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Influence of hypoxia on metabolism and activity in *Puntius sarana* (Hamilton) (Pisces: Cyprinidae)

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Abstract. In *Puntius sarana*, changes in metabolic rates in relation to ambient oxygen fell into the general pattern at 30 and 35°C, the differences being mainly in the levels of metabolism, both aerobic and anaerobic. The mean RQs at high ambient oxygen were 0.77 and 0.63 revealing that the fish were aerobic under adequate ambient oxygen. The hypoxic RQ were 1.42 and 1.90 suggesting that the fish derived considerable energy anaerobically. The aerobic AQs ranged between 0.06 and 0.18 depending on the utilization of protein and the hypoxic AQs were in consonance with the corresponding changes in hypoxic RQs. As judged from the asphyxial oxygen level, *P. sarana* can live, below air saturation, up to 0.41 and 0.49 mg O_2/l at 30° and 35°C respectively. The random activity of the fish increased with decrease of ambient oxygen.

Keywords. Metabolism; respiratory quotient; ammonia quotient; random activity; ambient oxygen; hypoxia; *Puntius sarana*.

1. Introduction

The multiplicity of invoivement between environment and metabolic rate illustrates that within the bounds of knowledge the factors of dissolved oxygen, temperature and activity exert the greatest effect on metabolism – in unpolluted waters. *Puntius sarana* (Hamilton) is one of the economically important carps not subjected to proper experimental study which describes the effects of dissolved oxygen and temperature. This paper deals with the influence of hypoxia on metabolic rates and quotients (RQ and AQ†) and random (spontaneous) activity in this species. Increase in RQ over unity was demonstrated in goldfish and rainbow trout (Kutty 1968), *Tilapia mossambica* (Kutty *et al* 1971a; Peer Mohamed and Kutty 1981) and in *Rhinomugil corsula* (Kutty and Peer Mohamed 1975) exposed to low ambient oxygen. To determine whether the ammonia produced is of use to freshwater fish under anaerobiosis (Peer Mohamed and Kutty 1981), relative changes in ammonia excretion and RQ have been estimated to study the utilization of proteins by fish.

† Respiratory quotient, RQ = volume of CO_2 produced/volume of O_2 consumed; ammonia quotient, AQ = the volume or mole relation of NH_3-N excreted to O_2 consumed as explained in Kutty (1972)

2. Material and methods

Minor carp, *Puntius sarana* were collected from the Vaigai Dam (Tamil Nadu, India) and kept in a tank (300 litre) for some days and then transferred to glass a aquaria (70 litre) for acclimation. Experimental fish was deprived of food for 36 hr (Peer Mohamed and Kutty 1981) and left in the respirometer overnight before experiments. Nine fish ranging in total length from 16.4 to 17.1 cm (mean 16.7 cm) and in weight from 26.6 to 27.9 g (mean 27.1 g) were tested at 30 ± 0.5°C and 35 ± 0.5° C.

The apparatus used was a modification of Fry's respirometer (Kutty *et al* 1971b) in which metabolic rates and random activity can be simultaneously measured. The annular transparent perspex respirometer (3 litre) was designed such that the diffusion of gases into and out of the water in the respirometer was minimised. Decarbonated tap-water, adjusted to a pH of 8.2 (Kutty 1968, 1972; Kutty *et al* 1971a), was used for experiments as explained in Kutty *et al* (1971a). From an overhead glass tank (70 litre) water flowed through the respirometer to a ground level tank of the same capacity from which it was pumped for recirculation.

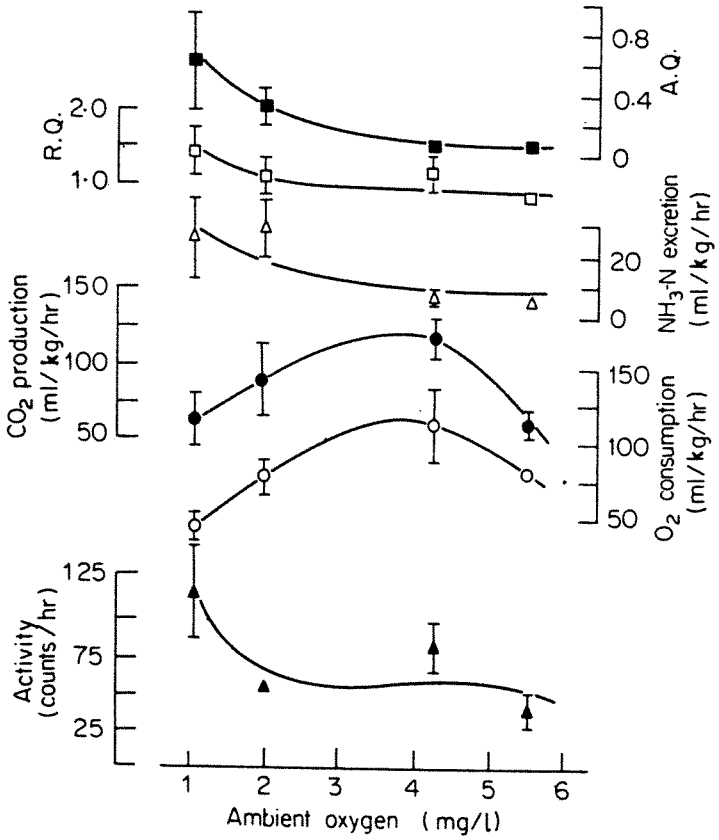

Figure 1. Oxygen consumption, carbondioxide production, ammonia excretion, RQ, AQ and random activity in relation to ambient oxygen below air saturation in the minor carp, *Puntius sarana* acclimated to and tested at 30°C. Each value plotted is a mean of (±S.E.)4 determinations. The SE is not indicated if it falls within the area of symbol shown in the figure.

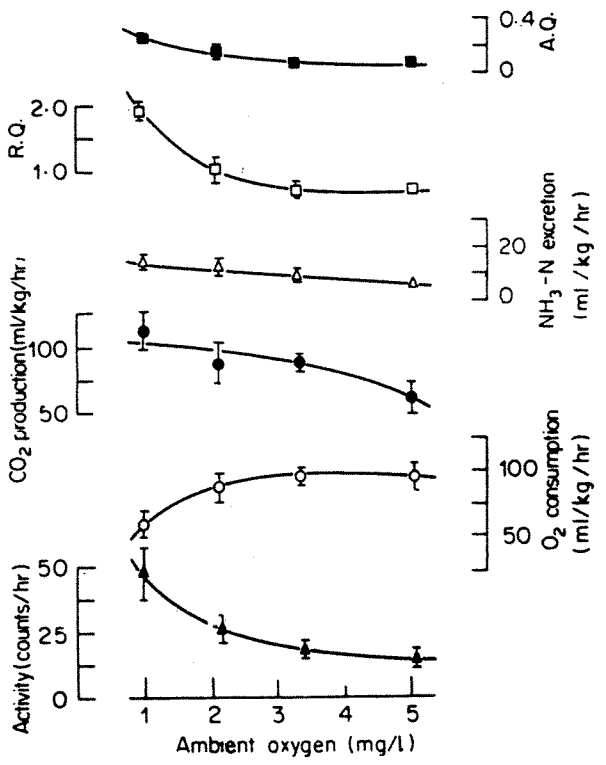

Figure 2. Oxygen consumption, carbon dioxide production, ammonia excretion, RQ, AQ and random activity in relation to ambient oxygen in *Puntius sarana* acclimated to and tested at 35°C. See legend to figure 1 for further explanation.

The experimental procedure followed was as described in Kutty and Peer Mohamed (1975) with closed and open periods which together constitute a run. Each experiment consisted of 5 -8 runs of 1 hr in duration when the respirometer remained closed. The ambient oxygen fell from air saturation to the asphyxial level (the oxygen concentration at which the fish begin to lose equilibrium) due to the depletion of oxygen by the respiration of the fish itself. Then the respirometer was flushed with air-saturated water to revive the animal.

2.1 *Methods of water analyses*

Dissolved oxygen was measured using unmodified Winkler technique (American Public Health Association 1965). The size of the sample for titration was 25 ml. Maros-Schulek technique (Maros *et al* 1961) modified for fish metabolism studies by Kutty *et al* (1971a) was followed for total carbondioxide estimation. The method of Stroganov (1962) as described by Kutty (1972) was followed for ammonia measurement. The size of the sample was 50 ml.

3. Results

The trend of oxygen consumption, carbondioxide production, ammonia excretion, RQ, AQ and random activity below air saturation in *P. sarana* acclimated to and tested at 30 and 35°C are graphically shown in figures 1 and 2 respectively. In table 1 are presented for comparison the mean values of metabolic rates, quotients and random activity at high (normoxic) and low (hypoxic) oxygen concentrations tested.

Table 1. Metabolic rates, RQ, AQ and random activity in *Puntius sarana* 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 O_2 –mg/l	5.45 ± 0.08	1.02 ± 0.10	5.14 ± 0.10	0.99 ± 0.30
Mean rate of O_2 consumption – ml/kg/hr	78.50 ± 1.50	42.3 ± 8.7	94.8 ± 8.6	61.0 ± 9.4
Mean rate of CO_2 production – ml/kg/hr	60.3 ± 6.9	63.0 ±17.4	6. ⁰ ±10.9	113.0 ±14.0
Mean rate of NH_3-N excretion–ml/kg/hr	4.7 ± 0.4	25.7 ±13.6	5.4 ± 0.6	15.6 ± 2.7
Mean respiratory quotient– RQ	0.77 ± 0.10	1.42 ± 0.31	0.63 ± 0.05	1.90 ± 0.13
Mean ammonia quotient – AQ	0.060± 0.006	0.626± 0.330	0.056± 0.007	0.256± 0.020
Mean random activity counts/hr	41.0 ±17.5	117.0 ±32.0	27.6 ± 1.5	57.5 ±11.8

The 'high' oxygen refers to a mean ambient oxygen concentration near air saturation and the 'low' oxygen refers to the lowest mean ambient oxygen concentration tested, *i.e.* the mean of initial and final oxygen value of a run, the final value of which is the asphyxial oxygen concentration. Each value is ±S.E. of four determinations.

The trend lines of oxygen consumption at both temperatures are similar at oxygen levels between 4 and 1 mg/l. At 30°C the oxygen consumption at the high ambient oxygen level tested (near air saturation) was low (80 ml/kg/hr), the lowest rate was 41 ml/kg/hr at 0.79 mg O_2/l and the highest was 178 ml/kg/hr at 3.9 mg O_2/l. At 35°C the oxygen consumption decreased with decrease of ambient oxygen; 108 ml/kg/hr at 4.9 mg O_2/l and 43 ml/kg/hr at 0.74 mg O_2/l.

The trend lines of carbondioxide production at 30°C (figure 1) resembles that of oxygen consumption, in that there was a decrease in carbondioxide production with decrease in ambient oxygen below the middle range.

The trend in ammonia excretion indicates that ammonia excretion increased with decrease in ambient oxygen at 30°C; the maximum rate being 20 ml/kg/hr during the hypoxic phase (1 mg O_2/l), and the lowest level being 10 ml/kg/hr during normoxic phase (air saturation).

The routine RQ increased with decrease in ambient oxygen and RQ was near unity at high oxygen concentrations. The RQ shot up above unity during hypoxia and reached up to 1.5 (30°C) and 2 (35°C) at 1 mg O_2/l.

The trend lines of random activity at both temperatures are similar. At 30°C during high ambient oxygen the random activity almost doubled that of 35°C. The maximum random activity recorded were 117 and 58 counts/hr during hypoxic phase at 30 and 35°C respectively.

4. Discussion

The routine oxygen consumption at both temperatures followed almost the same general pattern, displaying low rates at high and low oxygen concentrations test-

ed (table 1) and high rates in the middle range. This agrees with the observations made earlier on goldfish and rainbow trout (Kutty 1968), *R. corsula* (Kutty and Peer Mohamed 1975) and *T. mossambica* (Peer Mohamed and Kutty 1981).

The RQs of *P. sarana* at high ambient oxygen are 0.77 at 30°C and 0.63 at 35°C (table 1) which are close to unity. It can, however, be pointed out that routine RQs of *Puntius* are closest to fat RQ values. The fish tested may have used more fat than carbohydrates and proteins. The tolerance of hypoxia can also be indicated, at least partially, by the asphyxial oxygen level, anaerobic abilities of the species under hypoxia. The asphyxial level of *P. sarana* was 0.41 and 0.49 mg O_2/l at 30 and 35°C respectively.

The routine RQ values at the low oxygen concentrations tested are significantly above unity (table 1) which suggest that during the hypoxic phase considerable anaerobic metabolism has taken place, releasing extra carbondioxide. The intensity of anaerobic metabolism was higher at 35°C than at 30°C. In this case it is known that the hypoxic RQs (table 1) are sustained for about 1 hr. Kutty (1968) showed that goldfish at 20°C sustained an RQ of about 2 for months at 1.5 mg O_2/l and found that their anaerobic abilities as indicated by the RQ did not increase. But, in the present study no experiements were done on fish acclimated to low oxygen, and therefore the full hypoxic abilities of the species are perhaps not known. However, it must be stated that the present tests were conducted in a closed system. An open system, with continuous flow of low oxygenated water, might indicate tolerance of oxygen lower than that presently tested.

The AQ values at high ambient oxygen staying around an average of 0.1, agree with that reported for *Gambusia* (Stroganov 1962) and also for *R. corsula* (Kutty and Peer Mohamed 1975). Under the hypoxic conditions (low ambient oxygen tested) the AQ values of *P. sarana* were 0.63 and 0.26 at 30 and 35°C respectively, which clearly show that AQs under hypoxia are higher than those for fish in high oxygen, in consonance with the changes of RQ under similar conditions. The temperature effect on the hypoxic AQs shows that the relative protein utilization would be less at 35°C, as indicated by the AQ (table 1).

The increased ammonia excretion under hypoxia resulting from anaerobic degradation of protein/aminoacids, but not in all cases, is thus indicated by the magnitude of the AQs as a possible maximum of 0.33 is suggested from the activity considerations (Kutty 1978). As referred to earlier, the AQ and RQ increased under hypoxia, suggesting a coupling of the increased ammonia excretion and increased carbondioxide output at low oxygenated environment (Kutty 1972). This may have a special significance in acid-base balance and also in conserving Na^+ in freshwater fish (Kutty 1972; Kutty and Peer Mohamed 1975; Peer Mohamed and Kutty 1981). Hochachka and Somero (1973) suggested that anaerobic metabolism of carbohydrate and protein (aminoacids) can provide considerable amount of energy through pathways other than those for conventional glycolysis, such as those involved in the simultaneous breakdown of carbohydrates and aminoacids (Hochachka *et al* 1973). The high RQs and AQs in *P. sarana* may thus be of much significance in its survival.

P. sarana showed an increase in random activity on exposure to low oxygen (hypoxia), as described for several fish (Hamsa and Kutty 1972; Peer Mohamed and Kutty 1982). Increased activity induced by hypoxia might allow the fish to move out of the hypoxic environment to more oxygenated waters, if available. The increase of random activity to low ambient oxygen may have a major role

in the survival of *P. sarana* (Hamsa and Kutty 1972; Peer Mohamed and Kutty 1982).

The information obtained in the present study although provides some insight into the impact of low oxygen (hypoxia) on metabolism and activity, more information is necessary to elucidate the metabolic and behavioural responses of more species especially in view of their importance as cultivable warm-water fishes. However, the involvement of environmental interaction is not a deterrent to progress but a measure of the complexity to be met.

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