

GAS EXCHANGE WITH AIR AND WATER IN AN AIR-BREATHING CATFISH, *SACCOBRANCHUS* (\equiv *HETEROPNEUSTES*) *FOSSILIS*

BY G. M. HUGHES AND B. N. SINGH

Research Unit for Comparative Animal Respiration, The University, Bristol BS8 1UG

(Received 22 March 1971)

INTRODUCTION

Recent studies on air-breathing fish have shown that different species are adapted in varying degrees for gaseous exchange in both water and air. In the lungfishes these differences are correlated with different degrees of development of the gills, the efficiency of the lung in gas exchange, and the respiratory properties of the blood (Lenfant, Johansen & Grigg, 1966; Johansen & Lenfant, 1967, 1968; Lenfant & Johansen, 1968; Jesse, Shub & Fishman, 1967; McMahan, 1970). A similar situation exists among air-breathing teleosts, but there seems to be an even greater variation in the degree of dependence on water or air-breathing, which is again related to the structure of the gills and air-breathing organs and the normal environments in which they live (Farber & Rahn, 1970; Garey & Rahn, 1970; Hughes & Singh, 1970*a*, *b*; Johansen, 1966; Singh & Hughes, 1971).

Saccobranchus fossilis is an air-breathing fish which inhabits pools in tropical Asia. The structure and arrangement of the gills and air-breathing organs were described by Munshi (1962), the air-breathing organ in this case being a posterior diverticulum of the opercular cavity in the form of an air-sac. Studies on the gill respiratory epithelia showed that the gills of *Saccobranchus* are better adapted for gas exchange than those of other air-breathing teleosts from the same habitat (Munshi & Singh, 1968). Measurements of the air/blood pathway using electron microscopy (Hughes & Munshi, 1972) have shown longer distances than in *Anabas* (Hughes & Munshi, 1968) and other air-breathing fish from similar habitats. Although inhabiting similar environments, *Saccobranchus* differs from *Anabas* by aestivating in the mud when pools dry up in the summer.

The purpose of the present study was to investigate the relative roles of the gills and air-breathing organs in gas-exchange under varying environmental conditions. It also throws some light on the mechanism of metabolic adaptation in *Saccobranchus* under different conditions.

MATERIALS AND METHODS

Specimens of *Saccobranchus* (\equiv *Heteropneustes*) *fossilis* (Bloch) were collected in India, where this type of catfish is commonly called Singee, and transported by aeroplane to England. Fish were kept in aquaria at 25 ± 1 °C in the laboratory at Bristol University for several months. They were fed at regular intervals but not for at least 12 h before, or during, an experiment. The fish were acclimatized to the

experimental conditions at least overnight and no measurements were made until a few hours after the start of a given experiment; in this way the animals became well acclimatized to the respirometer and levels of oxygen consumption ($\dot{V}O_2$) were stabilized. All experiments were carried out at 25 ± 1 °C.

Most methods used were similar to those described for *Anabas* (Hughes & Singh, 1970a), but as *Saccobranchus* is able to survive in water alone, many of the experiments were conducted in a continuous-flow respirometer (Fig. 1). Samples (0.5 ml) were collected from the inflow and outflow water of the respirometer and their oxygen

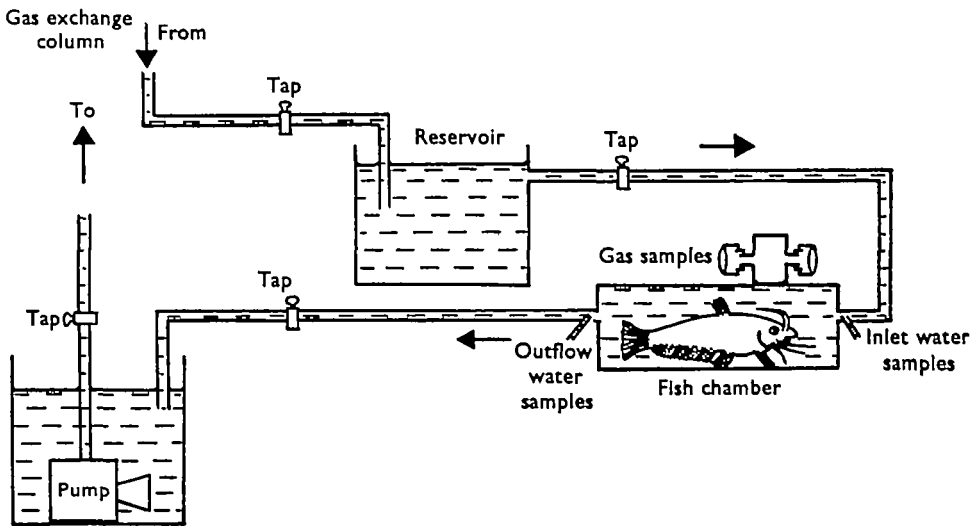


Fig. 1. Diagram of apparatus used for determining changes in oxygen tension in a continuous flow of water and the oxygen and carbon dioxide contents of the air above it. Samples of water and gas were taken for analyses from the rubber connectors as indicated.

tensions (WP_{O_2}) were measured using a Beckman oxygen macro-electrode. The rate of oxygen consumption in hypoxic water was also studied in this respirometer using water which had its oxygen tension reduced by bubbling nitrogen through the gas-exchange column. Allowances were made for the diffusion of oxygen from the air into the hypoxic water. Water was also equilibrated with gas mixtures containing 2–10% carbon dioxide in air to study the effect of carbon dioxide on the oxygen consumption of the fish. Gas mixtures were prepared using gas-mixing pumps. Total carbon dioxide content of the equilibrated water was determined for 1 ml samples by the method of van Slyke & Neill (1924).

Some experiments were also designed to measure skin respiration in water. The fish was placed in a respirometer with its head in one compartment and the rest of its body in another compartment for measurements of cutaneous gas exchange. The two chambers were made water-tight by means of a rubber barrier which also helped to fix the fish in an immobile posture. The respirometer was immersed in a water-bath and both compartments were continuously perfused with aerated water. $\dot{V}O_2$ via the skin was calculated from the reduction in P_{O_2} of the water leaving the body compartment.

RESULTS

When *Saccobranchus* is kept in tap water and allowed free access to air, it breathes from both media. The intervals between air-breaths are irregular and vary from a few minutes to about half-an-hour and are related to the oxygen pressure in the water and air. Variations are also found between individual fish. During these experiments it has been found that *Saccobranchus* ceases air-breathing but continues water-breathing when placed in a flow of air-saturated water. The fish shows no signs of stress or restlessness when placed in such a situation for periods of 6–12 h and rarely attempts air-breathing. The opercular frequencies are slightly raised above that when both water-breathing and air-breathing are allowed, but no significant change in amplitude is seen. When the oxygen content of the water is reduced below saturation, both water-breathing and air-breathing movements take place. The intervals between air-breaths are reduced in hypoxic water and also if the inspired air is low in oxygen.

Oxygen consumption (\dot{V}_{O_2}) in water when access to air is prevented

\dot{V}_{O_2} in air-saturated water. In air-saturated water ($P_{O_2} = 150\text{--}155$ mmHg) *Saccobranchus* rests at the bottom of the respirometer and exchanges gases with water alone. The mean \dot{V}_{O_2} in air-saturated water was 63.35 cc/kg/h (Table 1). The mean opercular frequency was 51/min but it varied between 46 and 58/min in different individuals. The amplitude of the gill ventilatory movements was slightly augmented in most individuals but appeared normal in others.

When breathing air-saturated water and not allowed to surface, the mean \dot{V}_{O_2} via the skin is 11.19 cc/kg/h. This constitutes 16.82% of the total \dot{V}_{O_2} in water and hence 83.18% enters via the gills when the fish is confined to water.

\dot{V}_{O_2} in water of low oxygen tensions. When the oxygen content is reduced below saturation, *Saccobranchus* shows an increased frequency and depth of breathing. Associated with this the fish is somewhat restless at the start of an experiment, but after some time it settles down and does not show any restlessness or attempted air-breathing until the oxygen tension has been reduced to about 100 mmHg. Both aquatic and aerial organs are used once the P_{O_2} is below 90 mmHg. There are individual variations in the level of WP_{O_2} (oxygen tension in water) at which air-breathing begins, but usually it is between 100 and 50 mmHg.

\dot{V}_{O_2} is reduced when the fish is in hypoxic water and denied free access to air (Fig. 2). \dot{V}_{O_2} in water at 110 mmHg is reduced to 44.05 cc/kg/h. This level of \dot{V}_{O_2} is maintained until the P_{O_2} is reduced to 50 mmHg. With a further lowering of WP_{O_2} the oxygen uptake from the water falls rapidly (Table 1, Fig. 2). The frequency and amplitude of the gill ventilatory movements increase with lowering WP_{O_2} . A significant increase in opercular frequency and amplitude occurred below 50 mmHg, but below 40 mmHg the opercular frequencies declined once more (Fig. 2).

\dot{V}_{O_2} from water in a closed respirometer without access to air. The fish was placed in a 2.8 l respirometer filled with air-saturated water. As it can only obtain oxygen from water, the ambient P_{O_2} falls and the fish is subjected to a gradual hypoxia over periods of 3–4 h. The ventilatory frequency and amplitude were a little high relative to those in fish with free access to air at the beginning of the experiment, but both increased significantly at lower oxygen tensions (Fig. 3). The fish made no attempt to breathe

Table 1. *Effect of environmental conditions on respiration of Saccobranchus in water and in air (25 ± 1 °C)*

Mean body wt (g)	Experimental conditions	W/P _{O₂} (mmHg)	Air P _{O₂} (mmHg)	Opercular frequency (min)	Depth of breathing	Total mean \dot{V}_{O_2} (cc/kg/h)	\dot{V}_{O_2} released to air (cc/kg/h)	RQ
A. Respiration in water without access to air								
59.86 (50-69)	Air-saturated water (cont. flow)	150-155		51 (46-58)	Slightly high or normal	65.35 (56.91-75.69)	—	—
57.54 (48-62)	Water with gradual hypoxia (closed respirometer)	Falls from 150 to 18		Inc. from 50-82	Increases with onset of hypoxia	96.40 (87.85-105.21)	—	—
55.0	Hypoxic water (cont. flow)	82 (80-84)		75.5 (68-86)	Heavy	42.18 (39.38-44.98)	—	—
55.1 (54-56)	Hypercarbic water 7.5% CO ₂ in air (cont. flow)	136.3		65.5 (59-74)	Normal or low	53.07 (47.20-58.94)	—	—
B. Respiration in water with access to air								
45.5	Air-saturated water (cont. flow of water)	155	Decreasing from 147.5 to 113.5	30-36	Very low	84.50 (72.63-96.47)	5.40	(0.17)*
55.0	Hypoxic water (cont. flow)	84 (80-88)	Decreasing from 110.03 to 83.01	47-48	Normal	56.67 (52.78-62.70)	—	—
57.3 (48.5-64.0)	De-oxygenated water (closed respirometer)					35.09 (30.15-42.88)	13.17	(0.38)*
58.9 (46-65)	Air-exposed					54.50 (49.10-69.45)	31.78	0.58
C. Respiration in air only								

In all cases the figures in parentheses indicate the range of measurements.

* Based upon release of CO₂ into air only.

air until WP_{O_2} was 90 mmHg. Three of the four fishes studied started to struggle and move towards the air chamber of the respirometer, presumably in search of air when the P_{O_2} was 60–100 mmHg. After the WP_{O_2} has fallen to below 60 mm, this behaviour increased and sometimes the fish made repeated unsuccessful attempts to breathe air from the air chamber. The depth of breathing was very high below this level of WP_{O_2} and opercular frequencies were reduced at WP_{O_2} 's below 30 mmHg (Fig. 3).

The mean \dot{V}_{O_2} under such conditions was 96.4 cc/kg/h (Table 1). The rate of oxygen consumption increased from this level to about 118 cc/kg/h, while WP_{O_2} decreased to about 102 mmHg. Then \dot{V}_{O_2} returned to its normal value of about 92 cc/kg/h which was maintained until WP_{O_2} fell to 40 mmHg. At lower O_2 tensions, \dot{V}_{O_2} was greatly reduced (Fig. 3).

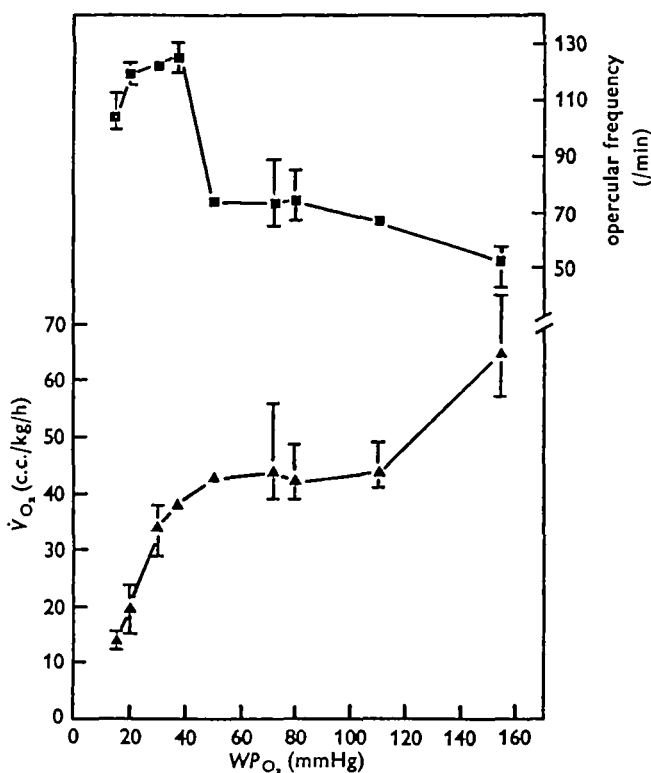


Fig. 2. Changes in the rate of O_2 consumption (\blacktriangle) and opercular frequency (\blacksquare) of *Saccobranchus* in a continuous flow of water of lowered oxygen tension. Note the levelling of \dot{V}_{O_2} where WP_{O_2} lies between 110–50 mmHg. This is followed by a sharp decline in \dot{V}_{O_2} as WP_{O_2} falls below this level. The high opercular frequency also declines below 40 mmHg. Vertical lines indicate the range of the determinations.

It is interesting to note that when this experiment was carried out with the fourth fish, it did not attempt air-breathing even when WP_{O_2} was as low as 10 mmHg. Even though the fish showed signs of stress and restlessness, it did not attempt to breathe air, and this experiment was repeated on several occasions with the same result.

\dot{V}_{O_2} in air-saturated but hypercarbic water

\dot{V}_{O_2} in three individuals was studied in water that was hypercarbic but with P_{O_2} close to air-saturation. The total carbon dioxide content of the water (C_{CO_2}) varied between 10.5 and 16.6 vols %. The oxygen tension of the water was in the range 119–151 mmHg (Table 2A).

Fish showed a little restlessness accompanied by an increased \dot{V}_{O_2} at the beginning of an experiment but after about 1 h a nearly constant level of \dot{V}_{O_2} was established. It was found that \dot{V}_{O_2} was reduced with an increase in C_{CO_2} (Table 2). However, air-breathing did not occur until the gas mixture being equilibrated with the water contained 7.5 % carbon dioxide in air. Of the three fishes studied, two did not start air-breathing until 10 % carbon dioxide in air was bubbled through the water.

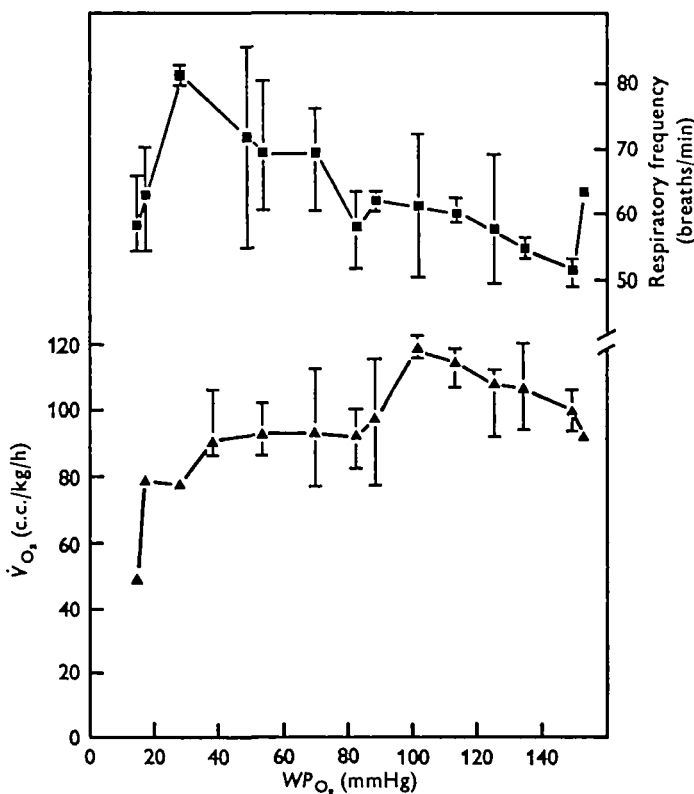


Fig. 3. \dot{V}_{O_2} (▲) and opercular frequency (■) of *Saccobranchus* in a closed respirometer when prevented from surfacing. \dot{V}_{O_2} is not reduced until WP_{O_2} falls to about 40 mmHg. Below this level \dot{V}_{O_2} is sharply reduced. Also note the difference in \dot{V}_{O_2} and opercular frequency at similar WP_{O_2} levels from fish exchanging gases in a continuous flow system (Fig. 2).

\dot{V}_{O_2} from hypercarbic water having C_{CO_2} of 16.6 vols % was reduced to 48.62 cc/kg/h before the fish was allowed to obtain oxygen from both water and air, when the total \dot{V}_{O_2} rose to 51.64 cc/kg/h (Table 2). However, one of the fish started to take air-breaths when the water was equilibrated with only 7.5 % carbon dioxide in air. The

Table 2. Effect of increasing carbon dioxide content of water on the respiration of *Saccobranchus* (25 ± 1 °C)

Mean body wt (g)	Gas mixture bubbled into the water	C _{CO₂} in water (vol %)	WP _{O₂} (mmHg)	Mean		Opercular frequency (min.)	Depth of water breathing	$\dot{V}O_2$ (water) (cc/kg/h)	$\dot{V}O_2$ (air) (cc/kg/h)	Total mean $\dot{V}O_2$ (cc/kg/h)
				C _{CO₂} in air (vol %)	O ₂ in air tube (vol %)					
59.9 (50.0-69.0)	Air	8.51	155	—	—	51 (46-58)	Slightly high	65.35 (56.91-75.69)	—	65.35
58.9 (58.2-59.6)	2% CO ₂ in air	10.51	151.2	—	—	60 (50-65)	Normal or slightly high	(I) 74.51 (66.68-75.02), (II) 65.14 (55.50-72.51)	—	65.14
57.0 (54.0-59.0)	5% CO ₂ in air	11.73	140.2	2.30	19.5	56.9 (53-61)	Normal or low	55.85 (46.92-62.79)	—	55.85
55.1 (54.2-56.0)	7.5% CO ₂ in air	14.50	136.3	2.60	19.0	65.5 (59-74)	Normal or low	53.07 (47.20-58.94)	—	53.07
54.9 (54.0-55.7)	10% CO ₂ in air	16.60	128.5 (119-132)	2.60	19.0	49.5 (42-57)	Very low	48.62 (41.96-55.21)	—	48.62
B. Respiration in hypercarbic water with access to air										
53	7.5% CO ₂ in air	14.50	136.0 (135-137)	2.85	18.1	52 (51-53)	Normal or low	23.97 (19.97-27.96)	25.44 (24.45-26.42)	49.41
54.9 (54.0-55.7)	10% CO ₂ in air	16.60	128.5 (119-132)	6.0	17.67	47.5 (47-48)	Very low	28.56 (21.38-34.06)	23.08 (21.72-27.29)	51.64

In all cases the figures in parentheses indicate the range of measurements.

C_{CO_2} was 14.50 vols % and WP_{O_2} was 136 mmHg (Fig. 4). The total \dot{V}_{O_2} from hypercarbic water and air in this fish was 49.41 cc/kg/h (Table 2 B). In the previous two fish the rate of oxygen uptake from water was slightly higher than that from the air, but in the third individual a little more than 50 % of its oxygen was obtained from the air. In these cases C_{CO_2} of the air in the air chamber was 2.85 and 6.0 vols % (Table 2 B). Part of this carbon dioxide had been released by the fish into the air during its air-breathing and the remainder had diffused from the hypercarbic water in the air chamber. The mean oxygen content of the air in the air chamber was 17.67 and 18.10 vols % (129.4 and 134.4 mmHg).

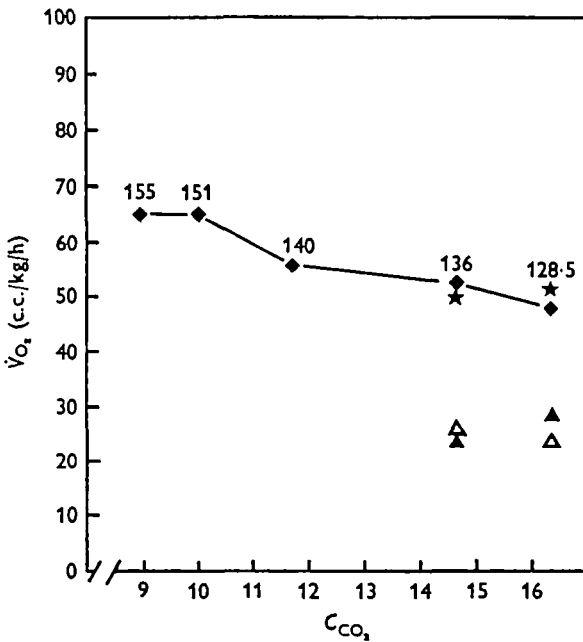


Fig. 4. Effect of increasing concentrations of carbon dioxide in water on \dot{V}_{O_2} of *Saccobranchus*. It exchanges gases with hypercarbic water alone (◆) until C_{CO_2} is raised to about 14.0 vols %. Above this level, and with WP_{O_2} of 128–136 mmHg, it breathes from both water and air following a short period breathing water alone at the start of the experiment. The amount of oxygen consumed from hypercarbic water (▲) and from air (△) is nearly equal. Only mean values for \dot{V}_{O_2} are plotted, and WP_{O_2} in each case is given above the line. The total \dot{V}_{O_2} from hypercarbic water and from air (★) is nearly the same as that from hypercarbic water alone (■) at similar WP_{O_2} levels.

The frequency of gill ventilation increased from 54 to 78 per min in hypercarbic water. In each individual the opercular frequency fell to 47–48/min once the fish had started to take air-breaths. The depth of breathing varied in individual fish but was slightly above normal in water having a C_{CO_2} of 10.5–11.7 vols %. Above this level of C_{CO_2} the gill ventilatory movements decreased in amplitude. The fish lost weight when it was subjected to highly hypercarbic waters. Thus the weight of one fish fell from 58.2 to 54 g during 1 week and the other from 59.6–55.7 g; the experiments being carried out at 1 to 2-day intervals on each fish. The third fish lost 1 g body weight during two such experiments.

\dot{V}_{O_2} from both water and air

When *Saccobranchus* is kept in a continuous flow of air-saturated water it does not normally take air-breaths or attempt to obtain air for periods of 6–12 h, and it shows no signs of stress during this period. The amplitude of gill ventilation is slightly higher than normal and the mean opercular frequency is 57/min. Thus six fish were kept in a continuous water-flow respirometer for periods of 6–12 h and only one of them (45.5 g) began air-breathing after 4–5 h of water-breathing. This fish showed similar behaviour when the experiment was repeated next day. Another fish (59.0 g) did not

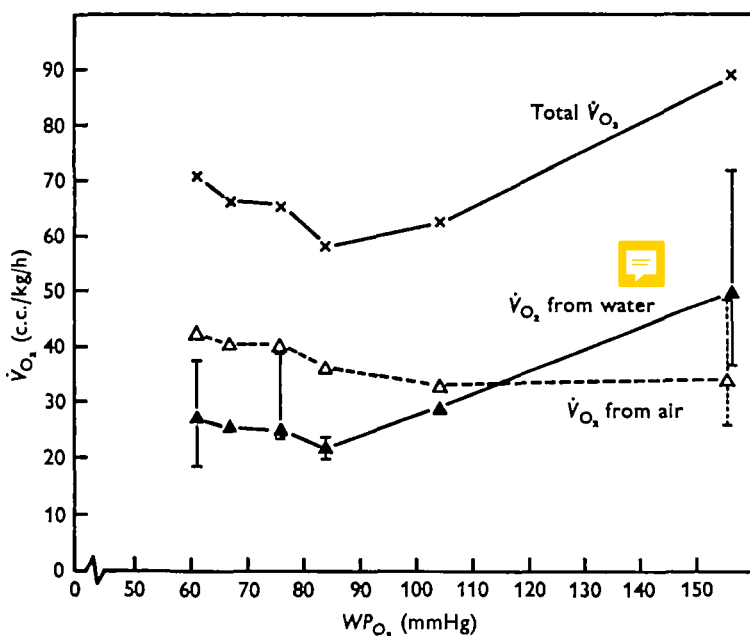


Fig. 5. Effect of lowering WP_{O_2} on the rates of oxygen consumption from water (\blacktriangle) and from air (\triangle) when the fish were allowed free access to air. In normoxic water ($WP_{O_2} = 156$ mmHg) it consumes more oxygen from water, but below 110 mmHg it consumes more from the air. In hypoxic water the total \dot{V}_{O_2} (\times) is reduced.

take any air-breaths on one day throughout about 7 h of observation in air-saturated water. The same fish started air-breathing next day after $4\frac{1}{2}$ h of gill ventilation in air-saturated water. But it discontinued air-breathing after about 1 h and in the later part of the experiment it exchanged oxygen and carbon dioxide with water alone.

The mean \dot{V}_{O_2} of the 45 g fish both from air-saturated water and from air was 84.55 cc/kg/h. This fish obtained more oxygen through the gills (mean 50.10 cc/kg/h) than through the air-breathing organs (34.45 cc/kg/h). \dot{V}_{O_2} from hypoxic water and from air is summarized in Table 1 and Fig. 5. Fig. 5 clearly suggests that *Saccobranchus* regulates its optimum level of \dot{V}_{O_2} in relation to P_{O_2} in water. It obtains more oxygen through the gills when the water is nearly air-saturated but nearly equal amounts enter through the gills and accessory organs when WP_{O_2} falls to about 100 mmHg. Below this level of WP_{O_2} a greater proportion of oxygen enters through the accessory

organs (Fig. 5). It should be noted that \dot{V}_{O_2} from air increases even though the P_{O_2} in the air chamber falls progressively.

\dot{V}_{O_2} from air only

\dot{V}_{O_2} of air-exposed fish. When *Saccobranchus* is exposed to air, its metabolic rate is reduced (Table 1 C). The mean \dot{V}_{O_2} is 54.5 cc/kg/h and the mean \dot{V}_{CO_2} is 31.78 cc/kg/h, hence the mean RQ is 0.58. It can be seen from Fig. 5 that \dot{V}_{O_2} from air is slightly

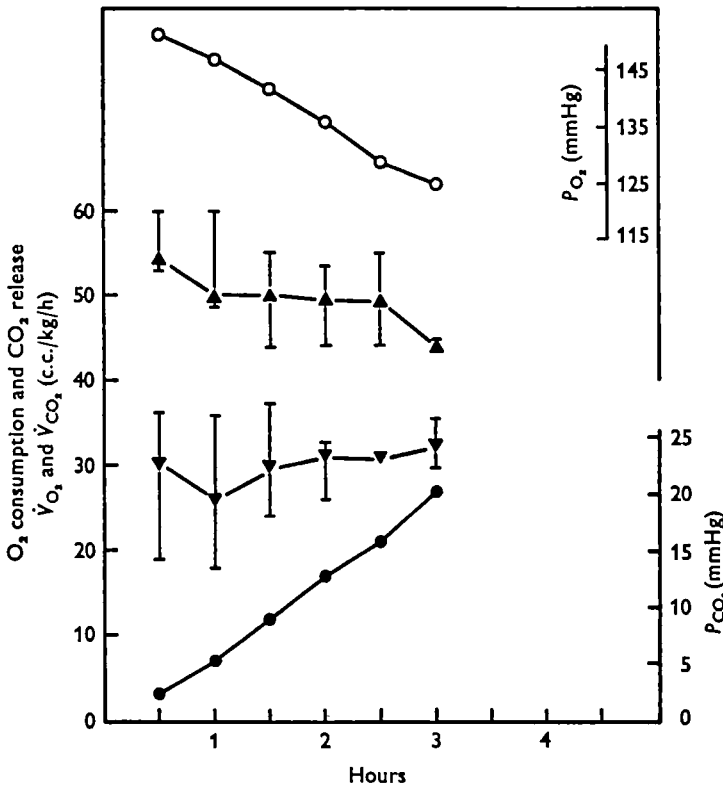


Fig. 6. Changes in \dot{V}_{O_2} (\blacktriangle) and \dot{V}_{CO_2} (\blacktriangledown) in fish exposed to air in a closed respirometer. The gradual decrease in P_{O_2} (\circ) and increase of P_{CO_2} (\bullet) in the respirometer air, as a result of breathing and re-breathing by the fish, is also shown.

reduced after about 1 h (30 min acclimation in air and 30 min after observation began) of air-exposure but it remains nearly constant when the air P_{O_2} is between 128 and 147 mmHg. A further reduction in \dot{V}_{O_2} occurs once more at P_{O_2} below 128 mmHg (Fig. 6).

The amount of carbon dioxide released is greater in the later part of the experiment so that the P_{CO_2} rises from 5 to 20 mmHg in a closed system but it does not appear to produce any marked change in \dot{V}_{O_2} until the air P_{CO_2} rises above 15 mmHg (Fig. 6).

\dot{V}_{O_2} from air only, the fish being in de-oxygenated water. When *Saccobranchus* is kept in de-oxygenated water and given free access to air, its oxygen consumption is very much reduced. The mean \dot{V}_{O_2} in this condition is 35.09 cc/kg/h. \dot{V}_{CO_2} is very low in air (13.1 cc/kg/h) but some carbon dioxide released into the air might have diffused into

the water. The RQ is apparently very low (0.38). It seems clear from Fig. 7 that when a fish is maintained in de-oxygenated water its \dot{V}_{O_2} is dependent on the P_{O_2} of the air.

When confined to de-oxygenated water *Saccobranchus* does not show any activity except air-breathing and occasional opercular movements for very short periods. Under such conditions the fish were always found waiting to take a breath from the air chamber of the respirometer.

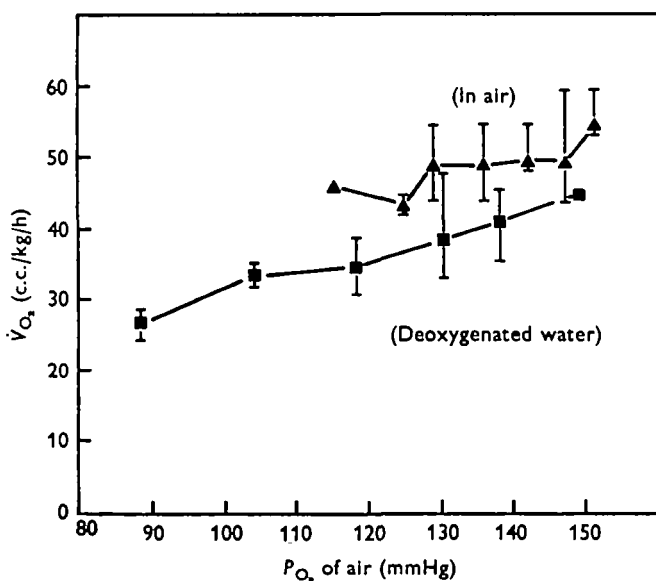


Fig. 7. A comparison of the effects of hypoxia on \dot{V}_{O_2} from air in fish exposed to air (▲) and in fish kept in de-oxygenated water but given free access to air (■). In de-oxygenated water the fish behaves like an oxygen conformer (dependent respiration), whereas during air exposure it shows a certain degree of independence.

DISCUSSION

This study has shown that the metabolic rate of *Saccobranchus fossilis* varies according to the experimental conditions, which were designed to simulate some of the seasonal conditions in tropical Asia. The total metabolic rate is reduced when the fish is exposed to air or submerged in water. Similar adjustments of total \dot{V}_{O_2} in different environmental conditions have been observed in *Anabas testudineus* (Hughes & Singh, 1970a) and *Clarias batrachus* (Singh & Hughes, 1971). However, it is apparent that *Saccobranchus* differs in some respects from *Anabas* and *Clarias*; for example, its total \dot{V}_{O_2} is low, and unlike the other two species, it takes up much more oxygen from water than from air (Table 3, Fig. 5). Furthermore, *Saccobranchus* can live in water with an oxygen tension as low as 50–70 mmHg and still exchange gases with water alone. In air-saturated water it shows little inclination for air-breathing whereas *Anabas* surfaces for air even when kept in air-saturated water (Hughes & Singh, 1970a, b). *Clarias batrachus* normally starts air-breathing once the WP_{O_2} is reduced to between 100 and 70 mmHg. As the metabolic rates are similar in

air-saturated water, this indicates that the resistance to oxygen transfer from water is less in *Saccobranchus* than in *Anabas* and *Clarias*.

Hughes & Munshi (1968) found that the water-to-blood distance for most of the gill of *Anabas testudineus* is 15–29 μm , but is thinner around the marginal channel of each secondary lamella (5–8 μm). The water/blood distance in *Clarias batrachus* is 8–16 μm whereas in *Saccobranchus fossilis* it is only 3–6 μm (Munshi & Singh, 1968). Moreover in *Clarias* only 52.47% of the vascular area of the secondary lamellae is effectively available for gaseous exchange, whereas in *Saccobranchus* it is about 66.6% (Munshi & Singh, 1968). It was suggested, therefore, that the *Saccobranchus* gill is more suitable for gas exchange than the gills of *Clarias* and other air-breathing fishes, such as *Channa striatus* and *Channa marulius* which are found in similar habitats. It has now been found that unlike the other species studied (Table 3), *Saccobranchus* obtains more of its total oxygen supply from water (59.17%) than from air (40.83%), a situation similar to that of *Amia calva*. Furthermore, *Saccobranchus* can obtain sufficient oxygen from water alone at oxygen tensions as low as 50–70 mmHg. Although *Anabas* and *Clarias* can survive for limited periods breathing air-saturated water alone, during which total \dot{V}_{O_2} is low (65–75 cc/kg/h), they cannot maintain this level for longer periods but become restless and attempt air-breathing. This greater ability of *Saccobranchus* to survive in water without surfacing is probably related to the thinner water/blood barrier.

Table 3. Proportion of oxygen consumption obtained from water (gill (G) and/or skin (S)) and from air in some air-breathing fishes

(Experimental conditions: water with access to air.)

Temp. (°C)	Species	% \dot{V}_{O_2}			Reference
		From water	(Organ)	From air	
20	<i>Amia calva</i>	65	(G)	35	Johansen <i>et al.</i> (1970)
25	<i>Saccobranchus fossilis</i>	59.2	(G, S)	40.8	Present authors
25	<i>Anabas testudineus</i>	46.4	(G)	53.6	Hughes & Singh (1970)
25	<i>Clarias batrachus</i>	41.6	(G, S)	58.4	Singh & Hughes (1971)
25–27	<i>Electrophorus electricus</i>	22	(S)	78	Farber & Rahn (1970)
20	<i>Protopterus aethiopicus</i>	11	(G)	89	Lenfant & Johansen (1968)
		10	(G)	90	McMahon (1970)

When exchanging gases with air-saturated water alone and without free access to air, both *Clarias* and *Saccobranchus* take up about 16% of their total \dot{V}_{O_2} via the skin and the rest through the gills. But in *Anabas* almost all of the oxygen enters via the gills when in water alone (Hughes & Singh, 1970a), the skin of this fish being scaly and thick. The functioning of the skin for aquatic gas exchange in *Clarias* and *Saccobranchus* appears to be very similar to that in the eel (*Anguilla vulgaris*), where about 10–15% of the oxygen also enters via the skin (Berg & Steen, 1965).

Effect of hypoxia

When *Saccobranchus* is kept in a continuous flow of hypoxic water the rate of oxygen consumption falls at a WP_{O_2} of about 110 mmHg, but the fish does not surface to breathe until WP_{O_2} is 50–100 mmHg. During this period the fish maintains a relatively constant level of oxygen consumption by augmenting the frequency and

Depth of gill ventilation (Fig. 2). However, one specimen of *Saccobranchus* did not attempt air-breathing even in water with an oxygen tension of 10 mmHg. *Clarias batrachus* also usually started air-breathing when the WP_{O_2} was 60–100 mmHg. Both species occupy similar habitats and normally exchange gases with both water and air over about the same range of WP_{O_2} , i.e. 50–100 mmHg.

A study of the effect of hypoxia in a closed system of water shows that, unlike *Anabas* and *Clarias*, *Saccobranchus* behaves like a water-breathing fish. Thus it consumes more oxygen as WP_{O_2} gradually decreases, but its normal total \dot{V}_{O_2} (92 cc/kg/h) is restored when WP_{O_2} is about 100 mmHg. (Fig. 3). *Saccobranchus* does not show a further reduction in total \dot{V}_{O_2} until WP_{O_2} falls to about 40 mmHg. But below this level the rate of oxygen consumption is greatly reduced and the effect of hypoxia is very clear. Marvin & Heath (1968) found that rainbow trout (*Salmo gairdneri*) and bluegill sunfish (*Lepomis macrochirus*) showed varying degrees of oxygen regulation in water down to 60–75 % saturation, below which \dot{V}_{O_2} was significantly reduced, but the brown bullhead catfish (*Ictalurus nebulosus*) showed dependent respiration. Thus it seems that *Saccobranchus* behaves more like rainbow trout and bluegill sunfish, whereas *Anabas* and *Clarias* resemble *Ictalurus nebulosus* and the toadfish (*Opsanus tau*) (Haschemeyer, 1969; Hall, 1929), which show dependent water respiration (oxygen conformers).

Tolerance of carbon dioxide

It is of great interest that *Saccobranchus* does not breathe air if maintained in nearly air-saturated water equilibrated with gas mixtures containing as much as 5 % carbon dioxide. Gas exchange with water continues even when C_{CO_2} is 14.5 vols %, but both water and air-breathing occur above this level. *Anabas* breathes both water and air in normal and hypercarbic water and air-breathing increased in water equilibrated with gas mixtures containing more than 2.5 % carbon dioxide (Hughes & Singh, 1970b). However, since *Saccobranchus* only begins air-breathing when about 7.5 % or more of carbon dioxide in air has been equilibrated with water, the carbon dioxide tolerance of this fish appears to be even higher than that of *Anabas*, although it was found that *Anabas* can tolerate very high carbon dioxide content compared with any other fish reported previously (Hughes & Singh, 1970b). It seems that above a certain level of blood P_{CO_2} , *Saccobranchus* starts breathing from both water and air and probably releases more carbon dioxide through the aerial route and thus prevents acidosis. The effect of carbon dioxide on the oxygen affinity of the blood of *Saccobranchus* has not yet been studied and hence we cannot say more in this context. However, a study of the blood of this fish and also of *Anabas* and *Clarius* is in progress and such studies should help elucidate the behaviour of these fish with regard to changes in carbon dioxide.

The rate of oxygen consumption of *Saccobranchus* in hypercarbic water is maintained at an optimal level (i.e. about 50 cc/kg/h) (Table 2, Fig. 4) and it exchanges gases with water alone. However, \dot{V}_{O_2} falls at certain levels of carbon dioxide and is associated with the initiation of air-breathing. Saunders (1962) found in water-breathing fishes (sucker, bullhead and carp) that \dot{V}_{O_2} is reduced if the water P_{CO_2} is raised by bubbling carbon dioxide gas mixtures. In *Saccobranchus* also, the rate of O_2 consumption is reduced if high levels of carbon dioxide (5–10 vols % in air) are equilibrated with the water. The rate and depth of breathing is increased when water is equilibrated

with low concentrations of carbon dioxide (i.e. 2.5–5 %) but the depth decreases in higher concentrations. These responses are similar to those found in *Anabas* (Hughes & Singh, 1970b).

Effect of air-exposure

It appears that a lowering of total \dot{V}_{O_2} following air-exposure is characteristic of several air-breathing teleosts (Berg & Steen, 1965; Hughes & Singh, 1970a; Singh & Hughes, 1971), but in other cases \dot{V}_{O_2} is not affected by air exposure e.g. the mud-skipper, *Periophthalmus sobrinus* (Gordon *et al.* 1969).

In *Saccobranchnus* it is also interesting to note that total \dot{V}_{O_2} is further reduced (54.50–35.09 cc/kg/h) when kept in de-oxygenated water with free access to still air. During such experiments P_{O_2} of the air soon fell because of re-breathing. The fish was observed with its head pointed towards the air-chamber as though waiting for an air-breath. The smaller \dot{V}_{O_2} of a fish in de-oxygenated water may be due to the absence of oxygen uptake through the skin. However, it must be remembered that a very low \dot{V}_{O_2} from air of fish in de-oxygenated water may also be due to a low P_{O_2} in the air chamber. The role of cutaneous respiration in *Saccobranchnus* becomes clearer when we study the effect of low P_{O_2} on air-exposed fish. From Fig. 7 it can be seen that \dot{V}_{O_2} in air-exposed fish is only slightly affected until P_{O_2} falls to about 128 mmHg but it is significantly reduced over the whole range with lowering of P_{O_2} in air when the fish is in de-oxygenated water. It suggests that in air-exposed fish at low P_{O_2} some oxygen may enter via the skin which helps the fish to maintain a relatively constant \dot{V}_{O_2} , a situation similar to that in *Clarias* (Singh & Hughes, 1971) and in *Anguilla* (Berg & Steen, 1965), but different from that in *Anabas*, which is more like an oxygen conformer (Hughes & Singh, 1970a).

Route of release of carbon dioxide

When *Anabas* breathes from both water and air, more carbon dioxide is released via the gills (RQ = 2.29) than via the air-breathing organs (RQ = 0.20) (Hughes & Singh, 1970a). In *Clarias* also, much more carbon dioxide is released into the water (RQ unknown) than into the air (RQ = 0.11) (Singh & Hughes, 1971). Farber & Rahn (1970) found a similar situation in the electric eel, *Electrophorus electricus*, the RQ into water being 3.65, whereas into air the RQ was 0.25. The overall RQ was about 1 in both *Electrophorus* and *Anabas*.

The experiments on gas exchange from water and air in *Saccobranchnus* were performed in a continuous flow of water and it was not possible to measure the small change in the carbon dioxide content of the water. However, if the overall RQ from both water and air is about 1, then it appears likely that much of the carbon dioxide is lost into the water, as deduced from the low gas-exchange ratio (0.17) with air. In animals exchanging gases with both water and air it has been suggested (Hughes, 1966) that much of the carbon dioxide may be lost through the gills and through the skin where this is an efficient gas-exchanging organ. It is possible that in *Saccobranchnus* some of the carbon dioxide is released into the water through the skin and the rest through the gills.

The amount of carbon dioxide released into the air during air-exposure is greater (31.78 cc/kg/h) than when the fish is kept in de-oxygenated water (13.10 cc/kg/h), the RQ being 0.58 and 0.38 respectively. However, it is possible that some of the carbon

Dioxide released into air might have diffused into the de-oxygenated water, and this might have resulted in a low RQ. In *Anabas* the RQ of air-exposed fish is about 0.71 (Hughes & Singh, 1970a), but in *Clarias* it is 0.52 (Singh & Hughes, 1971). The slightly higher RQ in air-exposed *Anabas* might result from the higher metabolic rate and activity of the fish; *Clarias* and *Saccobranchus* resemble each other very closely.

SUMMARY

1. Gas exchange of *Saccobranchus fossilis* with water and air has been studied under various experimental conditions which were designed to simulate some of the conditions of tropical Asia.

2. In tap water the fish exchanges gases with both water and air. When kept in air-saturated water it can exchange gases with water alone for periods of 6–12 h or even more. In de-oxygenated water, with free access to air, it obtains oxygen from the air and can live for several days under these conditions.

3. In air-saturated water more oxygen is obtained from water (60%) than from air (40%), but in hypoxic water this ratio is reversed.

4. When the fish is submerged in water, free access to air being prevented, the oxygen consumption is reduced, even in air-saturated water. In hypoxic and hypercarbic water oxygen consumption is further reduced. In air-saturated water about 17% of the oxygen enters via the skin and the rest via the gills. When exchanging gases with water alone and subjected to a gradual hypoxia, the fish shows a less dependent respirator.

5. When the fish is removed from the water its oxygen consumption is reduced. A greater reduction occurs when the fish is kept in de-oxygenated water but allowed to breathe air.

6. When the fish is exchanging gases with both water and air very little carbon dioxide is released into the air (RQ = 0.17). The total RQ in fish removed from the water is low, i.e. 0.58. The fish can survive in hypercarbic water only, provided that the content of carbon dioxide does not exceed 14.5 volumes %, when surfacing becomes necessary.

We wish to thank the Nuffield Foundation for their financial support. We are also grateful to the Smithsonian Institution, Washington, D.C., and especially to Dr. Stan Weitzman of Division of the Fishes, whose co-operation made this work possible.

REFERENCES

- BERG, T. & STEEN, J. B. (1965). Physiological mechanisms for aerial respiration in the eel. *Comp. Biochem. Physiol.* **15**, 469–84.
- FARBER, J. & RAHN, H. (1970). Gas exchange between air and water and the ventilation pattern in the electric eel. *Resp. Physiol.* **9**, 151–61.
- GAREY, W. F. & RAHN, H. (1970). Normal arterial gas tensions and pH and the breathing frequency of the electric eel. *Resp. Physiol.* **9**, 141–50.
- GORDON, M. S., BOËTIUS, I., EVANS, D. H., MCCARTHY, R. & OGLESBY, L. C. (1969). Aspects of the physiology of terrestrial life in amphibious fishes. I. The mudskipper *Periophthalmus sobrinus*. *J. exp. Biol.* **50**, 141–9.
- HALL, F. G. (1929). The influence of varying oxygen tensions upon the rate of oxygen consumption in marine fishes. *Am. J. Physiol.* **88**, 212–18.
- HASCHEMEYER, A. E. V. (1969). Oxygen consumption of temperature acclimated toadfish, *Opsanus tau*. *Biol. Bull. mar. biol. Lab., Woods Hole* **136**, 28–32.

- HUGHES, G. M. (1966). Evolution between air and water. *Ciba Foundation Symposium on the Development of the lung* (ed. A. V. S. de Reuck and R. Porter), pp. 64-80. London: Churchill.
- HUGHES, G. M. & MUNSHI, J. S. DATTA (1968). Fine structure of respiratory surfaces of an air-breathing fish, the climbing perch, *Anabas testudineus* (Bloch). *Nature, Lond.* **219**, 1382-4.
- HUGHES, G. M. & MUNSHI, J. S. DATTA (1972). Nature of the air-breathing organs of certain Indian fishes as shown by electron microscopy. (In preparation.)
- HUGHES, G. M. & SINGH, B. N. (1970a). Respiration in an air-breathing fish, the climbing perch *Anabas testudineus* (Bloch). I. Oxygen uptake and carbon dioxide release into air and water. *J. exp. Biol.* **53**, 265-80.
- HUGHES, G. M. & SINGH, B. N. (1970b). Respiration in an air-breathing fish, the climbing perch *Anabas testudineus*. II. Respiratory patterns, and the control of breathing. *J. exp. Biol.* **53**, 281-98.
- JESSE, M. J., SHUB, C. & FISHMAN, A. P. (1967). Lung and gill ventilation of the African lungfish. *Resp. Physiol.* **3**, 267-87.
- JOHANSEN, K. (1966). Air-breathing in teleosts, *Symbranchus marmoratus*. *Comp. Biochem. Physiol.* **18**, 383-95.
- JOHANSEN, K. & LENFANT, C. (1967). Respiratory function in the South American lungfish, *Lepidosiren paradoxa* (Fitz). *J. exp. Biol.* **46**, 205-18.
- JOHANSEN, K. & LENFANT, C. (1968). Respiration in the African lungfish *Protopterus aethiopicus*. II. Control of breathing. *J. exp. Biol.* **49**, 453-68.
- JOHANSEN, K., HANSON, D. & LENFANT, C. (1970). Respiration in a primitive air-breather *Amia calva*. *Resp. Physiol.* **9**, 162-74.
- LENFANT, C. & JOHANSEN, K. (1968). Respiration in an African lungfish *Protopterus aethiopicus*. Respiratory properties of blood and normal patterns of breathing and gas exchange. *J. exp. Biol.* **49**, 437-52.
- LENFANT, C., JOHANSEN, K. & GRIGG, C. (1966). Respiratory properties of blood and pattern of gas exchange in the lungfish *Neoceratodus forsteri* (Kreff). *Resp. Physiol.* **1**, 1-12.
- MARVIN, D. E. & HEATH, A. G. (1968). Cardiac and respiratory responses to gradual hypoxia in three ecologically distinct species of fresh-water fish. *Comp. Biochem. Physiol.* **27**, 349-55.
- MCMAHON, B. R. (1970). The relative efficiency of gaseous exchange across the lungs and gills of an African lungfish *Protopterus aethiopicus*. *J. exp. Biol.* **52**, 1-15.
- MUNSHI, J. S. DATTA (1962). On the accessory respiratory organs of *Heteropneustes fossilis*. *Proc. R. Soc. Edinb.* **B 68** 128-46.
- MUNSHI, J. S. DATTA & SINGH, B. N. (1968). A study of the gill-epithelium of certain fresh-water teleostean fishes with special reference to the air-breathing fishes. *Ind. J. Zool.* **9**, 91-107.
- 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**, 817-62.
- SINGH, B. N. & HUGHES, G. M. (1971). Respiration in an air-breathing catfish, *Clarias batrachus* (Linn.). *J. exp. Biol.* **55**, 421-34.
- VAN SLYKE, D. D. & NEILL, J. M. (1924). The determination of gases in blood and other solutions by vacuum extraction and manometric measurements. *J. biol. Chem.* **61**, 523-73.