Proc. Indian natn. Sci. Acad. **B49** No. 4 pp 303–310 (1983)

Respiratory Quotient and Ammonia Quotient in Goldfish, *Carassius auratus* (Linnaeus) with Special Reference to Ambient Oxygen*

M PEER MOHAMED and M N KUTTY**
Central Inland Fisheries Research Institute, Riverine and Lacustrine Division, 24, Pannalal Road, Allahabad 211002

(Received 18 February 1983; after revision 23 May 1983)

Changes in metabolic rates (O_2 consumption, CO_2 production and NH_3-N excretion) of goldfish, *Carassius auratus*, in relation to ambient oxygen below air saturation fell into the same general pattern at 30° and 35°C, the differences being mainly in the metabolism, aerobic and anaerobic levels. The respiratory quotient (RQ) and ammonia quotient (AQ) remained steady in high ambient oxygen, but increased at oxygen concentration below 4 mg O_2/l at 30° and 35°C, suggesting relative independence of carbon dioxide production and NH_3-N excretion with reference to ambient oxygen. The hypoxic RQs were 2.80 (30°C) and 2.48 (35°C) indicating that the fish derived considerable amount of energy anaerobically. The aerobic AQs were 0.10 and 0.11 at 30° and 35°C respectively depending on the degree of protein utilization. The hypoxic AQs were in consonance with the corresponding changes in hypoxic RQs. The random activity of the fish increased with decrease in ambient oxygen which plays a major role in the survival of goldfish.

Key Words: Respiratory quotient, Ammonia quotient, Random activity, Ambient oxygen, *Carassius auratus*

Introduction

Although the respiratory quotient (RQ) of fish has been studied in fair detail by earlier workers (see Kutty 1968) yet none of these studies specifically dealt with the influence of ambient oxygen on RQ. The relative CO_2 production in fish increased under hypoxic and anoxic conditions (Privolnev 1954, Blazka 1958).

*From a thesis (MPM) approved for the degree of Doctor of Philosophy, Madurai Kamaraj University, Madurai

**Fisheries College, Tamil Nadu Agricultural University, Tuticorin 628 003 (*Present address*: FAO African Regional Aquaculture Centre, PMB 6165, Port Harcourt, Nigeria)

Increase in RQ over unity was demonstrated in goldfish and rainbow trout (Kutty 1968), *Tilapia mossambica* (Kutty et al. 1971a, Kutty 1972, Peer Mohamed & Kutty 1981, Peer Mohamed 1981) and in *Rhinomugil corsula* (Kutty & Peer Mohamed 1975), at low oxygen level. The goldfish, subjected to extremely low oxygen levels, reportedly sustained partial anaerobiosis as indicated by an RQ = 2 for several months (Kutty 1968).

Since most of the nitrogen excreted by the freshwater fish is in the form of ammonia (Black 1957, Sukumaran & Kutty 1977), it is likely that fish make use of it under anaerobic conditions (Prosser & Brown 1961, Garcia Romeu & Motais 1966, Kutty 1972, 1978, Kutty & Peer Mohamed 1975, Peer Mohamed & Kutty 1981, Peer Mohamed 1981). Relative changes in the ratio of ammonia-excreted to oxygen-consumed by fish, i.e. ammonia quotient (AQ) (Stroganov 1956, Kutty 1972, Kutty & Peer Mohamed 1975, Sukumaran & Kutty 1977, Peer Mohamed & Kutty 1981, Peer Mohamed 1981) can serve as a potent tool to obtain more information on protein-utilization. The present study provides an insight on RQ and AQ of goldfish subjected to reducing ambient oxygen (from near air-saturation down to asphyxial oxygen level). The observations were made at 30° and 35°C because of the scarcity of information on goldfish energetics at these temperatures and because of their relevance to local conditions.

Material and Methods

Nine specimens of goldfish, *Carassius auratus* (L.), weighing 14.2–16.5 g (mean 15.5 g) were procured from Jamia Mosque Pond in Tuticorin and acclimated to 30° and 35°C (± 0.5°C) for 15 days before experimentation. Fish, in the acclimation tanks (70.1) were fed once daily with a formulated food: fish muscle, goat liver and wheat hearts in *pro rata* 2:1:1 (Hariharan 1973). The fish were deprived of food up to 36 hr before experimentation and subsequently left overnight in a modified, 3 l, Fry's respirometer (Kutty et al. 1971b) (figure 1). Decarbonated tap water, (*p*H 8.2) used (see Kutty et al. 1971a) was recirculated (cf. Peer Mohamed & Kutty 1981). Tap water was decarbonated by adding sulphuric acid to bring the *p*H down to 3–4 and aerating the water vigorously overnight. Subsequently the *p*H was brought up to 8.2 by adding sodium hydroxide solution.

Each experiment consisted of 5–7 runs of 1 hr duration during which period the respirometer remained closed. The experimental procedure laid down by Peer Mohamed and Kutty (1981) was followed for asphyxiating the fish. The respirometer was subsequently flushed with air-saturated water to revive the animal.

Methods of Water Analyses

Dissolved oxygen: This was measured by following unmodified Winkler technique (APHA 1965), using 25 ml sample.

Carbon dioxide: Total carbon dioxide was measured by following Maros-Schulek technique (Maros et al. 1961), as modified by Kutty et al. (1971a) (figure 2). The procedure is described as follows:

15 ml of 20% H_2SO_4 was taken in the receiver flask (RF) (500 ml) and made up to 250 ml with freshly boiled, double-distilled water. The distilled water was sucked up in the funnel (F) to a level just above stopcock (SC) and closed. The receiver flask was connected to the distillation unit. On heating by heating

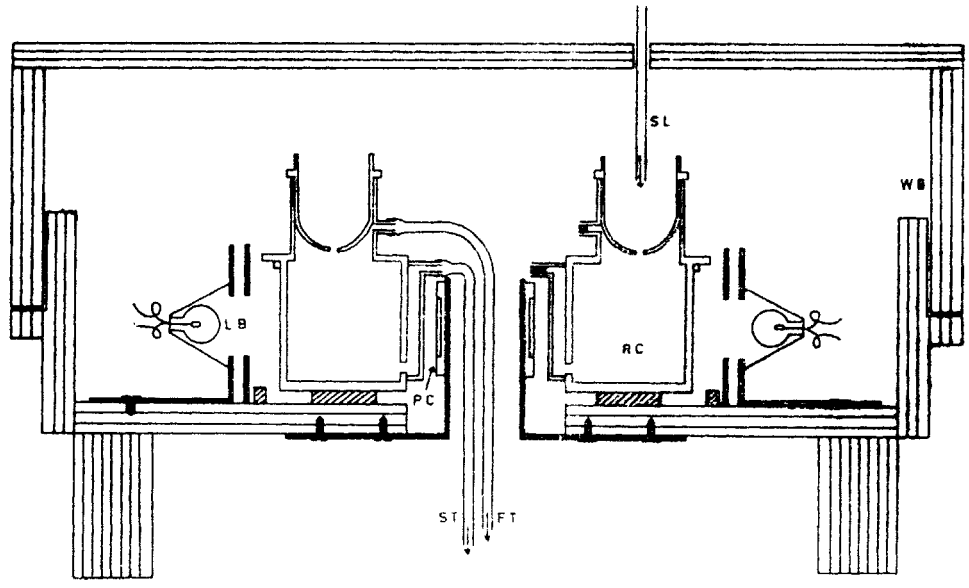

Figure 1 Schematic sectional view of modified Fry's respirometer assembly. The whole unit is housed inside a wooden box (**WB**). The two light blubs (**LB**) are fixed just outside the annular transparent 'Perspex' respirometer, so that the light beams fall on the photocells (**PC**) kept inside the hollow of the respiration chamber (**RC**). When fish moves through the annulus of the respirometer, it cuts the light beams and the events are recorded in the electronic counter (not shown in the figure, more details are given in Kutty et al. 1971b). Water from the supply line (**SL**) falls into one of the cups and flushes out of the respirometer through a flushing tube (**FT**) on the opposite side. Sampling of water is done through a sampling tube (**ST**) siphoning water from the bottom of the respirometer through a side tube fixed for this purpose. Note the provision for flushing and sampling from either halves of the respirometer, but in practice, the tube opposite to the inlet side only are used, while the tubes on the inlet side are kept closed, as shown in the figure. The sampling and flushing tubes, and, the photocells and light bulbs are fixed at 90° to each other, but are shown in the same axis in the figure for convenience in presentation. Parts made of wood are shown by parallel straight lines, those of metal are filled in and those of plastic (Perspex) are left blank

mantle (HM), when a vigorous plume of steam had come out through the condenser (C), the distillate collecting flask (DCF) (250 ml) was connected with condenser. The distillate collecting flask contained 10 ml of 0.01 N $Ba(OH)_2$ made up to 75 ml with distilled water and protected with a thick layer of hexane (10ml). Fifty ml of sample was taken into the receiver flask without upsetting the heating and condensation adjustment. This was followed with 25 ml of distilled water as rinse. When the level of condensed liquid with $Ba(OH)_2$ reached 200 ml mark, the flask was removed. This was titrated against 0.01 N HCl using thymol blue as indicator. The respiratory carbon dioxide is estimated from the difference in the contents of the two samples taken before and after closure of the respiration chamber, a blank determination could be avoided even though it is required for obtaining the total carbon dioxide content at each level accurately. The distilled water used was always double-distilled and freshly boiled. All the solutions were protected by a thick layer of hexane.

Ammonia: The method followed was

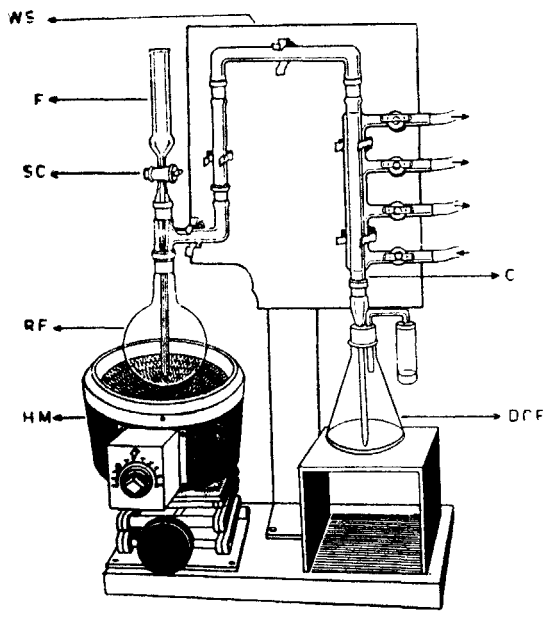

Figure 2 Modified Maros-Schulek apparatus for total carbon dioxide determination. HM, Heating mantle; RC, receiver flask; SC, stopcock; F, funnel; WS, wooden stand; C, condenser; DCF, distillate collecting flask. The inflow and outflow of water in the condenser are shown by arrow marks. Note a small tube containing $Ba(OH)_2$ solution, fixed in the rubber cork of the condenser, to protect the distillate

suggested by Stroganov (1962) as described by Kutty (1972). Fifty ml of sample was distilled, distillate nesslerised and the optical density read at 420 μ in a spectrophotometer (Spectronic-20). Ammonia-free water (APHA 1965) only was used for blank and preparing reagents.

Results

A comparison of figures 3 and 4 are made within the discussion itself. Mean values of metabolic rates and quotients at 'high' and 'low' ambient oxygen are presented in table 1.

O_2-consumption was similar both at 30° and 35°C, except for the higher values of O_2-consumption at high ambient oxygen (4–5 mg O_2/l) at 35°C. The level of O_2-consumption also varied at the peak phase (4 mg O_2/l), i.e. 100 and 150 ml/kg/hr at 30° and 35°C respectively. The low rate of O_2-consumption (below 50ml/kg/hr) is evident during the hypoxic phase at both the temperatures. CO_2-production at 30° and 35°C showed a peak (about

Table 1 *Metabolic rates, RQ, AQ, and random activity in* Carassius auratus *in 'high' and 'low' oxygen at 30° and 35°C*

	30°C				35°C			
	High O_2		Low O_2		High O_2		Low O_2	
Mean ambient oxygen (mg/l)	5.05 ±0.06	(6)	1.08 ±0.23	(5)	5.27 ±0.17	(6)	1.02 ±0.24	(5)
Mean rate of O_2 consumption (ml/kg/hr)	92.9 ±2.6	(6)	29.8 ±14.7	(5)	93.6 ±5.5	(6)	31.5 ±10.5	(5)
Mean rate of CO_2 production (ml/kg/hr)	89.3 ±2.8	(6)	74.5 ±31.5	(5)	92.0 ±5.8	(6)	67.0 ±17.2	(5)
Mean rate of NH_3-N excretion (ml/kg/hr)	6.6 ±4.7	(6)	4.3 ±0.9	(5)	10.3 ±1.2	(6)	7.0 ±1.7	(5)
Mean respiratory quotient (RQ)	0.96 ±0.02	(6)	2.8 ±0.55	(5)	0.99 ±0.03	(6)	2.48 ±0.39	(5)
Mean ammonia quotient (AQ)	0.104 ±0.024	(6)	0.219 ±0.094	(5)	0.111 ±0.012	(6)	0.248 ±0.056	(5)
Mean random activity (counts/hr)	47.0 ±18.0	(6)	33.0 ±7.4	(5)	14.5 ±4.0	(6)	37.3 ±4.7	(5)

Values are mean ± S.E.; Figures in the parentheses indicate no. of determinations.

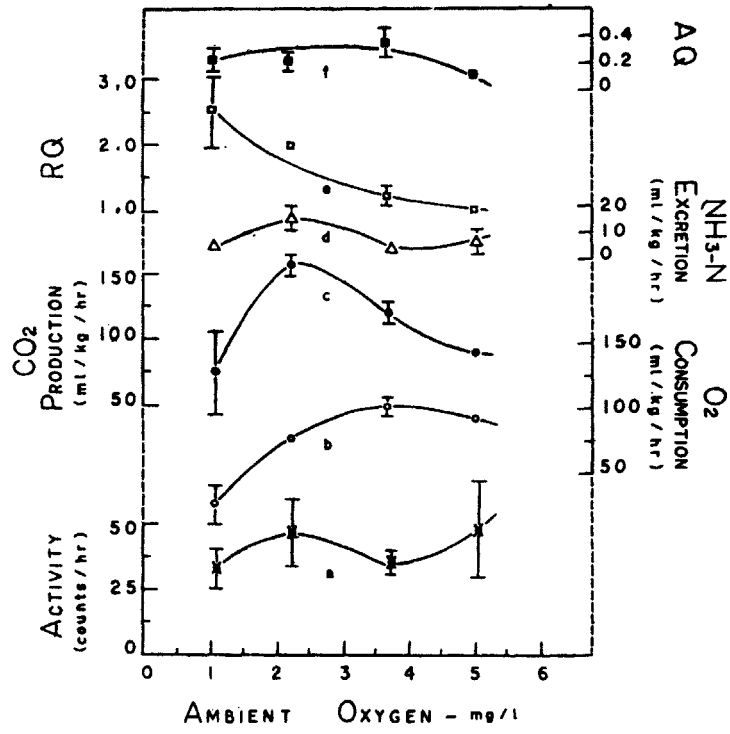

Figure 3 Random activity (*a*); O_2-consumption (*b*); CO_2-production; NH_3-N excretion (*d*); RQ (*e*); and AQ (*f*) in relation to ambient oxygen in *Carassius auratus* acclimated to and tested at 30°C. Ambient oxygen was reduced by the respiration of the fish itself. The experiment was terminated when the test fish lost its equilibrium. Each value plotted is a mean (± SE) of six determinations. SE is not indicated if it falls within the area of the symbol shown in the figure

150 ml/kg/hr) in the middle range of ambient oxygen.

NH_3-N excretion was similar at both the temperatures. However, during the hypoxic phase it was low in consonance with the steep fall in the CO_2 production (figures 3 & 4).

The RQ remained near unity from air-saturation up to 4 mg O_2/l at both the temperatures. The highest RQs at 30° and 35°C were 2.8 and 2.5 respectively at about 1 mg O_2/l.

At 35°C, the AQ increased with the decrease in ambient oxygen, falling in line with the general pattern of AQ change in relation to ambient oxygen.

Random activity at both the temperatures was similar and increased with decrease in ambient oxygen. At 30°C the random activity was high (about 50 counts/hr) at high ambient oxygen (5 mg O_2/l) and decreased slightly with increase of ambient oxygen and again shot at 2–3 mg O_2/l and decreased again. But none of these activity changes are significant. At 35 °C the activity increased with decrease of ambient oxygen and the highest activity (about 40 counts/hr) was observed below 1 mg O_2/l.

Discussion

The routine O_2-consumption at both the temperatures followed the general pattern i.e. low rates at the highest and lowest oxygen levels and, high rates in the middle

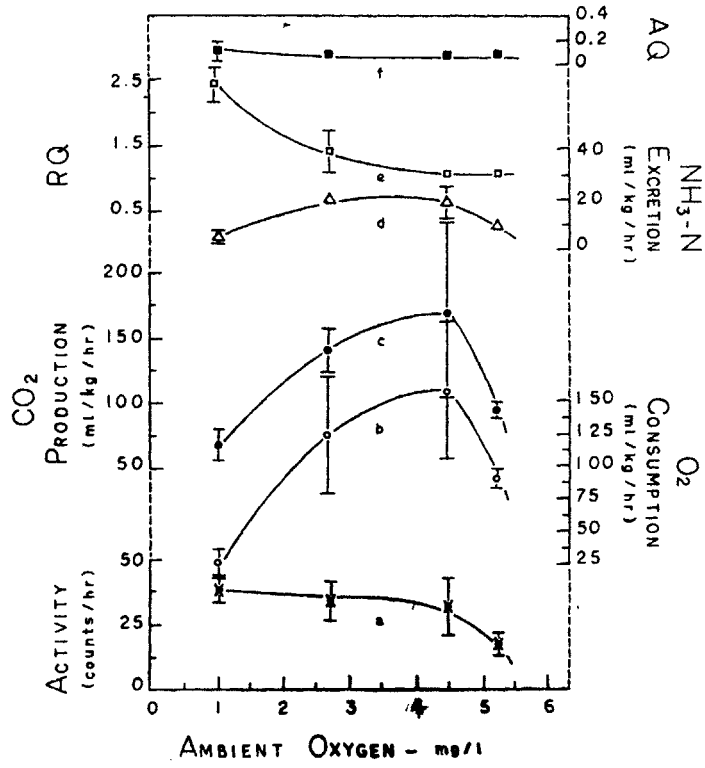

Figure 4 Random activity (*a*); O_2-consumption (*b*); CO_2-production (*c*); NH_3-N excretion (*d*); RQ (*e*); and AQ (*f*) in relation to ambient oxygen in *C. auratus* acclimated to and tested at 35°C (*see legend to figure* 3 *for further explanation*)

range. This pattern is in general agreement with the trends of O_2-consumption-ambient oxygen relation, described earlier for goldfish and rainbow trout (Kutty 1968), *R. corsula* (Kutty & Peer Mohamed 1975) and *T. mossambica* (Peer Mohamed 1981). The CO_2 production, below 50% ambient oxygen decreased at both 30° and 35°C indicating the limit of anaerobic metabolism of goldfish under hypoxic conditions. As also observed in earlier studies (Kutty 1968, 1972), the dependence of CO_2 is well-marked at low levels of ambient oxygen. NH_3-N excretion is similar at both the temperatures, particularly during hypoxic phase. The excretion was low, in consonance with the steep fall in CO_2 production, which leaves room for utilization of aerobic component, carbohydrate as substrate than protein.

The RQs at the highest ambient oxygen (near air-saturation) tested are close to unity and similar in magnitude to that observed for goldfish at 20°C (1.02) by Kutty (1968). To find the maximum influence of hypoxia on RQs the values determined at the lowest ambient oxygen concentrations (i.e. during the final run of the experiment in which the fish become asphyxiated) can be useful. In all the experiments, the fish being revived, subsequently, in air-saturated water, was observed to survive the hypoxic treatment. The routine RQ values at the lowest

oxygen concentration are significantly above unity and clearly suggest the occurrence of considerable amount of anaerobic metabolism during the hypoxic phase resulting in the release of extra CO_2. Kutty (1968) showed that goldfish at 20°C sustained an RQ of about 2 at 1.5 mg O_2/l for several months, whereas in the present tests the high RQs were sustained for a short period (about one hour only).

The AQs or routine protein utilization, were parallel to the RQ changes with ambient oxygen as observed earlier in *T. mossambica* (Kutty 1972, Peer Mohamed 1981), *R. corsula* (Kutty & Peer Mohamed 1975) and *Puntius sarana* (Peer Mohamed & Kutty 1983). The AQ value at the highest ambient oxygen (near air-saturation), around an average of 0.1, is in agreement with AQ reported for *Gambusia* (Stroganov 1962), *R. corsula* (Kutty & Peer Mohamed 1975) and *Mystus armatus* (Sukumaran & Kutty 1977).

The increased ammonia-excretion under hypoxia is certainly due to anaerobic degradation of protein/amino acids. The increase in AQ and RQ under hypoxia (mentioned earlier) suggest a coupling of the increased ammonia-excretion and increased CO_2 output in O_2-deficient environment (Kutty 1972, Kutty & Peer Mohamed 1975, Peer Mohamed & Kutty 1981, 1983, Peer Mohamed 1981). This may have a special significance in acid-base balance and also in conserving Na^+ in freshwater fish (Prosser & Brown 1961, Maetz & Garcia Romeu 1964, Forster & Goldstein 1969, Kutty 1972). Ammonia can neutralise the acid, CO_2 produced during anaerobic metabolism. Moreover, NH_4^+ can be exchanged with the Na^+ in ambient water through the gills. Thus anaerobic-metabolism and protein-degradation in ammonotelic animals may have a special significance. Hochachka and Somero (1973) suggested that anaerobic metabolism of carbohydrates and proteins can provide considerable energy through pathways other than the conventional glycolysis.

The increased random activity on exposure to low ambient oxygen is in agreement with the activity-ambient oxygen trends described in the same species and rainbow trout (Kutty 1968) and for *R. corsula* (Kutty & Peer Mohamed 1975). The random activity also decreases with the decrease in ambient oxygen, as reported for the milkfish, *Chanos chanos* (Hamsa & Kutty 1972) and *Tilapia* (Peer Mohamad & Kutty 1980, Peer Mohamed 1981). Such differences in behaviour on hypoxic treatment, have a special significance with regard to survival rate (Hamsa & Kutty 1972, Peer Mohamed & Kutty 1980). Increased activity induced by hypoxia might compell the fish to move out of the hypoxic environment to more oxygenated waters, if available (present study).

It must be stated that the present tests were conducted in a closed system and the anaerobic responses of fish, chronically exposed to low oxygen (Blazka 1958, Kutty 1968) have not been studied. It is possible that the anaerobic abilities of the species tested might be enhanced on full acclimation to low oxygen (hypoxia). On the other hand, this need not be so, for Kutty (1968) found that acclimation to low oxygen did not increase the RQs of goldfish.

Acknowledgements

Sincere thanks are due to Dr S V Job, for valuable suggestions; and to Professor S Krishnaswamy for the facilities provided. Appreciation is extended to Mr J Antony Basil for his help in collection and maintenance of fish stock.

References

American Public Health Association 1965 in *Standard Methods for the Examination of Water and Wastewater* 12th edn (New York: American Public Health Organisation) 769 pp

Black V S 1957 Excretion and osmoregulation; in *Physiology of Fishes* Vol 1 pp 163–205 ed M E Brown (New York: Academic Press Inc.)

Blazka P 1958 Anaerobic metabolism in fish; *Physiol. Zool.* **31** 117–128

Forster R P and Goldstein L 1969 Formation of excretory products; in *Fish Physiology* Vol 1 pp 313–345 eds W S Hoar and D J Randall (New York & London: Academic Press)

Garcia Romeu F et Motais R 1966 Mise en evidence d'echanges Na^+/NH_3^+ chez l'angille d'eau douce; *Comp. Biochem. Physiol.* **17** 1201–1204

Hamsa A K M S and Kutty M N 1972 Oxygen consumption in relation to spontaneous activity and ambient oxygen in five marine teleosts; *Indian J. Fish.* **19** 76–85

Hariharan K 1973 *Influence of feeding of protein-rich and protein-deficient diets on the growth, energy utilization and nitrogen balance in the freshwater fish, Tilapia mossambica*; Dissertation, Madurai Kamaraj University, Madurai

Hochachka P W and Somero G N 1973 in *Strategies of Biochemical Adaptation* (Philadelphia: W B Saunders & Co.)

Kutty M N 1968 Respiratory quotients in goldfish and rainbow trout; *J. Fish. Res. Bd. Can.* **25** 1689–1728

—— 1978 Ammonia quotient in sockeye salmon (*Oncorhynchus nerka*); *J. Fish. Res. Bd. Can.* **35** 1003–1005

—— 1972 Respiratory quotient and ammonia excretion in *Tilapia mossambica*; *Mar. Biol.* (*Springer-Verlag*) **16** 126–133

——, Karuppannan N V, Narayanan M and Peer Mohamed M 1971a Maros-Schulek technique for the measurement of carbon dioxide production in fish and respiratory quotient in *Tilapia mossambica*; *J. Fish. Res. Bd. Can.* **28** 1342–1344

——, Peer Mohamed M, Thiagarajan K and Leonard A N 1971b Modification of Fry's fish activity counter and respirometer; *Indian J. expl. Biol.* **9** 218–222

——, and Peer Mohamed M 1975 Metabolic adaptations of mullet *Rhinomugil corsula* (Hamilton) with special reference to energy utilization; *Aquaculture* **5** 253–270

Maetz J and Garcia Romeu F 1964 The mechanism of sodium and chloride uptake by the gills of a freshwater fish, *Carassius auratus*. III. Evidence for NH_4^+/Na^+ and HCO_3^-/C 1-exchanges; *J. gen. Physiol.* **47** 1209–1227

Maros L, Schulek E, Molnar-Perl I and Pinter-Szakacs M 1961 Einfaches Destillations verfahren zur titrimetrischen Bestimmung von Kohlendioxyd. (A simple distillation method for the titrimetric determination of carbon dioxide); *Analytica Chim. Acta* **25** 390–399 (Trans. from German., Fish. Res. Bd. Can. Trans. Ser. No. 596, 1965)

Peer Mohamed M and Kutty M N 1980 Influence of ambient oxygen on random activity in some freshwater teleosts; *Proc. Indian Acad. Sci.* (*Animal Sci.*) **89** 537–542

—— 1981 Metabolism of *Tilapia mossambica* (Peters) with emphasis on hypoxia; *Indian J. expl. Biol.* **19** 1098–1100

——, and Kutty M N 1981 Respiratory quotient and ammonia quotient in *Tilapia mossambica* (Peters) with special reference to hypoxia and recovery; *Hydrobiologia* **76** 3–10

——, and Kutty M N 1983 Influence of hypoxia on metabolism and activity in *Puntius sarana* (Hamilton); *Proc. Indian Acad. Sci.* (*Animal Sci.*) (in press)

Privolnev T I 1954 Physiological adaptations of fishes to new conditions of existence; *Tr. Sovesh Ikhtiologicheskoi Komis. Akad. Nauk. SSSR.* **3** 40-49 (Fish. Res. Bd. Can. Trans. Ser. No. 422)

Prosser C L and Brown Jr F A 1961 in *Comparative Animal Physiology* 2nd edn (Philadelphia & London: W B Saunders & Co.) 688 pp

Stroganov N S 1956 Physiological adaptability of fish to the temperature of surrounding medium; *Izd. Akad. Nauk. SSSR*, Moscow (Transl. from Russian by Israel Programme for Scientific Translations, Jerusalem, No. 619, 1956)

—— 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*, ed E N Pavlovskii *Izd. Akad. Nauk. SSSR.* 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 Kutty M N 1977 Oxygen consumption and ammonia excretion in the catfish *Mystus armatus*, with special reference to swimming speed and ambient oxygen; *Proc. Indian Acad. Sci.* **B 86** 195–206