	0.5	23	L210	0.6	25
L34	1.4	7	L66	1.5	23	L118	1.2	25	L151	0.8	20	L181	0.8	23	L211	1.7	15
L35	0.8	20	L67	1.8	15	L119	1.7	25	L152	2.1	25	L182	1.2	10	L212	0.7	9
L36	0.5	17	L68	2.4	22	L120	1.9	23	L153	2.0	28	L183	1.4	7	L213	1.9	7
L37	1.1	30	L69	1.3	18	L121	1.6	35	L154	2.4	28	L184	1.5	11	L214	2.1	8
L38	1.4	25	L70	2.1	28	L122	2.0	50	L155	3.0	23	L185	1.7	7	L215	2.5	8
L39	0.9	15	L71	1.7	13	L123	1.5	55	L156	2.7	20	L186	1.6	18	L216	0.7	7
L40	0.9	7	L72	0.9	11	L124	0.7	53	L157	2.7	15	L187	1.6	10	L217	2.1	8
L41	0.8	4	L73	1.2	8	L125	1.9	45	L158	1.1	12	L188	1.4	13	L218	1.9	8
L42	0.9	14	L74	1.0	15	L126	1.2	36	L159	1.0	8	L189	0.8	10	L219	2.2	15
L43	1.1	11	L75	0.9	18	L101	1.0	17	L160	0.9	14	L190	0.7	7	L220	2.3	16
L44	2.5	25	L76	1.0	8	L102	1.0	21	L161	0.9	6	L191	0.8	6	L221	2.1	5

Table 6. Oxygen consumption, carbon dioxide production, ammonia excretion, R.Q., A.Q. and random activity subjected to hypoxia (reducing ambient oxygen concentration in the closed respirometer by the respiration of the fish itself until it loses its equilibrium) and subsequent recovery in air-saturated water. The fishes were acclimated to and tested at 30°C in sea water. Each value shown is a mean of 15 determinations in each case (except the last run, 10 determinations) of the total of 70 determinations arranged as per the sequential order of runs (5) in hours in experiments for 15 fishes to facilitate the analysis. Each run in an experiment lasted for 60 minutes except the last run during which the fish lost its equilibrium. S.D. is also given.

Experimental runs (in hours)	Mean ambient oxygen. (ml/l)	Mean rate of oxygen consumption. (mg/kg/hr)	Mean rate of carbon dioxide production. (mg/kg/hr)	Mean rate of ammonia excretion. (mg/kg/hr)	Mean respiratory quotient (R.Q.)	Mean ammonia quotient (A.Q.)	Mean random activity. (counts/hour)	Remarks
I	2.84 ± 0.94	92.80 ± 48.88	149.28 ± 116.77	6.99 ± 5.24	1.65 ± 0.86	0.09 ± 0.07	21.29 ± 11.81	Hypoxia
II	1.89 ± 0.54	67.94 ± 39.62	173.12 ± 145.60	17.26 ± 12.53	2.71 ± 1.80	0.35 ± 0.40	28.70 ± 16.53	
III	2.47 ± 1.22	83.28 ± 39.55	179.11 ± 118.05	11.94 ± 9.10	2.48 ± 1.47	0.18 ± 0.12	32.43 ± 16.77	Recovery
IV	2.72 ± 1.01	80.47 ± 49.00	182.39 ± 148.55	15.15 ±12.43	2.17 ± 1.31	0.24 ± 0.22	34.43 ± 22.29	
V	3.13 ± 0.99	79.67 ± 29.48	203.40 ± 148.25	17.40 ± 17.53	2.58 ± 1.36	0.26 ± 0.16	36.60 ± 17.10	

Table 7. Metabolic rates, quotients and random activity subjected to hypoxia and subsequent recovery in air saturated water. The fishes were acclimated to and tested at 35°C in sea water. Each value is a mean of 15 determinations (except the last run, 7 determinations) of the total of 67 observations arranged in the sequential order of runs in hours in experiments for 15 fishes to facilitate the analysis. Each run lasted for 60 minutes except the last run where fish lost its equilibrium. S.D. is also given.

Experimental runs (in hours)	Mean ambient oxygen. (ml/l)	Mean rate of oxygen consumption. (mg/kg/hr)	Mean rate of carbon dioxide production. (mg/kg/hr)	Mean rate of ammonia excretion. (mg/kg/hr)	Mean respiratory quotient (R.Q.)	Mean ammonia quotient (A.Q.)	Mean random activity. (counts/hour)	Remarks
I	4.06 ± 1.01	101.33 ± 35.04	127.02 ± 96.76	7.22 ± 5.68	1.27 ± 0.82	0.17 ± 0.06	10.36 ± 7.78	Hypoxia
II	2.62 ± 0.64	91.78 ± 45.24	237.53 ± 109.34	21.30 ± 21.72	2.99 ± 1.35	0.22 ± 0.19	18.43 ± 11.37	
III	3.55 ± 0.79	91.50 ± 55.03	142.73 ± 62.73	9.47 ± 5.48	2.01 ± 1.06	0.11 ± 0.05	10.31 ± 6.63	Recovery
IV	3.51 ± 0.64	92.67 ± 38.21	187.07 ± 109.83	12.20 ± 5.04	2.16 ± 1.16	0.12 ± 0.04	16.43 ± 10.68	
V	3.99 ± 0.42	89.07 ± 27.14	289.83 ± 83.70	15.48 ± 8.81	3.44 ± 0.97	0.17 ± 0.11	30.67 ± 15.48	

FIGURE 13

Trends pertaining to oxygen consumption, carbon dioxide production, ammonia excretion, their corresponding quotients (R.Q. and A.Q.) and random activity of sea bass subjected to hypoxia (reducing ambient oxygen concentration in the closed respirometer by the respiration of the fish itself until the fish loses its equilibrium) and subsequent recovery in air saturated water. Acclimation of the fish and test at 30°C in sea water. The end of the hypoxic phase is indicated by the vertical line cutting across the curves fitted through the mean values based on Table 6. Each point shown is a mean of 15 observations in each case. The details of fishes used (15 fishes - Fish No.L_{30} to L_{44}) are given in Table 26.

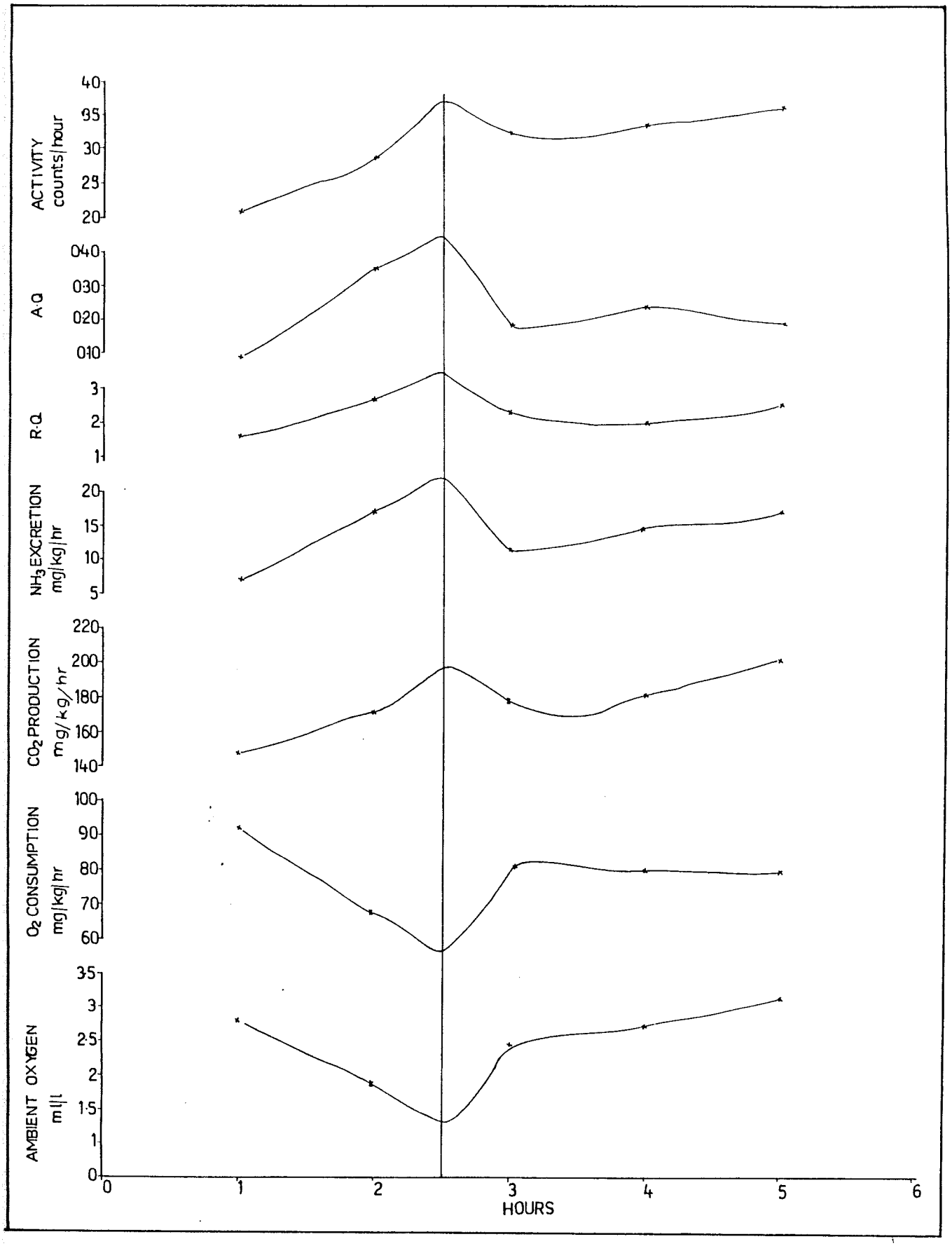
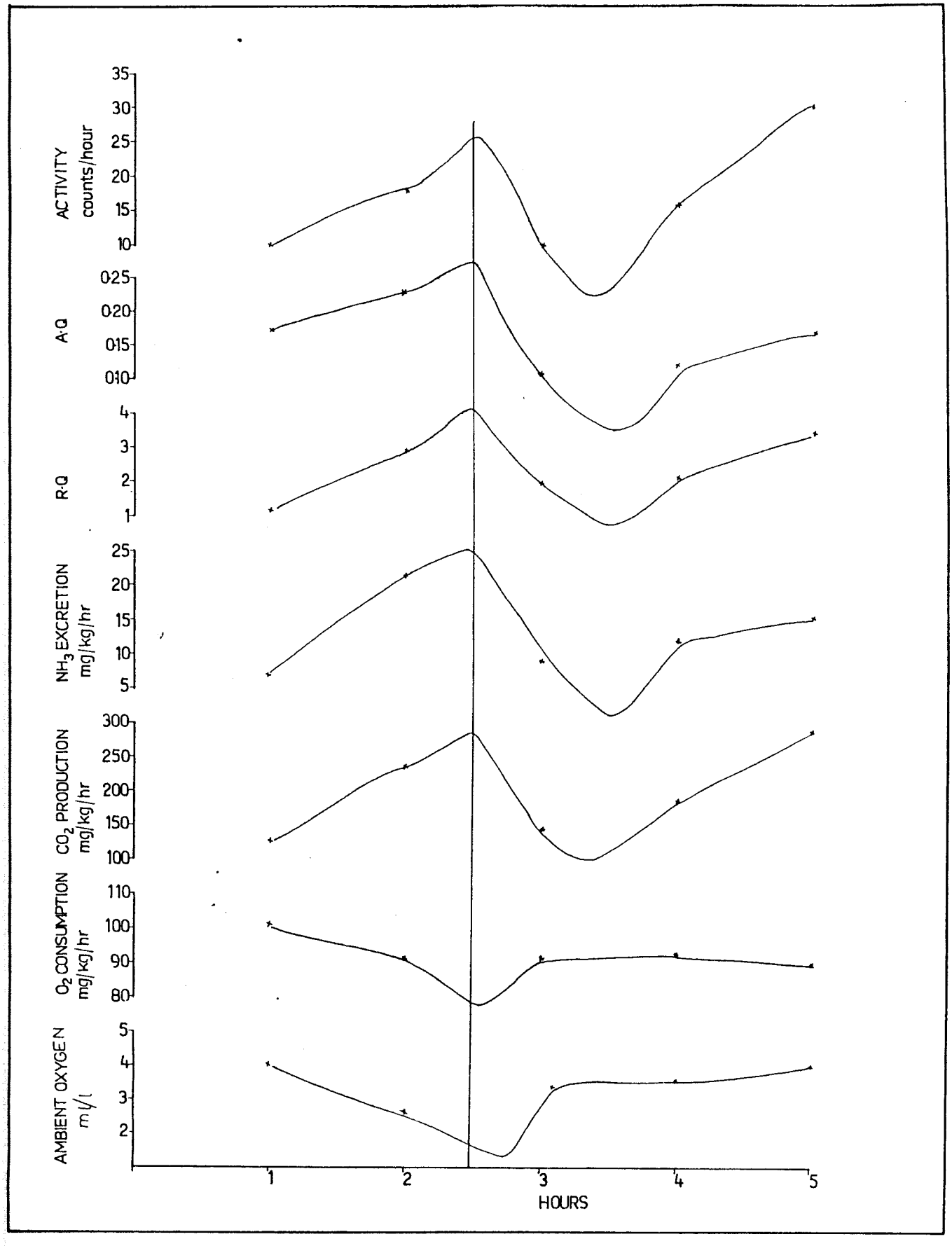

ACTIVITY counts/hour
40
35
30
25
20
A·Q
040
030
020
010
R·Q
3
2
1
NH3 EXCRETION mg/kg/hr
20
15
10
5
CO2 PRODUCTION mg/kg/hr
220
200
180
160
140
O2 CONSUMPTION mg/kg/hr
100
90
80
70
60
AMBIENT OXYGEN ml/l
3·5
3
2·5
2
1·5
1
0
0
1
2
3
4
5
6
HOURS

FIGURE 14

Trends pertaining to metabolic rates, quotients and random activity of sea bass subjected to hypoxia and subsequent recovery. Acclimation and test at 35°C in sea water. Plotted values are based on Table 7. The details of fishes used are (Fish No.L_{62} to L_{76}) given in Table 26.


ACTIVITY counts/hour
35
30
25
20
15
10
A·Q
0·25
0·20
0·15
0·10
R·Q
4
3
2
1
NH3 EXCRETION mg/kg/hr
25
20
15
10
5
CO2 PRODUCTION mg/kg/hr
300
250
200
150
100
O2 CONSUMPTION mg/kg/hr
110
100
90
80
AMBIENT OXYGEN m l/l
5
4
3
2
1
2
3
4
5
HOURS

and 35°C respectively. However, during recovery the parameter increased from 2.47 ml/l to 3.13 ml/l and 3.55 ml/l to 3.99 ml/l at 30 and 35°C respectively. Oxygen consumption showed a decreasing trend from 92.80 mg/kg/hr to 67.94 mg/kg/hr and 101.33 mg/kg/hr to 91.78 mg/kg/hr at 30 and 35°C respectively. The decrease in the availability of oxygen during hypoxia compared well with the hypoxic condition in Rhinomugil corsula (Peer Mohamed, 1974) at both the temperatures. However, during recovery it shot up first from 67.94 mg/kg/hr to 83.28 mg/kg/hr at 30°C and from 91.78 mg/kg/hr to 92.67 mg/kg/hr at 35°C with a minor decline towards the end of the experiment, in both the cases, to 79.67 mg/kg/hr and 89.07 mg/kg/hr respectively. Oxygen consumption during recovery at 30 and 35°C is comparable with that of Tilapia mossambica at the same temperatures (Peer Mohamed, 1974). These trends also resemble that of Tilapia mossambica at 30 and 35°C (Peer Mohamed and Kutty, 1981).

Carbondioxide production was initially high ('Prehypoxic' value) and was in the range of 149.28 mg/kg/hr to 173.12 mg/kg/hr at 30°C and 127.52 mg/kg/hr to 237.53 mg/kg/hr at 35°C showing a compensatory anaerobic mechanism for the supply of energy during periods of reduction in oxygen consumption. Again carbondioxide production rose to 203.40 mg/kg/hr and 289.83 mg/kg/hr respectively during recovery. Carbondioxide production at 30 and 35°C in hypoxic condition compares well with the same condition in Tilapia mossambica at 30°C (Peer Mohamed, 1974). Besides, the hypoxic condition at both temperatures also relate well with that of Tilapia mossambica (Peer Mohamed and Kutty, 1981). The rate of ammonia excretion

escalated during hypoxia (from 6.99 mg/kg/hr to 17.26 mg/kg/hr at 30°C and 7.22 mg/kg/hr to 21.30 mg/kg/hr at 35°C) and recovery (from 11.94 mg/kg/hr to 17.40 mg/kg/hr at 30°C and 9.47 mg/kg/hr to 15.48 mg/kg/hr at 35°C). The increasing trend of ammonia excretion at 30°C in hypoxic condition is in agreement with the report given by Kutty and Peer Mohamed (1975) in the case of Rhinomugil corsula at 30 and 35°C. The trend at 35°C also agrees with that for Rhinomugil corsula (Kutty and Peer Mohamed, 1975) and Tilapia mossambica (Peer Mohamed and Kutty, 1981) at 35°C.

Metabolic quotients

R.Q. and A.Q. showed an increasing trend at 30°C during hypoxia as in Rhinomugil corsula (Kutty and Peer Mohamed, 1975). The trends at 35°C also resemble the hypoxic condition of Rhinomugil corsula (Kutty and Peer Mohamed, 1975) and Tilapia mossambica (Peer Mohamed and Kutty, 1981) at 30 and 35°C. The R.Q. shot up during hypoxia and recovery - from 1.65 to 2.71 at 30°C and from 1.27 to 2.99 at 35°C during hypoxia, and from 2.48 to 2.58 at 30°C and 2.01 to 3.44 at 35°C during recovery - with a slight fall in the middle of the recovery phase at 30°C. The A.Q. values showed a sharper increase during hypoxia at both the temperatures from 0.09 to 0.35 and from 0.17 to 0.22 indicating that the relative protein utilization during hypoxia is higher at 30°C than at 35°C. It again increased from 0.11 to 0.17 during recovery at 35°C but at 30°C it first increased from 0.18 to 0.24 and then decreased to about 0.20 at the fifth hour. All the values of A.Q. are below unity.

Activity

During hypoxia an increase in random activity correlates well with a decrease in ambient oxygen at both the temperatures as in Rhinomugil

corsula (Kutty and Peer Mohamed, 1975). It increased from 21.29 counts/hour to 28.70 counts/hour at 30°C and from 10.36 counts/hour to 18.43 counts/hour at 35°C till the fish experienced asphyxia. During recovery also the activity increased from 32.43 counts/hour to 36.60 counts/hour and 10.31 counts/hour to 30.67 counts/hour respectively.

b) **EXPERIMENTS IN BRACKISH WATER**

(i) **Influence of random activity on metabolic rates and quotients at high ambient oxygen at 30 and 35°C**

Plots of routine oxygen consumption, carbondioxide production, ammonia excretion and R.Q. and A.Q. against random activity based on regression equations of metabolic rates and quotients of Lates calcarifer acclimated to and tested at 30°C at ambient oxygen concentration near air saturation are shown in Fig.3. Similar plots of Lates calcarifer acclimated to and tested at 35°C are given in Fig.4. Mean values of routine metabolic rates, quotients and activity at 30 and 35°C (Table 1) and their corresponding standard metabolic rates (Table 1) have also been presented. Regression coefficients of oxygen consumption, carbondioxide production, ammonia excretion and their corresponding quotients showed no significant difference at 30 and 35°C except the ammonia excretion at 35°C which showed significant difference at 5% level.

It is observed that the high and low rates of ambient oxygen were 4.5 ml/l and 2.18 ml/l at 30°C and 5.17 ml/l and 2.40 ml/l at 35°C. This is presented in Table 8. The related oxygen consumption, carbondioxide production, ammonia excretion, R.Q., A.Q. and activity at 30 and 35°C

FIGURE 3

Regression lines fitted for metabolic rates and quotients in relation to random activity of sea bass acclimated to and tested in air saturated brackish water at 30°C. The lines fitted, as per equations presented, are to show the trend lines and are calculated for pooled data for all fishes in each case. The details of fishes (22 fishes - Fish No.L_{77} to L_{98}) used are given in Table 26. 'r' value for parameter is also given.

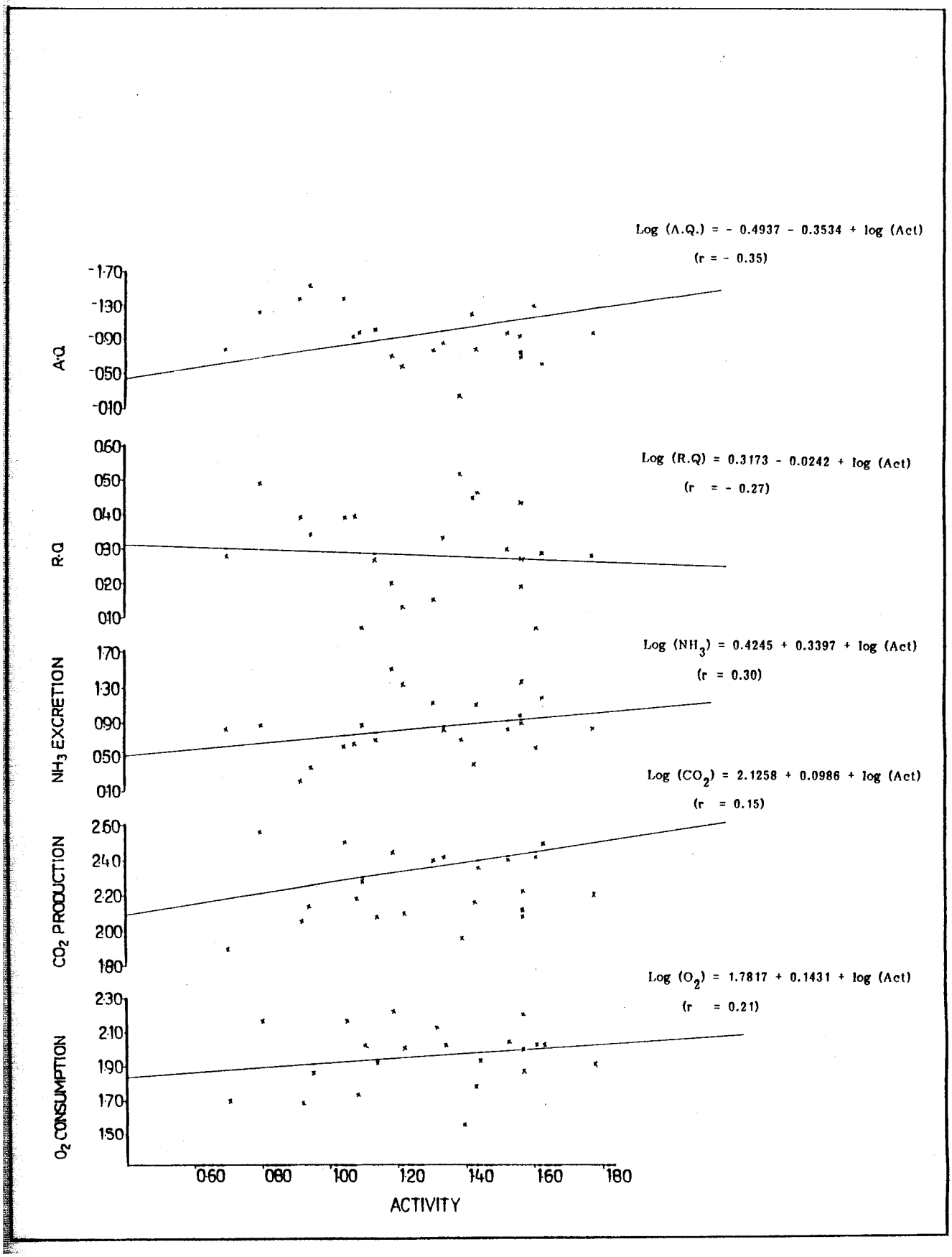

Log (A.Q.) = - 0.4937 - 0.3534 + log (Act)
(r = - 0.35)
A·Q
·170
·130
·090
·050
·010
Log (R.Q) = 0.3173 - 0.0242 + log (Act)
(r = - 0.27)
R·Q
060
050
040
030
020
010
Log (NH3) = 0.4245 + 0.3397 + log (Act)
(r = 0.30)
NH3 EXCRETION
170
130
090
050
010
Log (CO2) = 2.1258 + 0.0986 + log (Act)
(r = 0.15)
CO2 PRODUCTION
260
240
220
200
180
Log (O2) = 1.7817 + 0.1431 + log (Act)
(r = 0.21)
O2 CONSUMPTION
230
210
190
170
150
060
080
100
120
140
160
180
ACTIVITY

FIGURE 4

Regression fines fitted for metabolic rates and quotients in relation to random activity of sea bass acclimated to and tested in air saturated brackish water at 35°C. The lines drawn, based on the regression equations, to show the trends and calculated for the pooled data for all fishes in each case, are given. The details of fishes (17 fishes) used (Fish No. L_{129} to L_{146}) are shown in Table 26. Correlation coefficients are also given.

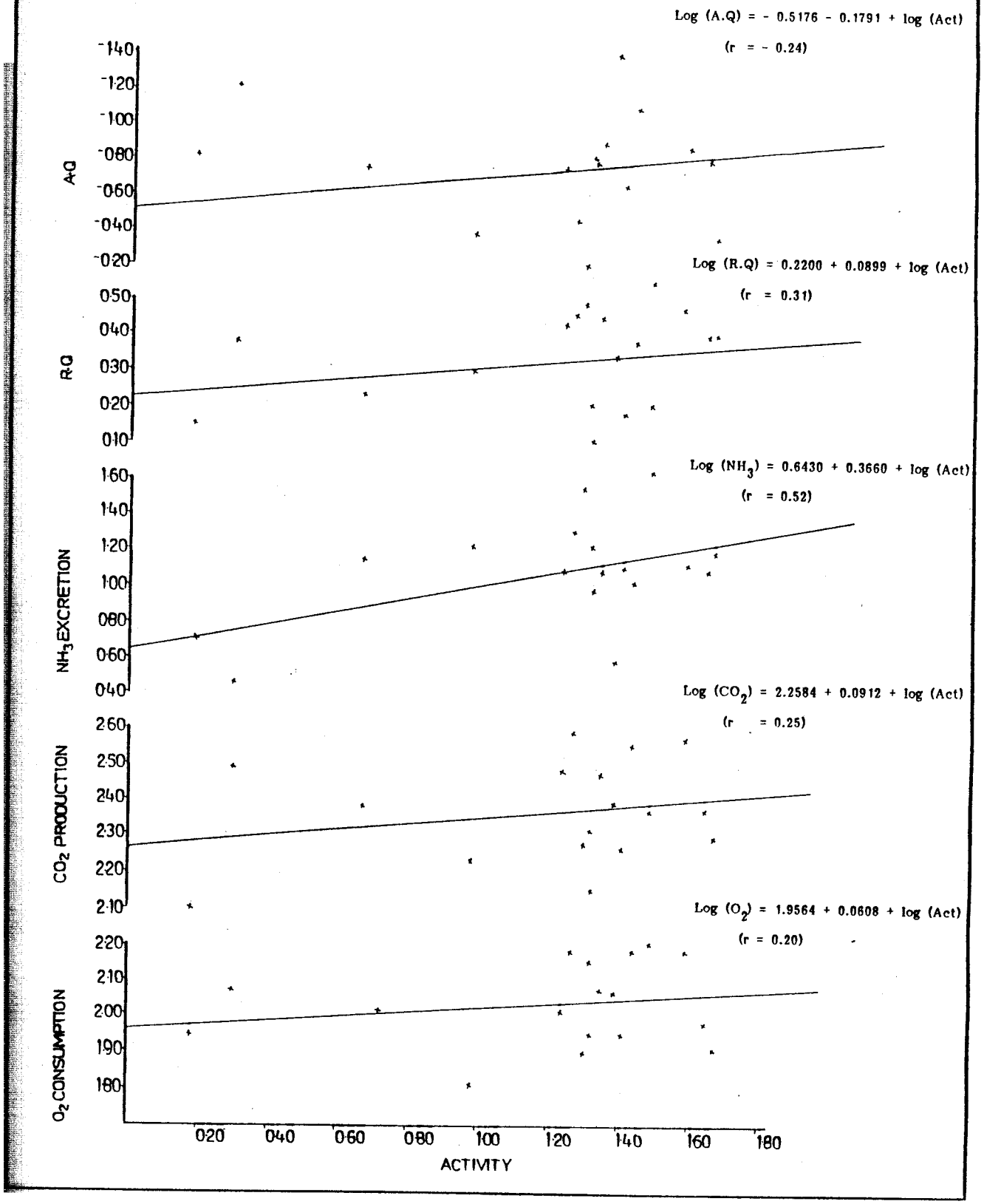

Log (A.Q) = - 0.5176 - 0.1791 + log (Act)
(r = - 0.24)
A·Q
·140
·120
·100
·080
·060
·040
·020
Log (R.Q) = 0.2200 + 0.0899 + log (Act)
(r = 0.31)
R·Q
050
040
030
020
010
Log (NH_3) = 0.6430 + 0.3660 + log (Act)
(r = 0.52)
NH_3 EXCRETION
160
140
120
100
080
060
040
Log (CO_2) = 2.2584 + 0.0912 + log (Act)
(r = 0.25)
CO_2 PRODUCTION
260
250
240
230
220
210
Log (O_2) = 1.9564 + 0.0608 + log (Act)
(r = 0.20)
O_2 CONSUMPTION
220
210
200
190
180
020
040
060
080
100
120
140
160
180
ACTIVITY

Table 8. Measurements of metabolic rates, quotients and random activity in sea bass at 'high' and 'low' ambient oxygen at 30 and 35°C in brackish water. The 'high' oxygen refers to mean ambient oxygen concentration near air saturation and 'low' oxygen refers to lowest mean ambient oxygen concentration tested. Each value is the mean of 15 determinations. S.D is also given.

	30°C		35°C	
	High oxygen	Low oxygen	High oxygen	Low oxygen
Mean of mean ambient oxygen (ml/l)	4.58(15) ± 0.59	2.18(15) ± 0.70	5.17(15) ± 1.10	2.40(15) ± 0.83
Mean rate of oxygen consumption (mg/kg/hr)	64.11(15) ± 40.54	166.69(15) ± 199.37	217.01(15) ± 151.69	161.81(15) ± 109.88
Mean rate of carbon dioxide production (mg/kg/hr)	221.74(15) ± 113.14	414.70(15) ± 344.84	257.09(15) ± 61.88	369.93(15) ± 150.21
Mean rate of ammonia excretion (mg/kg/hr)	198.25(15) ± 251.62	706.29(15) ± 547.48	626.04(15) ± 598.12	909.91(15) ± 682.26
Mean respiratory quotients (R.Q.)	5.89(15) ± 5.34	4.96(15) ± 3.54	2.82(15) ± 3.46	3.69(15) ± 3.03
Mean ammonia quotients (A.Q)	9.92(15) ± 25.86	16.02(15) ± 26.11	4.28(15) ± 3.70	9.01(15) ± 11.77
Mean random activity (c/hr)	16.67(15) ± 16.71	21.2(15) ± 11.66	13.85(15) ± 10.79	27.73(15) ± 17.82

estimated at high and low ambient oxygen rates are also given in Table 8.

(ii) **Influence of ambient oxygen (from air saturation down to asphyxial level) on metabolic rates, quotients and random activity at 30 and 35°C**

Table 9 and 10 present the data regarding the influence of ambient oxygen on metabolic rates, quotients and random activity of Lates calcarifer acclimated to and tested at 30 and 35°C and these are graphically shown in Figures 9 and 10 respectively.

Metabolism

With an increase in the ambient oxygen, the total oxygen consumption showed a corresponding increase from 47.28 mg/kg/hr to 217.02 mg/kg/hr at 35°C. But at 30°C it first increased from 85.11 mg/kg/hr to 166.82 mg/kg/hr and then decreased to 84.98 mg/kg/hr. Oxygen consumption trend resembles that of Mystus armatus (Sukumaran and Kutty, 1977) at 30°C. Carbondioxide output increased initially for both 30 and 35°C from 298.59 mg/kg/hr to 580.53 mg/kg/hr and from 121.98 mg/kg/hr to 306.70 mg/kg/hr and then decreased to 170.08 mg/kg/hr and 210.71 mg/kg/hr respectively. A further increase in carbondioxide output to 230.13 mg/kg/hr was noticed at 35°C with no corresponding increase at 30°C. Trend at 35°C is similar to the work done by Kutty and Peer Mohamed (1975) in Rhinomugil corsula at 30°C. At 30°C ammonia excretion showed a decreasing trend as in the case of Puntius sarana at 30°C (Peer Mohamed, 1974). The value declined from 30.72 mg/kg/hr to 7.91 mg/kg/hr with an increase in ambient oxygen.

Table 9. Oxygen consumption, carbon dioxide production, ammonia excretion, R.Q., A.Q. and random activity in relation to low ambient oxygen concentration below air saturation in sea bass acclimated to and tested at 30°C in brackish water. Each value is the average of groups of data pertaining to 23 determinations (except the last group, 24 determinations) after processing a total of 231 determinations tabulated in an ascending order of mean ambient oxygen to facilitate the analysis. Each run lasted for 60 minutes. S.D. is also given.

Mean ambient oxygen (ml/l)	Mean rate of oxygen consumption (mg/kg/hr)	Mean of carbon dioxide production (mg/kg/hr)	Mean rate of ammonia excretion (mg/kg/hr)	Mean respiratory quotient (R.Q)	Mean ammonia quotient (A.Q)	Mean random activity (counts/hour)
1.43 ± 0.24	85.11 ± 97.04	298.59 ± 293.28	30.72 ± 23.76	4.26 ± 3.18	0.78 ± 1.11	23.09 ± 19.73
1.94 ± 0.12	168.60 ± 184.39	580.53 ± 484.71	25.92 ± 27.36	3.83 ± 2.69	0.22 ± 0.27	29.91 ± 22.30
2.26 ± 0.07	136.16 ± 146.70	349.69 ± 415.97	18.95 ± 18.48	2.28 ± 1.31	0.68 ± 1.90	23.27 ± 18.96
2.46 ± 0.06	135.54 ± 111.82	444.94 ± 423.71	17.46 ± 20.87	3.56 ± 3.03	0.15 ± 0.17	26.48 ± 22.57
2.67 ± 0.06	159.45 ± 122.01	387.71 ± 331.82	18.69 ± 24.54	2.81 ± 1.81	0.14 ± 0.17	25.30 ± 17.47
2.84 ± 0.06	158.11 ± 126.59	247.57 ± 253.04	18.58 ± 23.58	1.78 ± 1.05	0.18 ± 0.23	22.78 ± 20.89
3.02 ± 0.05	166.82 ± 150.41	376.89 ± 364.70	15.05 ± 21.55	2.18 ± 0.84	0.20 ± 0.47	21.43 ± 12.18
3.27 ± 0.09	148.68 ± 92.44	388.54 ± 372.60	15.30 ± 22.30	2.54 ± 1.59	0.22 ± 0.43	26.18 ± 17.03
3.53 ± 0.11	90.55 ± 66.98	216.14 ± 251.65	9.81 ± 11.72	2.76 ± 2.16	0.12 ± 0.11	22.46 ± 16.43
4.30 ± 0.59	84.98 ± 60.23	170.08 ± 124.59	7.91 ± 9.74	2.02 ± 1.05	0.25 ± 0.67	15.09 ± 16.08

Table 10. Metabolic rates, quotients and random activity in relation to low ambient oxygen concentration below air saturation in sea bass acclimated to and tested at 35°C in brackish water. Each value is the mean of 15 determinations after arranging the total of 150 determinations in the ascending order of mean ambient oxygen to facilitate the analysis. Each run in an experiment lasted for 60 minutes. S.D. is also given.

Mean ambient oxygen. (ml/l)	Mean rate of oxygen consumption. (mg/kg/hr)	Mean of carbon dioxide production. (mg/kg/hr)	Mean rate of ammonia excretion. (mg/kg/hr)	Mean respiratory quotient (R.Q)	Mean ammonia quotient (A.Q)	Mean random activity. (counts/hour)
1.25 ± 0.22	47.28 ± 25.64	121.98 ± 122.45	27.96 ± 64.00	2.34 ± 2.04	0.68 ± 1.06	22.80 ± 15.56
1.69 ± 0.08	104.91 ± 63.94	306.70 ± 181.19	14.09 ± 11.45	2.96 ± 1.23	0.21 ± 0.23	34.20 ± 19.11
1.91 ± 0.06	104.91 ± 72.06	280.73 ± 206.75	10.75 ± 9.59	2.57 ± 1.59	0.20 ± 0.29	27.64 ± 19.21
2.07 ± 0.05	115.87 ± 48.30	269.92 ± 128.15	13.77 ± 11.02	2.22 ± 0.86	0.12 ± 0.12	22.92 ± 19.67
2.29 ± 0.09	163.74 ± 98.96	243.47 ± 146.95	11.26 ± 11.52	1.67 ± 0.83	0.16 ± 0.28	20.79 ± 18.69
2.52 ± 0.06	142.17 ± 75.63	291.61 ± 109.71	15.95 ± 15.83	2.33 ± 1.16	0.15 ± 0.18	30.46 ± 22.64
2.74 ± 0.10	112.82 ± 64.87	210.71 ± 147.38	22.44 ± 40.96	1.85 ± 1.12	0.32 ± 0.62	22.15 ± 16.46
3.13 ± 0.13	121.03 ± 74.02	220.94 ± 111.82	15.87 ± 16.27	2.20 ± 1.12	0.22 ± 0.36	23.29 ± 22.45
3.57 ± 0.17	143.24 ± 97.54	277.14 ± 158.99	24.88 ± 28.20	2.09 ± 0.90	0.19 ± 0.21	16.92 ± 12.63
5.18 ± 1.11	217.02 ± 151.68	230.13 ± 102.81	19.23 ± 17.13	1.53 ± 1.26	0.12 ± 0.11	13.85 ± 10.79

FIGURE 9

Trends pertaining to oxygen consumption, carbon dioxide production, ammonia excretion, R.Q., A.Q. and random activity in relation to ambient oxygen below air saturation in sea bass acclimated to and tested at 30°C in brackish water. Values plotted are based on Table 9. The details of fishes used (Fish No.L_{99} to L_{127} are given in Table 26).

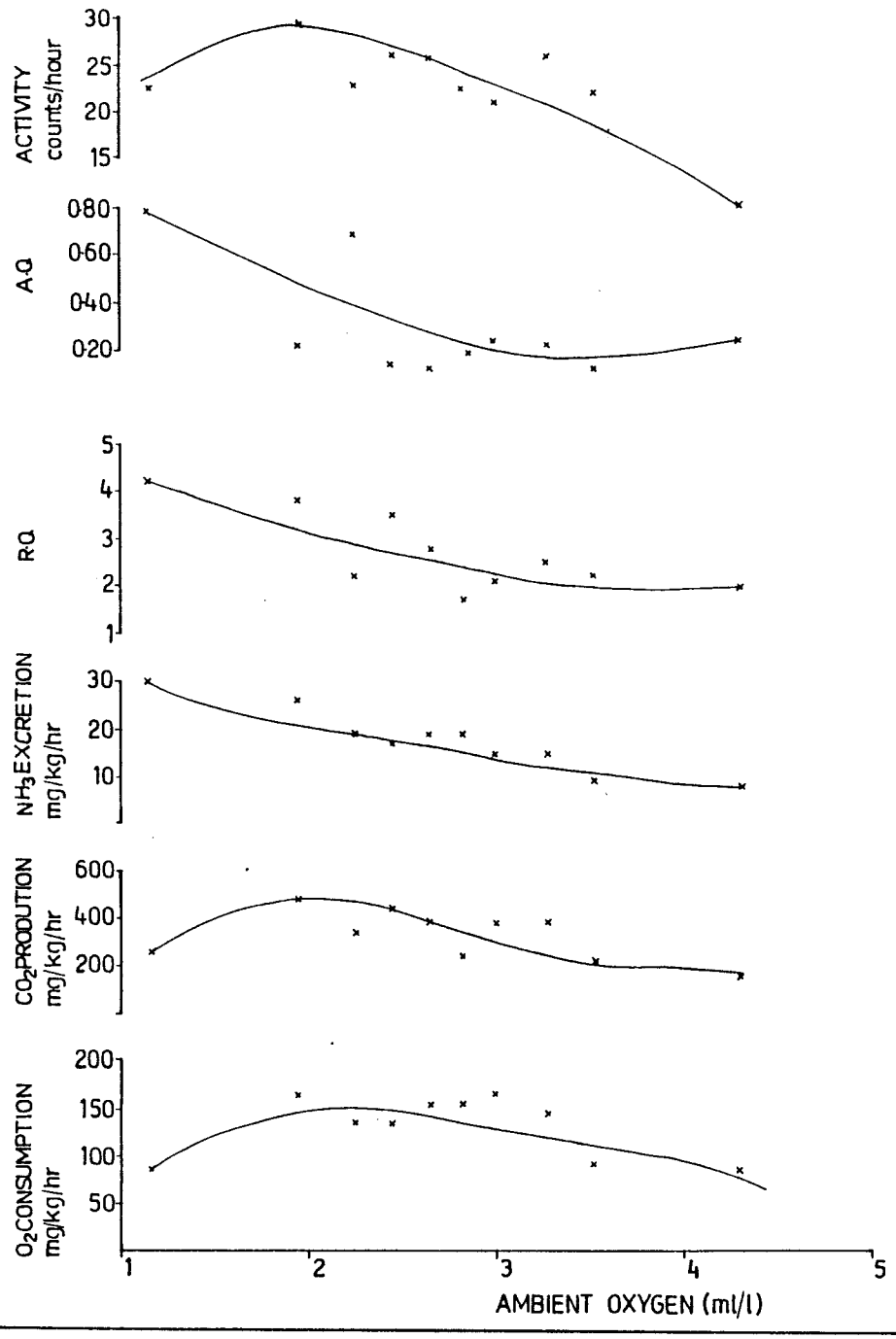
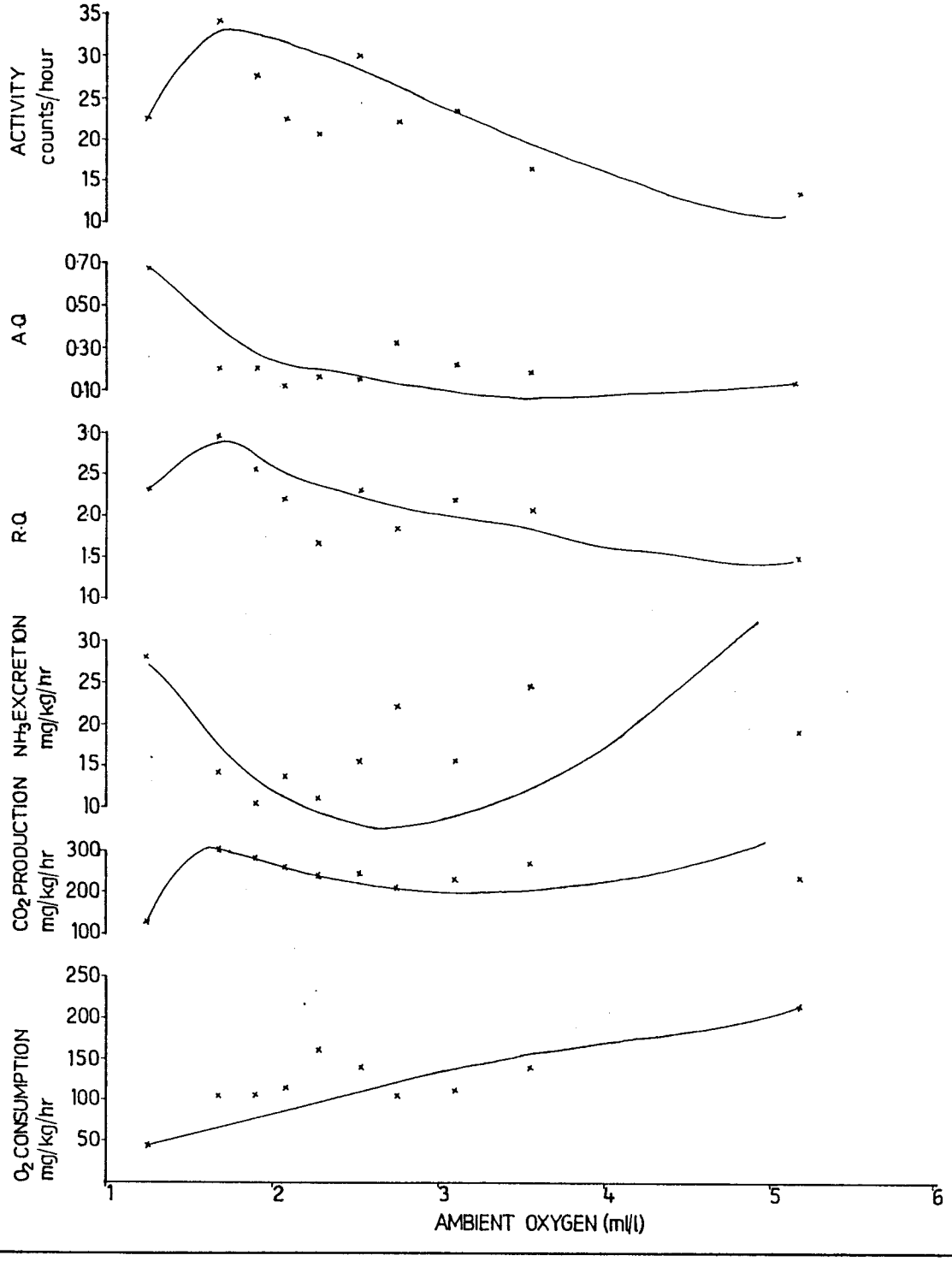

ACTIVITY counts/hour
30
25
20
15
AQ
0·80
0·60
0·40
0·20
RQ
5
4
3
2
1
NH3 EXCRETION mg/kg/hr
30
20
10
CO2 PRODUTION mg/kg/hr
600
400
200
O2 CONSUMPTION mg/kg/hr
200
150
100
50
1
2
3
4
5
AMBIENT OXYGEN (ml/l)

FIGURE 10

Trends pertaining to metabolic rates, quotients and random activity in relation to ambient oxygen below air saturation in sea bass acclimated to and tested at 35°C in brackish water based on Table 10. The details of fishes used (15 fishes - Fish No.L_{147} to L_{161}) are given in Table 26.


ACTIVITY counts/hour
35
30
25
20
15
10
A·Q
0·70
0·50
0·30
0·10
R·Q
3·0
2·5
2·0
1·5
1·0
NH3EXCRETION mg/kg/hr
30
25
20
15
10
CO2PRODUCTION mg/kg/hr
300
200
100
O2 CONSUMPTION mg/kg/hr
250
200
150
100
50
1
2
3
4
5
6
AMBIENT OXYGEN (ml/l)

However, at 35°C the ammonia values showed an initial declining trend from 27.96 mg/kg/hr to 11.26 mg/kg/hr and later rose to 19.23 mg/kg/hr.

Metabolic quotients

R.Q. at both the temperatures showed a declining trend as in gold fish at 30 and 35°C (Peer Mohamed, 1974). It declined from 4.26 to 2.02 at 30°C but at 35°C there was an initial increase from 2.34 to 2.96 and then a drop to 1.53. At both 30 and 35°C the trend obtained for A.Q. values in this experiment were comparable to that observed for Rhinomugil corsula (Kutty and Peer Mohamed, 1975). The trend in A.Q. obtained for Mystus armatus at a temperature of 30°C also compares well with the current values for Lates calcarifer at the same temperature. The A.Q. values declined from 0.78 to 0.25 and 0.68 to 0.12 at 30 and 35°C respectively.

Activity

Activity showed a similar trend at both the temperatures (30 and 35°C) with a rise in the beginning from 23.09 counts/hour to 29.91 counts/hour and from 22.80 counts/hour to 34.20 counts/hour respectively and a fall at the end to 15.09 counts/hour and 13.85 counts/hour respectively.

Asphyxial oxygen

The mean asphyxial oxygen (Table 5) was 1.53 ml/l at 30°C and 1.60 ml/l at 35°C in brackish water. The highest and lowest rates of oxygen during asphyxia were 2.6 ml/l (Fish weight, 38.69 g) and 0.7 ml/l (Fish weight, 15.70 g) at 30°C and 3.0 ml/l (Fish weight, 24.35 g) and 0.8 ml/l (Fish weight, 25.34 g) at 35°C.

(iii) **Metabolic rates, quotients and random activity during hypoxia and recovery at 30 and 35°C**

Figure 15 shows graphically the oxygen consumption, carbondioxide production, ammonia excretion, R.Q., A.Q. and random activity of Lates calcarifer (acclimation and test at 30°C) subjected to a hypoxic phase until the fish was asphyxiated in a closed respirometer and a subsequent recovery phase (in air saturated water). Similar values obtained for the fish acclimated to and tested at 35°C are given in Fig.16. The Tables 11 and 12 explain the mean values obtained for metabolism and random activity under hypoxia and recovery.

Metabolism

Mean ambient oxygen declined from 3.36 ml/l to 2.38 ml/l and from 3.54 ml/l to 3.11 ml/l during hypoxia at 30 and 35°C respectively but it again increased to 3.55 ml/l at the 5th hour during recovery. The hypoxic condition at 30°C compared well with hypoxic condition in Rhinomugil corsula (Kutty and Peer Mohamed, 1975), in Tilapia mossambica (Peer Mohamed and Kutty, 1981) and also in Mystus armatus (Sukumaran and Kutty, 1977). Similarly the hypoxic condition at 35°C agrees well with the same condition in Tilapia mossambica (Peer Mohamed and Kutty, 1981) and the recovery condition with that of Rhinomugil corsula (Kutty and Peer Mohamed, 1975). In the recovery phase of 30°C mean ambient oxygen rose to 3.09 ml/l at the 4th hour and then decreased to 2.88 ml/l. Oxygen consumption showed a decreasing trend as in Rhinomugil corsula (Kutty and Peer Mohamed, 1975) declining from 171.73 mg/kg/hr to 142.20 mg/kg/hr at 30°C but it

Table 11. Rates of oxygen consumption, carbon dioxide production, ammonia excretion, R.Q., A.Q. and random activity in sea bass acclimated to and tested at 30°C during hypoxia and recovery in brackish water. The values shown are means (± S.D) of 15 determinations in each case (except the last two runs, 14 and 8 determinations respectively) of the total of 67 determinations arranged in the sequential order of runs in hours in experiments for 15 fishes to facilitate the analysis. Each run lasted for 60 minutes except the last run where the fish lost its equilibrium.

Experimental runs (in hours)	Mean ambient oxygen. (ml/l)	Mean rate of oxygen consumption. (mg/kg/hr)	Mean rate of carbon dioxide production. (mg/kg/hr)	Mean rate of ammonia excretion. (mg/kg/hr)	Mean respiratory quotient (R.Q)	Mean ammonia quotient (A.Q.)	Mean random activity. (counts/hour)	Remarks
I	3.36 ± 1.23	171.73 ± 195.55	331.89 ± 358.73	25.97 ± 19.79	2.06 ± 0.63	0.45 ± 0.85	10.00 ± 8.94	Hypoxia
II	2.38 ± 0.70	142.20 ± 188.55	369.44 ± 350.44	27.05 ± 21.04	3.61 ± 2.02	0.94 ± 2.27	18.31 ± 15.27	
III	2.61 ± 0.92	175.64 ± 153.17	426.44 ± 401.54	38.65 ± 41.16	3.05 ± 2.86	0.86 ± 2.04	13.14 ± 8.55	
IV	3.09 ± 1.20	131.65 ± 102.30	427.19 ± 488.63	19.15 ± 22.69	3.52 ± 2.73	1.40 ± 2.92	14.17 ± 9.44	Recovery
V	2.88 ± 0.96	132.72 ± 78.40	391.85 ± 444.58	28.37 ± 23.89	2.65 ± 1.95	0.24 ± 0.22	17.25 ± 15.15	

Table 12. Metabolic rates, quotients and random activity in sea bass acclimated to and tested at 35°C during hypoxia and recovery in brackish water. Each value is the average of 15 determinations (except the last two runs, 14 and 8 determinations respectively) of the total of 67 determinations arranged in the sequential order of runs in hours in experiments for 15 fishes to facilitate the analysis. Each run lasted for 60 minutes except the last run where the fish lost its equilibrium. S.D. is also given.

Experimental runs (in hours)	Mean ambient oxygen (ml/l)	Mean rate of oxygen consumption (mg/kg/hr)	Mean rate of carbon dioxide production (mg/kg/hr)	Mean rate of ammonia excretion (mg/kg/hr)	Mean respiratory quotient (R.Q)	Mean ammonia quotient (A.Q)	Mean random activity (counts/hour)	Remarks
I	3.54 ± 1.67	165.79 ± 128.70	182.79 ± 116.42	16.61 ± 14.13	1.31 ± 0.62	0.10 ± 0.07	10.40 ± 10.67	Hypoxia
II	3.11 ± 1.49	173.35 ± 127.74	319.07 ± 154.57	26.43 ± 38.90	2.60 ± 1.81	0.25 ± 0.59	28.40 ± 16.48	
III	3.39 ± 1.65	176.78 ± 120.17	215.05 ± 181.86	21.93 ± 29.14	1.36 ± 0.85	0.22 ± 0.34	26.69 ± 13.41	
IV	3.06 ± 0.95	119.95 ± 84.67	207.16 ± 179.47	16.83 ± 19.86	1.54 ± 0.89	0.15 ± 0.12	21.82 ± 11.64	Recovery
V	3.55 ± 1.65	129.57 ± 97.58	343.83 ± 190.11	22.39 ± 22.95	2.67 ± 0.89	0.19 ± 0.11	19.00 ± 12.00	

FIGURE 15

Trends pertaining to metabolic rates, quotients and random activity of sea bass subjected to acclimation and test at 30°C in brackish water during hypoxia and recovery based on Table 11. The details of fishes used (Fish No.L_{99} to L_{127}) given in Table 26.

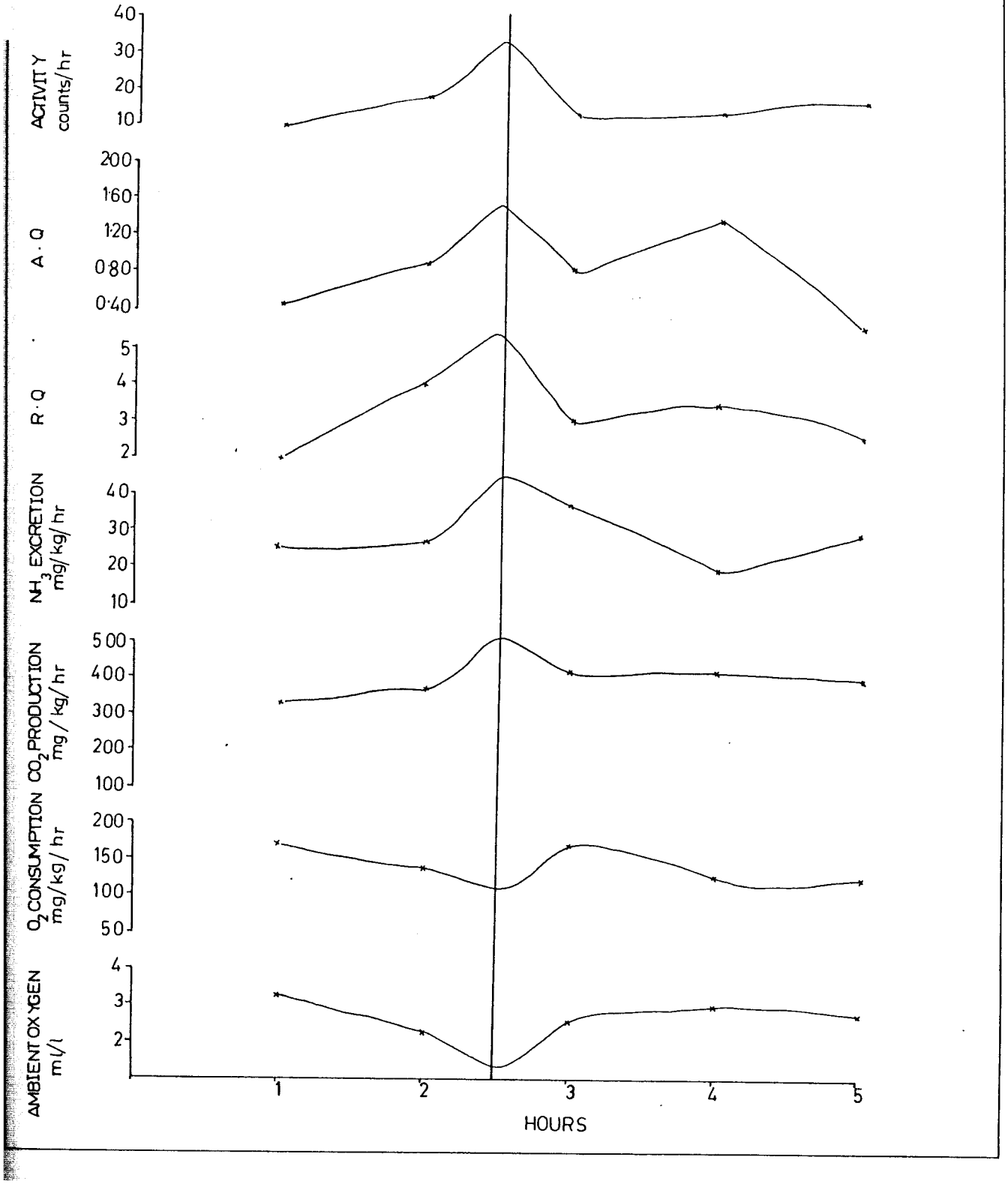
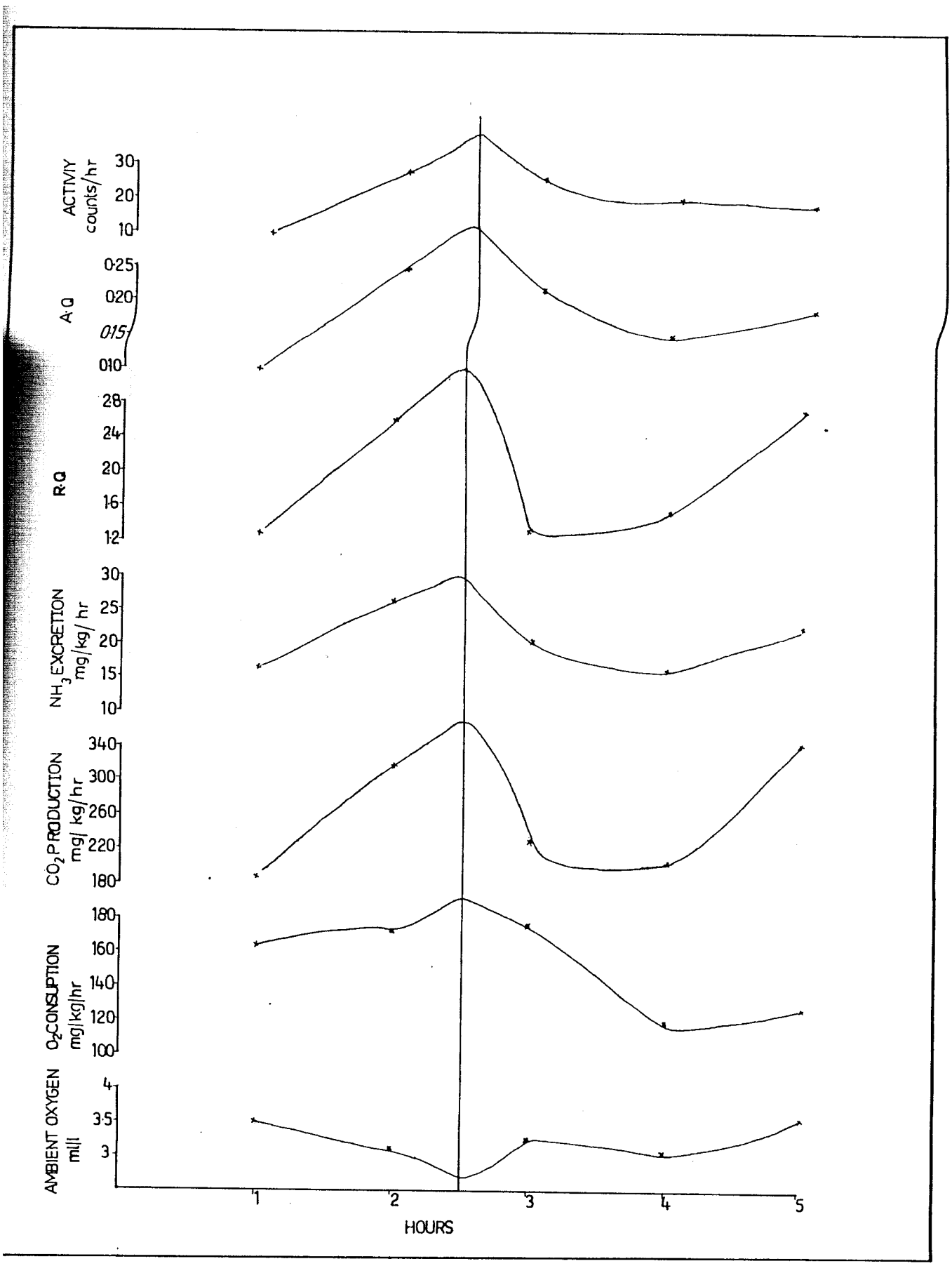

ACTIVITY counts/hr
40
30
20
10
A.Q
200
160
120
0·80
0·40
R.Q
5
4
3
2
NH3 EXCRETION mg/kg/hr
40
30
20
10
CO2 PRODUCTION mg/kg/hr
500
400
300
200
100
O2 CONSUMPTION mg/kg/hr
200
150
100
50
AMBIENT OXYGEN ml/l
4
3
2
1
2
3
4
5
HOURS

FIGURE 16

Trends pertaining to metabolic rates, quotients and random activity of sea bass subjected to acclimation and test at 35°C in brackish water during hypoxia and recovery based on Table 12. The details of fishes used (Fish No.L_{147} to L_{161}) are given in Table 26.


ACTIVIY counts/hr
30
20
10
A·Q
0·25
020
015
010
R·Q
28
24
20
16
12
NH3 EXCRETION mg/kg/hr
30
25
20
15
10
CO2 PRODUCTION mg/kg/hr
340
300
260
220
180
O2 CONSUPTION mg/kg/hr
180
160
140
120
100
AMBIENT OXYGEN ml/l
4
3·5
3
1
2
3
4
5
HOURS

increased from 165.79 mg/kg/hr to 176.39 mg/kg/hr at 35°C. In recovery phase, at both the temperatures, it dropped first from 175.64 mg/kg/hr to 131.65 mg/kg/hr and from 176.78 mg/kg/hr to 119.95 mg/kg/hr respectively and then rose to 132.72 mg/kg/hr and 129.57 mg/kg/hr. This rise is an indication of repayment of oxygen debt. Carbondioxide output was found to be increasing from 331.89 mg/kg/hr to 369.44 mg/kg/hr during hypoxia and decreasing from 426.44 mg/kg/hr to 391.85 mg/kg/hr during recovery phase at 30°C. This trend is similar to that for Tilapia mossambica at the same temperature, as reported by Peer Mohamed and Kutty (1981). When the temperature was increased to 35°C carbondioxide production increased from 182.79 mg/kg/hr to 319.09 mg/kg/hr during hypoxia, and during recovery decreased from 215.05 mg/kg/hr to 207.16 mg/kg/hr and again increased to 343.83 mg/kg/hr at the fifth hour.

Ammonia excretion rose from 25.97 mg/kg/hr to 27.05 mg/kg/hr at 30°C and from 16.61 mg/kg/hr to 26.43 mg/kg/hr at 35°C during hypoxia. As in the case of sea water, these trends resemble that for Rhinomugil corsula (Kutty and Peer Mohamed, 1975) at both temperatures. This also agrees well with the hypoxic condition in Tilapia mossambica (Peer Mohamed and Kutty, 1981) at 35°C. During recovery it showed a decrease from 38.65 mg/kg/hr to 19.15 mg/kg/hr and from 21.93 mg/kg/hr to 16.83 mg/kg/hr respectively, and again rose to 28.37 mg/kg/hr and 22.39 mg/kg/hr during the fifth hour.

Metabolic quotients

Quotients (R.Q. and A.Q.) showed an increasing trend during hypoxia at both temperatures. R.Q. increased from 2.06 to 3.61 at 30°C and from

1.31 to 2.60 at 35°C, while A.Q. rose from 0.45 to 0.94 at 30°C and 0.10 to 0.25 at 35°C. During recovery the R.Q. increased first from 3.05 to 3.52 and then declined to 2.65 at the fifth hour; and A.Q. declined from 0.86 to 0.24 at the fifth hour (both at 30°C). At 35°C the R.Q. increased from 1.36 to 2.67 but A.Q. decreased from 0.22 to 0.19. R.Q. at 30°C appeared to be similar to that of Rhinomugil corsula (Kutty and Peer Mohamed, 1975) and Tilapia mossambica (Peer Mohamed and Kutty, 1981) at the same temperature. Both R.Q. and A.Q. at 35°C compare well with the results for Tilapia mossambica at 35°C (Kutty and Peer Mohamed, 1975; Peer Mohamed and Kutty, 1981).

Activity

At 30°C during hypoxia, the fish showed trends similar to that of Rhinomugil corsula (Kutty and Peer Mohamed, 1975). At 30°C activity first increased from 10 counts/hour to 18.31 counts/hour during hypoxia, and it rose from 13.14 counts/hour to 17.25 counts/hour during recovery. However, at 35°C, although activity increased from 10.40 counts/hour to 28.40 counts/hour during hypoxia, it declined from 26.29 counts/hour to 19 counts/hour during recovery. The trend for 35°C in recovery phase is quite similar to that in Rhinomugil corsula (Kutty and Peer Mohamed, 1975) at 30°C.

C) EXPERIMENTS IN FRESHWATER

(i) Influence of random activity on metabolic rates and quotients at high ambient oxygen at 30 and 35°C

Regression lines fitted for plots of metabolic rates and quotients against random activity to show the trends at 30 and 35°C at ambient

oxygen concentration near air saturation are as shown in Figures 5 and 6. Average or mean values of routine and standard metabolic rates (extrapolated values to 0 activity), and random activity are included in Table 1. Regression equations of the same at 30 and 35°C are also shown in Figures 5 and 6. 'r' value or regression coefficient of carbondioxide production at 30°C was found to be significantly different at 1% level and A.Q. at 35°C was found to be significantly different at 5% level. All others did not show any significant difference.

The high rates of ambient oxygen in freshwater were found to be 4.26 ml/l and 4.15 ml/l at 30 and 35°C. Corresponding low rates were 1.88 ml/l and 1.89 ml/l. The related rates of oxygen consumption, carbon dioxide production, ammonia excretion, R.Q., A.Q. and activity observed at high and low rates of ambient oxygen at both temperatures are presented in Table 13.

(ii) **Influence of ambient oxygen (from air saturation down to asphyxial level) on metabolic rates, quotients and random activity at 30 and 35°C**

Trend lines of results for Lates calcarifer acclimated to 30 and 35°C and held under hypoxic condition are shown in Figures 11 and 12. The corresponding data are given in Tables 14 and 15. There is striking similarity in the patterns of data obtained for fish at both the temperatures.

Metabolism

Oxygen consumption was found to be increasing from 39.07 mg/kg/hr to 91.17 mg/kg/hr at 30°C and from 35.40 mg/kg/hr to 113.05 mg/kg/hr

Table 13. Measurements of metabolic rates, quotients and random activity in sea bass at 'high' and 'low' ambient oxygen at 30 and 35°C in freshwater. The 'high' oxygen refers to mean ambient oxygen concentration near air saturation and 'low' oxygen refers to lowest mean ambient oxygen concentration tested. Each value is the mean of 15 determinations. S.D. is also given.

	30°C		35°C	
	High oxygen	Low oxygen	High oxygen	Low oxygen
Mean of mean ambient oxygen (ml/l)	4.26(15) ± 0.44	1.88(15) ± 0.49	4.15(15) ± 0.40	1.89(15) ± 0.53
Mean rate of oxygen consumption (mg/kg/hr)	88.33(15) ± 30.34	51.52(15) ± 14.66	119.18(15) ± 29.35	37.76(15) ± 28.29
Mean rate of carbon dioxide production (mg/kg/hr)	168.87(15) ± 70.56	268.45(15) ± 97.43	275.25(15) ± 96.18	302.84(15) ± 72.93
Mean rate of ammonia excretion (mg/kg/hr)	317.37(15) ± 174.17	389.51(15) ± 325.16	862.55(15) ± 567.56	846.57(15) ± 412.48
Mean respiratory quotient (R.Q.)	2.62(15) ± 0.91	5.45(15) ± 1.83	2.36(15) ± 0.81	12.73(15) ± 9.93
Mean ammonia quotient (A.Q.)	3.22(15) ± 2.58	8.11(15) ± 6.13	7.91(15) ± 5.37	33.99(15) ± 28.08
Mean random activity (c/hr)	32.8(15) ± 17.19	17.33(15) ± 20.69	46.93(15) ± 19.42	15.2(15) ± 15.82

Table 14. Rates of oxygen consumption, carbon dioxide production, ammonia excretion, respiratory quotient, ammonia quotient and random activity in relation to mean ambient oxygen below air saturation in sea bass acclimated to and tested at 30°C in freshwater. Each value is the average of groups of data pertaining to 10 determinations (except the last group, 9 determinations) after arranging the total of 99 determinations in the ascending order of mean ambient oxygen to facilitate the analysis. Each run in an experiment lasted for 60 minutes. S.D. is also given.

Mean ambient oxygen (ml/l)	Mean rate of oxygen consumption (mg/kg/hr)	Mean of carbon dioxide production (mg/kg/hr)	Mean rate of ammonia excretion (mg/kg/hr)	Mean respiratory quotient (R.Q)	Mean ammonia quotient (A.Q.)	Mean random activity (counts/hour)
1.49 ± 0.28	39.07 ± 17.99	189.09 ± 92.16	10.69 ± 5.56	4.82 ± 2.32	0.35 ± 0.26	34.65 ± 18.92
2.06 ± 0.13	41.88 ± 17.02	177.00 ± 156.88	17.88 ± 17.40	3.70 ± 2.33	0.48 ± 0.40	28.56 ± 25.95
2.32 ± 0.06	70.71 ± 29.44	187.09 ± 101.21	17.74 ± 19.49	2.79 ± 1.51	0.30 ± 0.35	24.35 ± 19.89
2.58 ± 0.10	55.31 ± 21.98	131.91 ± 76.02	9.88 ± 7.07	2.61 ± 1.37	0.19 ± 0.13	26.40 ± 15.57
2.86 ± 0.07	88.73 ± 35.00	139.16 ± 86.23	9.98 ± 9.71	1.73 ± 1.12	0.15 ± 0.20	23.00 ± 12.19
3.03 ± 0.06	71.03 ± 19.43	131.98 ± 47.36	7.83 ± 3.90	1.99 ± 0.99	0.11 ± 0.07	23.60 ± 17.23
3.32 ± 0.11	102.61 ± 59.49	129.44 ± 48.04	9.10 ± 5.57	1.61 ± 1.12	0.10 ± 0.07	20.60 ± 13.03
3.59 ± 0.07	105.77 ± 56.67	173.32 ± 81.46	10.24 ± 11.82	1.84 ± 0.87	0.12 ± 0.17	32.00 ± 16.88
3.79 ± 0.07	87.27 ± 19.53	182.28 ± 45.48	13.78 ± 13.16	2.22 ± 0.74	0.16 ± 0.15	38.20 ± 13.77
4.54 ± 0.35	91.17 ± 35.16	160.24 ± 82.96	8.00 ± 4.11	1.87 ± 0.89	0.10 ± 0.07	26.22 ± 15.11

Table 15. Metabolic rates, quotients and random activity in relation to mean ambient oxygen below air saturation in sea bass acclimated to and tested at 35°C in freshwater. Each value is the average of groups of data pertaining to 7 determinations (except the last group, 13 determinations) after arranging the total of 76 determinations in the ascending order of mean ambient oxygen to facilitate the analysis. Each run lasted for 60 minutes. S.D. is also given.

Mean ambient oxygen (ml/l)	Mean rate of oxygen consumption (mg/kg/hr)	Mean of carbon dioxide production (mg/kg/hr)	Mean rate of ammonia excretion (mg/kg/hr)	Mean respiratory quotient (R.Q)	Mean ammonia quotient (A.Q.)	Mean random activity (counts/hour)
1.33 ± 0.37	35.40 ± 19.31	192.49 ± 140.84	29.81 ± 14.52	5.73 ± 3.32	1.06 ± 0.70	59.43 ± 11.06
2.15 ± 0.18	56.69 ± 57.59	144.37 ± 131.51	30.92 ± 14.57	3.29 ± 2.81	1.21 ± 1.08	50.71 ± 17.58
2.53 ± 0.13	62.41 ± 24.30	153.27 ± 48.81	17.27 ± 4.48	2.61 ± 0.74	0.32 ± 0.14	40.57 ± 18.09
2.86 ± 0.09	120.57 ± 43.82	249.80 ± 118.06	24.17 ± 8.93	2.28 ± 1.34	0.26 ± 0.24	50.57 ± 20.29
3.16 ± 0.09	134.91 ± 26.57	255.91 ± 122.87	21.36 ± 14.93	1.86 ± 0.66	0.17 ± 0.13	59.43 ± 37.43
3.39 ± 0.09	147.76 ± 23.09	276.41 ± 162.14	16.42 ± 8.78	1.86 ± 0.90	0.12 ± 0.08	55.14 ± 30.50
3.58 ± 0.03	143.02 ± 46.70	262.39 ± 151.80	12.22 ± 8.74	1.84 ± 0.79	0.09 ± 0.06	43.71 ± 15.64
3.68 ± 0.02	136.17 ± 19.03	277.86 ± 73.81	13.82 ± 8.42	2.05 ± 0.45	0.10 ± 0.06	48.86 ± 22.00
3.77 ± 0.04	135.40 ± 20.33	292.23 ± 89.39	11.95 ± 5.76	2.15 ± 0.55	0.09 ± 0.05	58.86 ± 30.98
4.21 ± 0.40	113.05 ± 25.68	211.38 ± 115.19	27.27 ± 15.16	1.84 ± 0.93	0.26 ± 0.15	44.92 ± 19.35

FIGURE 5

Regression lines fitted for metabolic rates and quotients in relation to random activity of sea bass acclimated to and tested in air saturated freshwater at 30°C. Each value plotted is a mean of observations obtained for 15 fishes. The lines fitted are based on the regression equations to show the trends. 'r' value for each parameter is also given. The details of fishes (15 fishes - fish No.L_{162} to L_{176}) are given in Table 26.

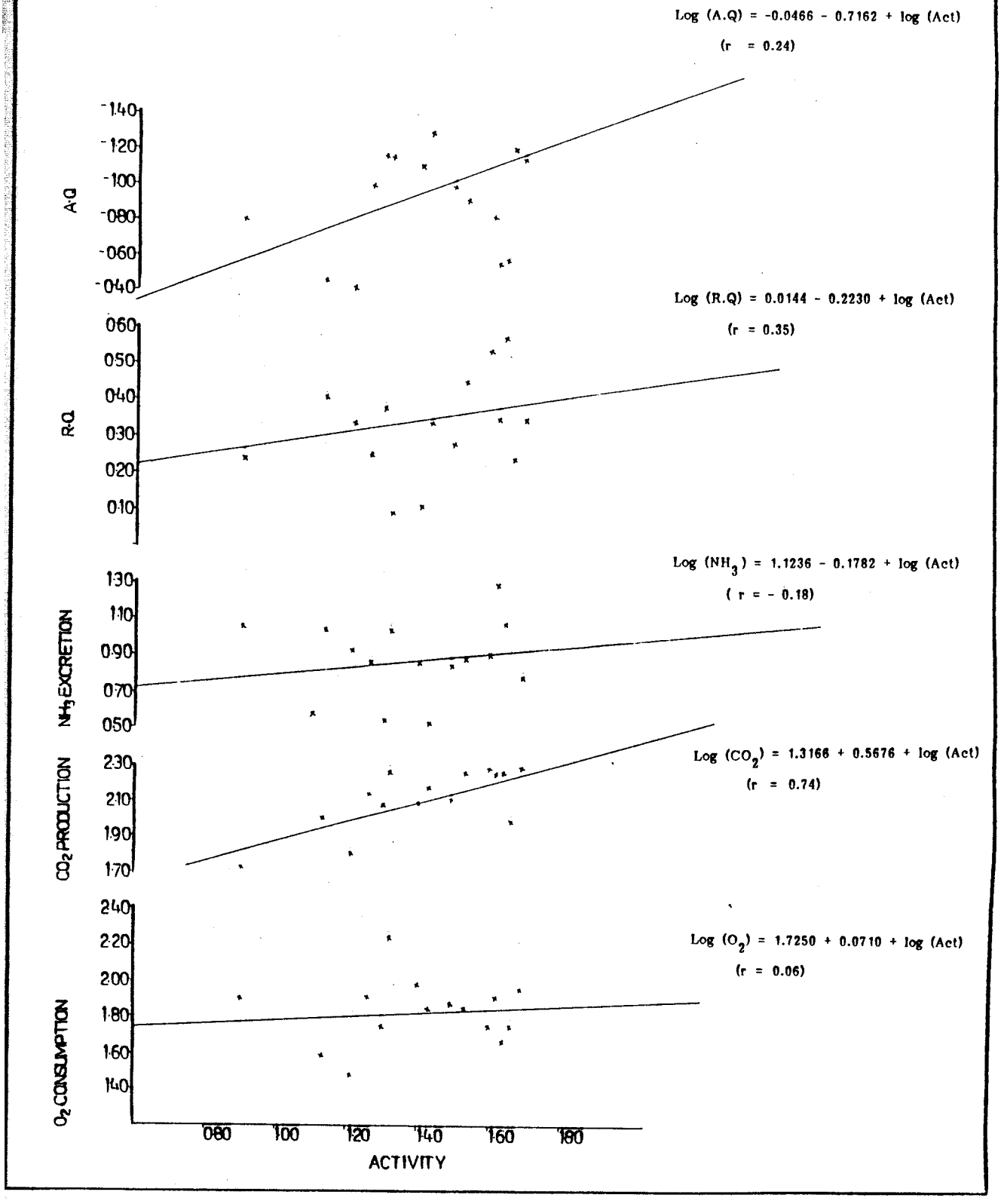

Log (A.Q) = -0.0466 - 0.7162 + log (Act)
(r = 0.24)
A·Q
·140
·120
·100
·080
·060
·040
Log (R.Q) = 0.0144 - 0.2230 + log (Act)
(r = 0.35)
R·Q
060
050
040
030
020
010
Log (NH_3) = 1.1236 - 0.1782 + log (Act)
(r = - 0.18)
NH_3 EXCRETION
130
110
090
070
050
Log (CO_2) = 1.3166 + 0.5676 + log (Act)
(r = 0.74)
CO_2 PRODUCTION
230
210
190
170
Log (O_2) = 1.7250 + 0.0710 + log (Act)
(r = 0.06)
O_2 CONSUMPTION
240
220
200
180
160
140
080
100
120
140
160
180
ACTIVITY

FIGURE 6

Regression lines fitted for metabolic rates and quotients in relation to random activity of sea bass acclimated to and tested in air saturated freshwater at 35°C, based on the equations given, are presented to show the trend lines. Each value plotted is the mean of observations obtained for 15 fishes. The details of fishes (15 fishes - fish No. L_{192} to L_{206}) are given in Table 26. Correlation coefficient ('r' value) for each parameter is also given.

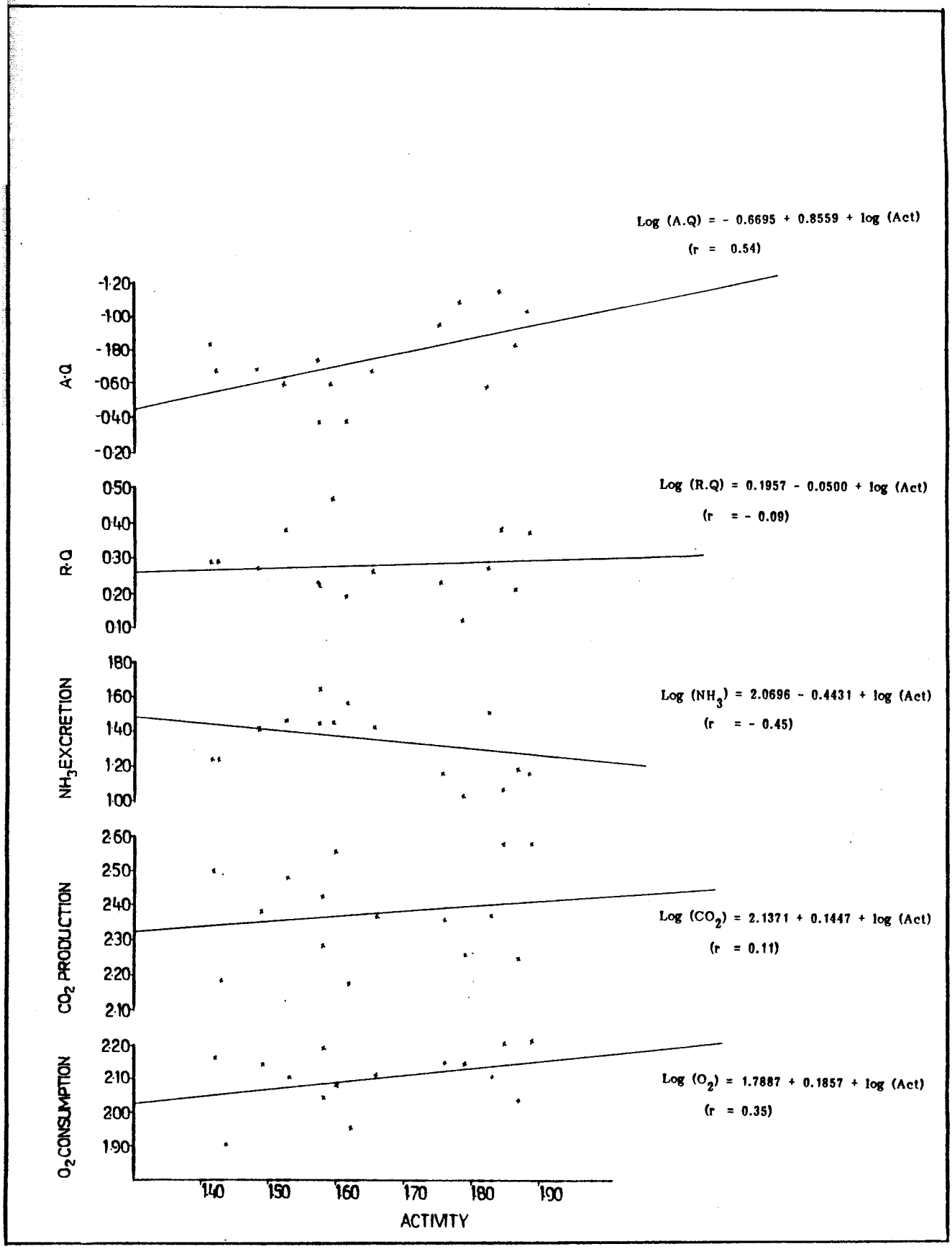

Log (A.Q) = - 0.6695 + 0.8559 + log (Act)
(r = 0.54)
A·Q
-1·20
-100
-180
-060
-040
-020
Log (R.Q) = 0.1957 - 0.0500 + log (Act)
(r = - 0.09)
R·Q
050
040
030
020
010
Log (NH3) = 2.0696 - 0.4431 + log (Act)
(r = - 0.45)
NH3 EXCRETION
180
160
140
120
100
Log (CO2) = 2.1371 + 0.1447 + log (Act)
(r = 0.11)
CO2 PRODUCTION
260
250
240
230
220
210
Log (O2) = 1.7887 + 0.1857 + log (Act)
(r = 0.35)
O2 CONSUMPTION
220
210
200
190
140
150
160
170
180
190
ACTIVITY

FIGURE 11

Trends pertaining to metabolic rates, quotients and random activity in relation to ambient oxygen below air saturation, in sea bass acclimated to and tested at 30°C in freshwater based on Table 14. The details of fishes used (15 fishes - Fish No.L_{177} to L_{191}) are shown in Table 26.

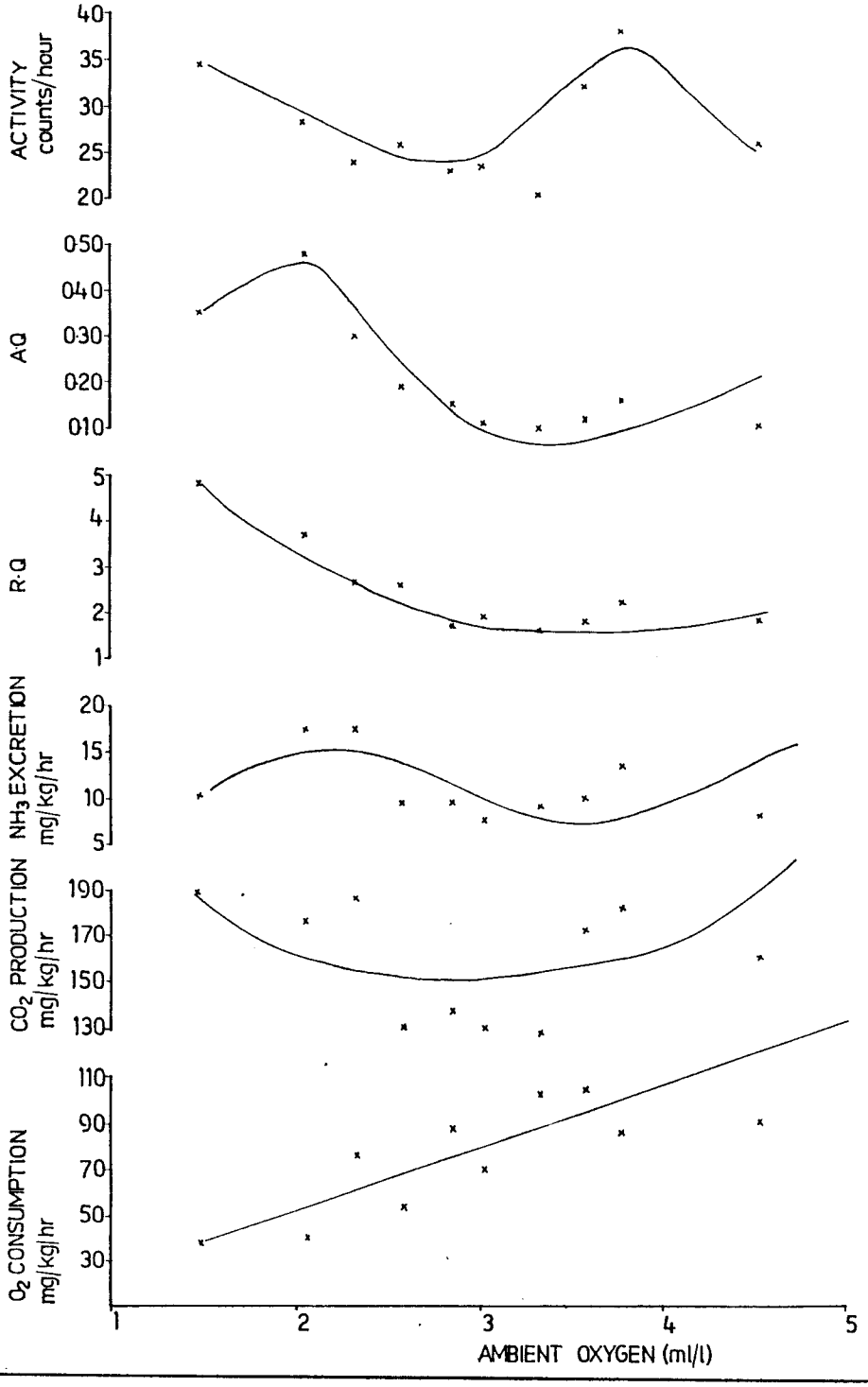
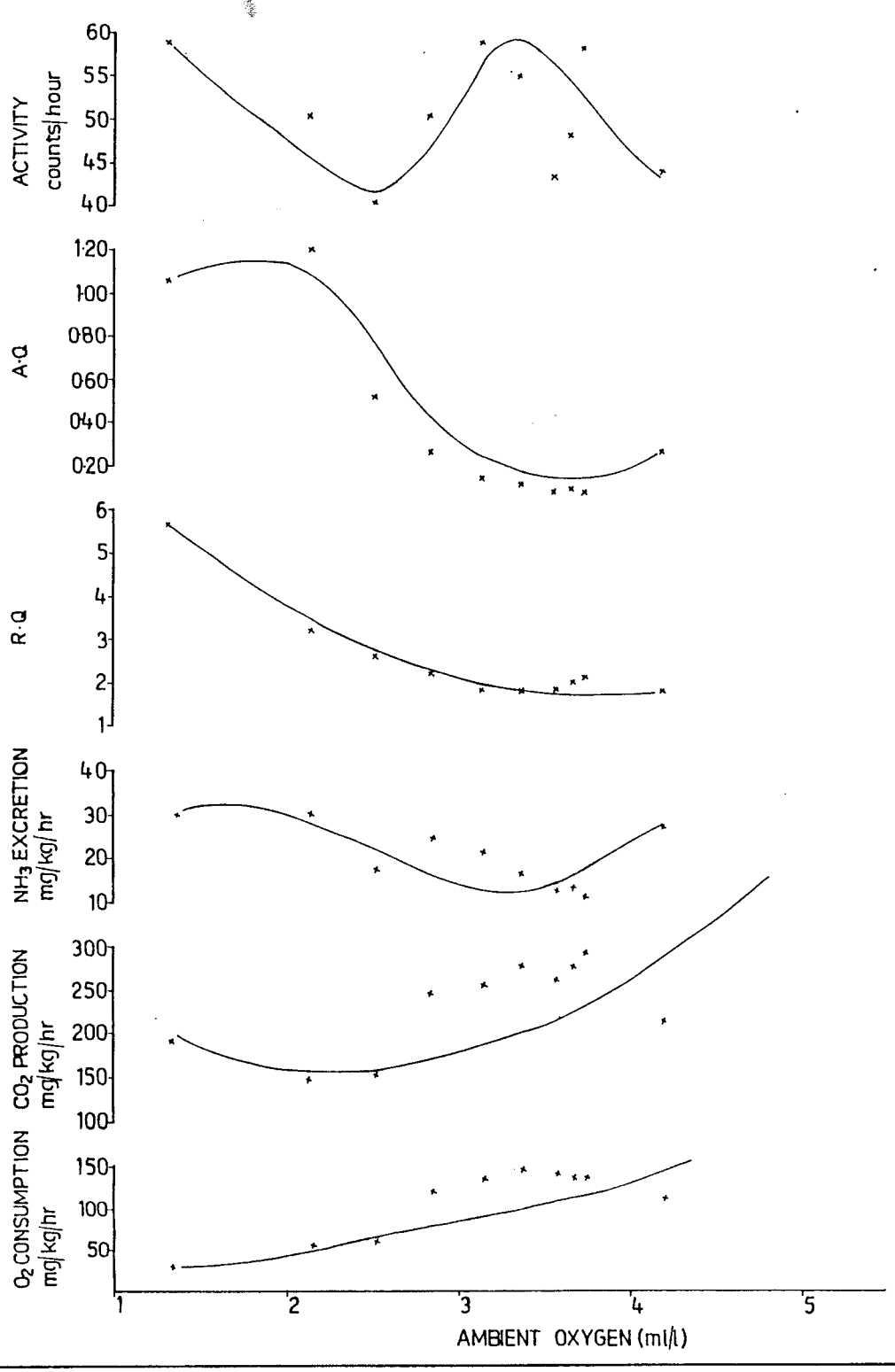

ACTIVITY counts/hour
40
35
30
25
20
AQ
0·50
0·40
0·30
0·20
0·10
R·Q
5
4
3
2
1
NH_3 EXCRETION mg/kg/hr
20
15
10
5
CO_2 PRODUCTION mg/kg/hr
190
170
150
130
O_2 CONSUMPTION mg/kg/hr
110
90
70
50
30
1
2
3
4
5
AMBIENT OXYGEN (ml/l)

FIGURE 12

Trends pertaining to metabolic rates, quotients and random activity in relation to ambient oxygen below air saturation, in sea bass acclimated to and tested at 35°C in freshwater based on Table 15. The details of fishes used (15 fishes - Fish No.L_{207} to L_{221}) are given in Table 26.


ACTIVITY counts/hour
60
55
50
45
40
A·Q
1·20
1·00
0·80
0·60
0·40
0·20
R·Q
6
5
4
3
2
1
NH3 EXCRETION mg/kg/hr
40
30
20
10
CO2 PRODUCTION mg/kg/hr
300
250
200
150
100
O2 CONSUMPTION mg/kg/hr
150
100
50
1
2
3
4
5
AMBIENT OXYGEN (ml/l)

at 35°C. Carbondioxide production showed a decreasing trend from 189.09 mg/kg/hr to 160.24 mg/kg/hr at 30°C whereas in the same medium at 35°C it showed an increase from 192.49 mg/kg/hr to 292.23 mg/kg/hr and then dropped down to 211.38 mg/kg/hr. Ammonia excretion was found to be deviating from a maximum of 10.69 mg/kg/hr to a minimum of 8.00 mg/kg/hr based on varying ambient oxygen. As the ambient oxygen increased, the ammonia excretion increased first from 10.69 mg/kg/hr to 17.88 mg/kg/hr and then decreased to 7.83 mg/kg/hr and further rose to 8 mg/kg/hr at 30°C in freshwater. When the temperature was increased to 35°C ammonia excretion was observed to decrease from 29.81 mg/kg/hr to 12.22 mg/kg/hr with a further increase to 27.27 mg/kg/hr.

Metabolic quotients

The trend at 30 and 35°C in R.Q. was in accordance with the trend in carbondioxide production. R.Q. also resembles the trend in Rhinomugil corsula (at 30 and 35°C) (Kutty and Peer Mohamed, 1975). It showed a decrease from 4.82 to 1.87 with the increase of ambient oxygen at 30°C and at 35°C this was found to decrease from 5.73 to 1.84. An alternate increase and decrease was shown by the A.Q. values at 30 and 35°C. At 30°C it first increased from 0.35 to 0.48 and then decreased to a minimum of 0.10. At 35°C and 0‰ salinity an increase was observed from 1.06 to 1.21. A.Q. then dropped to 0.09 from 0.32 and further rose to 0.26.

Activity

Activity showed an alternate decrease and increase from 34.65 counts/hour to 23 counts/hour and from 23.60 counts/hour to 38.20 counts/hour

respectively and again declined to 26.22 counts/hour at 30°C. A similar decreasing trend was also observed at 35°C from 59.43 counts/per hour to 40.57 counts/hour followed by an increase to 58.86 counts/hour and a further drop to 44.92 counts/hour.

Asphyxial oxygen

A value of 1.54 ml/l at 30°C and 1.60 ml/l at 35°C (Table 5) were observed as the mean values of asphyxial oxygen in freshwater. The maximum and minimum rates detected were 3.1 ml/l (Fish weight, 30.00 g) and 0.5 ml/l (Fish weight, 34.20 g) at 30°C. The corresponding rates at 35°C were 2.5 ml/l (Fish weight, 40.34 g) and 0.6 ml/l (Fish weight, 40.22 g).

(iii) **Metabolic rates, quotients and random activity during hypoxia and recovery at 30 and 35°C**

Mean values of metabolism and random activity of the test species at 30 and 35°C are summarised in Tables 16 and 17. The graphical representation of the same are presented in Figures 17 and 18.

Metabolism

As in the case of both sea water and brackish water, mean ambient oxygen showed a decreasing trend which resembles the trend in Rhinomugil corsula at 30 and 35°C during hypoxia. Mean ambient oxygen dropped during hypoxia, from 3.46 ml/l to 1.84 ml/l and from 3.34 ml/l to 1.89 ml/l at both temperatures respectively but during recovery at 30°C first it increased from 3.25 ml/l to 3.42 ml/l and then slightly declined to about 3.34 ml/l. At 35°C, it showed an opposite trend by decreasing from 3.66 ml/l to

Table 16. Metabolic rates, quotients and random activity in sea bass acclimated to and tested at 30°C in freshwater during hypoxia and recovery. Each value is the average of 15 determinations (except the last run, 13 determinations) of the total of 73 determinations after arranging them in sequential order of runs in hours in experiments for 15 fishes to facilitate the analysis. Each run lasted for 60 minutes except the last run where the fish lost its equilibrium. S.D. is also given.

Experimental runs (in hours)	Mean ambient oxygen (ml/l)	Mean rate of oxygen consumption (mg/kg/hr)	Mean rate of carbon dioxide production (mg/kg/hr)	Mean rate of ammonia excretion (mg/kg/hr)	Mean respiratory quotient (R.Q)	Mean ammonia quotient (A.Q.)	Mean random activity (counts/hour)	Remarks
I	3.46 ± 0.61	101.63 ± 40.75	198.18 ± 87.68	14.90 ± 13.47	2.12 ± 0.71	0.19 ± 0.22	29.87 ± 16.81	Hypoxia
II	1.84 ± 0.47	54.50 ± 14.45	266.94 ± 94.35	23.31 ± 18.34	5.14 ± 1.86	0.46 ± 0.38	40.07 ± 17.86	
III	3.25 ± 0.95	91.49 ± 41.37	166.03 ± 62.12	10.59 ± 10.77	2.33 ± 1.74	0.14 ± 0.15	38.27 ± 18.45	Recovery
IV	3.42 ± 0.89	89.41 ± 29.19	173.27 ± 70.81	18.82 ± 20.12	1.96 ± 0.67	0.29 ± 0.46	43.73 ± 19.86	
V	3.34 ± 0.48	79.37 ± 17.34	219.74 ± 49.86	22.04 ± 20.43	2.81 ± 0.52	0.30 ± 0.34	49.92 ± 20.45	

Table 17. Metabolic rates, quotients and random activity in sea bass acclimated to and tested at 35°C in freshwater during hpoxia and recovery. Each value is the average of 15 determinations of the total of 75 determinations arranged in the sequential order of runs in hours in experiments for 15 fishes to facilitate the analysis. Each run lasted for 60 minutes except the last run where the fish last its equilibrium. S.D. is also given.

Experimental runs (in hours)	Mean ambient oxygen (ml/l)	Mean rate of oxygen consumption (mg/kg/hr)	Mean rate of carbon dioxide produc- (mg/kg/hr)	Mean rate of ammonia excretion (mg/kg/hr)	Mean respiratory quotient (R.Q.)	Mean ammonia quotient (A.Q)	Mean random activity (counts/hour)	Remarks
I	3.34 ± 0.84	115.14 ± 48.49	178.03 ± 63.63	14.32 ± 10.22	1.71 ± 0.90	0.17 ± 0.19	38.13 ± 18.46	Hypoxia
II	1.89 ± 0.53	37.76 ± 28.29	147.62 ± 124.19	29.18 ± 13.79	4.20 ± 2.91	1.12 ± 0.84	56.33 ± 13.86	
III	3.66 ± 0.57	143.75 ± 30.87	217.45 ± 52.32	16.22 ± 11.04	1.59 ± 0.51	0.11 ± 0.07	41.07 ± 18.33	Recovery
IV	3.33 ± 0.68	126.05 ± 47.38	220.92 ± 83.87	21.43 ± 11.19	1.89 ± 0.83	0.22 ± 0.22	43.07 ± 14.42	
V	3.57 ± 0.53	119.70 ± 31.73	266.68 ± 65.87	27.03 ± 15.83	2.38 ± 0.84	0.24 ± 0.15	47.47 ± 11.58	

FIGURE 17

Trends pertaining to oxygen consumption, carbon dioxide production, ammonia excretion, R.Q., A.Q. and random activity of sea bass acclimated to and tested at 30°C in freshwater during hypoxia and recovery based on Table 16. The details of fishes used (Fish No.L_{177} to L_{191}) are given in Table 26.

AMBIENT OXYGEN
ml/l
1
2
3
4
O2CONSUMPTION
mg/kg/hr
50
70
90
110
CO2PRODUCTION
mg/kg/hr
160
200
240
280
NH3EXCRETION
mg/kg/hr
10
15
20
25
R·Q
2
4
6
A·Q
010
020
030
040
050
ACTIVITY
counts/hour
20
30
40
50
HOURS
1
2
3
4
5

FIGURE 18

Trends pertaining to metabolic rates, quotients and random activity of sea bass acclimated to and tested at 35°C in freshwater during hypoxia and recovery based on Table 17. The details of fishes used are (Fish No.L_{207} to L_{221}) given in Table 26.

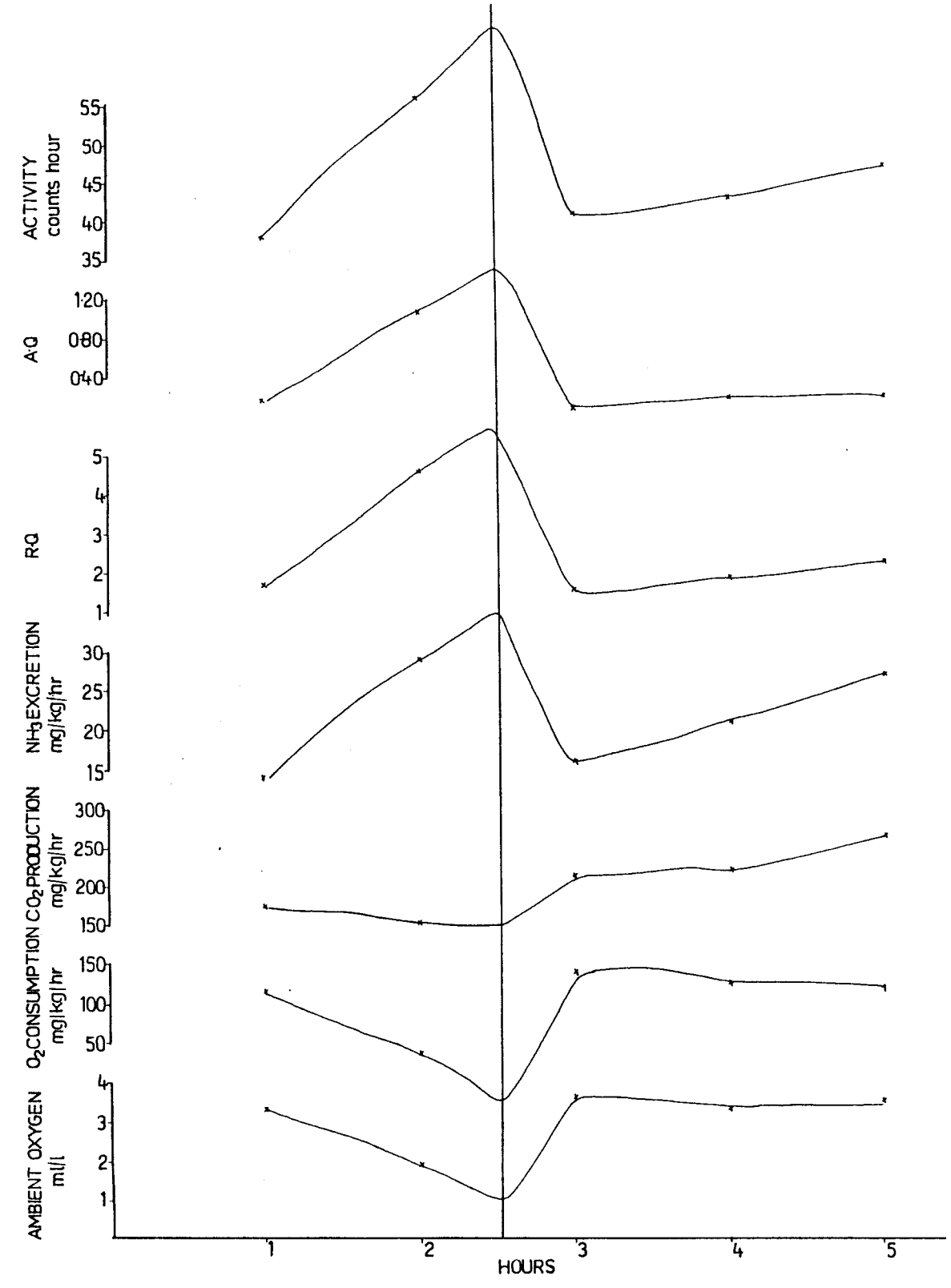

ACTIVITY counts hour
55
50
45
40
35
A:Q
1·20
0·80
0·40
R·Q
5
4
3
2
1
NH3 EXCRETION mg/kg/hr
30
25
20
15
CO2 PRODUCTION mg/kg/hr
300
250
200
150
O2 CONSUMPTION mg/kg/hr
150
100
50
AMBIENT OXYGEN ml/l
4
3
2
1
1
2
3
4
5
HOURS

Table 26. Details of fish used for experiments. Each fish is given a code number. 'L' denotes Lates calcarifer.

Fish No.	Fish length cm	Fish weight g	Fish No.	Fish length cm	Fish weight g
L_1	8.70	7.68	L_{23}	12.10	27.55
L_2	9.50	7.42	L_{24}	12.15	25.64
L_3	9.60	7.30	L_{25}	12.00	28.09
L_4	9.65	12.62	L_{26}	12.10	27.20
L_5	9.67	12.00	L_{27}	13.30	31.70
L_6	9.65	11.65	L_{28}	11.80	29.38
L_7	9.70	12.54	L_{29}	12.90	28.54
L_8	11.80	32.61	L_{30}	12.80	28.60
L_9	11.85	30.80	L_{31}	13.50	34.07
L_{10}	11.90	32.45	L_{32}	13.30	33.98
L_{11}	11.95	33.34	L_{33}	13.20	31.78
L_{12}	11.80	31.45	L_{34}	13.40	34.88
L_{13}	11.85	32.10	L_{35}	13.30	32.68
L_{14}	11.80	32.33	L_{36}	15.00	43.35
L_{15}	11.95	34.61	L_{37}	13.00	32.92
L_{16}	11.80	33.78	L_{38}	13.20	32.10
L_{17}	11.80	31.50	L_{39}	15.00	46.19
L_{18}	11.90	32.63	L_{40}	13.40	35.00
L_{19}	12.00	26.26	L_{41}	13.50	38.14
L_{20}	12.10	27.43	L_{42}	13.30	32.86
L_{21}	12.10	25.80	L_{43}	13.40	33.81
L_{22}	12.05	24.32	L_{44}	13.40	33.04

Table 26. contd.

Fish No.	Fish length cm	Fish weight g	Fish No.	Fish length cm	Fish weight g
L_{45}	11.90	32.95	L_{68}	15.00	38.10
L_{46}	11.95	30.80	L_{69}	13.20	32.33
L_{47}	11.90	32.40	L_{70}	13.50	34.29
L_{48}	11.80	31.33	L_{71}	15.20	45.52
L_{49}	11.85	32.10	L_{72}	13.70	34.33
L_{50}	11.90	31.70	L_{73}	13.60	38.01
L_{51}	13.20	32.60	L_{74}	13.70	35.20
L_{52}	13.25	30.50	L_{75}	13.70	34.21
L_{53}	13.20	31.20	L_{76}	13.80	39.24
L_{54}	13.30	29.90	L_{77}	10.80	15.98
L_{55}	12.20	23.77	L_{78}	10.90	14.43
L_{56}	13.30	33.96	L_{79}	7.00	5.77
L_{57}	15.20	44.80	L_{80}	15.30	45.89
L_{58}	13.50	35.10	L_{81}	13.70	31.94
L_{59}	13.50	34.90	L_{82}	13.80	28.95
L_{60}	13.10	30.52	L_{83}	13.70	24.35
L_{61}	11.80	28.86	L_{84}	12.00	16.85
L_{62}	13.30	32.02	L_{85}	12.20	18.85
L_{63}	15.10	44.14	L_{86}	12.10	19.78
L_{64}	13.30	31.01	L_{87}	15.40	41.05
L_{65}	15.00	41.80	L_{88}	15.50	40.93
L_{66}	13.20	33.40	L_{89}	14.45	40.80
L_{67}	15.00	40.20	L_{90}	10.50	19.98

Table 26. contd.

Fish No.	Fish length cm	Fish weight g	Fish No.	Fish length cm	Fish weight g
L_{91}	14.70	33.30	L_{114}	13.00	27.74
L_{92}	14.60	33.25	L_{115}	13.50	24.32
L_{93}	15.70	33.34	L_{116}	14.10	40.88
L_{94}	11.40	15.13	L_{117}	13.20	36.80
L_{95}	11.45	13.77	L_{118}	14.70	46.17
L_{96}	11.40	16.92	L_{119}	15.00	45.98
L_{97}	14.20	37.26	L_{120}	16.90	66.28
L_{98}	14.10	34.77	L_{121}	11.00	18.02
L_{99}	10.90	5.80	L_{122}	11.10	17.70
L_{100}	11.10	5.72	L_{123}	13.80	32.89
L_{101}	11.10	4.68	L_{124}	11.10	15.70
L_{102}	11.05	5.80	L_{125}	10.50	11.60
L_{103}	10.90	15.52	L_{126}	10.30	23.46
L_{104}	7.32	7.90	L_{127}	13.30	30.10
L_{105}	8.10	6.37	L_{128}	14.30	38.69
L_{106}	8.00	6.60	L_{129}	11.20	19.88
L_{107}	8.05	6.00	L_{130}	11.25	19.59
L_{108}	7.80	7.15	L_{131}	14.20	35.89
L_{109}	9.60	12.28	L_{132}	14.30	35.89
L_{110}	10.00	9.07	L_{133}	11.90	20.00
L_{111}	10.30	13.17	L_{134}	11.30	18.27
L_{112}	12.00	12.72	L_{135}	11.40	18.20
L_{113}	11.40	18.62	L_{136}	11.70	20.39

Table 26. contd.

Fish No.	Fish length cm	Fish weight g	Fish No.	Fish length cm	Fish weight g
L 137	13.50	28.89	L 160	12.20	24.74
L 138	11.80	22.90	L 161	12.00	26.09
L 139	11.90	23.60	L 162	16.10	49.54
L 140	13.00	20.12	L 163	16.20	61.54
L 141	13.10	28.39	L 164	17.00	80.65
L 142	13.20	26.31	L 165	16.00	59.40
L 143	12.80	19.70	L 166	16.20	60.43
L 144	12.90	18.85	L 167	16.10	41.54
L 145	11.10	21.29	L 168	16.20	60.42
L 146	12.30	27.60	L 169	16.25	59.10
L 147	13.30	24.31	L 170	15.90	62.00
L 148	12.40	28.19	L 171	15.80	60.00
L 149	12.30	26.46	L 172	16.00	59.30
L 150	12.20	25.42	L 173	15.90	61.12
L 151	12.30	25.34	L 174	15.80	61.04
L 152	11.50	22.54	L 175	16.10	58.82
L 153	11.80	22.50	L 176	15.90	59.25
L 154	10.30	15.62	L 177	14.50	35.00
L 155	12.40	24.35	L 178	14.40	33.80
L 156	11.90	21.59	L 179	13.20	30.00
L 157	12.00	21.23	L 180	14.20	34.20
L 158	12.00	25.66	L 181	14.30	34.00
L 159	12.10	23.81	L 182	14.80	58.20

Table 26. contd.

Fish No.	Fish length cm	Fish weight g	Fish No.	Fish length cm	Fish weight g
L_{183}	14.60	56.92	L_{207}	14.70	38.00
L_{184}	14.80	60.00	L_{208}	13.80	33.00
L_{185}	14.70	55.40	L_{209}	14.80	41.00
L_{186}	14.80	60.23	L_{210}	14.70	40.22
L_{187}	14.60	58.41	L_{211}	14.80	41.43
L_{188}	14.70	46.64	L_{212}	14.90	42.00
L_{189}	14.60	57.92	L_{213}	14.00	37.61
L_{190}	14.80	61.29	L_{214}	14.70	39.00
L_{191}	14.80	60.83	L_{215}	14.80	40.34
L_{192}	14.30	33.71	L_{216}	14.10	38.00
L_{193}	14.60	36.20	L_{217}	14.60	57.04
L_{194}	14.50	34.70	L_{218}	14.80	42.84
L_{195}	13.70	31.12	L_{219}	14.70	38.39
L_{196}	14.70	36.51	L_{220}	14.90	43.82
L_{197}	14.00	37.52	L_{221}	14.70	39.26
L_{198}	14.70	39.10			
L_{199}	14.80	40.21			
L_{200}	14.10	37.90			
L_{201}	14.80	39.80			
L_{202}	14.60	57.01			
L_{203}	14.70	40.32			
L_{204}	14.80	38.14			
L_{205}	14.70	36.58			
L_{206}	14.70	39.18			

3.33 ml/l and then increasing to about 3.57 ml/l during recovery. Hypoxia at 30°C is quite similar to that of Tilapia mossambica (Peer Mohamed, 1974; Peer Mohamed and Kutty, 1981) and Mystus armatus at the same temperature (Sukumaran and Kutty, 1977). Hypoxia at 35°C also is similar to that of hypoxia in Tilapia mossambica at 35°C (Peer Mohamed, 1974 and Peer Mohamed and Kutty, 1981).

Oxygen consumption at both the temperatures declined in a similar pattern to the values for sea water. This was also the case for Rhinomugil corsula as reported by Kutty and Peer Mohamed, 1975. During hypoxia oxygen consumption declined from 101.63 mg/kg/hr to 54.50 mg/kg/hr and 115.14 mg/kg/hr to 37.76 mg/kg/hr at 30 and 35°C; and during recovery from 91.49 mg/kg/hr to 79.37 mg/kg/hr and from 143.75 mg/kg/hr to 119.70 mg/kg/hr respectively. Hypoxia at 30°C resembles that of Tilapia mossambica (Peer Mohamed and Kutty, 1981) and recovery at 30°C resembles the pattern for Tilapia mossambica (Peer Mohamed and Kutty, 1981) as well as Mystus armatus (Sukumaran and Kutty, 1977). Hypoxia at 35°C is similar to that of Rhinomugil corsula (Kutty and Peer Mohamed, 1975) and Tilapia mossambica (Peer Mohamed and Kutty, 1981) and the recovery compares well with that of Tilapia mossambica (Kutty and Peer Mohamed, 1975).

The trend in carbondioxide production at 30°C showed an increase from 198.18 mg/kg/hr to 266.94 mg/kg/hr during hypoxia. This resembles the hypoxic phase in Tilapia mossambica (Peer Mohamed and Kutty, 1981). But when the temperature was raised to 35°C it showed a decrease from 178.03 mg/kg/hr to 147.62 mg/kg/hr; whereas in recovery phase at 30°C

it increased from 166.03 mg/kg/hr to 219.74 mg/kg/hr and at 35°C from 217.45 mg/kg/hr to 266.68 mg/kg/hr.

As in sea water, ammonia excretion showed an increasing trend in hypoxia which resembles the trend in Rhinomugil corsula at both the temperatures. Ammonia excretion shot up from 14.90 mg/kg/hr to 23.31 mg/kg/hr at 30°C and from 14.32 mg/kg/hr to 29.18 mg/kg/hr at 35°C during hypoxia and it also showed a similar trend in recovery metabolism by increasing from 10.59 mg/kg/hr to 22.04 mg/kg/hr at 30°C and from 16.22 mg/kg/hr to 27.03 mg/kg/hr at 35°C. Hypoxia at 35°C compares well with the results for Tilapia mossambica (Peer Mohamed and Kutty, 1981).

Metabolic quotients

With an increase in the carbondioxide output at 30°C the R.Q. also showed a corresponding increase from 2.12 to 5.14 during hypoxia whereas in recovery phase it first decreased from 2.33 to 1.96 and again increased to 2.81 at the fifth hour. When the temperature was increased to 35°C R.Q. showed an increase from 1.71 to 4.20 during hypoxic phase and from 1.59 to 2.38 during recovery phase. In all the treatments at 30 and 35°C the trend in A.Q. also showed a corresponding increase from 0.19 to 0.46 at 30°C and from 0.17 to 1.12 at 35°C with the increase of ammonia excretion. During recovery it showed an increase from 0.14 to 0.30 at 30°C and from 0.11 to 0.24 at 35°C. R.Q. at 30 and 35°C during hypoxia resembles values for Tilapia mossambica (Peer Mohamed, 1974; Peer Mohamed and Kutty, 1981) and the R.Q. at 30°C during recovery resembles that of

Tilapia mossambica (Peer Mohamed and Kutty, 1981). Similarly A.Q. at 30°C during recovery resembles that of Tilapia mossambica (Peer Mohamed and Kutty, 1981) and A.Q. at 30°C during hypoxia resembles values obtained for Mystus armatus (Sukumaran and Kutty, 1977) and at 35°C it agrees well with the results for Rhinomugil corsula (Kutty and Peer Mohamed, 1975).

Activity

In freshwater, activity showed a similar increasing trend during hypoxia and recovery at both the temperatures, as in sea water and brackish water. At 30°C during hypoxia, activity showed an increase from 28.87 counts/hour to 40.07 counts/hour and during recovery it showed an increase from 38.27 counts/hour to 49.92 counts/hour. But when the temperature was changed to 35°C the activity also increased from 38.13 counts/hour to 56.33 counts/hour during hypoxia and 41.07 counts/hour to 47.47 counts/hour during recovery. The increasing trend in hypoxic phase at both the temperatures and the increasing trend in recovery phase at 35°C are in agreement with the results for Rhinomugil corsula (Kutty and Peer Mohamed, 1975). Activity at 35°C also resembles that of Tilapia mossambica (Peer Mohamed and Kutty, 1981).

d. **Statistical analysis**

To test the differences between means of different environments, temperature levels and experiments, analysis of variance was carried out for each study character using the following model:

$$Y_{ijkl} = \mu + a_i + b_j + c_k + [ab]_{ij} + [ac]_{ik} + [bc]_{jk} + e_{ijkl}$$ where

Y ijkl is the l th observation value of the study character of the k th experiment at the j th temperature level in the i th environment.

[i = 1,2,3 ; j=1,2 ; k=1,2,3]

u = overall mean

ai = effect of i th environment

bj = effect of j th temperature level

c k = effect of k th experiment

(ab)ij = interaction-effect of environment and temperature

(ac)ik = interaction-effect of environment and experiment

(bc)jk = interaction-effect of temperature and experiment

e ijkl = random error component

The results of ANOVA are summarized in Tables 18 a to 18 g.

Analysis of variance (Table 18a) showed that mean ambient oxygen was found to be significantly different at 1% level between the experiments and the interaction of temperature to experiment. Environment, temperature and the interactions between them were not significant.

The average oxygen consumption (Table 18b) was found to be significantly different between environments and so also among temperature levels. However among experiments there was no significant difference.

The effects of environments and its (carbondioxide production) interaction with environment and the interaction of temperature with experiment were found to be significant which indicates that mean carbondioxide production (Table 18c) was significantly different between environments.

The ammonia excreted (Table 18d) by the fish was significantly

Table 18a. Analysis of Variance : ANOVA

Mean ambient oxygen

Source	Degree of freedom	Sum of squares	Mean sum of squares	F	
Environment	2	2.39	1.19	1.45	NS
Temperature	1	0.90	0.90	1.08	NS
Experiment	2	14.76	7.38	8.84	**
Environment by temperature	2	0.01	0.005	0.01	NS
Environment by Experiment	4	6.58	1.64	1.97	NS
Temperature by Experiment	2	17.69	8.85	10.60	**
Within cells	277	231.25	0.83		

NOTE: ** Indicates significance at 1% level

NS indicates Non-significance

Table 18b. Analysis of variance : ANOVA

Oxygen consumption

Source	Degree of freedom	Sum of squares	Mean sum of squares	F	
Environment	2	132779.93	66389.97	16.86	**
Temperature	1	19005.84	19005.84	4.83	**
Experiment	2	12570.20	6285.10	1.60	NS
Environment by temperature	2	7155.56	3577.78	0.90	NS
Environment by Experiment	4	61981.20	15495.30	3.94	*
Temperature by Experiment	2	2744.25	1372.12	0.35	NS
Within cells	277	1090521.30	3936.90		

NOTE : * indicates significance at 5% level

** indicates significance at 1% level

NS indicates Non-significance

Table 18c. Analysis of Variance : ANOVA

Carbon dioxide production

Source	Degree of freedom	Sum of squares	Mean sum of squares	F	
Environment	2	470899.90	235449.95	12.64	**
Temperature	1	796.27	796.27	0.04	NS
Experiment	2	42570.95	21285.48	1.14	NS
Environment by temperature	2	152005.57	76002.78	4.08	*
Environment by experiment	4	95395.27	23848.82	1.28	NS
Temperature by experiment	2	163547.08	81773.54	4.39	*
Within cells	277	5159170.50	18625.16		

NOTE : * indicates significance at 5% level

** indicates significance at 1% level

NS indicates Non-significance

Table 18d. Analysis of Variance : ANOVA

Ammonia Excretion

Source	Degree of freedom	Sum of squares	Mean sum of squares	F	
Environment	2	2784.43	1392.21	6.98	**
Temperature	1	29.15	29.15	0.15	NS
Experiment	2	1272.55	636.28	3.19	*
Environment by Temperature	2	1794.07	897.04	4.50	*
Environment by Experiment	4	3506.73	876.68	4.40	**
Temperature by Experiment	2	1491.35	745.67	3.74	*
Within cells	277	55230.64	199.39		

NOTE : * indicates significance at 5% level

** indicates significance at 1% level

NS indicates Non-significance

Table 18e. Analysis of Variance : ANOVA

R.Q. (Respiratory Quotient)

Source	Degree of freedom	Sum of squares	Mean sum of squares	F	
Environment	2	10844.80	5422.40	1.09	NS
Temperature	1	6049.95	6049.95	1.22	NS
Experiment	2	10724.84	5362.42	1.08	NS
Environment by Temperature	2	10316.82	5158.41	1.04	NS
Environment by Experiment	4	23838.32	5959.58	1.20	NS
Temperature by Experiment	2	11555.05	5777.52	1.16	NS
Within cells	277	1379274.89	4979.33		

NOTE : NS indicates Non-significance

Table 18f. Analysis of Variance : ANOVA

A.Q. (Ammonia Quotient)

Source	Degree of freedom	Sum of squares	Mean sum of squares	F	
Environment	2	1.58	0.79	2.24	NS
Temperature	1	1.09	1.09	3.10	NS
Experiment	2	0.79	0.39	1.12	NS
Environment by Temperature	2	2.12	1.06	3.00	NS
Environment by experiment	4	4.30	1.08	3.04	*
Temperature by experiment	2	1.51	0.75	2.13	NS
Within cells	277	97.86	0.35		

NOTE : * indicates significance at 5% level

NS indicates Non-significance

Table 18g. Analysis of Variance : ANOVA

Activity

Source	Degree of freedom	Sum of squares	Mean sum of squares	F	
Environment	2	21660.98	10830.49	48.95	**
Temperature	1	694.88	694.88	3.14	NS
Experiment	2	3649.71	1824.86	8.25	**
Environment by Temperature	2	2918.29	1459.14	6.59	**
Environment by experiment	4	5356.60	1339.15	6.05	**
Temperature by experiment	2	2834.38	1417.19	6.41	**
Within cells	277	61287.02	221.25		

NOTE : ** indicates significance at 1% level

NS indicates Non-significance

different both with the environment and the interaction of ammonia excretion with the experiment at 1% level. Similarly it was significantly different with experiment and the interactions of temperature with both environment and experiment at 5% level. The temperature individually did not show any significant difference.

However, the analysis of covariance showed that environment and experiment individually; and the interaction of environment with temperature and experiment; and the interaction of temperature with experiment alone were found to be significantly different at 1% level (Table 18h).

R.Q., A.Q. and their respective interactions (Table 18e and 18f) did not show any significant difference. But in the case of A.Q., the interaction between environment and experiment showed significant difference at 5% level. In order to compare R.Q., and A.Q. the analysis of covariance also was carried out with activity as the auxiliary variable and the results are summarized in Tables 18i and 18j.

All the parameters and their interactions at 30°C and 35°C had significant difference at 1% level with activity (Table 18g). But the temperature showed no significant difference individually. At both the test temperatures, the rates are in close proximity and the analysis of variance and covariance showed that the values at 30°C and 35°C are not significantly different suggesting that effect of temperature (30°C-35°C) is minor. This is possible because these temperatures are close to each other and also within the upper range, to which sea bass is adapted for the greater part of the year.

Table 18h. Analysis of Covariance : ANCOVA

Activity Vs. Ammonia

Source	Degree of freedom	Sum of squares	Mean sum of squares	F	
Environment	2	21495.31	10747.65	48.61	**
Temperature	1	714.33	714.33	3.23	NS
Experiment	2	3414.56	1707.28	7.72	**
Regression	1	264.19	264.19	1.19	NS
Environment by temperature	2	3136.24	1568.12	7.09	**
Environment by experiment	4	5599.45	1399.86	6.33	**
Temperature by experiment	2	3041.21	1520.60	6.88	**
Within cells	276	61022.83	221.10		

NOTE : ** Indicates significance at 1% level

NS indicates Non-significance

Table 18i. Analysis of Covariance : ANCOVA

Activity Vs R.Q.

Source	Degree of freedom	Sum of squares	Mean sum of squares	F	
Environment	2	21349.91	10674.95	48.16	**
Temperature	1	655.79	655.79	2.96	NS
Experiment	2	3579.59	1789.80	8.07	**
Regression	1	110.45	110.45	0.50	NS
Environment by Temperature	2	2920.01	1460.00	6.59	**
Environment by experiment	4	5342.57	1335.64	6.03	**
Temperature by experiment	2	2902.31	1451.16	6.55	**
Within cells	276	61176.56	221.65		

NOTE : ** indicates significance at 1% level

NS indicates Non-significance.

Table 18j. Analysis of Covariance : ANCOVA

Activity Vs. A.Q.

Source	Degree of freedom	Sum of squares	Mean sum of squares	F	
Environment	2	21536.57	10768.28	48.54	**
Temperature	1	645.48	645.48	2.91	NS
Experiment	2	3550.34	1775.17	8.00	**
Regression	1	59	59	0.27	NS
Environment by temperature	2	2963.07	1481.53	6.68	**
Environment by experiment	4	5413.91	1353.48	6.10	**
Temperature by experiment	2	2890.12	1445.06	6.51	**
Within cells	276	61228.02	221.84		

NOTE : ** indicates significance at 1% level

NS indicates Non-significance

Mean, standard deviation and the number of cases for each experiment at both temperatures in sea water, brackish water, and freshwater are presented in detail in Tables 19, 20 and 21.

Table 19. Mean, standard deviation and the number of fishes used for routine metabolism, hypoxic metabolism, and hypoxia - recovery metabolism in sea bass acclimated to and tested at 30 and 35°C in the sea water are given.

Temperature	Variable	Routine			Hypoxia			Hypoxia and Recovery		
		Mean	Std. Deviation	Cases	Mean	Std. Deviation	Cases	Mean	Std. Deviation	Cases
30°C	Mean ambient oxygen	3.6659	1.8503	29	2.2636	0.6993	15	2.8740	0.9278	15
	O_2	78.2607	34.8853	29	76.3614	36.7193	15	84.0753	33.5488	15
	CO_2	144.0228	99.0057	29	152.0550	97.0868	15	168.3893	128.3197	15
	NH_3	16.9359	11.3428	29	13.0121	7.5616	15	14.2553	12.6106	15
	R.Q.	2.1893	1.5687	29	2.4786	0.9227	15	2.0347	1.0358	15
	A.Q.	0.3507	0.2865	29	0.2407	0.1906	15	0.1760	0.1399	15
	Activity	34.7934	18.5573	29	27.7321	13.3440	15	30.0887	17.2288	15
35°C	Mean ambient oxygen	2.4976	0.8550	17	3.2947	0.7140	15	3.6380	0.6269	15
	O_2	86.5471	31.6013	17	96.1093	31.4400	15	89.7193	41.4775	15
	CO_2	168.6688	76.0000	17	185.0893	97.0535	15	176.3867	89.7315	15
	NH_3	15.4435	16.8607	17	10.8500	12.0605	15	6.7480	7.1163	15
	R.Q.	2.0000	1.0117	17	2.1787	0.8663	15	2.2780	0.9060	15
	A.Q.	0.2424	0.3261	17	0.1207	0.1182	15	0.0800	0.0747	15
	Activity	46.3800	21.4495	17	14.3887	8.5222	15	14.1333	10.2674	15

Table 20. Mean, standard deviation and the number of fishes used for routine metabolism, hypoxia metabolism and hypoxia - recovery metabolism in sea bass acclimated to and tested at 30 and 35°C in brackish water are given.

Temperature	Variable	Routine			Hypoxia			Hypoxia and Recovery		
		Mean	Std. Deviation	Cases	Mean	Std. Deviation	Cases	Mean	Std. Deviation	Cases
30°C	$M.A.O_2$	2.9941	0.3242	22	2.6687	0.7853	15	2.9900	0.6987	15
	O_2	100.5718	40.8687	22	161.9000	191.6047	15	128.8267	69.7585	15
	CO_2	195.2209	82.3200	22	369.1667	348.0900	15	335.5893	290.8910	15
	NH_3	9.6845	8.1075	22	30.4120	19.0873	15	23.8260	25.5919	15
	R.Q.	2.0241	0.6413	22	3.1727	1.3291	15	83.5127	313.8477	15
	A.Q.	0.1414	0.1065	22	0.5847	0.8219	15	0.9947	2.3004	15
	Activity	23.7209	14.6216	22	14.9880	8.7701	15	14.3220	9.4204	15
35°C	$M.A.O_2$	2.5311	0.5647	18	3.0033	1.2675	15	3.4627	1.1847	15
	O_2	111.4567	27.9106	18	166.8107	96.1804	15	139.3307	98.6835	15
	CO_2	248.0422	78.0207	18	242.8807	143.7749	15	193.5254	166.0239	15
	NH_3	17.5856	12.8737	18	25.4387	25.4536	15	15.5387	13.7017	15
	R.Q.	2.2872	0.6624	18	1.8767	1.0930	15	1.5140	1.0711	15
	A.Q.	0.2412	0.1880	18	0.2247	0.3155	15	0.1533	0.1126	15
	Activity	25.0011	17.8120	18	22.3560	11.5938	15	17.6220	11.0392	15

Table 21. Mean, standard deviation and the number of fishes used for routine metabolism, hypoxic metabolism, and hypoxia - recovery metabolism in sea bass acclimated to and tested at 30 and 35°C in freshwater are given.

Temperature	Variable	Routine			Hypoxia			Hypoxia and Recovery		
		Mean	Std. Deviation	Cases	Mean	Std. Deviation	Cases	Mean	Std. Deviation	Cases
30°C	$M.A.O_2$	3.3320	0.7541	15	2.6187	0.5053	15	3.3973	0.5328	15
	O_2	72.5907	33.4474	15	76.7156	24.9450	15	91.8120	29.3032	15
	CO_2	139.0193	47.1217	15	201.5960	97.8068	15	179.1480	46.4464	15
	NH_3	8.2853	4.4079	15	19.0713	14.9995	15	15.7433	16.1624	15
	R.Q.	2.1660	0.8275	15	3.7660	1.0473	15	2.1020	0.5076	15
	A.Q.	0.2153	0.2436	15	0.3313	0.2835	15	0.2300	0.2954	15
	Activity	28.6667	12.7262	15	35.9667	17.5260	15	42.1780	14.5811	15
35°C	$M.A.O_2$	3.5013	0.5199	15	2.6173	0.5785	15	3.5193	0.4948	15
	O_2	126.3600	23.1712	15	76.6520	31.4742	15	129.8313	32.9619	15
	CO_2	249.8887	77.7357	15	162.2473	71.1283	15	235.0140	54.7292	15
	NH_3	22.6100	9.7545	15	21.7513	10.4100	15	21.5593	9.5250	15
	R.Q.	1.9373	0.4243	15	2.9927	1.3879	15	1.9540	0.5618	15
	A.Q.	0.2040	0.1087	15	0.6473	0.4889	15	0.1913	0.1180	15
	Activity	48.7867	17.7621	15	47.2333	15.5378	15	43.8660	12.5040	15

V. DISCUSSION

Eventhough fishes live in a fantastic variety of habitats from the darkest depths of the ocean to the boundless surface of the open sea, they have become marvelously adapted to the different environments wherever they live. In the present investigation, since the experimental conditions and experimental procedures for the tests in all the media are identical, the results obtained can be accepted and the adaptability in different environments (media) can be compared.

It is very interesting to compare the metabolism of fishes on exposure to normoxia and hypoxia. Most of the fishes remove about 40-80% of the available oxygen in the water they breathe under normoxic conditions (Shelton, 1970). In Lates calcarifer the mean rate of oxygen consumption were 78.26 mg/kg/hr at 30°C and 86.55 mg/kg/hr at 35°C in sea water (Table 19). But in brackish water (Table 20) the consumption rates observed were 100.57 mg/kg/hr at 30°C and 111.46 mg/kg/hr at 35°C whereas in freshwater (Table 21) the values were found to increase from 72.59 mg/kg/hr (at 30°C) to 126.36 mg/kg/hr (at 35°C) in normoxic condition. In all the media the consumption rates or metabolic rates increased when temperature was increased.

The corresponding comparable rates during the hypoxic condition were 76.72 mg/kg/hr at 30°C and 76.65 mg/kg/hr at 35°C in freshwater (Table 21); 76.36 mg/kg/hr at 30°C and 96.11 mg/kg/hr at 35°C in sea water (Table 19) and 161.90 mg/kg/hr at 30°C and 166.81 mg/kg/hr at 35°C in brackish water (Table 20). In 30‰ and 15‰ the consumption rate increased at 35°C as in normoxic condition but in freshwater medium there was

a slight decrease in rate at 35°C which may be due to the change of environment from sea water and brackish water to freshwater. As suggested by Saunders (1962); Holten and Randall (1967); Shelton (1970); Randall and Jones (1973); Itazawa and Takeda (1978); in hypoxic conditions fish appear to be unable to maintain a high oxygen extraction efficiency especially when hypoxia is severe. Under severe hypoxia the amount of water respired increases in higher proportion than the proportionate drop in environmental oxygen. and also it is not unusual for a fish to increase respiratory water flow rate by a factor of 10-20 fold in response to severe hypoxia (Shelton, 1970; Itazawa and Takeda, 1978). Hughes and Johnstons (1978) and Hochachka (1980) have observed that there is an increased oxygen uptake as the available oxygen in the water becomes less in the respirometer but eventually the point is reached where the anaerobic mechanisms are insufficient to satisfy these requirements and either the oxygen must fall with consequent reductions in activity, or there must be an increase in other mechanisms which enable the continuation of adequate supply of energy. They have also confirmed that the presence of pathways and their importance varies according to the species presumably in relation to their mode of life or their particular life habits. It is significant that the order of hypoxic tolerances of the fish tested in different media at different temperatures were checked from the anaerobic abilities judged from the intensity of the high R.Qs. Increments in carbon dioxide and R.Q. indicate the anaerobic metabolism (Peer Mohamed, 1974) and the increase of A.Q. and ammonia indicate the protein utilization. The link of increased protein degradation to anaerobic energy utilization may have a significance in combating acidosis and also

in conserving sodium during hypoxia which may be specially required due to hypoxic stress of iono-osmotic mechanism in different media. The tremendous effect of random activity on metabolism, the ambient oxygen influence on metabolism and activity, the relationship of the quotients with the anaerobic metabolism and protein utilization, the hypoxic tolerance limits during asphyxia, the post hypoxic repayment of oxygen debt in recovery metabolism, the comparative adaptability of this euryhaline species in different saline media, and finally the estimates of energy utilization from different substrates are also dealt with in detail.

a) **Influence of random activity on metabolism in sea water, brackish water and freshwater at 30 and 35°C**

Activity to metabolism interaction is of much importance in organismal physiology. Scope for activity has been considered as a means to assess environmental stress on fishes (Brett, 1958), as an index of the energy available for swimming fish (Brett, 1964) and as an index to the total energy expenditure by fish (Beamish and Dickie, 1967; Dickson and Kramer, 1971).

Influence of random activity on metabolism can be found out from the regression lines drawn with the plots of metabolic rates and quotients against activity. Extrapolations of regression lines drawn through such plots of metabolic rates to 'zero' activity can be used for estimating the standard metabolism (Spoor, 1946; Beamish and Mookherjii, 1964; Smit, 1965; Brett, 1964; Kutty, 1968a; Kutty et al., 1971; Peer Mohamed, 1974; Kutty and Peer Mohamed, 1975).

In the present study the data obtained from simultaneous measurements of metabolism, metabolic quotients and activity of different fishes tested under similar oxygen conditions were pooled and the regression lines drawn. In normoxic condition in sea water, the regression lines of oxygen consumption at 30 and 35°C were not similar. At 30°C it had a positive slope which resembles the regression line in Tilapia mossambica at 30°C (Peer Mohamed, 1981b) but at 35°C it had a negative slope. In contrast to oxyygen consumption, for carbon dioxide production the slope observed at 30°C was negative, while the slope was positive at 35°C. In the case of ammonia excretion, the situation was reverse. At 30°C the ammonia excretion plots showed a positive trend but it was negative when the temperature was raised to 35°C as in the case of oxygen consumption. The slope of R.Q. was negative at 30°C but the R.Q. at 35°C and A.Q. at both the temperatures showed a positive trend.

In brackish water, the regression lines fitted for plots of oxygen consumption and carbon dioxide production are almost parallel at 30 and 35°C, suggesting the change in R.Q. with changes in random activity observed earlier in mullet, Rhinomugil corsula (Kutty and Peer Mohamed, 1975) and in Tilapia mossambica (Peer Mohamed, 1981b, 1982). At both the temperatures the slopes of ammonia excretion plots are positive (Figures 3 and 4) which resemble the plots in Tilapia mossambica at either temperature (Peer Mohamed, 1981b). Also the A.Q. plots at both the temperatures yield regressions which have positive slopes (Figures 3 and 4). R.Q. plots showed negative slope at 30°C but the slope was positive as in Tilapia

mossambica at 35°C (Peer Mohamed, 1981b) when the temperature was raised to 35°C.

As in brackish water, the regression lines observed for oxygen consumption and carbon dioxide production were found to be parallel at 30 and 35°C in the freshwater medium which also resembles the regression lines in Tilapia mossambica (Peer Mohamed, 1981b). But the slope of ammonia excreted by the fish showed a positive trend at 30°C but negative at 35°C. It was noticed that the R.Q. plots at both the temperatures showed positive trends comparable to conditions in Tilapia mossambica at 30 and 35°C (Peer Mohamed, 1981b). The slopes of A.Q. rates showed similar positive trends at both the temperatures.

Except the negative slope at 35°C in sea water, the oxygen consumption showed a positive (increasing) trend when the activity increased in all the media at 30 and 35°C. This could be attributed to the variability between individual fishes in their metabolism to random activity relation as suggested by Smit (1965). Peer Mohamed (1974) reported that mullet had the lowest activity at high ambient oxygen at 30°C, while it displayed the highest oxygen consumption. In all the media when activity increased the carbon dioxide production also increased except at 30°C in sea water. The R.Q. plots showed a negative trend at 30°C in sea water and brackish water and a positive trend at 35°C in all the media. The A.Q. in normoxic condition showed an increasing trend at 30 and 35°C in sea water, brackish water and freshwater with increase in activity. Ammonia showed an increasing trend at 30°C in all the three media and at 35°C in brackish water;

but it decreased at 35°C in sea water and freshwater with increase in activity. Saunders and Kutty (1974) obtained the results in several tests with smolting Atlantic salmon that lower random activity was associated with higher A.Q. i.e. the less active the fish, the proportionately higher its protein use.

On hypoxic exposure, the activity first increased and then decreased at 30 and 35°C in sea water and brackish water. But in freshwater at 30 and 35°C the activity showed a decreasing trend towards the end of the experiment with an increase in the middle. This showed that temperature has no influence on activity in hypoxic condition.

During hypoxia and recovery, in hypoxic condition the activity increased with the decrease in ambient oxygen in all the media. In recovery metabolism, the activity showed an increasing trend in all the cases except the condition at 35°C in brackish water. This was similar to conditions observed in Rhinomugil corsula, Puntius sarana and Carassius auratus by Peer Mohamed (1974). The trends of oxygen consumption and R.Q. in relation to random activity suggested a compensatory increase in anaerobic energy utilizations. Peer Mohamed (1987) stated that activity has a tremendous effect on metabolism, frequently elevating the oxygen uptake by a factor of four times at optimum temperatures and reaching a maximum of eight times in some fish species, but in the case of active metabolism, under-nourished, unexercised disease-inhibited, sluggish or lazy fish could reduce the potential level.

During studies on the relation of respiratory metabolism to swimming speed in young sockeye salmon, Oncorhynchus nerka, it was observed that excitable nature of the fish resulted in considerable variability in the metabolic rate accompanying all the highest bursts of speed (Brett, 1964; Brett and Sutherland, 1965).

Both swimming and the accumulation of lactic acid may continue until the glycogen deposits are depleted or the end product of anaerobic glycolysis exerts a detrimental effect on activity. In such conditions exercise is extreme in its severity and death may result during the recovery period (Parker and Black, 1959; Parker et al., 1959; Beamish, 1966; Caillouet, 1967). The actual cause of death is uncertain but may result from interference with the acid-base equilibrium coupled with a reduced affinity of haemoglobin for oxygen and in the presence of excess acid, lowered affinity for carbon dioxide (Black, 1958). When death does not follow strenuous exercise, the elevated rate of oxygen consumption serves not only to meet the routine metabolic requirements but also to replace supplies of adenosine tri phosphate, creatine phosphate and glycogen (Bilinski, 1974).

Analysis of variance showed that all the parameters and their respective interactions at both the temperatures had significant difference at 1% level with activity.

b) **Influence of ambient oxygen on metabolism - comparison in the three different media at 30 and 35°C**

Graphic representations of oxygen consumption, carbon dioxide production, ammonia excretion, random activity and quotients against ambient

oxygen taken from the respective figures in 'Results' are given together in Figures 19 and 20 (30°C) and Figures 21 and 22 (35°C) to compare the observations in three different media. The values of metabolic rates, quotients, and activity at the highest (near air saturation) and the lowest (near asphyxial) ambient oxygen concentrations in three different media at two different temperatures are also shown (Tables 2, 8 and 13).

The influence of ambient oxygen on metabolism can be analysed from the trend lines of hypoxia because in the graphic representations, metabolic rates and quotients are plotted against ambient oxygen. Under hypoxia, oxygen consumption increased in all the cases at 30 and 35°C except the oxygen consumption curve in brackish water at 30°C in which it first increased and then decreased. This must be due to the excitement in the new medium as suggested by Brett (1965). The carbon dioxide production showed a similar trend at 30°C in sea water and at 35°C in brackish water with the increase of ambient oxygen. Similarly the curves at 30°C in brackish water and 35°C in sea water showed an increase in the beginning and decrease at the end whereas in freshwater they showed opposite trends of decrease and increase at 30 and 35°C. Ammonia excretion at both the temperatures in sea water and freshwater showed similar trends of increase at the ends with a decrease in the middle. But in brackish water it showed a decreasing trend at 30°C while it first decreased and then increased at 35°C. The respiratory quotient at 30 and 35°C in sea water showed opposite trends. In brackish water at 30°C and in freshwater at both the temperatures it showed a decreasing trend while at 35°C in brackish water it first increased

FIGURE 19

Comparison of oxygen consumption, carbon dioxide production and ammonia excretion in relation to low ambient oxygen below air saturation, in sea bass acclimated to and tested at 30°C in sea water, brackish water and freshwater. In figure, S = seawater,

B = brackish water,

F = freshwater .

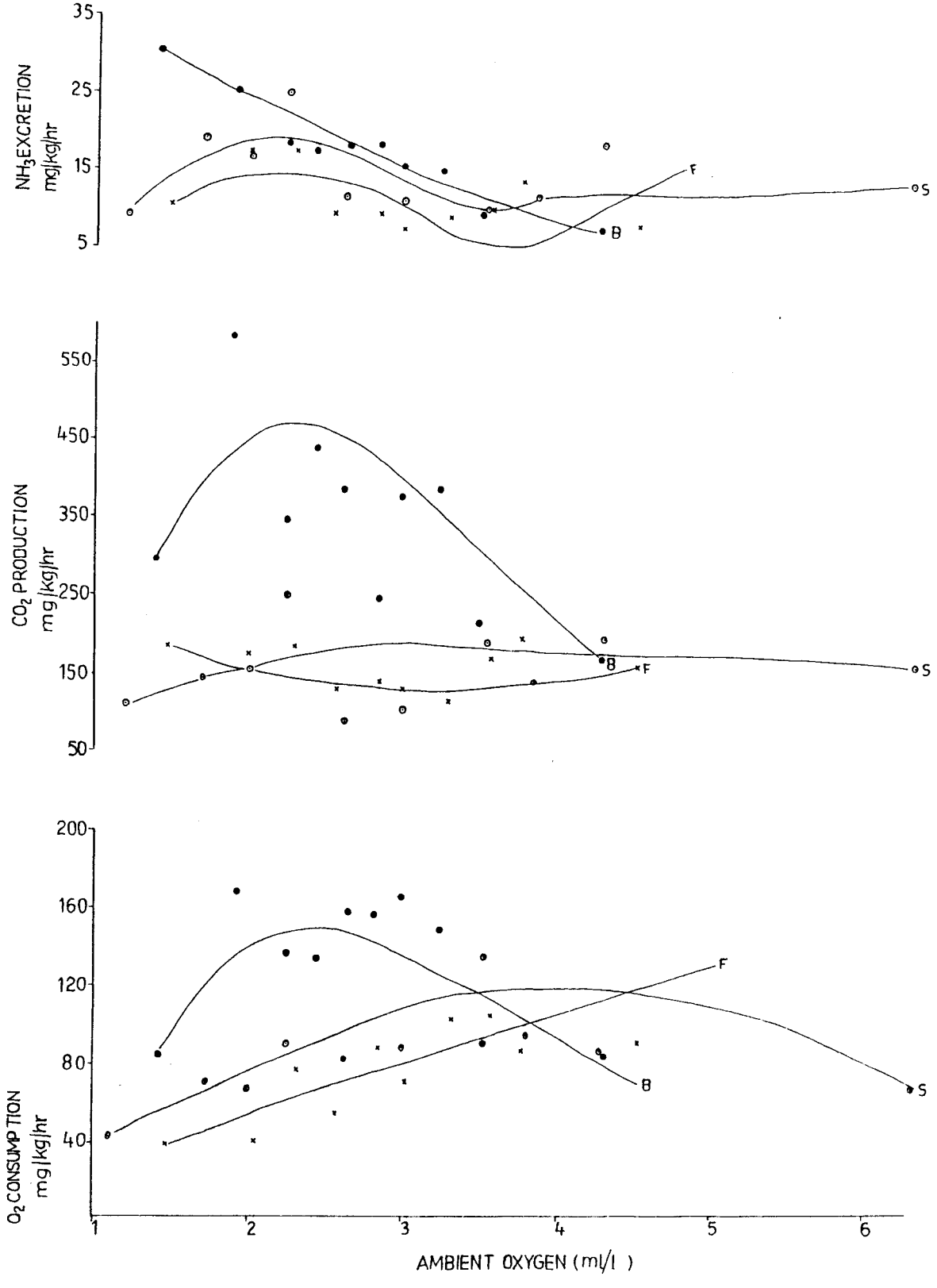

35
25
15
5
NH_3EXCRETION
mg/kg/hr
F
S
B
550
450
350
250
150
50
CO_2 PRODUCTION
mg/kg/hr
B
F
S
200
160
120
80
40
O_2CONSUMPTION
mg/kg/hr
F
B
S
1
2
3
4
5
6
AMBIENT OXYGEN (ml/l)

FIGURE 20

Comparison of respiratory quotient (R.Q.), ammonia quotient (A.Q.) and activity in relation to low ambient oxygen below air saturation, in sea bass acclimated to and tested at 30°C in sea water, brackish water and freshwater. In figure, S = sea water,

B = brackish water,

F = freshwater.

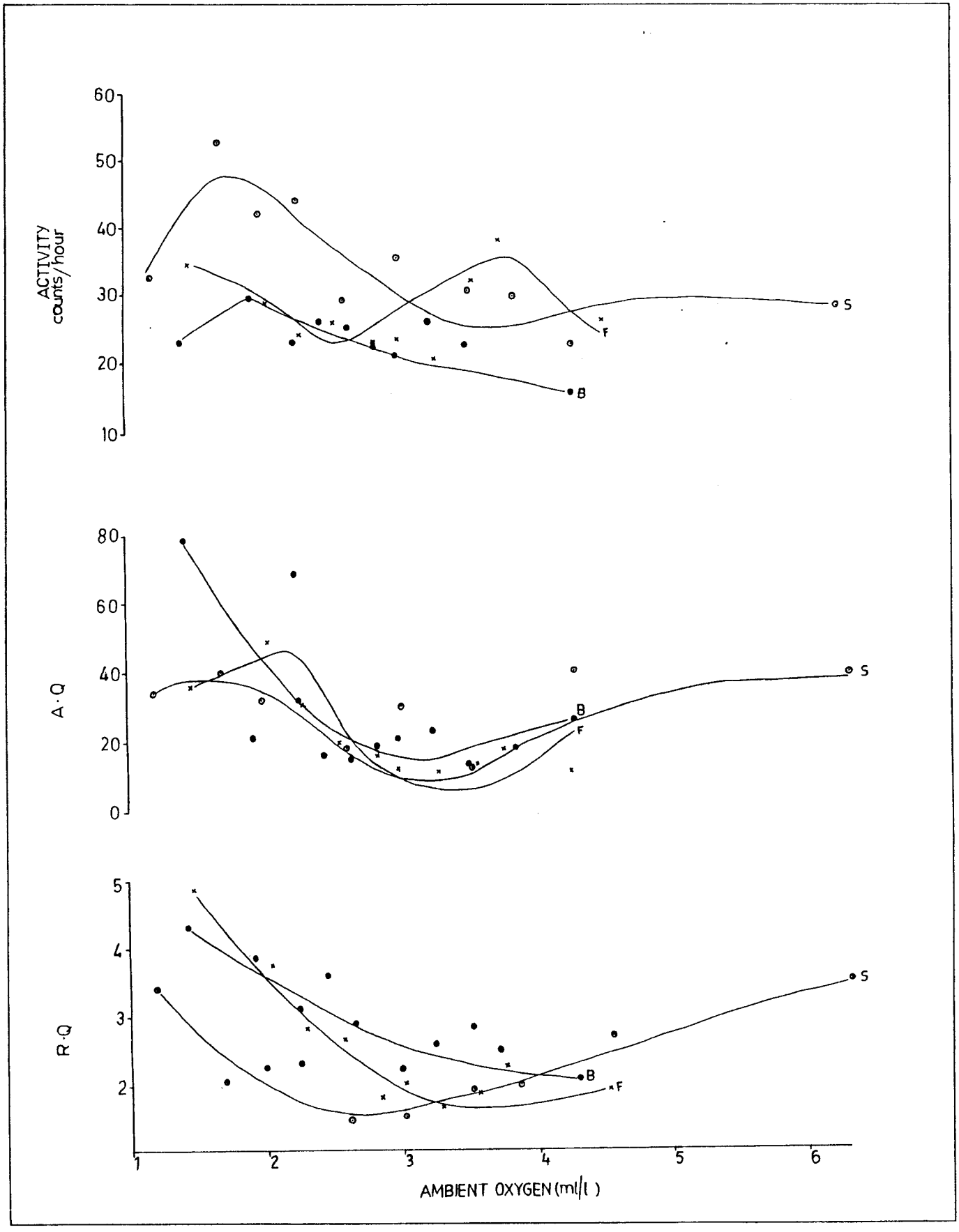

ACTIVITY counts/hour
60
50
40
30
20
10
S
F
B
A·Q
80
60
40
20
0
S
B
F
R·Q
5
4
3
2
S
B
F
1
2
3
4
5
6
AMBIENT OXYGEN (ml/l)

FIGURE 21

Comparison of oxygen consumption, carbon dioxide output and ammonia excretion in relation to low ambient oxygen below air saturation, in sea bass acclimated to and tested at 35°C in sea water, brackish water and freshwater. In figure, S = sea water,

B = brackish water,

F = freshwater.

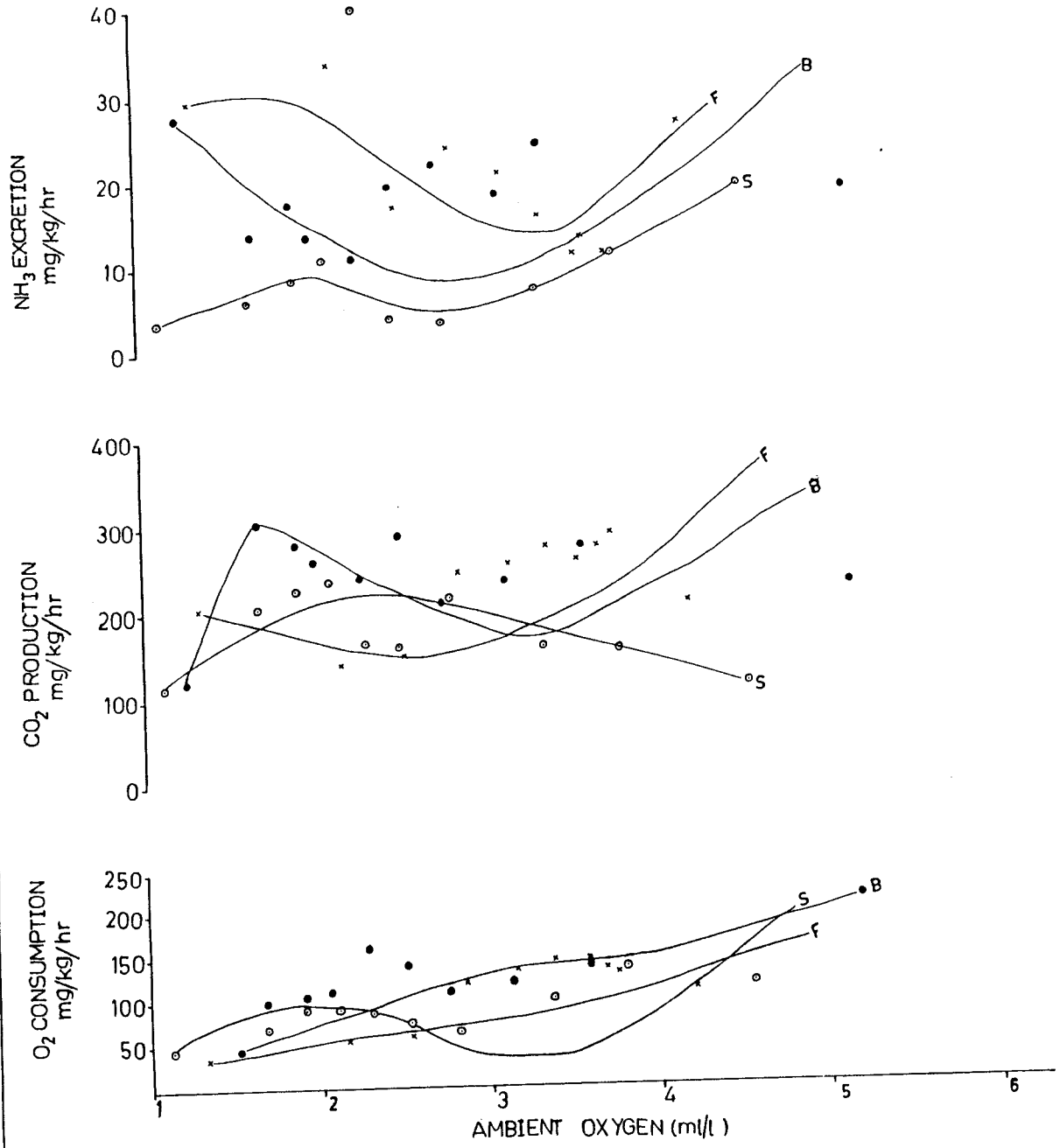
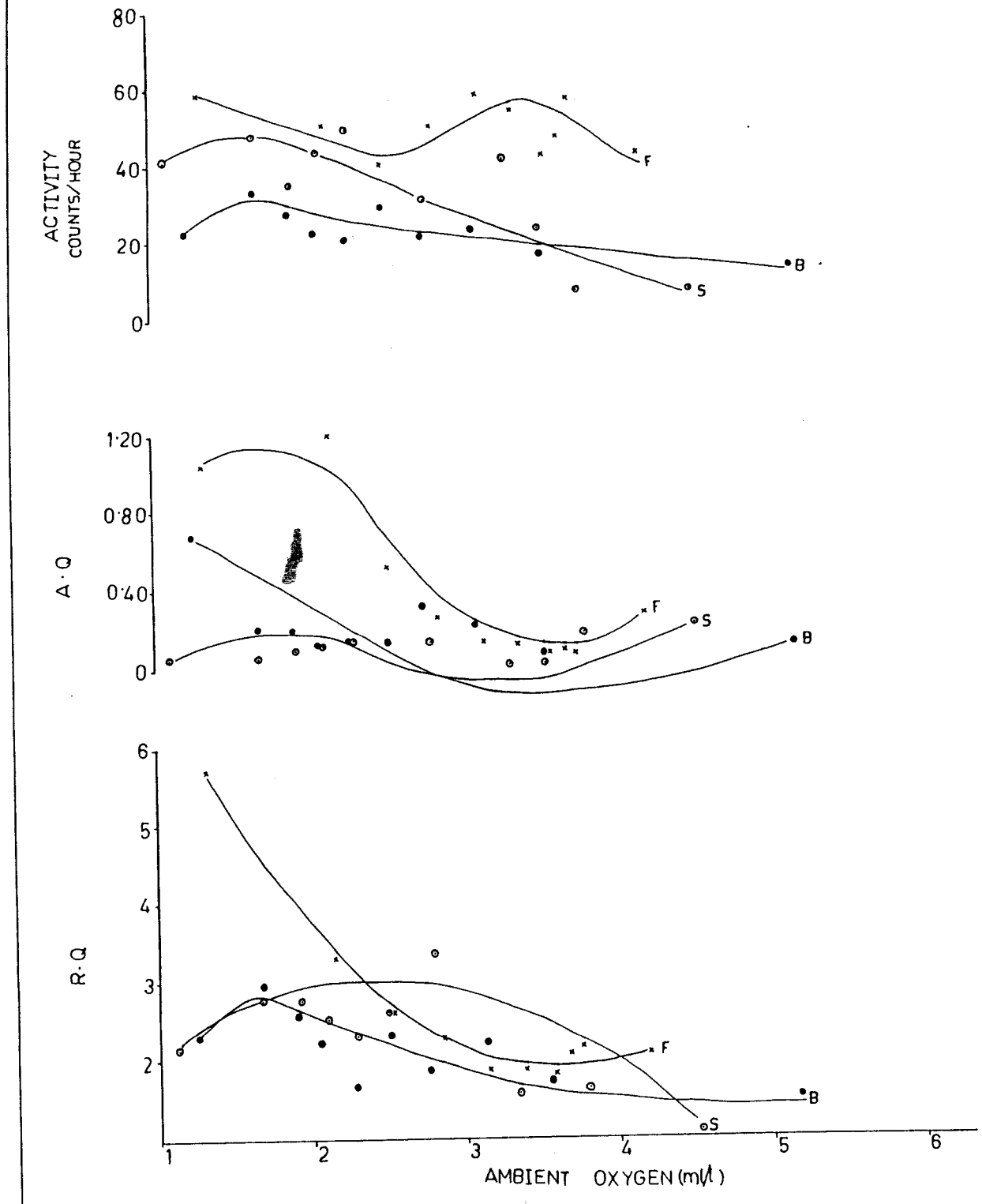

NH_3 EXCRETION mg/kg/hr
40
30
20
10
0
B
F
S
CO_2 PRODUCTION mg/kg/hr
400
300
200
100
0
F
B
S
O_2 CONSUMPTION mg/kg/hr
250
200
150
100
50
S
B
F
1
2
3
4
5
6
AMBIENT OXYGEN (ml/l)

FIGURE 22

Comparison of metabolic quotients (R.Q. and A.Q.) and random activity in relation to low ambient oxygen below air saturation, in sea bass acclimated to and tested at 35°C in sea water, brackish water and freshwater. In figure, S = sea water,

B = brackish water,

F = freshwater.


ACTIVITY
COUNTS/HOUR
80
60
40
20
0
F
B
S
A·Q
1·20
0·80
0·40
0
F
S
B
R·Q
6
5
4
3
2
F
B
S
1
2
3
4
5
6
AMBIENT OXYGEN (ml/l)

and then decreased with the increase of ambient oxygen. The A.Q. at 30°C in sea water, brackish water and freshwater resembled the trends at 35°C in the three media respectively.

In the hypoxia-recovery phase, the ambient oxygen followed a general pattern of decreasing trend during hypoxia and increasing trend during recovery in all the media at both the temperatures showing that ambient oxygen influence is similar in all the cases. The increase in oxygen consumption with the increase in ambient oxygen in recovery metabolism is markedly due to excitement as reported by Brett (1965), Smit (1965) and Kutty (1968a). The analysis of variance showed that with experiment, and the interaction of temperature to experiment, the mean ambient oxygen was found to be significantly different at 1% level.

The resemblance seen in the R.Q. - ambient oxygen curve of sea bass at 30°C in brackish water and freshwater, and also at 35°C in freshwater with those of gold fish and rainbow trout (Kutty, 1968a) and Tilapia mossambica (Kutty, 1972) suggest that this fish passes into the clear anaerobic phase at a much lower ambient oxygen level and can probably sustain a higher level of anaerobic metabolism as reported by Kutty (1972). Active metabolic rate of fish is far more sensitive to the availability of environmental oxygen than is standard metabolism (Job, 1955; Brett, 1964; and Ali, 1979). Shepard (1955) and Anderson (1975) reported that the rate at which hypoxic and anoxic conditions occur has an effect on the ultimate resistance of the fish. The lethal threshold for hypoxia of Salvelinus fontinalis was lowered considerably, when the fish was acclimated to an intermediate

level of hypoxia (Shepard, 1955). Many fish which can breathe air facultatively switch over predominantly to air breathing in response to hypoxic conditions in water (Hughes and Singh, 1970; Stevens and Holeton, 1978).

In cases where the fish are subjected to chronic very low oxygen levels, continued oxygen uptake is physiologically not possible. This continued survival depends on resistance strategies, and most of the strategies involved possess mechanisms such as exploiting body oxygen stores, using anaerobic metabolism or slowing down of metabolism (oxygen dependance). Anderson (1975) found that Carassius auratus could survive for extended periods in water containing less than 0.1 mgO_2, particularly at low temperature and the resistance of these fish to anoxia was quite sensitive to seasonal effects, being 11 hrs in autumn at 5°C and 75 hrs in spring at 5°C. Oxygen extracting efficiency of fishes tend to remain constant under hyperoxic conditions while respiratory flow rate drops commensurately (Holeton, 1972; De jours, 1973; Randall and Jones, 1973; De jours et al., 1977).

Among the routine active rates of metabolism and their standard rates given in Table 1, except the case in sea water at 35°C, the active rate of oxygen uptake was higher in routine metabolism than in standard metabolism as observed by Brett (1962) and by Brett and Groves (1979) that the active rate of oxygen uptake of most of the fishes ranges from 2.5 to 10 times the standard rate. Brett (1964) demonstrated that at warmer temperatures, sockeye salmon Oncorhynchus nerka could increase their active rate of oxygen uptake under hyperoxic conditions. Job (1955) stated that even slight reductions in oxygen levels below air saturation reduced

the active oxygen uptake rate of brook trout Salvelinus fontinalis. Sea bass showed a comparative reduction in standard rate of oxygen uptake in sea water at 35°C (Table 1). Laurence (1973) and Fry (1957) demonstrated that the active metabolic rate can decline at the upper limits of temperature tolerance even below the lethal level.

c) **Influence of ambient oxygen on random activity in sea water, brackish water and freshwater at 30 and 35°C**

It is interesting to note that the random activity first increased and then declined at 30 and 35°C in sea water and brackish water on exposure to low oxygen whereas at both the temperatures in freshwater it showed decreasing trends towards the end with a rise in between. A decreasing trend in random activity on exposure to low oxygen was observed in Tilapia mossambica by Peer Mohamed (1974) whereas the reverse trend was observed in gold fish and rainbow trout by Kutty (1968a); in four marine teleosts among the five species tested by Hamsa and Kutty (1972); and in Rhinomugil corsula, Puntius sarana and Carassius auratus by Peer Mohamed (1974). Milk fish, Chanos chanos displayed lower activity at low oxygen concentrations (Peer Mohamed, 1974). On exposure to low ambient oxygen Chanos chanos showed a decrease in breathing rate whereas Mugil cephalus indicated the reverse trend (Usha Devi, 1987).

Random activity which decreased under hypoxia, increased immediately on exposure of fish to water near saturation at 30°C in brackish water as in Tilapia mossambica (Peer Mohamed, 1974) but at 35°C it increased during hypoxia and declined on exposure to water near air saturation. In

all the other media both at 30 and 35°C the activity increased during hypoxia as well as recovery.

The variability in the rate of oxygen uptake is a major difficulty particularly for fish where activity markedly alters oxygen consumption (Sprague, 1971; Peer Mohamed and Gupta, 1983). Davis et al. (1963) found that swimming performance of juvenile chinook and coho salmon was dependent on oxygen at all levels below air saturation but this was especially evident at all levels below 50% air saturation and similar explanations have been proposed by Kutty and Saunders (1973) that the swimming performance of salmon smolts is highly dependent on ambient oxygen. The fact that oxygen limited the swimming performance was also noted previously in brook trout (Salvelinus fontinalis) at all levels below 6.0 mg/l at 8°C by Graham (1949). When oxygen gradually decreased in the respirometer some fish seem to become more passive and some more active thereby establishing a dichotomy in behaviour of fishes at low oxygen levels (Peer Mohamed and Kutty, 1982). The increased activity induced by hypoxia might allow some species (mullet, Rhinomugil corsula; minor carp, Puntius sarana; and gold fish, Carassius auratus to move out of the hypoxic environment to more oxygenated waters while the decreased activity under hypoxic exposure would allow certain species (Tilapia mossambica, Chanos chanos) to conserve energy. With measurement of activity, Peer Mohamed (1974) recorded that there is an inverse relation between ambient oxygen and activity.

In the present observation, in Lates, during hypoxia in the hypoxia - recovery phase, activity was increased towards asphyxia in all the experi-

ments at both the temperatures in sea water, brackish water and freshwater. This may be in order to escape the hypoxic environment, as supported by Peer Mohamed (1974) in the case of marine teleosts, Rhinomugil corsula, Puntius sarana and Carassius auratus. In the recovery phase also in all the experiments, activity showed an increasing trend except the case at 35°C in brackish water in which the activity showed a decreasing trend. These differences in responses of random activity to ambient oxygen may have resulted from a dichotomy in behavioural evolution and may have a major role in the survival of the species (Hamsa and Kutty, 1972; Anantha Krishnan and Kutty, 1974; Peer Mohamed, 1974; Peer Mohamed and Kutty, 1982; Usha Devi, 1987). With some tolerance to oxygen, the limited source of oxygen and the energy conserved might be of value in survival (Peer Mohamed, 1974; Usha Devi, 1987).

Mean rates of random activity at 35°C seemed to be higher than the rates at 30°C in routine metabolism, hypoxia and also in hypoxia and recovery metabolism in sea water (Table 19), brackish water (Table 20) and freshwater (Table 21) except in the cases of hypoxia and hypoxia - recovery phase in sea water.

d) **Respiratory quotient and anaerobic metabolism**

It is well recognized that R.Q. under aerobic conditions has to be between 0.7 and 1.0 (unity), if no special circumstances are involved, such as lipogenesis or gluconeogenesis (Peer Mohamed, 1974; Usha Devi, 1987). An R.Q. of 1 is ascribed to the aerobic degradation of carbohydrate, 0.8 to protein and 0.7 to fat (Peer Mohamed, 1974; Usha Devi, 1987). Under

the present experimental conditions R.Q. values above 1 were obtained both in routine metabolism and hypoxic metabolism. Based on the statement that the magnitude of R.Q.'s over unity is indicative of the intensity of anaerobic metabolism, there must be the anaerobic metabolism in aerobic and anaerobic condition.

In routine metabolism at 30°C, R.Q. showed a decreasing trend in sea water and brackish water, but an increase in freshwater. When the temperature was raised to 35°C, R.Q. showed an increasing trend in all the three media.

In sea water, brackish water and freshwater at 30 and 35°C the R.Q. showed a similar decreasing trend in hypoxic condition.

As for hypoxia and recovery, in all the media at both 30 and 35°C R.Q. showed an increasing trend during hypoxia while during the recovery metabolism at 30°C in both sea water and freshwater it first dropped and then increased, the trend being vice-versa in brackish water. In the case of 35°C, in all the media the R.Q. showed an increasing trend.

The mean R.Q.s of Rhinomugil corsula, Tilapia mossambica, Puntius sarana and Carassius auratus tested at the highest oxygen concentration (near air saturation) were respectively 1.0, 1.01, 0.77 and 0.96 at 30°C and 0.98, 1.03, 0.63 and 0.99 at 35°C suggesting that these fish were aerobic under adequate oxygen concentrations (Peer Mohamed, 1974). In the case of Lates calcarifer the R.Q. values tested at the highest ambient oxygen were 6.67 at 30°C and 1.01 at 35°C in sea water, 5.89 at 30°C and 2.82 at 35°C in brackish water, and 2.62 at 30°C and 2.36 at 35°C in fresh-

water. All these values are above unity and it can be taken that in all the media the fish seem to show anaerobic metabolism as in the case of Tilapia mossambica observed by Kutty (1972).

To find out the maximum influence of hypoxia on R.Qs., the values determined at the lowest ambient oxygen concentrations (ie. during the final run of the experiment in which the fish became asphyxiated) can be taken. In all the cases the fact that the fish subsequently recovered in air saturated water indicate that all of them had survived the hypoxic exposure. The routine R.Qs at the lowest oxygen concentrations in different media were 3.96 at 30°C and 2.92 at 35°C in sea water, 4.96 at 30°C and 3.69 at 35°C in brackish water and 5.45 at 30°C and 12.73 at 35°C in freshwater. Just like the values at the highest ambient oxygen, these values are also significantly above unity and clearly suggest that during the hypoxic phase considerable amount of anaerobic metabolism has taken place resulting in the release of extra carbon dioxide as in the case of fry and fingerlings of Mugil cephalus and Chanos chanos reported by Usha Devi (1987). The intensity of anaerobic metabolism seem to be higher at 30°C than at 35°C. This is in agreement with the findings of Peer Mohamed (1974) on mullet (Rhinomugil corsula - 1.96 at 30°C and 1.69 at 35°C); tilapia (Tilapia mossambica - 2.36 at 30°C and 2.09 at 35°C); and gold fish (Carassius auratus - 2.80 at 30°C and 2.50 at 35°C). However, in the present study, the routine R.Q. at the lowest oxygen concentration in freshwater differs from the rest in that the R.Q. at 35°C (12.73) is higher than the value (5.95) at 30°C. Kutty (1968a) showed that gold fish at 20°C sustained an R.Q. of about 2 at about 1.5 mg O_2/l and this was sustained by gold fish for months

probably producing large amounts of organic carbon dioxide whereas in the present observation, the high R.Qs were sustained only for a short period. Anaerobic abilities of certain carps, such as crucian carp (Blazka, 1958) and Rasbora daniconius (Mathur, 1967) are very pronounced that they have been reported to survive under complete anoxic conditions for months. In crucian carp the complete anoxic condition was found at 5°C, but in Rasbora, it was claimed at the ambient temperature of 28°C. All the species tested by Peer Mohamed (1974), became asphyxiated at some low oxygen concentrations and so complete anoxic tolerance cannot be claimed. It must be stated that the experiments were conducted in a closed respirometer but an open system with continuous flow of low oxygenated water might indicate tolerance of oxygen lower than that presently indicated here. Thus a high magnitude of R.Q. can only indicate the intensity of anaerobiosis and a complete picture can be had only with additional information on the period for which such hypoxic R.Qs are sustained.

The 'extra' or 'anaerobic' carbon dioxide produced by fish under hypoxia can result from two sources (Ekberg, 1962; Kutty, 1968a; Peer Mohamed and Kutty, 1981; Usha Devi, 1987). Among them, one is that resulting from the 'bicarbonate' or 'alkaline reserve' consequent to the production of lactic acid by glycolysis (Black et al., 1961) which is referred to as the acidic or non metabolic carbon dioxide, and the other is that resulting from metabolic break down of the substrate molecule itself which is referred to as the organic or metabolic carbon dioxide (Peters and Van Slyke, 1946; Ekberg, 1962; Kutty, 1968a; Peer Mohamed, 1981b; Usha Devi,

1987). Hochachka (1961) using labelled carbon dioxide confirmed that gold fish excrete organic carbon dioxide when held in free water. Ekberg (1962) showed that excised gills of crucian carp produce both organic and acidic carbon dioxide. During anaerobiosis, it appears that the anaerobic glycolysis and amino acid break down are linked together (Kutty, 1972; Hochachka and Somero, 1973; Kutty and Peer Mohamed, 1975). Anaerobic abilities of Atlantic salmon are little known, the rainbow trout does have slight anaerobic ability (Kutty, 1968b; Kutty and Saunders, 1973). Mechanisms of simultaneous breakdown of glucose and amino acids anaerobically resulting in organic carbon dioxide and accumulation of alanine and producing adequate energy for fish have been suggested by Hochachka and Somero (1973). It is known that fish under hypoxic and anoxic conditions, produce both 'acidic' and 'organic' carbon dioxide and actual proportions of these or pathways of their production are not known. Kutty (1972), based on his calculations on mammalian energetics, observed that Tilapia mossambica whose metabolism was measured immediately after handling at 30°C had an anaerobic component in the metabolism and concluded that 20% of the carbon dioxide produced resulted from anaerobic sources. There is possibly a large amount of metabolic carbon dioxide production in Lates calcarifer during anaerobiosis as in mullet and gold fish reported by Hochachka, 1961 and Kutty, 1968b. Thus in agreement with the observations made by earlier workers (Kutty, 1968a; Peer Mohamed, 1974; Usha Devi, 1987) it can be taken that the magnitude of the R.Qs above unity in the present study indicates the intensity of anaerobiosis - i.e. the higher the R.Q. over unity, the higher the extent of anaerobic metabolism.

Accumulation of lactic acid in blood and tissues of fishes consequent to anaerobiosis, whether due to exercise (Black, 1955, 1957a,b,c; Black et al., 1959, 1960, 1966; and Hayashi et al., 1964 in salmonids; Heath and Pritchard, 1962 in blue gill sun fish; Caillouet, 1964 in carp) or due to hypoxia (Heath and Pritchard, 1965 in blue gill sun fish and cut throat trout) are well documented. There is also evidence to show that fish under hypoxic or anoxic conditions do not accumulate lactic acid (Prosser et al., 1957 in gold fish; Blazka, 1958 in crucian carp).

e) **Ammonia quotient and protein utilization**

Participation of proteins in the metabolism can be checked from measures of ammonia excretion. The term A.Q. was proposed by Stroganov (1962) for the proportion of ammonia excreted (in weight) to oxygen consumed (in volume) by fish, but Kutty (1972) used it as a proportion of volume of ammonia excreted to volume of oxygen consumed (Peer Mohamed, 1974) and this was claimed to be a potent tool for further understanding energy utilization (Kutty, 1972, 1978; Kutty and Peer Mohamed, 1975; Sukumaran and Kutty, 1987). Generally in fish, as the swimming speed increases carbon dioxide output and ammonia excretion or protein utilization also increase. From the A.Q., the concentration of ammonia in the medium can be estimated and if A.Q. is more it suggests high excretion of ammonia.

The changes in A.Q. can be taken as reflecting changes in protein degradation, although the exact ways of amino acid breakdown and ammonia release especially under anaerobic systems such as hypoxia and anoxia in fish are not known (Forster and Goldstein, 1969; Kutty, 1972; Karuppannan,

1972; Saunders and Kutty, 1974; Peer Mohamed, 1974, 1981b; Kutty and Peer Mohamed, 1975; Kutty, 1975; Sukumaran and Kutty, 1977; Peer Mohamed and Kutty, 1981; Usha Devi, 1987). In the present study, in routine metabolism A.Q. plots showed similar (positive) trends at 30 and 35°C in sea water, brackish water and freshwater. The A.Q. values tested in different media (sea water, brackish water and freshwater) at the highest ambient oxygen (near air saturation) are found to be 12.59, 9.92, 3.22 at 30°C and 6.41, 4.28 and 7.91 at 35°C (Tables 2, 8, 13) respectively. A.Q. values are higher in Lates calcarifer compared to other species showing that it is relatively using up a considerable amount of protein. The mean A.Q. of Rhinomugil corsula, Tilapia mossambica, Puntius sarana and Carassius auratus at adequate oxygen concentrations were 0.11, 0.08, 0.06, 0.10 at 30°C and 0.11, 0.18, 0.06 and 0.11 at 35°C; thus the aerobic or normoxic A.Qs ranged between 0.06 and 0.18 depending on the intensity of relative protein utilization in species concerned (Peer Mohamed, 1974). The A.Q. reported for Gambusia by Stroganov (1962) was only an average A.Q. of 0.1 while Kutty (1972) obtained a routine A.Q. of 0.23 in Tilapia mossambica at 30°C and Peer Mohamed observed the highest routine A.Q. of 0.18 under adequate oxygen concentration in Tilapia mossambica at 35°C. If under certain circumstances the A.Q. goes above 0.33, it can be taken that anaerobic degradation of proteins or possibly interconversion of substrates definitely takes place (Kutty, 1975, 1978). This is well in agreement with the present study showing the A.Q. values above 0.33.

Under hypoxia, in all the three media, the A.Q. plots show more or less similar trends at 30 and 35°C. At the lowest oxygen concentrations

or under hypoxic conditions, the A.Q. values observed in Lates calcarif are 6.78, 16.02, 8.11 at 30°C and 4.55, 9.01 and 33.99 at 35°C in sea wate brackish water and freshwater respectively. In brackish water and sea wate the A.Q. values under hypoxia seem to be higher at 30°C than at 35° but in freshwater the values are higher at 35°C. These values clearly sho that A.Qs under hypoxia are higher than those of fish in high oxygen consonance with the changes of A.Q. under similar conditions. This is i agreement with the findings of Peer Mohamed (1974) in mullet, tilapia barbus and goldfish. The hypoxic A.Qs corresponding to the hypoxic R.Q referred to for fishes such as Rhinomugil corsula, Tilapia mossambica, Puntiu sarana, and Carassius auratus were respectively 0.38, 0.25, 0.63 and 0.2 at 30°C and 0.19, 0.33. 0.26 and 0.25 at 35°C, all the values being highe than those in high oxygen in consonance with the changes in R.Qs unde hypoxia (Peer Mohamed, 1974). If A.Q. is below the aerobic maximum valu of 0.33 it suggests involvement of other substrates, thus reducing the relative contribution of protein catabolism in energy release (Kutty, 1978).

The temperature effect on hypoxic A.Q. seems to be different for different species. It appears that except in freshwater the relative protein utilization values were less at 35°C than at 30°C as indicated by the A.Q. in mullet and barbus by Peer Mohamed (1974). Kutty (1972) measured hypoxic A.Qs of Tilapia mossambica at 0.6 mg O_2/l and found that A.Q. would go up as high as 1.0. In this case the experimental set up he used was different and the species not a carnivore. Being a carnivore, naturally, Lates calcarifer would utilize more energy.

In hypoxia and recovery, at 30 and 35°C, A.Q. plots showed an increasing trend under hypoxia. Similarly during recovery they showed similar pattern of decreasing in the beginning and then increasing towards the end at 30 and 35°C in sea water, brackish water and freshwater. Hence salinity does not have any influence on A.Q. since the trends at these two temperatures are similar in all respects in all the media. In hypoxia and recovery the A.Q. and R.Q. increase under hypoxic condition suggest a coupling of the increased ammonia excretion and increased carbon dioxide output at low ambient oxygen environment (Kutty, 1972; Peer Mohamed, 1974). This may have a specific significance in acid-base balance and also in conserving sodium in freshwater (Prosser and Brown, 1961; Garcia Romeu and Motais, 1966; Maetz and Garcia Romeu, 1964; Forster and Goldstein, 1969; Kutty, 1972; Sukumaran and Kutty, 1977).

Ammonia as a base can neutralise acid and the carbon dioxide resulting from anaerobic metabolism. The ammonium can be exchanged at the gills with sodium in ambient water and this was also observed in gold fish, Carassius auratus and eel, Anguilla anguilla by Maetz and Garcia Romeu (1964) and Garcia Romeu and Motais (1966) and this is a more complex process than a simple exchange of ions (De Vooys, 1968). The relative increase in ammonia production during anaerobiosis helps to prevent acidosis (Prosser and Brown, 1961). Metabolic production and the release of ammonia into the blood exceeded the combined rates of excretion and detoxification of ammonia in rainbow trout (Fromm and Gillete, 1968). The activity of renal glutaminase in mammals is enhanced by acidosis (Rector et al., 1955)

and in the neural tissues increased ammonia concentration induces more glycolysis (Fromm and Gillete, 1968). Usually fish excretes most of the ammonia branchially and the activities of glutaminase and glutamic acid dehydrogenase are high in fish gills (Forster and Goldstein, 1969). Thus the ammonia excretion and ammonia quotient with protein degradation in ammonotelic animals have a special significance. Since ammonia is the nitrogenous end product investigated in much of the excretion studies in fish, it is important for ionic regulation and acid-base balance in the aquatic organism (Maetz and Garcia Romeu, 1964; Garcia Romeu and Motois, 1966; Kutty, 1972, 1978). The A.Q. values are lower in initial phase where R.Q. is more showing the relative utilization of protein; and this may be due to the sparing action of carbohydrate on proteins (Kutty, 1981; Sukumaran and Kutty, 1987).

f) **Asphyxial oxygen concentration in three different media at 30 and 35°C**

Asphyxial oxygen concentration is the low lethal level of oxygen below which the fish cannot survive. The difference in the tolerance limit to low ambient oxygen (asphyxiation) is species specific as observed in earlier studies on certain marine teleosts by Hamsa and Kutty (1972); certain fresh-water teleosts by Peer Mohamed and Kutty (1982) and the fry and finger-lings of the milk fish, Chanos chanos and the grey mullet, Mugil cephalus by Usha Devi (1987). The maximum hypoxic tolerance in Carassius auratus, Tilapia mossambica, Puntius sarana and Rhinomugil corsula are 0.28 ml/l, 0.36 ml/l, 0.41 ml/l and 0.86 ml/l at 30°C and 0.28 ml/l, 0.56 ml/l, 0.49

ml/l and 0.84 ml/l at 35°C respectively. Usha Devi (1987) observed that in the case of the fry of milk fish Chanos chanos, the maximum tolerance to low ambient oxygen (at 35°C and 15‰) was 0.56 ml/l, while for the fry of grey mullet (at 30°C and 30‰), it was 0.68 ml/l.

In the present study, in sea water (30‰), the asphyxial oxygen concentration observed was 1.17 ml/l at 30°C and 1.45 ml/l at 35°C. But when the salinity was decreased to 15‰ (Brackish water) and the temperature was increased to 35°C (from 30°C), the asphyxial oxygen concentration was found to increase from 1.53 ml/l to 1.60 ml/l whereas when the salinity was again decreased to 0‰ (freshwater), the values observed were 1.54 ml/l and 1.60 ml/l at 30 and 35°C respectively. The order of hypoxic tolerance is the same as that of the anaerobic abilities as judged from the magnitude of the respiratory quotients (Peer Mohamed and Kutty, 1982). According to the asphyxial oxygen levels obtained by them, the tolerance of hypoxia in different media can be arranged as follows, sea water $>$ brackish water $>$ freshwater at 30 & 35°C(Table 14). Comparatively, the hypoxic tolerance was more in sea water at 30°C. When the temperature was increased to 35°C, it was observed that the tolerance limit was significantlyy lower in sea water, brackish water and freshwater. However, at both the temperatures, the order of tolerance was similar. It is evident from Table 5 that the tolerance was higher at 30°C than at 35°C in all the media.

During hypoxia, decrease in ambient oxygen from air saturation down to the asphyxial oxygen concentration can cause different behavioural res-

ponses. The difference in behaviour pattern of hypoxic exposure may hav a special significance for survival (Hamsa and Kutty, 1972; Peer Mohame and Kutty, 1980). Gold fish withstands low oxygen concentrations wel (Fry, 1947; Prosser et al., 1957; Kutty, 1968a) but rainbow trout is relativel intolerant of low oxygen concentrations (Gutshell, 1929; Parry and Holliday 1960; Kutty, 1968a).

In Lates, as in the case of Chanos chanos and Mugil cephalus (Ush Devi, 1987), complete anoxic tolerance cannot be claimed since the experi ments were conducted in a closed system. A number of fish show definit avoidance responses to low oxygen but there is considerable variation i the threshold oxygen level required between species and within a species with respect to temperature and season (Jones, 1952; Whitmore et al., 1960; Hoglund, 1961; Ali, 1979). It is also possible for fish to acquire or 'learn' behavioural responses with manipulation of environmental oxygen as the primary conditioning stimulus (Sommers, 1962).

The minimum rate of asphyxial oxygen observed in Lates calcarifer was 1.17 ml/l which was in sea water at 30°C and hence below this rate it cannot survive. Severe oxygen depletion can be lethal to fish but the actual lethal level may change at different stages in the life history (Ali, 1979). The size makes a difference since smaller fish can exploit smaller microhabitats such as oxygen rich surface films and shallow water but the large fish can move further and faster and can resist the effects of poten- tially lethal oxygen concentrations for longer (Shepard, 1955). Longer resist- ance times of larger fish to potentially lethal oxygen concentrations may be due to lower weight - specific oxygen consumption.

g) **Recovery metabolism and oxygen debt in sea water, brackish water and freshwater at 30 and 35°C**

After asphyxiation, in all the experiments, the fish recovered subsequently in air saturated water, thus indicating that the anaerobic ability to survive hypoxia exists. An oxygen debt which is repaid can be measured in animals immediately after the stress of hypoxia or exercise is relieved (Peer Mohamed, 1974). Certain species of fish such as the trouts accumulate an oxygen debt while others such as the crucian carp do not (Blazka, 1958).

The increase in oxygen consumption during the initial phase of recovery is an indication of oxygen debt (Sukumaran and Kutty, 1977). In this species, in all the experiments, except at 35°C in sea water, there is post hypoxic increase in oxygen consumption during the initial phase of recovery. In sea water at 30°C, the consumption rate increased from the asphyxial level of 67.94 mg/kg/hr to 83.28 mg/kg/hr (Table 6); in brackish water it rose from 142.20 mg/kg/hr to 175.64 mg/kg/hr at 30°C (Table 11) and from 173.35 mg/kg/hr to 176.78 mg/kg/hr at 35°C (Table 12); and in freshwater the rate shot up from 54.50 mg/kg/hr to 91.49 mg/kg/hr at 30°C (Table 16) and from 37.76 mg/kg/hr to 143.75 mg/kg/hr at 35°C (Table 17). However, at 35°C (Table 7) in sea water it remained steady (91.78 mg/kg/hr to 91.50 mg/kg/hr - the difference being only marginal, i.e. 0.28 mg/kg/hr) initially and then rising to 92.67 mg/kg/hr to repay the oxygen debt. Finally the consumption rate decreased to 89.07 mg/kg/hr showing the accumulation of oxygen debt as has been shown in trout (Blazka, 1958) in which however, there was no record of activity measurements. Trout does not repay an oxygen debt as in Rhinomugil corsula (Peer Mohamed, 1974). The trend

lines of oxygen consumption in the recovery phase resembles the trend lines in hypoxic phase. The post hypoxic increase in oxygen consumption in Lates at 30 and 35°C is quite pronounced and it is noted that the fish repays almost entirely all the oxygen debt accumulated as in Tilapia mossambica reported by Peer Mohamed (1974). Tilapia mossambica is known to accumulate lactic acid (also excrete a negligible amount) when exercised at 30°C (Karuppannan, 1972; Peer Mohamed, 1974) but the lactic acid accumulation in Lates is not yet known. Carbon dioxide showed an increasing trend in all the media except at 30°C in brackish water. Similarly ammonia also showed an increasing trend at 30 and 35°C in sea water and freshwater but in the case of brackish water ammonia first decreased and then rose. The corresponding metabolic quotients at both the temperatures resemble each other. In all the media, the values of R.Q. and A.Q. are comparatively very high (above unity) suggesting that some anaerobic energy utilization persists. R.Q. during the fifth hour of the experiment, but third hour of recovery phase, had risen to above the pre-hypoxic level in all the media at both the temperatures, suggesting that the recovery processes were almost complete. The activity showed an increasing trend in recovery metabolism, except at 35°C in brackish water in which the trend was the reverse.

The differences between recovery at 30 and 35°C might account for the differences in oxygen debt accumulation and also the changes in metabolic mechanisms in Tilapia at two different temperatures. In Lates there is not much difference between recovery at 30 and 35°C and so the temperature has no influence on recovery metabolism.

The anaerobic contribution is perhaps most conveniently assessed by continued measurement of oxygen consumption on completion of swimming until it returns to pre-exercise levels, at which time the oxygen debt is presumably repaid (Heath and Pritchard, 1962; Brett, 1964; Smit et al., 1971; Hoar and Randall, 1978). This procedure assumes that the products of anaerobic metabolism such as lactate are not excreted but subsequently oxidized during the recovery phase following exercise. In sockeye salmon the rate of replacement of oxygen debt following fatigue was in excess of 3 hr, and independent of temperature (Brett, 1964; Hoar and Randall, 1978). The magnitude of the oxygen debt accumulated during fatigue was highly influenced by the temperature.

h) **Comparative metabolic adaptability of Lates calcarifer in sea water, brackish water and freshwater at 30 and 35°C**

Metabolic rate is of major significance because, it affects the speed of all vital processes. The metabolic rates are linked directly with oxygen requirements, and these must be understood in order to solve many problems associated with rearing fish, handling fish, shipping live fish etc. (Winberg, 1960).

Among the euryhaline fishes, Lates calcarifer showed a remarkable adaptability to the wide variations in salinity. Food intake is higher in the salinity range of 5-20‰ and this indicates that for young specimens the ideal salinity range for better metabolism is between 5-20‰ and it also indicates that the apetite of fry is influenced by the ambient salinity (Mukhopadhyay and Karmarkar, 1981). Similar observations had been made

in Mugil cephalus (De Silva and Perera, 1976) and Cyprinodon macularis (Kinne, 1960).

The metabolic rate in fish, as in other animals, is most often evaluated by the rate of respiration, chiefly by the rate of oxygen consumption. While comparing the oxygen consumption rates in different media, the mean rates (mean of means) at both the temperatures during hypoxia are as follows: 82.87 mg/kg/hr at 30°C (Table 3) and 89.80 mg/kg/hr at 35°C (Table 4) in sea water; 133.4 mg/kg/hr at 30°C (Table 9) and 127.30 mg/kg/hr at 35°C (Table 10) in brackish water; and 75.36 mg/kg/hr at 30°C (Table 14) and 108.54 mg/kg/hr at 35°C (Table 15) in freshwater. Similarly, in hypoxia-recovery phase the mean rates are, 80.83 mg/kg/hr at 30°C (Table 6) and 93.27 mg/kg/hr at 35°C (Table 7) in sea water; 150.79 mg/kg/hr at 30°C (Table 11) and 153.70 mg/kg/hr at 35°C (Table 12) in brackish water and 83.28 mg/kg/hr at 30°C (Table 16) and 108.48 mg/kg/hr at 35°C (Table 17) in freshwater. In all the experiments, except the condition in brackish water at 30°C during hypoxia, the oxygen consumption rates are higher at 35°C than at 30°C. Oxygen consumption rate is known to increase in marine organisms transferred to dilute media, as also in freshwater organisms transferred to saline media (Parvatheswararao, 1965 and Madanmohan Rao, 1968) and this enables the organisms to meet the additional energy demands for osmoregulation in the new media (Bashamohideen and Parvatheswararao, 1972). Similarly while transferring this marine species to brackish water and also to freshwater the oxygen consumption rates seem to be increased. The mean values estimated in brackish water and freshwater are higher than the values in sea water at both the temperatures. This was also

supported by the view that oxygen consumption increases due to excitement (Brett, 1965; Smith, 1965; Kutty, 1968a).

The serum salt concentration of fish is less than that of sea water. In a marine environment water is lost at the gills and other body surfaces (Black, 1951; Potts, 1954;; Hoar and Randall, 1978). Conversely freshwater homeostasis is dependant on the elimination of absorbed water, the concentration of the body fluids being greater than that of the environment (Black, 1957; Hoar and Randall, 1978). The mechanism by which osmoregulation is achieved may vary among species (Parry, 1958; Gordon, 1963; Threadgold and Houston, 1964; Hoar and Randall, 1978) but each requires the expenditure of energy. Dehnel (1960) postulated that a reduction in salinity causes increased oxygen uptake because of increased osmotic load but Gordon et al. (1965) stated that salinity has no effect on oxygen consumption in certain marine fishes (Ritakumari and Sreelatha, 1987). Farmer and Beamish (1969) observed the lowest oxygen utilization in isosmotic salinities. After reviewing the literature on teleost fishes, Fry (1971) concluded that the most consistent pattern shows minimum metabolic rates at salinities which are closest to the osmotic content of the body fluids. Chanchal et al. (1977) postulated that the effect of salinity on metabolic rate is both stimulation and depression and it varies from species to species (Ritakumari and Sreelatha, 1987).

Adaptability of Lates calcarifer to wide variations in salinity show that there is a possibility of culturing this species commercially in sea water, brackish water and freshwater. In the euryhaline fish Aholehole

(Kuhlia sandvicensis) the values for both active and standard oxygen consumption were higher in salt water than in freshwater but the differences were not statistically significant (Muir and Niimi, 1972). Madanmohan Rao, in 1968, reported a higher oxygen consumption for rainbow trout in 30‰ salt water than in freshwater at all swimming speeds and attributed the difference to a higher cost of osmoregulation in salt water.

The temperature dependence may be achieved through suitable adjustment of the metabolism, and not by an adjustment of the milieu as noted previously in Etroplus maculatus (Prosser, 1955; Ritakumari and Sreelatha, 1987). Regarding the size of the fish, the metabolic rates should be high since young ones were used for the present study. Bashamohideen and Parvatheswararao (1972) suggested that the smaller individuals are osmotically more efficient than larger fish. Body size is known to influence osmoregulation in fishes although its direction may not be the same in all cases (Parry, 1958; Houston, 1961; Kinne, 1964, 1971; Parvatheswararao, 1965; 1967). Fishes could respire upto a level as high as the active metabolic rate due to excitement (Fry, 1967; Peer Mohamed, 1981a). Metabolic rates related to racial behaviour in an arctic whitefish, and to season in the blue gill, have been found to show significant differences when body weight, temperature and activity are taken into account (Wohlschlag, 1957; Wohlschlag and Juliano, 1959; Brett, 1962).

In Lates, the activity has no change in varying salinity which is in concordance with the observations made by Dizon et al. (1977) on the relationship between salinity and swimming performances of skip jack and

yellow fin tuna. He found that a consistent pattern of change in the swimming speed of these species did not occur in response to salinity decrease from 34‰ to 29‰. The main direction in adaptable metabolic phenomena in the course of evolution was one leading the path of active metabolism and the capacity to increase it to this or another level. The determination of maximal active metabolism of the fish and its comparison with standard metabolism are of great importance and this holds good for the determination of energy expenditure during movements performed over long periods of time.

i) **Estimates of energy utilization in sea water, brackish water and freshwater**

Energy yielding metabolism is one of the main biochemical criteria organically linked with the vital functions of animals (Pavlovskii, 1964). Like other animals, fish also tend to eat to meet their energy requirements (Rozin and Mayer, 1961).

From the data on normoxia and hypoxia it is possible to estimate the proportion of energy derived aerobically and anaerobically; and to some extent the relative amount of substrates degraded could also be checked, as suggested by Kutty and Peer Mohamed (1975). Basing his calculations on mammalian energetics, Kutty (1972) observed that the metabolism of Tilapia mossambica, immediately after handling, had an anaerobic component in the metabolism at 30°C and concluded that 20% of carbondioxide produced resulted from anaerobic sources. Similarly Kutty and Peer Mohamed (1975) found out that mullet at 30 and 35°C did not derive any energy,

anaerobically. Further, they expressed that the values from mammalian energetics need not be true for fish since the end product of fish metabolism is predominantly ammonia, and the structure and caloric content of the fish protein may be different from those of mammals (Krueger et al., 1968). Hence an attempt to get an estimate of energy utilization in the case of Lates in different media is made, based on approximations made from data on fish by Kutty and Peer Mohamed (1975).

In fish tissue free amino acid pool is an important source of energy (Hochachka and Somero, 1973; Kutty and Peer Mohamed, 1975) as shown in the case of other groups of animals (Awapara, 1962; Florkin, 1966; Peer Mohamed, 1974). Free amino acids available would indicate the type of proteins available for degradation (Peer Mohamed, 1974). In carp muscle about 80% of the free amino acid pool is made up of glycine (48%) and histidine (32%) (Creac'h, 1966; Love, 1970; Kutty and Peer Mohamed, 1975). The muscle amino acids are the major energy source (muscle constitutes about 50% of body weight in most fish) and hence the energy derivable from proteins can be estimated (Peer Mohamed, 1974). Brafield and solomon (1972) pointed out that since amino acids are completely broken down to carbon dioxide, water and ammonia the oxycalorific value of proteins in fish metabolism should differ from that of mammalian metabolism and an R.Q. of unity could be expected if alanine is oxidized. Similarly, if glycine is used as a substrate, the R.Q. and A.Q. will be:

$$C_2H_5O_2N + 1.5\ O_2 \longrightarrow 2\ CO_2 + H_2O + NH_3$$

$$\text{R.Q.} = CO_2/O_2 = 2/1.5 = 1.33$$

$$\text{A.Q.} = NH_3/O_2 = 1/1.5 = 0.667$$

Accordingly histidine would yield R.Q. and A.Q. values of 1.2 and 0.6 respectively. Hence it is obvious that aerobic break down of these amino acids, as expected in ammonotelic animals, yields R.Q. values of above unity and A.Q. values upto 0.67 or higher, as in the case of arginine which yields an A.Q. of 0.9. Generally, while estimating R.Q. and A.Q. values of protein break down in fish, it is assumed that always a mixture of glycine and histidine is used as a substrate in the ratio of 3:2 and the degradation of this mixture would result in R.Q. and A.Q. values of 1.24 and 0.621 respectively (Peer Mohamed, 1974). With these basic assumptions the energy yield from proteins and other substrates in sea bass has been calculated as given below.

i) **Estimates of energy utilization in routine metabolism at 30 and 35°C**

Using the routine values of metabolism tested in air saturated sea water at 30°C the estimates of energy utilization has been worked out (data from Table 22).

Routine Oxygen consumption	=	126.55 mg/kg/hr	
Routine A.Q.	=	0.77	
Routine A.Q.	=	0.09	
Routine Carbon dioxide production	=	126.55x0.77	= 97.44 mg/kg/hr
Routine Ammonia excretion	=	126.55 x 0.09	= 11.39 mg/kg/hr
Oxygen needed to degrade proteins (glycine + histidine) to produce 11.39 mg/kg/hr of Ammonia	=	11.39/0.621	= 18.34 mg/kg/hr

Table 22. Metabolic rates, quotients and activity used for the estimates of energy utilized by sea bass in sea water, brackish water and freshwater at 30 and 35°C during routine metabolism are given.

	30°C			35°C		
	Sea water	Brackish water	Fresh water	Sea water	Brackish water	Freshwater
Oxygen (mg/kg/hr)	126.55 ± 42.72	100.14 ± 93.15	135.90 ± 58.43	79.65 ± 65.22	94.87 ± 87.73	129.63 ± 28.40
Carbon dioxide (mg/kg/hr)	97.23 ± 32.70	76.09 ± 69.67	105.57 ± 47.77	64.84 ± 69.03	76.56 ± 74.51	104.55 ± 51.06
Ammonia (mg/kg/hr)	10.51 ± 10.46	8.92 ± 5.24	11.43 ± 5.85	3.59 ± 4.70	12.37 ± 12.68	13.34 ± 8.57
R.Q.	0.77 ± 0.08	0.80 ± 0.15	0.77 ± 0.10	0.72 ± 0.16	0.82 ± 0.17	0.76 ± 0.26
A.Q.	0.09 ± 0.09	0.12 ± 0.07	0.10 ± 0.06	0.04 ± 0.02	0.14 ± 0.06	0.12 ± 0.11
Random activity (counts/hour)	31.5 ± 10.96	23.5 ± 5.74	11.56 ± 10.04	39.00 ± 22.77	32.50 ± 13.00	43.00 ± 30.70

Carbon dioxide equivalent of 18.34 mg/kg/hr oxygen in protein breakdown	=	18.34 x 1.24	= 22.74 mg/kg/hr
Non protein oxygen	=	126.55 - 18.34	= 108.21 mg/kg/hr
Non protein carbon dioxide	=	97.44 - 22.74	= 74.70 mg/kg/hr
Non protein R.Q.	=	74.7/108.21	= 0.690

Since the non protein R.Q. is clearly below unity, it appears that the sea bass was completely aerobic in air saturated water at 30°C. It is also possible to calculate the proportionate energy derivations from the three substrates proteins, carbohydrates and fats in Lates from the values already obtained. As protein yields 4.57 k cal/l O_2 (4.57 cal/ml O_2) (corrected value for ammonotelic animals, Brafield and Solomon, 1972), the net caloric yield from protein is 83.81 cal/kg/hr (4.57 x 18.34). Non protein R.Q. derived for sea bass at 30°C is below 0.7 which is quite below the conventional R.Q. values for fat (Dowban, 1969). Therefore it is assumed that non protein substrate is only fat and, glucose is not involved in oxidative energy release (Sukumaran and Kutty, 1987). The energy yields from fat is 4.665 cal/ml/O_2 (Cantarow and Scheparts, 1967). From this it can be estimated that the total yield from fat is 504.80 cal/kg/hr (4.665x108.21). Hence the percentage of energy utilized by the breakdown of proteins and fats in sea bass exposed to high oxygen levels at 30°C in sea water are 14.24% and 85.76% respectively.

Based on the above calculations, the estimates of caloric yield from three substrates proteins, carbohydrates and fats, and, their percentage

composition along with non protein R.Q. are given in Table 23 (based on data from Table 22) for sea bass tested at 30 and 35°C in sea water, brackish water and freshwater. When tested at 30°C the caloric yield from protein metabolism had increased from 83.81 cal/kg/hr in sea water to 88.48 cal/kg/hr in brackish water and then increased to 99.99 cal/kg/hr in freshwater. As suggested by Brett and Groves (1979) that the principal energy source in carnivorous fish appear to be lipid and protein rather than lipid and carbohydrate, here also the energy yield from protein and fat are more than the energy from carbohydrate in aerobic condition. In all the three media non protein R.Q. values are below 0.7 which is quite below the conventional R.Q. values for fat (Sukumaran and Kutty, 1987) suggesting that the fish has not derived any energy from carbohydrate. The caloric yield from fat metabolism has declined in brackish water (377.24 cal/kg/hr) from the level of sea water (504.80 cal/kg/hr) and it increased in freshwater (530.42 cal/kg/hr) from that of sea water.

When sea bass was exposed to 35°C, the caloric yield from proteins showed a continued increase from sea water to brackish water and then further to freshwater (23.49 cal/kg/hr → 97.75 cal/kg/hr → 114.52 cal/kg/hr). Since the non protein R.Qs in sea water, brackish water and freshwater are 0.684, 0.698 and 0.645, the fish has not used carbohydrate as the energy source in all the media. However, the caloric yield from fat slightly declined from 346.99 cal/kg/hr to 343.52 cal/kg/hr and then increased to 481.96 cal/kg/hr in freshwater. A similar trend was observed in percentage of caloric yield also.

Table 23. Caloric yield and percentage of energy derived from different substrates (protein and fat) by sea bass during routine metabolism, in sea water, brackish water and freshwater at 30 and 35°C are given. Corresponding non protein R.Q. values in each medium are also given.

Substrate	30°C						35°C					
	Sea water		Brackish water		Freshwater		Sea water		Brackish water		Freshwater	
	Caloric yield cal/kg/hr	%	Caloric yield cal/kg/hr	%	Caloric yield cal/kg/hr	%	Caloric yield cal/kg/hr	%	Caloric yield cal/kg/hr	%	Caloric yield cal/kg/hr	%
Protein	83.81	14.24	88.48	19.00	99.99	15.86	23.49	6.34	97.75	22.15	114.52	19.20
Carbohydrate	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Fat	504.80	85.76	377.24	81.00	530.42	84.14	346.99	93.66	343.52	77.85	481.96	80.80
Total energy	588.61		465.72		630.41		370.48		441.27		596.48	
Non protein R.Q.	0.690		0.694		0.680		0.684		0.698		0.645	

(ii) **Estimates of energy utilization under hypoxia at 30 and 35°C**

To show the estimates of energy derived during hypoxic metabolism, the metabolic rates obtained at the lowest ambient oxygen level (based on Table 24) at 30°C in sea water is given as an example.

Oxygen consumption	=	75.53 mg/kg/hr
R.Q.	=	1.46
A.Q.	=	0.184
Carbon dioxide production	=	75.53x1.46 = 110.27 mg/kg/hr
Ammonia excretion	=	75.53x0.184 = 13.90 mg/kg/hr
Oxygen equivalent of Ammonia excreted	=	13.90/0.621 = 22.38 mg/kg/hr
Carbon dioxide equivalent of oxygen consumed	=	22.38x1.24 = 27.75 mg/kg/hr
Non protein oxygen	=	75.53-22.38 = 53.15 mg/kg/hr
Non protein carbon dioxide	=	110.27-27.75= 82.52 mg/kg/hr
Non protein R.Q.	=	82.52/53.15 = 1.55
Anaerobic carbon dioxide produced	=	0.55x53.15 = 29.23 mg/kg/hr

Since the non protein R.Q. is above unity, it is suggestive that sea bass at 30°C is anaerobic. As suggested by Kutty and Peer Mohamed (1975), since the A.Q. has not exceeded 0.62 (aerobic maximum for the mixture of glycine and histidine, as assumed), it is impossible to judge whether any ammonia is produced anaerobically. An A.Q. near unity was obtained at an ambient oxygen level of 0.6 mg/l (Kutty, 1972) in the case of Tilapia mossambica, and in such cases anaerobic ammonia production must have occurred. It is quite possible that the anaerobic glycolysis and amino acid

breakdown are linked together during anaerobiosis (Kutty, 1972; Hochachka and Somero, 1973; Kutty and Peer Mohamed, 1975).

The proportion of energy derived anaerobically from proteins and carbohydrates cannot be calculated directly from the data on hypoxic metabolism. As assumed by Kutty and Peer Mohamed, this can be approximated, if an energy estimate for the mean level of random activity concerned for the hypoxia (Fig.1) is known. Based on that, the mean random activity for the corresponding hypoxic metabolism is 34.79 counts/hr. An extrapolated oxygen consumption for the random activity (34.79 counts/hr), is 74.13 mg/kg/hr from the regression line of oxygen consumption against random activity at 30°C (Fig.1). With the assumption of Kutty and Peer Mohamed (1975) that the caloric value of oxygen for randomly active sea bass exposed to high oxygen is also applicable to the extrapolated value of 74.13 mg/kg/hr, it can be calculated that the latter is equivalent to 516.07 cal/kg/hr (588.61 x 74.13/84.55). The energy derived from proteins is 102.28 cal/kg/hr (4.57x 22.38 mg/kg/hr). The amount of energy derived from carbohydrates aerobically, equivalent to 53.15 ml O_2/kg/hr, is 268.41 cal/kg/hr (5.05x53.15). Since fat is not known to be used anaerobically (Hochachka and Somero, 1973; Kutty and Peer Mohamed, 1975), the non protein energy source in hypoxic condition is mainly carbohydrate, and from the total energy of 516.07 cal/kg/hr the energy derived anaerobically can be calculated as 145.38 cal/kg/hr [516.07-(102.28+268.41)] which is about 28.17% (145.38x100/516.07). The estimate of anaerobic carbon dioxide is 29.23 mg/kg/hr of the total carbon dioxide produced.

Similarly, the estimates of caloric yield from proteins, carbohydrates and anaerobic source, and their percentage composition alongwith non protein R.Q. were calculated and presented in Table 25 (based on the data given in Table 24) for sea bass tested at 30 and 35°C in three different media.

The energy derived from proteins at 30°C in hypoxic metabolism was found to increase from 102.28 cal/kg/hr in sea water to 190.75 cal/kg/hr in brackish water, which then decreased to 132.48 cal/kg/hr in freshwater. In contrast to protein, the energy derived from carbohydrate first decreased from 268.41 cal/kg/hr in sea water to 163.17 cal/kg/hr in brackish water, and then increased to 266.79 cal/kg/hr in freshwater. The anaerobic energy derived, are in increasing order in sea water, brackish water and freshwater such as 145.38 cal/kg/hr, 67.7 cal/kg/hr and 170.24 cal/kg/hr respectively. From the non protein R.Q. of 1.55, 2.89 and 2.53 the anaerobic carbon dioxide produced can be calculated as (0.55 x 53.15) 29.23 mg/kg/hr in sea water, (1.89 x 32.31) 61.07 mg/kg/hr in brackish water, and (1.53 x 52.83) 80.83 mg/kg/hr in freshwater.

At 35°C, the energy derived from protein is in increasing order from sea water to brackish water to freshwater (45.61 cal/kg/hr to 147.34 cal/kg/hr to 182.89 cal/kg/hr). As in 30°C the energy derived from carbohydrate decreased from 262.55 cal/kg/hr in sea water to 211.55 cal/kg/hr in brackish water and then increased to 221.94 cal/kg/hr. The anaerobic energy continuously increased from 11.33 cal/kg/hr in sea water to 74.28 cal/kg/hr in brackish water and then to 172.56 cal/kg/hr in freshwater. The percentage of caloric yield also showed a continued increase from sea water to brackish

Table 24. Metabolic rates, quotients and random activity used for the estimates of energy derived by sea bass during hypoxic metabolism in sea water, brackish water and freshwater at 30 and 35°C are given.

	30°C			35°C		
	Sea water	Brackish water	Freshwater	Sea water	Brackish water	Freshwater
Oxygen (mg/kg/hr)	75.53 ± 14.32	74.05 ± 42.41	81.82 ± 16.87	61.97 ± 19.13	74.14 ± 34.82	83.97 ± 30.54
Carbon dioxide (mg/kg/hr)	109.72 ± 27.26	150.78 ± 121.13	166.25 ± 48.07	119.37 ± 44.78	141.75 ± 67.31	179.44 ± 42.42
Ammonia (mg/kg/hr)	13.85 ± 7.80	22.22 ± 4.97	15.78 ± 14.79	6.07 ± 1.42	12.13 ± 12.84	24.24 ± 15.86
R.Q.	1.46 ± 0.23	1.96 ± 0.57	2.07 ± 0.56	1.90 ± 0.15	1.90 ± 0.22	2.23 ± 0.38
A.Q.	0.184± 0.09	0.35 ± 0.13	0.22 ± 0.23	0.10 ± 0.01	0.27 ± 0.44	0.296± 0.14
Random activity (counts/hour)	25.28 ± 17.29	18.25 ± 10.72	24.89 ± 12.69	6.0 ± 4	25.75 ± 19.32	40.8 ± 30.36

Table 25. Caloric yield and percentages of energy derived from different substrates (proteins and carbohydrates) by sea bass during hypoxic metabolism at 30 and 35°C in sea water, brackish water and freshwater are given. Anaerobic energy derived under hypoxia and non protein R.Q. values in each media are also given.

	30°C						35°C					
	Sea water		Brackish water		Freshwater		Sea water		Brackish water		Freshwater	
	Caloric yield cal/kg/hr	%	Caloric yield cal/kg/hr	%	Caloric yield cal/kg/hr	%	Caloric yield cal/kg/hr	%	Caloric yield cal/kg/hr	%	Caloric yield cal/kg/hr	%
Proteins	102.28	19.82	190.75	45.24	132.48	23.26	45.61	14.28	147.34	34.01	182.89	31.68
Carbohydrates	268.41	52.01	163.17	38.70	266.79	46.85	262.55	82.17	211.55	48.84	221.94	38.43
Fat	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Anaerobic energy	145.38	28.17	67.7	16.06	170.24	29.89	11.33	3.55	74.28	17.15	172.56	29.89
Total energy	516.07		421.62		569.51		319.49		433.17		577.40	
Non protein R.Q.	1.55		2.89		2.53		2.03		2.41		3.13	

water and then further to freshwater (3.55% to 17.15% and then to 29.89%). The total energy derived were 319.49 cal/kg/hr in sea water, 433.17 cal/kg/hr in brackish water and 577.40 cal/kg/hr in freshwater which was also in increasing order. The non protein R.Q. also showed an increasing trend from 2.03 to 2.41 and then to 3.13. So the anaerobic carbon dioxide produced are 53.55 mg/kg/hr (1.03 x 51.99) in sea water, 59.07 mg/kg/hr (1.41x41.89) in brackish water and 93.61 mg/kg/hr (2.13x43.95) in freshwater.

The sea bass derives energy from protein in both aerobic and anaerobic condition. Carbohydrate is not used as the energy source in aerobic condition. In routine metabolism, lipid or fat seems to be the major energy source. Brett and Groves (1979) suggested that lipids are the principal non protein energy source in the natural diets of both carnivorous and omnivorous fish provided, dietary fats are liquid at ambient temperatures and fats are both highly digestible and readily metabolized. The fact that "the presence of large amounts of oil in fish means that lipids rather than carbohydrate is the favoured energy reserve of most aquatic animals in natural environment" apply to all fish, especially to marine fish (Hoar et al., 1979). The larvae of sea bass nourishes well with oil globule, and the long persistence of the oil globule in this species explains the robustness of the larvae and the stabilization of survival rates at a very early stage of its life history (Hiroshi Kohno et al., 1986). During the present study, R.Q. values were found to be greater than unity in the case of sea bass. R.Q. values greater than unity have been reported in several cases where there was active synthesis of body fat. Kutty (1972) reported that in the case of fish, anaerobic metabolism can result in R.Q. values between 1 and 2. Usually the caloric

equivalent of oxygen uptake "Qox" consequently varies with the physiological state and substrate utilized. Blazka (1958) and Nagai and Ikeda (1971) suggested that in crucian carp under ammonia, glycogen is converted to fat resulting in the release of carbon dioxide and energy. Nagai and Ikeda (1971) who studied carbohydrate metabolism in carp, showed the incorporation of glucose C^{14} in amino acids (protein) and in lipids clearly suggesting the conversion of glucose into other energy substrates namely fat and proteins.

The energy necessary for the osmotic work in stress media seems to be derived predominantly through oxidative degradation of blood glucose as evidenced by the increase in caloric yield of carbohydrate in anaerobic condition and the accompanying increase in the oxygen consumption in fish acclimated to these media. The rate of utilization of blood glucose by sea bass in various acclimation media appears to be governed by the combined effects of media, salinity and the osmotic gradient of blood medium as observed in Tilapia mossambica by Bashamohideen and Parvatheswararao (1972).

j) General discussion

The solution to a number of practical problems such as the amount of food needed for the existence and growth of fish in natural and artificial conditions cannot be arrived at without a knowledge of active metabolism (Pavlovskii, 1964). Numerous factors may influence the standard or active metabolic rates, or both, and can therefore affect the scope for activity (Fry, 1957, 1967; Dickson and Kramer, 1971).

One of the significant observations of the present investigation is the higher rate of oxygen consumption in freshwater and brackish water

than in sea water in the case of sea bass. This is supported by the fact that oxygen consumption is known to increase in marine organisms transferred to dilute media, as also in freshwater organisms transferred to saline media (Parvatheswararao, 1965; Madanmohan Rao, 1968). Madanmohan Rao (1968) reported a higher oxygen consumption for rainbow trout in 30‰ than in freshwater at all swimming speeds, and attributed the difference to a higher cost of osmoregulation in salt water. The increase in heterosmotic media was attributed to the difference in salinity (Hickman, 1959) or increased osmotic gradient (Sarojini Devi, 1960; Parvatheswararao, 1965; Lotan, 1966; Madanmohan Rao, 1968) or to the pattern of metabolic osmotic relation (Bashamohideen and Parvatheswararao, 1972). This difference in the relation of metabolic rate to the salinity of the medium or the osmotic gradient, assumes considerable significance in the light of the suggestion that during the evolution of animals, osmotic independence appears to have originated a number of times and radiated in a number of directions, leading to the development of diverse regulatory mechanisms to solve the osmotic problems encountered in nature (Hickman, 1959). Such variance may be pronounced in the teleost fishes, whose chequered phylogeny involving a series of trans-migrations between fresh and salt waters, exposed them to a variety of osmotic situations to which they could adapt successfully, possibly through varied means (Parvatheswararao, 1967; 1970). Statistical results indicate that with oxygen consumption the interaction between environment and experiment were significantly different at 5% level. Active oxygen consumption for a 30 g euryhaline fish Aholehole (Kuhlia sandvicensis) is about 12% higher in salt water than in freshwater, but analysis of co-variance (F-test) indicates

that the samples are not significantly different. Freeman (1950) postulated that the metabolic activity of the brain is the predominating factor governing the rate of oxygen consumption in fish and, probably excitement is effective through the central nervous system, causing increased muscle tone and increased rate of oxygen consumption (Smit et al., 1971).

Another significant observation is the anaerobic ability of the sea bass to survive hypoxia seen in all the media at both the temperatures. In agreement with the findings of Kutty (1968a), and Kutty and Peer Mohamed (1975), it is assumed that the magnitude of R.Qs can only indicate the intensity of anaerobic metabolism. R.Q. values during the current study were significantly above unity, clearly suggesting that during hypoxia, there was a considerable amount of anaerobic metabolism resulting in the release of excess carbon dioxide, as in the case of Rhinomugil corsula noted by Kutty and Peer Mohamed (1975) and fry and fingerlings of Mugil cephalus and Chanos chanos as confirmed by Usha Devi (1987).

It is very interesting to note that in routine metabolism at highest ambient oxygen, R.Q. value rose to a maximum of 6.67 and at lowest ambient oxygen to 12.73 as mentioned earlier. The higher the R.Q. over unity, the higher the extent of anaerobic metabolism (Kutty and Peer Mohamed, 1975). The R.Q. values above unity recorded by Kutty (1972) in Tilapia mossambica suggested that the observed routine R.Q. of unity has a protein component since there is some amount of protein degradation and, this then leaves room for an anaerobic component in the routine R.Q. and, the corresponding anaerobic carbon dioxide produced also can be estimated. The R.Q. values above unity indicate the involvement of anaerobic energy

at high ambient oxygen concentrations during routine and active metabolism that can be at least partially attributed to the fact that the fish were handled just prior to the experiment as indicated by the studies of Kutty (1972) in Tilapia mossambica. Since the R.Q. values are above unity, sea bass seems to be showing partial anaerobiosis during normoxia similar to the partial anaerobiosis at high ambient oxygen noticed in gold fish, which sustained for a long period (Kutty, 1968a); and Tilapia mossambica (Kutty, 1972) which has mechanisms to produce metabolic carbon dioxide even in the complete absence of oxygen possibly like its kin, the crucian carp (Blazka, 1958; Hochachka, 1961). Routine R.Q. value of above unity was estimated by Kutty (1968a) in gold fish (maximum mean R.Q. of 2.43) and in the initial phase of exercise in Rhinomugil corsula (Sukumaran and Kutty, 1987) suggesting that anaerobic energy utilization is high during initial phase. Tilapia mossambica acclimated to and tested in freshwater at 30°C maintained a routine R.Q. of above unity and an A.Q. of about 0.2 at high ambient oxygen concentrations. At low oxygen concentrations (below 2 ppm), R.Q. and A.Q. increased sharply to values of 8 and 1 respectively at 0.6 ppm, indicating a close relationship between increase in anaerobic energy utilization and increase in protein metabolism at inadequate oxygen concentrations. R.Q., A.Q. and their interactions did not show any significant difference statistically. Privolnev (1954) found out a R.Q. of 4.3 at 20°C for the crucian carp in the hypoxic condition. Kutty (1972) showed a maximum R.Q. of 8 in Tilapia mossambica and Peer Mohamed (1974) 3.56 in gold fish and 3.28 in Tilapia mossambica under hypoxic conditions.

Even under conditions of least stress, an R.Q. consistently above unity, signifying an anaerobic fraction, was found in gold fish forced to swim for hours at low swimming speed in adequate ambient oxygen (Kutty, 1968a). Tilapia mossambica could live in low oxygen conditions through aerobic means for long periods, but its capacity for continued anaerobic energy utilization appears to be limited (Kutty, 1972).

The anaerobic carbon dioxide or additional carbon dioxide produced are in increasing order from sea water to decreasing salinities right down to freshwater. This carbon dioxide production under hypoxia can be metabolic or non metabolic carbon dioxide as has already been discussed under "R.Q. and anaerobic metabolism". The accumulation of lactic acid in animal tissues causes the release of carbon dioxide from the bicarbonate reserve (Black, 1958; Black et al., 1959, 1966), thereby resulting in an R.Q. above unity (Kutty, 1968a). It is not known whether extra stress would increase the R.Q. (at higher levels of activity) as suggested by Kutty (1968a). Analysis of variance showed that carbon dioxide had significant difference with environment at 1% level. In most of the studies, the experimental fish was kept in the respirometer for sometime for recovery from the handling effects, if any, because handling results in excitement and causes randon activity to increase (Brett, 1964; Fry, 1967). It has also been reported that R.Q. of gold fish and rainbow trout (Kutty, 1968a) and Tilapia mossambica (Kutty, 1972) are frequently above unity during periods of excitement. In the present study also, resultant routine R.Q. above unity must be due to the handling of fishes just before the experiments.

Thirdly, there was a clear cut increase in the rates of ammonia excretion with the decrease in rates of oxygen consumption under hypoxic conditions observed during hypoxia and recovery, as in Mystus armatus reported by Sukumaran and Kutty (1977). The end product of protein metabolism is taken as ammonia and calculations based on this fact indicate the prominent changes in R.Q., A.Q. and also in the energy derived from proteins (Brafield and Solomon, 1972). Statistical analysis showed that ammonia excretion was significantly different with the experiment, and the interactions of temperature with both environment and the experiment at 5% level. Increased ammonia excretion during hypoxia and the extra release of ammonia may be either from anaerobic ammonia production, or from the trapped or bound ammonia in muscle cell and blood as suggested by Rosado et al. (1962) in mammals. This ammonia may be of advantage to the fish in acid-base regulaltion (preventing acidosis) and iono-osmotic regulation (conservation of sodium) (Prosser and Brown, 1961; Maetz and Garcia Romeu, 1964; Garcia Romeu and Motais, 1966; Forster and Goldstein, 1969; Kutty, 1972). Goldstein et al. (1964) postulated that it is possible for non-ionic ammonia to be excreted by passive diffusion down a concentration gradient across the gill surface from blood to water and, that the activities of glutaminase and glutaminic acid dehydrogenase are high in fish gills. Release and excretion of ammonia may have a much more important role in buffering blood in fish, since the mechanism of acid-base balance in fish is less known and hitherto unknown buffering systems may be in operation (Albers, 1970).

Another salient point which comes out from the present study is the rise in A.Q. in routine metabolism, hypoxic metabolism and hypoxia and recovery in sea water, brackish water and freshwater clearly indicating the extra release of ammonia anaerobically as proved by Kutty (1972) in Tilapia mossambica and Peer Mohamed (1974) in Tilapia mossambica, Rhinomugil corsula, Puntius sarana and Carassius auratus. This is also supported by Driedzic and Hochachka (1975) that amino acids were utilized as an anaerobic energy source in carp white muscle although adenylate is the potential source of anaerobic ammonia (Sukumaran and Kutty, 1977). In all the cases at 30 and 35°C the A.Q. values obtained were more during hypoxia than in routine metabolism. The A.Q. values of aerobic phase such as 0.09, 0.12 and 0.10 at 30°C increased to 0.184, 0.35 and 0.22 at the same temperature during hypoxia in sea water, brackish water and freshwater respectively, showing the increased utilization of proteins as energy source. Similarly, the A.Q. values such as 0.04, 0.14 and 0.12 at 35°C shot up to about 0.10, 0.27 and 0.296 in sea water, brackish water and freshwater respectively. This is evidenced by the report given by Kutty (1972) in the case of Tilapia mossambica that the A.Q. values of the fish during aerobic phase remained at about 0.27 but shot up to a value of 1.0 at low oxygen concentrations (Kutty, 1972), which represents the extra release of ammonia anaerobically. Similarly, routine A.Q. value of 0.17 shot up to about 0.3 in Mystus armatus (Sukumaran and Kutty, 1977). The rise in A.Q. indicates the extra release of ammonia anaerobically (Kutty, 1972). In the case of A.Q., the interaction between environment and the experiment showed significant difference at 5% level.

Yet another significant observation is that more tolerance was observed in sea water at 30°C (asphyxial oxygen concentration, 1.17 ml/l O_2) and at 35°C (asphyxial oxygen concentration, 1.45 ml/l O_2) as stated previously. So in sea water, the natural habitat of sea bass, capacity of tolerance is more compared to other media, may be due to less osmoregulation, suggesting the operation of metabolic homeostasis alongside regulation as pointed out by Bashamohideen and Parvatheswararao (1972). There is considerable evidence to indicate the metabolic homeostasis as proved in the case of prawn Metapenaeus monoceros by Panampathi Rao, 1958; in the fish Etroplus maculatus by Sarojini Devi, 1960; Parvatheswararao, 1971; and in Tilapia mossambica by Bashamohideen and Parvatheswararao, 1972. Low oxygen acclimation increased blood oxygen capacity and efficiency in the utilization of oxygen, but not oxygen tolerance and anaerobic capacity (Shepard, 1955; Prosser et al., 1957, Kutty, 1968a,b; Peer Mohamed and Kutty, 1981). Shepard (1955) demonstrated that brook trout (Salvelinus fontinalis) could improve their tolerance to severe hypoxia through prolonged acclimation to hypoxia. The acclimation to low oxygen did not increase the R.Qs of gold fish exposed to low oxygen in the experiment conducted by Kutty (1968a). The energy necessary for osmotic work in stress media seems to be derived predominantly through oxidative degradation of blood glucose, as evidenced by the sharp increase in the blood glucose level and the accompanying increase in oxygen consumption and cytochrome oxidase activity in fish acclimated to these media. The negative slopes in hypoxic condition during the periods of hypoxia and recovery of the oxygen consumption - activity relations in sea water

and freshwater can perhaps be explained as due to the changes in acid loading or acidosis, and the consequent reduction in oxygen capacity of the blood, as in the case of hypoxic mullet explained by Peer Mohamed (1974). In crucian carp the ability to live anaerobically was much different at two different temperatures (Blazka, 1958; Kutty, 1968a) - while it can survive in anoxic conditions for months at 5°C, its hypoxic existence at 20°C is no more than a few hours. Anaerobic condition and the tolerance limit will not change the blood constituents (Shepard, 1955). There must be a complex interaction of water balance, ionic retention, tissue tolerance, irritability, oxygen transport, osmotic work etc., involved in the final metabolic balance (Brett, 1962).

A fifth significant observation is that in recovery metabolism, in all the three media, the fish recovered subsequently in air saturated water indicating that the anaerobic ability to survive hypoxia exists in this species. During recovery phase, sea bass showed a post hypoxic repayment of oxygen debt. It is noted that fish repays almost entirely all the oxygen debt accumulated. There is a clear correlation between metabolic and behavioural recovery (Head and Baldwin, 1986). Increased lactate concentrations in the tail muscle in Cherax destructor during the recovery period imply that anaerobic glycolysis makes a significant contribution to the metabolic recovery process and, the activation of glycolysis in this muscle has been attributed to the effects of decreased energy change in the regulatory enzymes phosphofructokinase and pyruvate kinase (England and Baldwin, 1985). The differences in recovery metabolism, in different media at the two temperatures, may be due to changes in metabolism and pathways due

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to temperature as suggested by Peer Mohamed (1974) in the case of Tilapia mossambica. Excitement and oxygen debt replacement make large demands on metabolism (Peer Mohamed, 1987). As swimming fish approach critical velocity, the anaerobic energy contribution increases forcing the fish into a cumulative oxygen debt (Brett, 1964) and this debt is repaid in the post exercise recovery period. In the present study Lates is seen showing post hypoxic repayment of oxygen debt.

Dickson and Kramer (1971) provided considerable scientific and statistical evidence in support of the hypothesis that males have higher active metabolic rates than females. However, in sea bass, the sex cannot be differentiated since they mature only after attaining a total length of 46 cm (Moore, 1979). Small individuals were found to have high rates of oxygen consumption (Moshiri and Cummins, 1969) and generally utilized less blood glucose and muscle glycogen than large ones in the various acclimation media, suggesting that the energy cost of osmoregulation is less in smaller fish as reported by Bashamohideen and Parvatheswararao (1972) that smaller individuals of Tilapia mossambica are osmotically efficient. The time required for completion of osmotic adjustments increases with age and decreases with increasing metabolic rate, and the gross efficiency of fish in stress media tends to decrease with increasing age (Kinne, 1964).

The temperature range (30-35°C) does not show any marked metabolic differences in the species either in the same medium or in different media. The test of significance also showed that the values at 30 and 35°C are not significantly different, suggesting that the temperature effect is minor.

This corroborates well with the findings of Peer Mohamed (1981b) in the case of Tilapia mossambica. While considering the fact that the rate of oxygen uptake of fish increases by a factor of 2 to 2.3 fold for every 10°C rise in temperature (Fry and Hochachka, 1970; Evans, 1977; Brett and Groves, 1979), substantial differences in metabolism at 30 and 35°C in the present study cannot be expected since there is only a difference of 5°C.

It was pointed out that among the three media tested in sea bass during hypoxia the activity first increased and later decreased towards the end of hypoxia in sea water and brackish water at either temperatures. This decrease in activity under hypoxic exposure, though it may not lead the fish to escape, would allow it to conserve energy as in Rhinomugil corsula (Kutty and Peer Mohamed, 1975; Peer Mohamed and Kutty, 1980) and Chanos chanos (Hamsa and Kutty, 1972; Usha Devi, 1987). In fresh-water, at both the temperatures, activity showed a reverse trend with a decrease at the end. The increased activity induced by hypoxia (in hypoxia and recovery) in all the cases, except 30°C in brackish water, might allow the fish to move out of the hypoxic environment to more oxygenated waters as in the case of Rhinomugil corsula, Puntius sarana and Carassius auratus reported by Peer Mohamed and Kutty (1980, 1982) and four marine teleosts, highlighted by Hamsa and Kutty (1972). Thus the difference in behaviour during hypoxic exposure may have a special significance for survival (Hamsa and Kutty, 1972). High blood glucose levels are correlated to high levels of activity (Gray and Hall, 1930).

The sixth main observation is the energy utilization of sea bass in all the media. Hoar et al. (1979) reported that the rate at which the energy is expended by fish can be seen to vary greatly according to the species, climatic zone, temperature, size, level of activity; and the energy utilization in the fish resembles that of a diabetic mammal. Glucose dehydrogenase activity in livers of carnivorous fish is said to be 4-7 times that in mammalian livers whereas the ability in herbivorous fish (grass carp, silver carp) is similar to that of mammals (Nagayama et al., 1973). Fish, very muscular animals, rely on glycolytic fermentation or so-called 'anaerobic metabolism' to cover their short term energy requirements. For a period of about 20 seconds fish may spend energy at a rate of about 100 times the basal rate (Brett, 1979). It has been proved by Blazka (1958, 1960); and Blazka and Kopecky (1961) that crucian carp (Carassius carassius) can survive without oxygen for months, obtaining energy by adequately converting glycogen to fat, thereby showing that the end product of the anaerobic metabolism is fat . Hinton et al. (1972) found that the liver of large mouth bass has a low fat content and that glycogen is the primary storage product (Heidinger and Crawford, 1977). The observation of Blazka and Kopecky (1961) that fish excrete organic carbon dioxide was confirmed by Hochachka (1961) in gold fish. Ekberg (1962) showed that under anoxic conditions the exercised gills excrete both organic and acidic carbon dioxide. There may have been a mixture of anaerobic and aerobic metabolism and the value of R.Q. may not be an indication that carbohydrate was the sole fuel used (Kutty, 1968a).

Energy derived aerobically and anaerobically by sea bass from different substrates in different media at 30 and 35°C were also estimated based

on the assumptions of Kutty and Peer Mohamed (1975). In aerobic condition, the non protein R.Q. was below 0.7 and so the fish has used only protein and fat as the energy sources. In anaerobic metabolism, the non protein R.Q. values are above unity which indicate that anaerobic energy has been derived and is mainly from protein and carbohydrate. Lates has used comparatively more energy in freshwater medium, in routine and hypoxic metabolism. This agrees well with the findings of Unnikrishnan and Laxminarayana (1984) in prawn, Penaeus indicus, that it spent more energy in lower salinities than in higher salinities. The lack of ions in freshwater could impose a fair energy demand to maintain osmotic balance in fish (Peer Mohamed, 1987). Carnivorous fish feeding on other fish, excrete an average of 27% of the energy of ingested food (Brett and Groves, 1979). Based on different views, this extra energy derived may be utilized for increased activity of the organism arising from its attempts to escape from the stress medium (Potts, 1954; Gross, 1957), or is utilized for increased transport of electrolytes in the media (Hickman, 1959), or is used towards increased osmotic work in stress media as the increase in oxygen consumption has been found to be directly proportional to the increase in the blood medium osmotic gradient (Sarojini Devi, 1960; Parvatheswararao, 1965; Madanmohan Rao, 1968). Analysis of variance showed that the interaction between the environment and the experiment was significantly different at 5% level but the others did not show any significant difference.

As stated by Kutty and Peer Mohamed (1975) in the estimates of energy utilized by Rhinomugil corsula under hypoxia, it is assumed that

in sea bass also the fish derives considerable amounts of energy anaerobically much more efficiently by pathways other than the glycolytic path, such as those involved in the simultaneous breakdown of carbohydrate and amino acids (Hochachka and Somero, 1973) resulting in increased carbon dioxide and ammonia excretion. Both the Embden - Mayerhoff and the Pentose phosphate (hexose mono phosphate shunt) pathways are reported to be taking part in the metabolism of fishes (Brown, 1960; Hochachka, 1961, 1962; Hochachka and Hayes, 1962). Although pathways are unknown, Hochachka (1961) and Ekberg (1962) suggested that gold fish has mechanisms for the production of metabolic carbon dioxide even in the complete absence of oxygen at low temperatures. These mechanisms apparently can adequately supply all the energy demands of the organism. Carbon dioxide has long been known to reduce the affinity of blood for oxygen (Root, 1931) and to influence the metabolic rate of fish (Basu, 1959; Beamish, 1964; Hoar and Randall, 1978).

Hochachka observed the accumulation of pyruvate and alanine as the end products of glycolysis in carp white muscle (Sukumaran and Kutty, 1987). Pyruvate and lactate are important metabolites which appear in both anaerobic and aerobic metabolism; however, if the combustion of metabolites through the Kreb's cycle is impaired through a reduction in oxygen supply, then lactate accumulates. Accumulation of lactate is one of the indications of disturbed homeostasis i.e. fatigue (Black et al., 1966). Energy lack is compensated by increased anaerobic energy utilization which is also accompanied by increased protein degradation, production and excretion of ammonia as suggested by the sharp increase in A.Q. with increase in

random activity. Kutty (1972) pointed out that terminal deaminations (Forster and Goldstein, 1969) can possibly account for a large portion of ammonia produced, in which case, the relative energy available from proteins may be much less. This type of ammonia excretion in Tilapia mossambica was not observed during forced activity (Karuppannan, 1972) and the ammonia produced in Mystus armatus (Sukumaran and Kutty, 1977) also denotes energy derived fully from the protein metabolism.

The present study indicates that among the euryhaline fishes, sea bass shows remarkable adaptability to wide variations in salinity. The results of energy utilization during the current study have shown that the energy expenditure of this species is minimal in sea water and brackish water compared to freshwater (Table 23 and 25). Hence it is expected that growth rates would be best in these media. It has also been established through the current study that activity of the fish is relatively least in brackish water leading to less energy expenditure, and hence better food conversion to stored energy. This should contribute significantly to high production rates. Eventhough the growth rate will not be as high as in the other two media, this species can also be cultured in freshwater impoundments of inland waters. The prospects of culturing this species on a commercial scale in sea water, and brackish water impoundments should prove very successful due to the remarkable versatility and adaptability of the species to changing salinities, its voracious feeding habits, its phenomenal growth rate, and the commercial value that the fish commands in Indian and overseas markets.

VI. SUMMARY

1. Metabolic rates, quotients (R.Q. and A.Q.) and random swimming activity of sea bass (acclimation and test at 30 and 35°C) subjected to routine metabolism (at high ambient oxygen near air saturation), hypoxic metabolism (from air saturation down to asphyxial level) and recovery metabolism (recovery after hypoxia) were determined in three different media such as sea water (30‰), brackish water (15‰) and freshwater (0‰).

2. Measurements of oxygen consumption, carbon dioxide production, ammonia excretion, quotients (R.Q. and A.Q.) and random activity of sea bass analysed in all the experiments (routine, hypoxia, and hypoxia-recovery) are presented in Figures 1 to 22 based on the corresponding Tables to show trends in each medium at different temperatures.

3. The experimental set-up and experimental procedure for all the experiments in different salinities are similar and were conducted with the help of a respirometer. For transitional acclimatization of the fishes from 30‰ saline medium to 15‰ and 0‰ media, the 'drip method' was used and this was found suitable because of the gradual increase or decrease in salinity. It can easily be adapted to by the animal since the change in salinity is within the 'zone of tolerance' (Perry et al., 1984).

4. It was noticed that oxygen consumption rates were high in the media to which the experimental fish were transferred (brackish water and freshwater) than in natural medium (sea water) and, also the rates were high at 35°C as compared to 30°C in all the media. The mean routine rate of oxygen consumption increased, from 78.26 mg/kg/hr to 86.55 mg/kg/hr (Table 19) in sea water; 100.57 mg/kg/hr to 111.46 mg/kg/hr (Table 20)

in brackish water; and 72.59 mg/kg/hr to 126.36 mg/kg/hr (Table 21) in freshwater, when the temperature was raised. The interaction between the experiment and environment was significantly different at 5% level with oxygen consumption.

5. All the experimental media were decarbonated before use in order to get an accurate estimate of total carbon dioxide. Carbon dioxide production during hypoxia compares well with the same condition in Tilapia mossambica (Peer Mohamed, 1974) at 30 and 35°C in sea water. Carbon dioxide production and ammonia excretion showed similar trends at both the temperatures in sea water during hypoxia. This increasing trend of ammonia excretion at 30 and 35°C is in agreement with the report given in the case of Rhinomugil corsula (Kutty and Peer Mohamed, 1975). The relative increase in ammonia production during anaerobiosis helps to prevent acidosis (Prosser and Brown, 1961). The changes in the rates of oxygen consumption, carbon dioxide output and ammonia excretion at different temperatures and salinities are reflected in R.Qs and A.Qs.

6. The R.Q. values were significantly above unity in normoxia and hypoxia clearly suggesting that considerable amount of anaerobic metabolism has taken place, resulting in the release of excess carbon dioxide as in Rhinomugil corsula reported by Kutty and Peer Mohamed (1975); in gold fish by Kutty (1968a); in Tilapia mossambica by Kutty (1972), Sukumaran and Kutty (1987); in crucian carp by Privolnev (1954) and fry and fingerlings of Mugil cephalus and Chanos chanos as reported by Usha Devi (1987). The size of the R.Qs over unity is indicative of the intensity of anaerobic metabolism.

7. The rise in A.Q. in all the experiments, in all the media, at 30 and 35°C clearly indicate the extra release of ammonia anaerobically. A.Q. values increased along with R.Q. under low oxygen suggesting a coupling of increased ammonia excretion with increased carbon dioxide output. The A.Q. values obtained were higher during hypoxia than during normoxic condition, in all the cases at both the temperatures.

8. The random activity of the fish increased when the temperature was raised from 30 to 35°C in all the media during routine metabolism. The mean rates which were 35.98 c/hr, 24.19 c/hr and 23.17 c/hr in sea water, brackish water and freshwater respectively at 30°C, rose to 39.93 c/hr, 24.27 c/hr and 53.00 c/hr at 35°C (Table 1). When the oxygen was gradually reduced in the respirometer during hypoxia in sea water and brackish water, the activity of the fish first increased and then decreased apparently to escape from the medium as in the case of Tilapia mossambica and Chanos chanos. The increased activity induced by hypoxia (in hypoxia - recovery phase) might allow the fish to move out of the hypoxic environment to more oxygenated waters as in the case of Rhinomugil corsula, Puntius sarana and Carassius auratus, reported by Peer Mohamed and Kutty (1980 and 1982) and, four marine teleosts as reported by Hamsa and Kutty (1972). Thus sea bass cannot be grouped singularly under passive or active group, since it is showing the characters of both depending on the situation.

9. During hypoxia, the asphyxial oxygen concentration (concentration at which the fish begins loosing its equilibrium) observed in sea water (30‰) was 1.17 ml/l at 30°C and 1.45 ml/l at 35°C, but when the salinity was

decreased to 15‰ (brackish water) the asphyxial oxygen concentration was found to be 1.53 ml/l at 30°C and 1.60 ml/l at 35°C, while when the salinity was again decreased to 0‰ (freshwater) the asphyxia was noted at 1.54 ml/l oxygen concentration at 30°C and 1.60 ml/l at 35°C. Hence the decreasing tolerance of hypoxia in the three media can be arranged in the following order : sea water $\geq$ brackish water $\geq$ freshwater at 30°C. The fish also showed relatively more tolerance in sea water at 35°C.

10. In recovery metabolism, in all the three media, after asphyxiation, the fish recovered subsequently in air saturated water, thus indicating that the anaerobic ability to survive hypoxia exists in this species. During the recovery phase, sea bass showed a post hypoxic repayment of oxygen debt. It is noted that fish repays almost entirely all the oxygen debt accumulated. The trends at 30 and 35°C are similar in the hypoxic phase and subsequently the recovery phase.

11. Collected data was statistically analysed by different methods. Regression lines fitted for plots of metabolic rates and quotients against random activity at 30 and 35°C at ambient oxygen concentration near air saturation are presented. Analysis of variance showed that mean ambient oxygen was found to be significantly different with oxygen consumption; carbon dioxide production also had significant difference with the environment; and results of ammonia excreted by the fish were significantly different both with the environment and the interaction of the same with the experiment. R.Q., A.Q. and their respective interactions did not show any significant difference. All the parameters and their interactions had significant difference with activity. But temperature showed no significant

difference individually with activity. All the regression coefficients of metabolic rates and quotients at 30 and 35°C did not show significant difference.

12. The observations were made at 30 and 35°C; the higher temperatures were chosen because of the scarcity of information on fish energetics at these temperatures and also because of their relevance to tropical conditions. Temperature did not show any significant influence in all the three media. It is also statistically proved that the temperature range (30°C-35°C) does not cause a marked metabolic difference in the species, either in the same medium or in different media.

13. The amount of energy utilized, both aerobically and anaerobically from different substrates by sea bass in sea water, brackish water and freshwater at 30 and 35°C were estimated based on the assumptions by Kutty and Peer Mohamed (1975). In aerobic condition, the non protein R.Q. was below 0.7 which is quite below the conventional R.Q. values for fat. Hence the fish has used only protein and fat, and glucose is not involved in oxidative energy release. In anaerobic metabolism, the non protein R.Q. values were above 1 which showed that anaerobic energy has been derived and that it is mainly from protein and carbohydrate. Energy utilization by sea bass under hypoxia suggest that the fish derives considerable amounts of energy anaerobically, much more efficiently by pathways other than the glycolytic path, such as those involved in the simultaneous breakdown of carbohydrates and amino acids (Hochachka and Somero, 1973) resulting in increased carbon dioxide and ammonia excretion, as in the case of Rhinomugil corsula reported by Kutty and Peer Mohamed (1975).

14. The present investigation indicates the possibility of culturing the marine species, Lates calcarifer in freshwater and brackish water without adopting any transitional acclimatization which is necessary in the case of many other species. The energy utilization studies have shown that eventhough it derives considerable amount of energy anaerobically in all the three media, the energy expenditure and the activity of the species are minimal in sea water and brackish water compared to freshwater. Hence the growth rate would be more in these two media. However, due to the remarkable versatility and adaptability of this species to changing salinities, its voracious feeding habits, its phenomenal growth rate and the commercial value in Indian and overseas markets, the culture prospects of this species on a commercial scale in any of the three media namely sea water, brackish water and freshwater should prove very successful.

VII. REFERENCES

ALBERS, C., 1970. Acid-base balance. In: Fish physiology, Vol.VI. W.S. Hoar and D.J. Randall (Eds.), Academic Press, New York. pp. 173-208.

ALI, M.A., 1979. Environmental physiology of fishes. Plenum, New York.

ANANTHA KRISHNAN, K.R. and **M.N. KUTTY,** 1974. Mortality and breathing rate at ambient temperatures in the cichlid fish, Tilapia mossambica (Peters). Indian. J. Exp. Biol., **12:** 55-59.

ANDERSON, J.R., 1975. The anaerobic resistance of Carassius auratus (L.). Ph.D. Thesis, Australian Nat. Univ., 424 pp.

ARSJAD, M., 1982. Sea bass and grouper culture programme in Jakarta province. In: Report of training course on sea bass spawning and larval rearing, Songkhla, Thailand; 1-20 June, Manila: SCS/GEN/82/39. 79 pp.

AURE, R.C., 1982. The culture of sea bass and related species in Iloilo province. In: Ibid. SCS/GEN/82/39. pp. 88-89.

AWANG, A., 1982. Programme for the culture of sea bass and related species by the Fisheries Division, at the west coast of peninsular Malaysia. In: Ibid. SCS/GEN/82/39. pp. 84-85.

AWAPARA, J., 1962. Free amino acids in invertebrates: a comparative study of their distribution and metabolism. In: J.T. Holden (Ed.). Amino acid pools. Elsevier, New York. pp. 158-175.

BAGARINAO, T., and **P. KUNGVANKIJ,** 1986. An incidence of swim bladder stress syndrome in hatchery-reared sea bass (Lates calcarifer) larvae. Aquaculture, **51(3-4):** 181-188.

BANNO, J. and **E. AMAR,** 1984. Culture of sea bass, Lates calcarifer (Bloch), in combination with different stocking densities of milk fish, Chanos chanos (Forskal) and Tilapia, Oreochromis mossambicus (Peters) in brackish water grow out ponds. Annu. Rep. Aquacult. Dep. SEAFDEC pp. 39-46.

BARLOW, C.G., 1981. Breeding and larval rearing of Lates calcarifer (Bloch) (Pisces: Centropomidae) in Thailand. Report to NSW States Fisheries, Sydney. 9 pp.

BASHAMOHIDEEN, M. and **V. PARVATHESWARARAO,** 1972. Adaptations to osmotic stress in the freshwater euryhaline teleost Tilapia mossambica. IV. Changes in blood glucose, liver glycogen and muscle glycogen levels. Marine Bilogy, **16:** 68-74.

BASU, S.P., 1959. Active respiration of fish in relation to ambient concentrations of oxygen and carbon dioxide. J. Fish. Res. Bd. Can., **16:** 175-212.

BEAMISH, F.W.H., 1964. Respiration of fishes with special emphasis on standard oxygen consumption. III. Influence of oxygen. Can. J. Zool., **42:** 355-366.

BEAMISH, F.W.H., 1966. Muscular fatigue and mortality in haddock, Melanogrammus aeglefinus, caught by otter trawl. J. Fish. Res. Bd. Can., **23:** 1507-1521.

BEAMISH, F.W.H. and **P.S. MOOKERJII,** 1964. Respiration of fishes with special emphasis on standard oxygen consumption. I. Influence of weight and temperature on respiration of gold fish, Carassius auratus L. Can. J. Zool., **42:** 161-175.

BEAMISH, F.W.H. and **L.M. DICKIE,** 1967. Metabolism and biological production in fish. In: The biological basis for freshwater fish production. S.D. Gerking (Ed.), Blackwell Scientific Publication. Oxford. 215 pp.

BILINSKI, E., 1974. Biochemical aspects of fish swimming. In: "Biochemical and biophysical perspectives in Marine Biology". D.C. Malins and J.R. Sargent (Eds.), Vol.1: 247-288. Academic Press, New York.

BLACK, V.S., 1951. Changes in body chloride, density and water content of chum and coho salmon fry when transferred from freshwater to sea water. J. Fish. Res. Bd. Can., **8:** 164-177.

BLACK, E.C., 1955. Blood levels of haemoglobin and lactic acid in some freshwater fishes following exercise. J. Fish. Res. Bd. Can., **12(6):** 917-928.

BLACK, V.S., 1957. Excretion and osmoregulation. In: Physiology of fishes. M.E. Brown (Ed.), Vol.1: 163-205. Academic Press Inc., New York.

BLACK, E.C., 1957a. Alterations in the blood level of lactic acid in certain salmonid fishes following muscular activity. 1. Kamloops trout, Salmo gairdneri. J. Fish. Res. Bd. Can., **14(2):** 117-134.

BLACK, E.C., 1957b. Alterations in the blood level of lactic acid in certain salmonid fishes following muscular activity. II. Lake trout, Salvelinus namayoush. J. Fish. Res. Bd. Can., **14(4):** 645-649.

BLACK, E.C., 1957c. Alterations in the blood level of lactic acid in certain salmonid fishes following muscular activity. III. Sockeye salmon, Oncorhynchus nerka. J. Fish. Res. Bd. Can., **14(6):** 807-814.

BLACK, E.C., 1958. Energy stores and metabolism in relation to muscular activity in fishes. pp. 51-67. In : The investigation of fish-power problems. H.R. Mac Millan Lectures, University of British Columbia Institute of Fisheries, Vancouver, B.C.

BLACK, E.C., W.G. CHIU, F.D. FORBES and **A. HANSLIP,** 1959. Changes in pH, carbonate and lactate of the blood of yearling Kamloops trout, Salmo gairdneri during and following severe muscular activity. J. Fish. Res. Bd. Can., **16(4):** 391-402.

BLACK, E.C., A.C. ROBERTSON, A.R. HANSLIP and **W.G. CHIU,** 1960. Alterations in glycogen, glucose and lactate in rainbow and Kamloops trout, Salmo gairdneri, following muscular activity. J. Fish. Res. Bd. Can., **17(4):** 487-500.

BLACK, E.C., A.C. ROBERTSON and **F.R. PARKER,** 1961. Some aspects of carbohydrate metabolism in fish. In: Physiology of carbohydrate metabolism in heterothermic animals. A.M. Martin (Ed.), Univ. Washington Press, Seattle, Washington, pp. 89-134.

BLACK, E.C., G.T. MANNING and **K. HAYASHI,** 1966. Changes in levels of haemoglobin, oxygen, carbon dioxide, pyruvate and lactate in venous blood of rainbow trout (Salmo gairdneri) during and following severe muscular activity. J. Fish. Res. Bd. Can. **23(6):** 783-795.

BLAZKA, P., 1958. The anaerobic metabolism of fish. Physiol. Zool., **31:** 117-128.

BLAZKA, P., 1960. Anaerobini metabolismus nizsich obratloviu (Anaerobic metabolism of lower vertebrates) Babakova Sbirka, **16:** 132-138. Abstract in "Sbornik" - Papers Inst. Chem. Tech. Prague, 1963: 576(24).

BLAZKA, P. and **M. KOPECKY,** 1961. Basic mechanisms of adaptations to anoxia and hypoxia. Proc. 5th Natl. Congr. Czechoslovak physiol. Soc. Karlovy Vary, June 13-16, 111. The physiology of adaptive phenomena. pp. 103-106.

BOSWORTH, N.W., H.O. BIREN and **W.R. AMBERSON,** 1936. Determination of R.Q. in marine animals. J. Cell. Comp. Physiol., **9:** 77-87.

BOUNHIOL, J.P., 1905. Experimental studies in aquatic respiration. Respiration of fish. Bull. Sci. France Belg., **39:** 277-305.

BOWER, C.E. and **J.P. BIDWELL,** 1978. Ionization of ammonia in sea water: Effects of temperature, pH and salinity. J. Fish. Res. Bd. Can., **35:** 1012-1016.

BRAFIELD, A.E. and **D.J. SOLOMON,** 1972. Oxycalorific coefficient for animals respiring nitrogenous substrates. Comp. Physiol. Biochem., **43 A:** 837-841.

BRETT, J.R., 1958. Implications and assessment of environmental stress, pp. 69-83. In: P.A. Larkin (Ed.) H.R. Mac Millan Lectures in Fisheries, Univ. of British Columbia, Vancouver, B.C.

BRETT, J.R., 1962. Some considerations in the study of respiratory metabolism in fish, particularly salmon. J. Fish. Res. Bd. Can., **19(6):** 1025-1038.

BRETT, J.R., 1964. The respiratory metabolism and swimming performanc of sockeye salmon. J. Fish. Res. Bd. Can., **21**: 1183-1226.

BRETT, J.R., 1965. The relation of size to rate of oxygen consumptio and sustained swimming speed of sockeye salmon (Oncorhynchus nerka) J. Fish. Res. Bd. Can., **22**: 1491-1501.

BRETT, J.R., 1973. Energy expenditure of sockeye salmon. Oncorhynchus nerka, during sustained performances. J. Fish. Res. Bd. Can., **30(12)**: 1799-1809.

BRETT, J.R., 1979. Environmental factors and growth. In: Fish physiology, Vol.IX. W.S. Hoar, D.R. Randall and J.R. Brett (Eds.), Academic Press, New York, pp. 599-667.

BRETT, J.R. and **D.B. SUTHERLAND**, 1964. Respiratory metabolism of pumpkin seed (Lepomis gibbosus) in relation to swimming speed at 20°C. MS, Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C.

BRETT, J.R. and **D.B. SUTHERLAND**, 1965. Respiratory metabolism of pumpkin seed (Lepomis gibbosus) in relation to swimming speed. J. Fish. Res. Bd. Can., **22(2)**: 405-409.

BRETT, J.R. and **T.D.D. GROVES**, 1979. Physiological energetics. In: Fish physiology, Vol. VII. W.S. Hoar, D.J. Randall and J.R. Brett (Eds.). Academic Press, New York, pp. 280-352.

BROWN, W.D., 1960. Glucose metabolism in carp. J. Comp. Cell. Physiol., **55(1)**: 81-86.

BURROWS, R.E., 1964. Effects of accumulated excretory products on hatchery - reared salmonids. Bureau of Sport Fish and Wild life. Res. Rep. No.**66**: 12 pp.

CAILLOUET, C.W.Jr., 1964. Blood lactic acid concentration of unexercised and exercised mature carp in winter and summer. Iowa State J. Sci., **38(3)**: 309-322.

CAILLOUET, C.W. Jr., 1967. Hyperactivity, blood lactic acid and mortality in channel cat fish. Iowa State Univ. Sci. Tech. Res. Bull., **551**: 897-915.

CANTAROW, A. and **B. SCHEPARTZ,** 1967. Biochemistry. W.B. Saunders Co., Philadelphia, Pa. and London, 4 edn., 898 pp.

CHAN, W.L., 1982. Management of the nursery of sea bass fry. In: Report of training course of sea bass spawning and larval rearing. SCS/GEN/82/39, UNDP/FAO, Rome, Italy. pp. 34-37.

CHANCHAL, A.K., P.N. PANDAY and **S. NATH,** 1977. Effect of salinity on oxygen consumption from water in an air-breathing fish Channa punctatus (Bloch). J. Inl. Fish. Soc. India, **9**: 199.

CHAO, T.M., 1984. Studies on the transmissibility of lymphocystis disease occurring in sea bass (Lates calcarifer, Bloch). Singapore J. Prim. Ind., **12 (1)**: 11-16.

CHOU, R., 1984. Effect of dietary water content on the feed intake, food conversion efficiency and growth of young sea bass (Lates calcarifer, Bloch). Singapore J. Prim. Ind., **12(2)**: 120-127.

CREAC'H, Y., 1966. Protein thiols and free amino acids of carp tissues during prolonged starvation. Archs. Sci. Physiol., **20:** 115-121.

DANAYADOL, Y. and **J. BURANAPANIDGIT**, 1984. Study on the areas of occurrence and symptoms of a kidney disease in cultured sea bass, Lates calcarifer. In: Report of Thailand and Japan joint coastal aquaculture research project, Songkhla. Thailand, Apr. 1981-1984. Japan International Co-operation Agency, Tokyo. pp. 145-155.

DANAYADOL, Y., S. DIRECKBUSSARAKOM and **J. BURANAPANIDGIT,** 1984. Columnaris disease in sea bass, Lates calcarifer. Technical paper No.14/1984 of National Institute of Coastal Aquaculture, Songkhla, Thailand, National Institute of Coastal Aquaculture, 9 pp.

DAVIS, T.L.O., 1984. Estimation of fecundity in barramundi, Lates calcarifer (Bloch) using an automatic particle counter. Aust. J. Mar. Freshwat. Res., **35(1):** 111-118.

DAVIS, T.L.O., 1985a. Seasonal changes in gonad maturity and abundance of larvae and early juveniles of barramundi, Lates calcarifer in Van Diemen Gulf and the Gulf of Carpentaria. Aust. J. Mar. Freshwat. Res., **36(2):** 177-190.

DAVIS, T.L.O., 1985b. Food of barramundi, Lates calcarifer (Bloch), in coastal and inland waters of Van Diemen Gulf and the Gulf of Carpentaria, Aust. J. Fish. Biol., **26:** 669-682.

DAVIS, T.L.O. and **D.D. REID**, 1982. Estimates of Tag shedding rates for Floy FT-2 part and FD-67 anchor tags in baramundi, Lates calcarifer

DAVIS, T.L.O. and **G.P. KIRKWOOD**, 1984. Age and growth studies on barramundi, Lates calcarifer (Bloch), in Northern Australia. Aust. J. Mar. Freshw. Res., **35**: 673-689.

DAVIS, D.E., J. FORSTER, C.E. WARREN and **P. DOUDOROFF**, 1963. The influence of oxygen concentration on the swimming performance of juvenile pacific salmon at various temperatures. Trans. Amer. Fish. Soc., **92**: 111-124.

DE, G.K., 1971. On the biology of post-larval and juvenile stages of Lates calcarifer (Bloch). J. Indian Fish. Ass., **1(2)**: 51-64.

DEHNEL, P.A., 1960. Effect of temperature and salinity on the oxygen consumption of two intertidal crabs. Biol. Bull., Mar. Biol. Lab., Woods Hole, **118(2)**: 215-249.

DE JOURS, P., 1973. Problems of control of breathing in fishes. In: "Comparative physiology". Bolis, K. Schmidt-Nielson and S.H.P. Maddrel (Eds.), North Holl and Publ. pp. 117-133.

DE JOURS, P., A. TOULMOND and **J.P. TRUCHO**, 1977. The effect of hyperoxia on the breathing of marine fishes. Comp. Biochem. Physiol., **58A**: 409-411.

DE SILVA, S.S. and **P.A.B. PERERA**, 1976. Studies on the young grey mullet, Mugil cephalus. 1. Effect of salinity on food intake, growth and food conversion. Aquaculture, **7**: 327-338.

DE VOOYS, G.G.N., 1968. Formation and excretion of ammonia in teleosts. 1. Excretion of ammonia through gills. Archs. int. Physiol. Biochem., **76(2)**: 268-272.

DICKSON, I.W. and **R.H. KRAMER,** 1971. Factors influencing scope for activity and active and standard metabolism of rainbow trout (Salmo gairdneri). J. Fish. Res. Bd. Can., **28:** 587-596.

DIZON, A.E., W.H. NEILL and **J.J. MAGNUSON,** 1977. Rapid temperature compensation of volitional swimming speeds and lethal temperatures in tropical tunas (Scombridae). Env. Biol. Fish., **2:** 83-92.

DOUDOROFF, P. and **D.L. SHUMWAY,** 1970. Dissolved oxygen requirements of freshwater fishes. FAO Fish. Tech. Rep. 86, FIRI/T86 Wat. Pollut.

DOWBAN, R.M., 1969. General physiology. A molecular approach. Harper International edition. (New York: Harper and Row; London: Evanston; Tokyo: John Weatherhill Inc.).

DRIEDZIC, W.R. and **P.W. HOCHACHKA,** 1975. The unanswered question of high anaerobic capabilities of carp white muscle. Can. J. Zool., **53:** 706-712.

DRUMMOND, G.I. and **E.C. BLACK,** 1960. Comparative physiology: Fuel of muscle metabolism. Ann. Rev. Physiol., **22:** 169-190.

EKBERG, D.R., 1962. Anaerobic and aerobic metabolism in gills of the crucian carp adapted to high and low temperatures. Comp. Biochem. Physiol., **5:** 123-128.

ENGLAND, W.R. and **J. BALDWIN,** 1985. Anaerobic energy metabolism in the tail musculature of the Australian Yabby Cherax destructor (Crustacea, Decapoda, Parastacidae). Regulation of anaerobic glycolysis. Comp. Biochem. Physiol., **80B:** 327-335.

EVANS, D.O., 1977. Seasonal changes in standard metabolism, upper and lower thermal tolerance and thermoregulatory behaviour of the pumpkin seed, Lepomis gibbosus (Linnaeus). Ph.D. Thesis, Univ. Toronto. 429 pp.

FARMER, G.J. and **F.W.H. BEAMISH,** 1969. Oxygen consumption of Tilapia nilotica in relation to swimming speed and salinity. J. Fish. Res. Bd. Can., **26:** 2807-2821.

FLORKIN, M., 1966. Nitrogen metabolism. In: Physiology of Mollusca, Vol.2. K.M. Wilbur and C.M. Yonge (Eds.), Academic Press, New York, pp. 309-351.

FORSTER, R.P. and **L. GOLDSTEIN,** 1969. Formation of excretory products. In: Fish Physiology, Vol.1. W.S. Hoar and D.J. Randall (Eds.), Academic Press, New York. pp. 313-345.

FREEMAN, J.A., 1950. Oxygen consumption, brain metabolism and respiratory movements of gold fish during temperature acclimatization, with special reference to lowered temperatures. Biol. Bull., **99(3):** 416-424.

FROMM, P.O. and **J.R. GILLETE,** 1968. Effect of ambient ammonia on nitrogen excretion of rainbow trout, Salmo gairdneri. Comp. Biochem. Physiol., **26:** 887-896.

FRY, F.E.J., 1947. Effects of the environment on animal activity. University Toronto Stud., Biol. Ser., No.**55,** Pub. Ontario Fish. Res. Lab., No.**68:** 62 pp.

FRY, F.E.J., 1957. The aquatic respiration of fish. In: Physiology of fishes, Vol.1. M.E. Brown (Ed.), Academic Press, New York. pp. 1-63.

FRY, F.E.J., 1967. Thermobiology. A.A. Rose (Ed.), Academic Press, London, 375 pp.

FRY, F.E.J., 1971. The effect of environmental factors on the physiology of fish. In: Fish physiology, Vol.VI. W.S. Hoar and D.J. Randall (Eds.), Academic Press, New York. pp. 1-98.

FRY, F.E.J. and **J.S. HART,** 1948. Cruising speed of gold fish in relation to temperature. J. Fish. Res. Board. Can., **7:** 169-175.

FRY, F.E.J. and **P.W. HOCHACHKA,** 1970. Fish. In: "Comparative physiology of Thermoregulation, Vol.1, Invertebrates and Non mammalian Vertebrates"., G.C. Whittow (Ed.), pp. 79-134. Academic Press, New York.

GARCIA ROMEU, F. and **R. MOTAIS,** 1966. Mise en evidence d'echanges Na^+/NH_3^+ chez l'anguille d'eau douce. Comp. Biochem. Physiol., **17:** 1201-1204.

GARDNER, J.A. and **C. LEETHAM,** 1914. On the respiratory exchange in freshwater fish. Part I. On brown trout. Biochem. J., **8:** 374-390.

GARDNER, J.A., G. KING and **E.B. POWERS,** 1922. The respiratory exchange in freshwater fish. III. Gold fish. Biochem. J., **16:** 523-529.

GARDNER, J.A. and **G. KING,** 1923. The respiratory exchange in freshwater fish. IV. Pike. Biochem. J., **17:** 170-173.

GHOSH, A., 1971. Observations on acclimatization and growth of the Bhetki, Lates calcarifer (Bloch) in freshwater ponds. J. Inl. Fish. Soc. India., **3:** 123-124.

GHOSH, A., 1973. Observations on the larvae and juveniles of the Bhetki, Lates calcarifer (Bloch) from the Hooghly-Matlah estuaries system. I.J.F., **20(2):** 372-379.

GHOSH, A., A.K. SINHA and **P.R. ROY,** 1977. Structure of the vertebra and its role in age determination of Lates calcarifer (Bloch). J. Inl. Fish. Soc. India, **9:** 186-189.

GHOSH, A. and **P.K. PANDIT,** 1979. On the rearing of fry of Bhetki, Lates calcarifer (Bloch) in [illegible]ckish water ponds. Matsya, **5:** 50-55.

GOLDSTEIN, L., R.P. FORSTER and **G.M. FANELLI, Jr.**, 1964. Gill blood flow and ammonia excretion in the marine teleost Myxocephalus scorpius. Comp. Biochem. Physiol., **12:** 489-499.

GORDON, M.S., 1963. Chloride exchange in rainbow trout (Salmo gairdneri) adapted to different salinities. Biol. Bull., **124:** 45-54.

GORDON, M.S., I. BOETIUS, D.H. EVANS, R. Mc CARTHY and **L.C.OGLESDY,** 1965. Salinity adaptation in the mud-skipper fish. Hvalrad. Skr., **48:** 85-93.

GRAHAM, J.M., 1949. Some effects of temperature and oxygen pressure on the metabolism and activity of the speckled trout, Salvelinus fontinalis. Canadian J. Res., Sect. **D, 27:** 270-288.

GRAY, E.F. and **F.G. HALL,** 1930. Blood sugar and the activity of fishes. Biol. Bull., Mar. Biol. Lab., Woods Hole., **58:** 217-223.

GREENWOOD, P.H., 1976. A review of the family Centropomidae (Pisces, Perciformes). Bull. Br. Mus. (Nat. Hist.) Zool., **29:** 1-81.

GREHANT, M., 1870. Cited by Jolyet and Regnard (1877) in Recherches physiologiques sur la respiration des animaux aquatiques., I^re^ partie. Arch. Physiol. Norm. Pathol., Ser., **2,4:** 44-62.

GROSS, W.J., 1957. A behavioural mechanism for osmotic regulation in a semiterrestrial crab. Biol. Bull., Mar. Biol. Lab., Woods Hole., **113:** 268-273.

GUMBMANN, M., W.D. BROWN and **A.L. TAPPEL,** 1958. Intermediary metabolism of fishes and other aquatic animals. Spec. Sci. Rept., Fish. No.**288,** U.S. Fish. Wildlife Serv., Washington, D.C., 51 pp.

GUTSHELL, J.A., 1929. Influence of certain freshwater conditions, especially dissolved gases on trout. Ecology., **10:** 77-96.

HAMSA, A.K.M.S. and **M.N. KUTTY,** 1972. Oxygen consumption in relation to spontaneous activity and ambient oxygen in five marine teleosts. I. J. F., **19:** 76-85.

HARVEY, B. and **J. NACARIO,** 1985. Induced spawning of sea bass, Lates calcarifer, and rabbit fish, Siganus guttatus, after implantation of pelleted LH-RH analogue. Aquaculture, **47:** 53-59.

HAYASHI, K., N.W. GREEN and **E.C. BLACK,** 1964. Carbohydrate metabolism during transportation of live rainbow trout, Salmo gairdneri. Rept. Fac. Fish., Prefectural Univ. Mie., **5(1):** 51-125.

HEAD, G. and **J. BALDWIN,** 1986. Energy metabolism and the fate of lactate during recovery from exercise in the Australian freshwater Cray fish Cherax destructor. Aust. J. Mar. Freshw. Res., **37:** 641-646.

HEATH, A.G. and **A.W. PRITCHARD,** 1962. Changes in the metabolic rate and blood lactic acid of blue gill sunfish, Lepomis macrochirus, Raf., following severe muscular activity. Physiol. Zool., **35(4):** 323-329.

HEATH, A.G. and **A.W. PRITCHARD,** 1965. Effects of severe hypoxia on carbohydrate energy stores and metabolism in two species of freshwater fish. Physiol. Zool., **38(4):** 325-334.

HEIDINGER, R.C and **S.D. CRAWFORD,** 1977. Effect of temperature and feeding rate on the liver-somatic index of the large mouth bass, Micropterus salmoides. J. Fish. Res. Bd. Can., **34:** 633-638.

HICKMAN, C.P., 1959. The osmoregulatory role of the thyroid gland in the starry flounder, Platichthys stellatus. Can. J. Zool., **37:** 997-1060.

HINTON, D.E., R.L. SNIPES and **M.L. KENDALL,** 1972. Morphology and enzyme histochemistry in the liver of the large mouth bass(Micropterus salmoides). J. Fish. Res. Board Can., **29:** 531-534.

HIRAYAMA, K., 1974. Water control by filtration in closed culture systems. Aquaculture, **4:** 369-385.

HIROSHI KOHNO, SHIRO HARA and **YASUHIKO TAKI,** 1986. Early larval development of the sea bass, Lates calcarifer with emphasis on the transition of energy sources. Bulletin of the Japanese Society of Scientific Fisheries, **52(10):** 1719-1725.

HOAR, W.S. and **D.J. RANDALL,** 1978. Fish Physiology. Volume VII. Locomotion. Academic Press, New York.

HOAR, W.S., D.J. RANDALL and **J.R. BRETT,** 1979. Fish Physiology. Volume VIII. Bioenergetics and growth. Academic Press, New York.

HOCHACHKA, P.W., 1961. Glucose and acetate metabolism in fish. Can. J. Biochem. Physiol., **39:** 1937-1941.

HOCHACHKA, P.W., 1962. Thyroidal effects on pathways for carbohydrate metabolism in a teleost. Gen. Comp. Endocrinol., **2(6):** 499-505.

HOCHACHKA, P.W., 1980. Living without oxygen (Cambridge Mass: Harvard University Press) pp. 1-173.

HOCHACHKA, P.W. and **F.R. HAYES,** 1962. The effect of temperature acclimation on pathways of glucose metabolism in trout. Canadian J. Zool., **40:** 261-270.

HOCHACHKA, P.W. and **G.N. SOMERO,** 1973. Strategies of Biochemical Adaptation. W.B. Saunders Co., Philadelphia., Pa. 358 pp.

HOGLUND, L.B., 1961. The reactions of fish in concentration gradients. Rep. Inst. Freshwater Res. Drottningholm, **43:** 1-47.

HOLETON, G.F., 1972. Gas exchange in fish with and without haemoglobin. Respir. Physiol., **14:** 142-150.

HOLETON, G.F. and **D.J. RANDALL,** 1967. The effect of hypoxia upon the partial pressure of gases in the blood and water afferent and efferent to the gills of rainbow trout. J. Exp. Biol., **46:** 317-327.

HOUSTON, A.H., 1961. Influence of size upon the adaptation of steelhead trout, (Salmo gairdneri) and chum salmon, (Oncorhynchus keta) to sea water. J. Fish. Res. Bd. Can., **18:** 401-415.

HUGHES, G.M. and **B.N. SINGH,** 1970. Respiration in an air-breathing fish, the climbing perch Anabas testudineus (Bloch). 1. Oxygen uptake and carbon dioxide release into air and water. J. Exp. Biol., **53:** 265-280.

HUGHES, G.M. and **I.A. JOHNSTONS,** 1978. Some responses of the electric ray (Torpedo marmorata) to low ambient oxygen tensions. J. Exp. Biol., **73:** 107-117.

ITAZAWA, Y. and **T. TAKEDA,** 1978. Gas exchange in the carp gills in normoxic and hypoxic conditions. Respir. Physiol., **35:** 263-269.

JAMES, P.S.B.R. and **R. MARICHAMY,** 1987. Status of sea bass (Lates calcarifer) culture in India. In: "Management of wild and cultured sea bass/barramundi (Lates calcarifer)", J.W. Copland and D.L. Grey (Eds.), Proceedings of an international workshop held at Darwin, N.T., Australia, 24-30 Sep. 1986., A.C.I.A.R. Proc. No.**20:** 74-79.

JHINGRAN, V.G. and **A.V. NATARAJAN,** 1969. A study of the fisheries and fish populations of the Chilka lake during the period 1957-65. J. Inl. Fish. Soc. India, **1:** 49-126.

JOB, S.V., 1955. The oxygen consumption of Salvelinus fontinalis. Publs. Ont. Fish. Res. Lab., **73:** 1-39.

JOLYET, F. and **P. REGNARD,** 1877a. Recherches physiologiques sur la respiration des animaux aquatiques, 1re partie. Arch. Physiol., Norm. Pathol., Ser., **2, 4:** 44-62.

JOLYET, F. and **P. REGNARD,** 1877b. Recherches physilogiques sur la respiration des animaux aquatiques, 2 me partie. Arch. Physiol., Norm. Pathol., Ser., **2, 4:** 44-62.

JONES, J.R.E., 1952. The reactions of fish to water of low oxygen concentration. J. Exp. Biol., **29:** 403-415.

KARUPPANNAN, N.V., 1972. Studies on the locomotory metabolism of Tilapia mossambica (Peters). Ph.D. Thesis, Madurai Univ., Madurai.

KASIM, H.M. and **P.S.B.R. JAMES,** 1987. Distribution and fishery of Lates calcarifer in India. "Management of wild and cultured sea bass/barramundi (Lates calcarifer)". J.W. Copland and D.L. Grey (Eds.), Proceedings of an international workshop held at Darwin, N.T., Australia, 24-30 Sept. 1986. A.C.I.A.R. Proc. No.**20:** 109-114.

KATAYAMA, M. and **Y. TAKI,** 1984. Lates japonicus, a new centropomid fish from Japan. Japan. J. Icthyol., **30(4):** 361-367.

KAWAI, A., Y. YOSHIDA and **M. KIMATA,** 1965. Biochemical studies on the bacteria in the aquarium with a circulating system. II. Nitrifying activity in the filter sand. Bull. Jpn. Soc. Sci. Fish., **31:** 65-71.

KAWAMOTO, N.Y., 1961. The influence of excretory substances of fish on their own growth. Progressive Fish Culturists. **23:** 70-75.

KHUDA-BUKHSH, A.R., 1979. Chromosomes in 3 species of fishes, Aplocheilus panchax (Cyprinodontidae), Lates calcarifer (Percidae) and Gadusia chapra (Clupeidae). Caryologia, **32(2):** 161-169.

KINNE, O., 1960. Growth, food intake, and food conversion in a euryplastic fish exposed to different temperatures and salinities. Physiol. Zool., **33**: 288-317.

KINNE, O., 1964. The effect of temperature and salinity on marine and brackish water animals. II. Salinity and temperature-salinity combinations. Oceanogr. Mar. Biol., A. Rev., **2**: 281-339.

KINNE, O., 1971. Marine Ecology. Vol.I. Environmental factors. London: Wiley-Interscience., Pt. 2. pp. 683-1244.

KNAUTHE, K., 1898. Zur Kenntnis des Stoffwechsels der Fische. Pfluger's Arch. Ges. Physil., **73**: 490-500.

KOSUTARAK, P., 1984. Effect of supplemental vitamins on feeding fish meat to juvenile sea bass, Lates calcarifer. In: Report of Thailand and Japan joint coastal aquaculture Research project, Songkhla, Thailand, Apr. 1981-March 1984. Japan International Co-operation Agency, Tokyo. pp. 88-92.

KOSUTARAK, P. and **T. WATANABE**, 1984a. Growth and survival of nearly-hatched larvae of sea bass, Lates calcarifer in starved condition. In: Ibid. pp. 81-82.

KOSUTARAK, P. and **T. WATANABE**, 1984b. Notes on development of larval and juvenile stages of sea bass, Lates calcarifer. In: Ibid. pp. 36-45.

KOSUTARAK, P., **T. PECHMANEE** and **T. WATANABE**, 1984. Survival and growth of early stage of sea bass, Lates calcarifer under different conditions of salinity. In: Ibid. pp. 58-67.

KOWTAL, G.V., 1977. Some observations on the breeding of Lates calcarifer (Bloch) from the Chilka lake. J. Inl. Fish. Soc. India, **9**: 191-192.

KREUGER, M.H., J.B. SADDLER, J.J. TINSLEY and **R.R. LOWRY**, 1968. Bioenergetics, exercise and fatty acids of fish. An. Zool., **8**: 119-129.

KULDEEP KUMAR LAL, 1991. Studies on the reproductive physiology of Lates calcarifer (Bloch). Ph.D. Thesis, Cochin University of Science and Technology.

KUNGVANKIJ, P., and **N. SUTHEMECHAIKUL**, 1986. Mass production of sea bass, Lates calcarifer (Bloch) by environmental manipulation. In: The first Asian Fisheries Forum, Manila, Philippines, 25-31. May 1986. pp. 7.

KUNGVANKIJ, P., B.J. PUDADERA, L.B. TIRO and **I.O. POTESTAS**, 1986. Biology and culture of sea bass (Lates calcarifer). NACA Training manual, Series No.3.

KUTTY, M.N., 1966. Some studies on the respiratory quotient in gold fish and rainbow trout. Ph.D. Thesis, Univ. Toronto, Toronto, Ontario, VII : 104 pp. (National Library of Canada, Canadian Theses on micro-film, No.646).

KUTTY, M.N., 1968a. Respiratory quotients in gold fish and rainbow trout. J. Fish. Res. Bd. Can., **25(8)**: 1689-1728.

KUTTY, M.N., 1968b. Influence of ambient oxygen on the swimming per-formance of gold fish and rainbow trout. Can. J. Zool., **46**: 647-663.

KUTTY, M.N., 1970. Respiratory quotient and ammonia excretion in Tilapia mossambica. Abstr. Pap. Presented Amer. Fish. Soc. 100th Annu. Meeting. 7 pp.

KUTTY, M.N., 1972. Respiratory quotient and ammonia excretion in Tilapia mossambica. Mar. Biol. (Springer-Verlag), **16(2)**: 126-133.

KUTTY, M.N., 1975. Energy metabolism in mullet. Invited contribution. Chapter in IBP synthesis, Volume on grey mullets.

KUTTY, M.N., 1978. Ammonia quotient in sockeye salmon (Oncorhynchus nerka). J. Fish. Res. Bd. Can., **35**: 1003-1005.

KUTTY, M.N., 1981. Energy metabolism in mullet. In: Aquaculture of grey mullets. O.H. Oren (Ed.), London, Cambridge University Press. pp. 219-253.

KUTTY, M.N. and **R.L. SAUNDERS**, 1973. Swimming performance of young Atlantic salmon (Salmo salar) as affected by reduced ambient oxygen concentration. J. Fish. Res. Bd. Can., **30**: 223-227.

KUTTY, M.N. and **M. PEER MOHAMED**, 1975. Metabolic adaptations of mullet, Rhinomugil corsula (Hamilton) with special reference to energy utilization. Aquaculture, **5**: 253-270.

KUTTY, M.N., M. NARAYAN and **N. SUKUMARAN**, 1976. Use of a biological filter for removal of ammonia in small aquaria. J. Mad. Univ., **5**: 61-65.

KUTTY, M.N., N.V. KARUPPANNAN, M. NARAYAN and **M. PEER MOHAMED**, 1971. Maros-Schulek technique for measurement of carbon dioxide production in fish and respiratory quotient in Tilapia mossambica. J. Fish. Res. Bd. Can., **28**: 1342-1344.

LAURENCE, G.C., 1973. Influence of temperature on energy utilizatio of embryonic and prolarval tautog, Tautoga onitis. J. Fish. Res Bd. Can., **30:** 435-442.

LEONG, T.S., and **S.Y. WONG,** 1986. Parasitic fauna of sea bass Late calcarifer imported from Thailand and those cultured in cages Penang. In: The first Asian Fisheries Forum, Manila, Philippines 25-31 May 1985. 145 pp.

LIMSUWAN, C., S. CHINABUT and **Y. DANAYADOL,** 1983. Lymphocysti disease in sea bass (Lates calcarifer), Thailand, National Island Fisheri Institute. (Technical paper/Natl. Inland Fish. Inst., Fresh Wat. Fish Div., Dep. Fish., No.**21**). 6 pp.

LOTAN, R.,1966. Oxygen consumption in the gills of Tilapia aurea (Stein dachner) (Pisces, Chichlidae) in various saline conditions. Israel J Zool., **15:** 33-37.

LOVE, R.M., 1970. The Chemical Biology of Fishes. Academic Press, Ne York, N.Y., 545 pp.

MADANMOHAN RAO, G., 1968. Oxygen consumption in rainbow trou (Salmo gairdneri) in relation to activity and salinity. Can. J. Zool., **46:** 781-786.

LAURENCE, G.C., 1973. Influence of temperature on energy utilization of embryonic and prolarval tautog, Tautoga onitis. J. Fish. Res. Bd. Can., **30:** 435-442.

LEONG, T.S., and **S.Y. WONG,** 1986. Parasitic fauna of sea bass Lates calcarifer imported from Thailand and those cultured in cages in Penang. In: The first Asian Fisheries Forum, Manila, Philippines, 25-31 May 1985. 145 pp.

LIMSUWAN, C., S. CHINABUT and **Y. DANAYADOL,** 1983. Lymphocystis disease in sea bass (Lates calcarifer), Thailand, National Island Fisheries Institute. (Technical paper/Natl. Inland Fish. Inst., Fresh Wat. Fish. Div., Dep. Fish., No.**21**). 6 pp.

LOTAN, R.,1966. Oxygen consumption in the gills of Tilapia aurea (Steindachner) (Pisces, Chichlidae) in various saline conditions. Israel J. Zool., **15:** 33-37.

LOVE, R.M., 1970. The Chemical Biology of Fishes. Academic Press, New York, N.Y., 545 pp.

MADANMOHAN RAO, G., 1968. Oxygen consumption in rainbow trout (Salmo gairdneri) in relation to activity and salinity. Can. J. Zool., **46:** 781-786.

MAETZ, J., and **F. GARCIA ROMEU,** 1964. Mechanism of sodium and chloride uptake by the gills of a freshwater fish, Carassius auratus. II. Evidence of NH_4^+/ Na^+ and HCO_3^-/Cl^- exchanges. J. Gen. Physiol., **47:** 1209-1227.

MANEEWONG, S., and **N. RUANGPANIT,** 1977. Experiment on feeding 3-20 days old sea bass, (Lates calcarifer, Bloch) larvae by using 3 kinds of zooplankton. Annu. Rep. Songkhla Fish. Stn. Dep. Fish, pp. 184-193.

MANEEWONGSA, S., and **T. TATTANON,** 1982a. Food and feeding of sea bass larvae and juveniles. In: Report of Training course on sea bass spawning and larval rearing, Songkhla, Thailand. 1-20 June 1982. FAO/UNDP SCS/GEN/82/39. 25 pp.

MANEEWONGSA, S., and **T. TATTANON,** 1982b. Care and maintenance of sea bass brood stock. In: Ibid. 42 pp.

MANEEWONG, S., T. TATTANON and **T. WATANABE,** 1984. Studies on the seed production of sea bass, Lates calcarifer. 1. Present status of production and some results of rearing in 1982 at National Institute of Coastal Aquaculture, Thailand. In: Report of Thailand and Japan Joint Coastal Aquaculture Research Project, Songkhla, Thailand, Apr. 1981-Mar. 1984. Japan International Co-operation Agency, Tokyo. pp. 13-35.

MANEEWONG, S., N. RUANGPANIT, D. PECHMANEE and **T. TATTANON,** 1977. On the mass production of sea bass (Lates calcarifer, Bloch) in 1977. Annu. Rep. Songkhla Fish. Stn. Dep. Fish. pp. 174-183.

MANEEWONG, S., N. RUANGPANIT, T., PECHMANEE and **T. TATTANON,** 1981. On the mass production of sea bass (Lates calcarifer, Bloch) in 1981. Annu. rep. Natl. Inst. Coast. Aquaculture. Dep. Fish. (Thailand). pp. 71-81.

MAROS, L., E. SCHULEK, I. MOLUARPER and **M. PINTER-SZAKACS,** 1961. Einfaches Distillations verfahren zur titrimetrischen Bestimmung von kohlendioxyd. (A simple distillation method for the titrimetric determination of carbon dioxide). Anal. Chim. Acta. **25.** pp. 390-399 (Trans. from German, Fish. Res. Bd. Can. Trans. Ser. No.596-1965).

MATHUR, G.B., 1967. Anaerobic respiration in cyprinoid fish, Rasbora daniconius (Ham). Nature, pp. 214-318.

MOORE, R., 1979. Natural sex inversion in the giant perch (Lates calcarifer). Aust. J. Mar. Freshw. Res., **30:** 803-813.

MOORE, R., 1980. Reproduction and migration in Lates calcarifer (Bloch). Ph.D. Thesis, University of London.

MOORE, R., 1982. Spawning and early life history of barramundi, Lates calcarifer (Bloch) in Papua New Guinea. Aust. J. Mar. Freshw. Res., **33:** 647-661.

MOORE, R. and **L.F. REYNOLDS,** 1982. Migration pattern of barramundi, Lates calcarifer (Bloch) in Papua New Guinea. Aust. J. Mar. Freshw. Res., **33(4):** 671-682.

MOSHIRI, G.A. and **K.W. CUMMINS,** 1969. Calorific values for Leptodora kindtii (Focke) (Crustacea : Cladocera) and selected food organisms. Arch. Hydrobiol., **66:** 91-99.

MUIR, B.S. and **A.J. NIIMI,** 1972. Oxygen consumption of the euryhaline fish aholehole (Kuhlia sandvicensis) with reference to salinity, swimming and food consumption. J. Fish. Res. Bd. Can., **29:** 67-77.

MUKHOPADHYAY, M.K. and **P.U. VERGHESE,** 1978. Observations on the post larvae of "Bhekti" Lates calcarifer (Bloch) from Hooghly estuary with a note on their collection. J. Inl. Fish. Soc. India , **10:** 138-141.

MUKHOPADHYAY, M.K. and **H.C. KARMARKAR,** 1981. Effect of salinity on food intake, growth and conversion efficiency in juveniles of Lates calcarifer (Bloch); J. Inl. Fish. Soc. India, **13(1):** 8-14.

MUKHOPADHYAY, M.K. and **H.C. KARMARKAR,** 1982. Effect of food and space on the growth and survival of the fry of Bhetki, Lates calcarifer (Bloch). J. Inl. Fish. Soc. India, **14(1):** 23-27.

MUKHOPADHYAY, M.K., P.U. VERGHESE and **H.C. KARMARKAR,** 1983. Pigmentation in juvenile Bhetki Lates calcarifer. Environ. and Ecol., **1:** 265-267.

NACARIO, J.P., 1985. Biology and ecology of Lates calcarifer. Tigbauan, Iloilo, Training and Extension, Aquaculture Department, Southeast Asian Fisheries Development Centre.

NAGAI, M. and **S. IKEDA,** 1971. Carbohydrate metabolism in fish II. Effect of Dietary composition on metabolism of glucose-6-^{14}C in carp; Bull. Jpn. Soc. Sci. Fish., **37:** 410-414.

NAGAYAMA, F., H. OHSHIMA and **K. UMEZAWA,** 1973. Activities of hexokinase and glucose dehydrogenase in fish liver. Nippon Suisan Gakkaishi. **39:** 1439.

PANAMPATHI RAO, K., 1958. Oxygen consumption as a function of size and salinity in Metapenaeus monoceros Fab. from marine and brackish water environments. J. Exp. Biol., **35:** 307-313.

PARKAR, R.R. and **E.C. BLACK**, 1959. Muscular fatigue and mortality in troll-caught chinook salmon (Oncorhynchus tshawytscha). J. Fish. Res. Bd. Can., **16(1)**: 95-106.

PARKAR, R.R., E.C. BLACK and **P.A. LARKIN**, 1959. Fatigue and mortality in troll-caught Pacific salmon (Oncorhynchus). J. Fish. Res. Bd. Can., **16(4)**: 429-448.

PARRY, G., 1958. Size and osmoregulation in salmonid fishes. Nature, Lond., **181**: 1218-1219.

PARRY, G., and **F.G.T. HOLLIDAY**, 1960. An experimental analysis of the pseudobranch in teleosts. J. Exptl. Biol., **37**: 344-354.

PARVATHESWARARAO, V., 1965. Influence of different temperature-salinity combinations on the oxygen consumption in the freshwater fish, Etroplus maculatus (Teleosti). Helgolander wiss. Meeresunters., **12**: 301-314.

PARVATHESWARARAO, V., 1967. Some mechanisms of salinity acclimation in the euryhaline teleost, Etroplus maculatus. Mar. Biol., **1**: 97-101.

PARVATHESWARARAO, V., 1970. Adaptations to osmotic stress in fishes. Indian Biol., **2**: 16-36.

PARVATHESWARARAO, V., 1971. Compensatory metabolic regulation to seasonal thermal stress in a tropical freshwater fish, Etroplus maculatus (Bleeker). Indian J. exp. Biol., **9**: 40-44.

PATNAIK, S. and **S. JENA**, 1976. Some aspects of biology of Lates calcarifer (Bloch) from Chilka Lake. I. J. F., **23**: 65-72.

PAVLOVSKII, E.N., 1964. The techniques for the investigation of fish physiology. Izd. Akad. Nauk. SSSR. Podossenski per. Moscow, U.S.S.R.

PEER MOHAMED, M., 1974. Studies on the influence of hypoxia on fish metabolism and activity. Ph.D. Thesis, Madurai Univ., Madurai, India.

PEER MOHAMED, M., 1981a. Effects of handling on oxygen consumption and random activity in the freshwater mullet, Rhinomugil corsula (Hamilton). Proc. Indian. Acad. Sci. (Anim. Sci.), **91(5)**: 469-472.

PEER MOHAMED, M., 1981b. Metabolism of Tilapia mossambica (Peters) with emphasis on hypoxia. Indian J. Exp. Biol., **19**: 1098-1100.

PEER MOHAMED, M., 1982. Metabolic rates and quotients in the cichlid fish, Tilapia mossambica (Peters) in relation to random activity. Proc. Indian Acad. Sci. (Anim. Sci.), **91**: 217-223.

PEER MOHAMED, M., 1987. Determination of metabolic rates and quotients in fish. Tech. Paper 22. Presented at the Summer Institute in recent advances in fin fish and shell fish nutrition. 11-30 May, 1987.

PEER MOHAMED, M. and **M.N. KUTTY,** 1980. Influence of ambient oxygen on random activity in some freshwater teleosts. Proc. Indian Acad. Sci. (Anim. Sci.), **89(6)**: 537-542.

PEER MOHAMED, M. and **M.N. KUTTY,** 1981. Respiratory quotient and ammonia quotient in Tilapia mossambica (Peters) with special reference to hypoxia and recovery. Hydrobiologia, **76**: 3-9.

PEER MOHAMED, M. and **M.N. KUTTY**, 1982. Low ambient oxygen tolerance in some freshwater teleosts. Experentia, **38**, Birkhauser Verlag, CH-4010 Basel/Switzerland. 587 pp.

PEER MOHAMED, M. and **M.N. KUTTY,** 1983. Respiratory quotient and ammonia quotient in gold fish, Carassius auratus (Limnaeus) with special reference to ambient oxygen. Proc. Indian Natn. Sci. Acad. **B49(4):** 303-310.

PEER MOHAMED, M. and **R.A. GUPTA,** 1983. Effects of sublethal concentration of ethyl parathion on oxygen consumption and random swimming activity of Cirrhinus mrigala (Hamilton). Indian. J. Exp. Biol., **22:** 42-44.

PEER MOHAMED, M., D. NATH, G.N. SRIVASTAVA and **R.A. GUPTA,** 1978. Influence of sublethal DDT on standard (Basal) metabolism of the freshwater fishes Cirrhina mrigala (Hamilton), Labeo rohita (Hamilton) and Colisa fasciata (Bloch and Schneider). Indian J. Exp. Biol., **16(3):** 385-386.

PERRY, S.F., P.S. DAVIE, C. DAXBOECK, A.G. ELLIS and **D.G. SMITH,** 1984. Perfusion methods for the study of gill physiology. In: Fish physiology, Vol.X. Part B. W.S. Hoar and D.J. Randall (Eds.). Academic Press, New York. 325 pp.

PETERS, J.P. and **D.D. VAN SLYKE,** 1946. Quantitative clinical chemistry Vol.1. 2nd Ed.

PIETERSZ, U.L.C., 1983. Cage culture of barramundi successful in Thailand. Aust. Fish., **42(12):** 18-20.

POTTS, W.T.W., 1954. The energetics of osmotic regulation in brackish and freshwater animals. J. Exp. Biol., **31:** 618-630.

PRIVOLNEV, T.I., 1954. (Physiological adaptations of fishes to new conditions of existence). Tr. Soveshch. Ikhtiologicheskoi Komis. Akad. Nauk SSSR, No.3. pp. 40-49. (Fish. Res. Bd. Can., Transl. Ser. No.4222).

PROSSER, C.L., 1955. Physiological variation in animals. Biol. Rev., **30:** 229-262.

PROSSER, C.L. and **F.A. BROWN, Jr.,** 1961. Comparative animal physiology. 688 pp. 2nd Ed. Philadelphia and London, W.B. Saunders and Co.

PROSSER, C.L., L.M. BARR, R.A. PINC and **C.Y. LERNER,** 1957. Acclimation of gold fish to low concentrations of oxygen. Physiol. Zool., **30:** 137-141.

PROVENCAL and **HUMBOLDT,** 1809. Recherches sur la respiration des poissons. Mem. Soc. Arceuil Physique Chim., **2,** 1807-1809: 359-404.

RAJYALEKSHMI, T. and **D.M. REDDY,** 1984. A note on growth and production of Lates calcarifer (Bloch) in brackish water in India. J. World. Maricul. Soc., **15:** 244-245.

RANDALL, D.J. and **D.R. JONES,** 1973. The effect of deafferentation of the pseudobranch on the respiratory response to hypoxia and hyperoxia in the trout (Salmo gairdneri). Respir. Physiol., **17:** 291-301.

RECTOR, F.C., D.W. SELDIN and **J.H. COPENHAVER,** 1955. The mechanism of ammonia excretion during ammonia chloride acidosis. J. Clin. Invest., **34:** 20-26.

REYNOLDS, L.F. and **R. MOORE,** 1982. Growth rates of barramundi, Lates calcarifer (Bloch), in Papua New Guinea. Aust. J. Mar. Freshwat. Res., **33(4):** 663-670.

RITAKUMARI, S.D. and **K.S. SREELATHA,** 1987. Respiratory metabolism in Etroplus maculatus (Bloch) under estuarine conditions and pesticide stress. J. Mar. Biol. Ass. India, **29(1** and **2):** 226-231.

ROOT, R.W., 1931. The respiratory function of the blood of marine fishes. Biol. Bull. (Woods Hole, Mass.), **61:** 427-456.

ROSADO, A., G. FLORES, J. MORA and **G. SOBERSON,** 1962. Distribution of and ammonia load in the normal rate. Am. J. Physiol., **203:** 37-42.

ROZIN, P. and **J. MAYER,** 1961. Regulation of food intake in the gold fish. Am. J. Physiol., **201:** 968-974.

RUANGPAN, L., 1982. Disease and parasites of sea bass, Lates calcarifer. Technical paper of National Institute of Coastal Aquaculture, Songkhla, Thailand, National Institute of Coastal Aquaculture. pp. 46-52.

RUSSELL, D.J. and **R.N. GARRETT,** 1983. Use by juvenile barramundi, Lates calcarifer (Bloch), and other fishes of temporary supralittoral habitats in a tropical estuary in northern Australia. Aust. J. Mar. Freshw. Res., **34:** 805-811.

RUSSELL, D.J. and **R.N. GARRETT,** 1985. Early life history of barramundi, Lates calcarifer (Bloch), in North eastern Queensland. Aust. J. Mar. Freshwat. Res., **36(2):** 191-201.

SAEKI, A., 1958. Studies on fish culture in filtered closed circulation aquaria. Bull. Japan. Soc. Sci. Fish., **23:** 684-695. (Defence Res. Bd. Canada Transl. T & J by E.R. Hope, 1964. 15 pp).

SAHA, A.K., P. GHOSH and **P.R. ROY,** 1978. Observation on the growth rate of Lates calcarifer (Bloch). J. Inl. Fish. Soc. India, **10:** 113-119.

SAKARAS, W. and **S. SUKBANTEANG,** 1984. Growth and production of sea bass, Lates calcarifer (Bloch), cultured in pen. Annual Rep. Rayong. Brackishwat. Fish. Stn. Dep. Fish., pp. 108-117.

SAROJINI DEVI, C., 1960. Studies on the effects of salinity on the oxygen consumption of Etroplus maculatus. M. Phil. Dissertation, Sri Venkateswara University, Tirupati.

SAUNDERS, R.L., 1962. The irrigation of the gills in fishes. II. Efficiency of oxygen uptake in relation to respiratory flow activity and concentrations of oxygen and carbon dioxide. Can. J. Zool., **40(5):** 817-862.

SAUNDERS, R.L. and **M.N. KUTTY,** 1974. Oxygen consumption and ammonia excretion in Atlantic salmon smolts (Ms).

SHAKLEE, J.B. and **J.P. SALINI,** 1985. Genetic variation and population subdivision in Australian Barramundi, Lates calcarifer (Bloch). Aust. J. Mar. Freshw. Res., **36:** 203-218.

SHELTON, G., 1970. The regulation of breathing. In: Fish physiology, Vol.IV. W.S. Hoar and D.J. Randall (Eds.), Academic Press, New York. pp. 293-359.

SHEPARD, M.P., 1955. Resistance and tolerance of young speckled trout (Salvelinus fontinalis) to oxygen lack, with special reference to low oxygen acclimation. J. Fish. Res. Bd. Can., **12:** 387-446.

SMIT , H., 1965. Some experiments on the oxygen consumption of gold fish (Carassius auratus L.) in relation to swimming speed. Can. J. Zool., **43:** 623-633.

SMIT , H., J.M. AMELINK - KOUTSTAAL , J. VIJVERBERG and **J.C. VON VAUPEL- KLEIN,** 1971. Oxygen consumption and efficiency of swimming gold fish. Comp. Biochem. Physiol., **A 39:** 1-28.

SOLARZANO, L., 1969. Determination of ammonia in natural waters by the phenol hypochlorite method. Limnol. Oceanogr., **14:** 749-801.

SOMMERS, P. VAN., 1962. Oxygen-motivated behaviour in the gold fish. Science, **137:** 678-679.

SPOOR, W.A., 1946. A quantitative study of the relationship between the activity and oxygen consumption of the gold fish, and its applications to the respiratory metabolism in fishes. Biol. Bull., **91:** 312-325.

SPRAGUE, J.B., 1971. Measurement of pollutant toxicity to fish. 3. Sublethal effects and 'safe' concentrations. Wat. Res., **5:** 245-246.

STEVENS, E. DON and **G.F. HOLETON,** 1978. The partitioning of oxygen uptake from air and from water by eurythrinids. Can. J. Zool., **56:** 965-969.

STRICKLAND, J.D.H. and **T.R. PARSONS,** 1968. A practical handbook of sea water analysis. Fish. Res. Bd. Can., Bull., **167:** 311 pp.

STROGANOV, N.V., 1962. Methods of study of respiration of fish and methods for ammonia determination used in studies on fish metabolism. In: Techniques for the investigation of fish physiology (E.N. Pavlovskii. (Ed.), Izd. Akad. Nauk. SSSR., pp. 106-111. (Trans.Natn.Sci.Fdn. Washington, D.C. PST Cat No.1130 by the Israel Programme for Scientific Translations, Jerusalem, 1964).

SUKUMARAN, N. and **M.N. KUTTY,** 1977. Oxygen consumption and ammonia excretion in the cat fish Mystus armatus, with special reference to swimming speed and ambient oxygen. Proc. Indian Acad. Sci., **B 3:** 195-206.

SUKUMARAN, N. and **M.N. KUTTY,** 1987. Energy utilization in freshwater mullet, Rhinomugil corsula (Hamilton) under exercise. Proc. Indian. Acad. Sci. (Anim. Sci.), **96(6):** 705-714.

THREADGOLD, L.T. and **A.H. HOUSTON,** 1964. An electron microscope study of the "chloride cell" of Salmo salar L. Exp. Cell Res., **34:** 1-23.

UNNIKRISHNAN, T.N. and **A. LAXMINARAYANA,** 1984. Oxygen consumption and ammonia excretion of Penaeus indicus H. Milne Edwards, in different salinities. CMFRI special publication No.**19:** 10-13.

USHA DEVI, V.K., 1987. Low ambient oxygen tolerance in fry and fingerlings of Chanos chanos (Forskal) and Mugil cephalus Linnaeus. M.Sc. Dissertation. Cochin University of Science and Technology.

WEBB, P.W., 1971. The swimming energetics of trout. II. Oxygen consumption and swimming efficiency. J. Exp. Biol., **55:** 521-540.

WHITMORE, C.M., C.E. WARREN and **P. DOUDOROFF,** 1960. Avoidance reactions of salmonids and centrarchid fishes to low oxygen concentrations. Trans. Am. Fish. Soc., **89:** 17-26.

WINBERG, G.G., 1960. Rate of metabolism and food requirements of fishes. Published in Nauchnye Trudy Belorusskovo Gosudarstvennovo Universiteta imeni V.S. Lenina, Minsk, 253 pp.

WOHLSCHLAG, D.E., 1957. Differences in metabolic rates of migratory and resident freshwater forms of an Arctic whitefish. Ecology, **38:** 502-510.

WOHLSCHLAG, D.E. and **R.O. JULIANO,** 1959. Seasonal changes in blue gill metabolism. Limnol. Oceanogr., **42:** 195-209.

WONG, S.Y., 1986. Bacterial flora of sea bass, Lates calcarifer, imported from Thailand for culture on floating cages in Penang. In: The first Asian fisheries forum, Manila, Philippines, 25-31 May 1985. 122 pp.

YOUNG, C., 1985. Commercial barramundi culture - a possibility. Aust. Fish., **44(9):** 16-17.

ZUNTZ, N., 1901. Ein Respirationsapparat fur Wasserthiere. Arch. Anat. Physiol., Jahrgang, pp. 543-551.