RESPIRATION IN AN AIR-BREATHING FISH, THE CLIMBING PERCH *ANABAS TESTUDINEUS* BLOCH

I. OXYGEN UPTAKE AND CARBON DIOXIDE RELEASE INTO AIR AND WATER

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INTRODUCTION

Respiratory mechanisms of fish are adapted to a variety of environments. The adaptive features of those inhabiting water of low O_2 and high CO_2 content have been described (Carter, 1957), and include structural modifications which enable their possessors to breathe air. The three living dipnoan genera are of especial interest in this connexion because they are closest to the line of evolution from which the tetrapods are believed to have originated. Comprehensive studies of air-breathing fish, using modern techniques, have recently been made on these genera (Jesse, Shub & Fishman, 1967; Johansen & Lenfant, 1967, 1968; Lenfant, Johansen & Grigg, 1966; Lenfant & Johansen, 1968; McMahon, 1969), but currently more attention is being directed to the adaptations of teleosts that breathe air. Studies of these forms, while not so significant from the evolutionary standpoint, nevertheless are proving invaluable by indicating possible solutions to the basic physiological problems which arise during the transition from water to land (Hughes, 1966).

Many of these studies have shown that when a fish breathes air alone there is a fall in the total oxygen consumption; absorption of oxygen may take place through the skin, as in eels, but in this fish, as in the mudskipper, the role of the gills in oxygen uptake has been shown to be important on land. When out of water most fish show bradycardia, but the reverse was found in the Australian mudskipper (Garey, 1962). However, the South African mudskipper studied by Gordon *et al.* (1969) did not behave in this way and its low oxygen consumption remained constant when removed from water. The South American teleost, *Symbranchus*, also uses its gills for air-breathing (Johansen, 1966); elimination of CO_2 to the water being more important than to air under conditions where both media are available.

The climbing perch, *Anabas*, is another well-known air-breathing fish, which represents a whole group of so-called labyrinthine fish which come to the surface to obtain air, and pass it into a suprabranchial cavity that contains the characteristic labyrinthine organs. Gaseous exchange takes place in this cavity between the inhaled air and the blood that circulates through very fine sets of capillaries in the labyrinthine plates, and in the lining of the suprabranchial cavity. The labyrinthine plates were thought to be modified gills, but electron microscopic studies (Hughes & Munshi, 1968, 1970), showed that

this is not the case, since they do not contain the characteristic pillar cells as had previously been thought. The water-to-blood distances in these fish are comparatively great over most of the surface of the secondary lamellae (15–29 μm) but are much shorter (5–8 μm) in the marginal region (Hughes & Munshi, 1970). These distances contrast with the very short diffusion distance found in the air-breathing organs, which may be less than 0.3 μm. The area of the secondary lamellae in *Anabas* is relatively small (Saxena, 1962), and that of the labyrinthine plates and inner surface of the suprabranchial cavities amounts to about $\frac{1}{2}$–$\frac{1}{4}$ that of the secondary lamellae (Hughes, 1970).

The purpose of the present study is to investigate the relative roles of the gills and accessory organs in the respiration of this fish when the tensions of the respiratory gases were altered in the water and/or air and under different conditions of access to these two media. Experiments were restricted to ones not interfering with the fish.

The classical studies of Willmer (1934), on *Erythrinus*, indicated that the use of aerial or aquatic respiration was governed not only by the O_2 content but also by the CO_2 content of the air and water, and this led to his plotting the first O_2/CO_2 diagram. From a functional point of view, and in relation to the physical properties of the media, it has been suggested (Hughes, 1966) that the gills of *Anabas* might be more involved in the elimination of CO_2 than the accessory organs and the latter might be of greater importance from the point of view of oxygen uptake. It has become clear that the air-breathing organs are very effective in supplying the oxygen needs of the animal, and that in addition to this function the gills are particularly important for the release of carbon dioxide at least when the fish is in water.

MATERIALS AND METHODS

Living specimens were collected in India and transported by aeroplane in oxygen-charged Polythene bags containing water. Fishes were kept in still-water aquaria at Bristol and maintained at a temperature of $25 \pm 1°$ C. Unfortunately large specimens suffered heavy casualties during transportation and acclimatization so that the surviving individuals were of only small and average size (28–51 g) and hence study was restricted to this size range.

(1) Five specimens were used in studies of O_2 uptake and CO_2 release under conditions where the fish could breathe either air or water. Routine metabolism was measured with the fish in a closed Perspex box of 6 l capacity which was filled with air-saturated water (Fig. 1 a). The fish was first kept in the tank for 12–24 h and could only obtain air from a special air chamber. At the commencement of an experiment both inlet and outlets were closed. The water and air remained in direct contact and the fish were able to come freely to the surface to inhale air without being disturbed. The fish was therefore respiring in still water and in air, and was re-breathing the same water and air during the course of the experiment. Consequently there was a progressive fall in the P_{O_2} of both these media which were more or less in equilibrium with each other (Fig. 4). Each fish was used for at least two experiments. The fish remained in the closed respirometer for periods of 6–8 h during which samples of water and air were collected in syringes at intervals of 30 min to 2 h.

With nitrogen in the air chamber the fish could only obtain oxygen from the water,

and such experiments were continued for 3 h, during which time O_2 uptake through the accessory organs was prevented.

The P_{O_2} of the water samples was determined using a P_{O_2} macro-electrode and a Beckman 160 physiological gas analyser. Gas samples were analysed for O_2 and CO_2 using a Scholander 0·5 ml gas analyser and/or Beckman P_{O_2} electrode.

Total CO_2 content of the water in these studies was determined by means of a Van Slyke apparatus using the method of Van Slyke & Neill (1924). Determinations were carried out in duplicate and two readings from the same water sample agreed within about 0·3 ml/l.
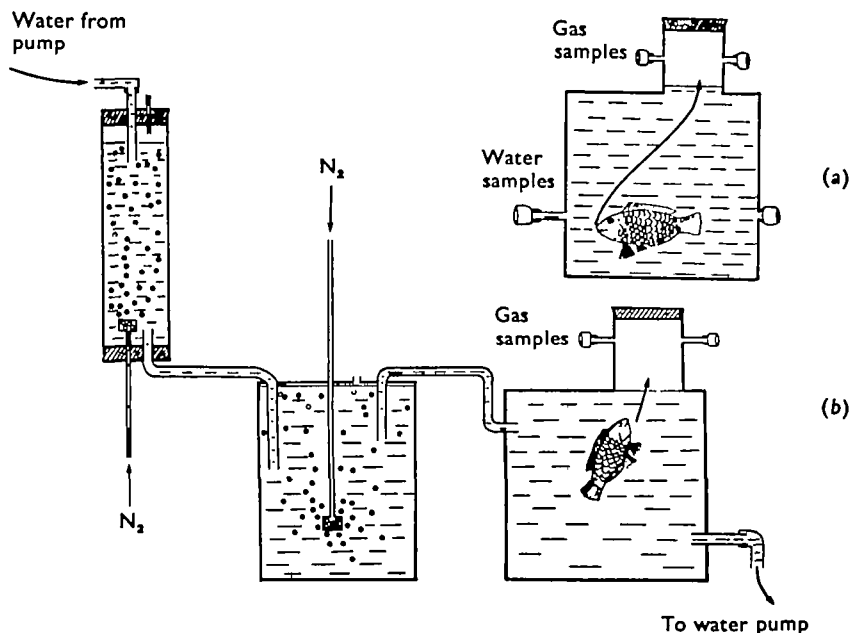

Fig. 1. Diagram of apparatus used for measurements of changes in P_{O_2} in water and air during respiration of *Anabas*. (a) Closed respirometer: air and water samples are taken from the rubber connectors as indicated. (b) Flow-through respirometer used to study respiration in de-oxygenated water.

Opercular frequencies were counted for periods of 2–4 min at half-hour intervals and the amplitude of their movements was estimated by visual observations only.

(2) O_2 consumption of two fish (29, 35 g) in a continuous flow of de-oxygenated water was determined. De-oxygenation was achieved by bubbling nitrogen through the exchange column (Fig. 1 b). The possibility of oxygen diffusing from the air into the water was relatively slight, so the fish was only able to obtain its oxygen from the air, and it remained swimming most of the time.

(3) Three specimens were kept in a Perspex respirometer of 340 ml capacity and could only breathe air. Their O_2 uptake and CO_2 liberation were followed over a period of 3–4 h. The fish was kept moist during these observations in order to avoid any effects of dehydration. No measurements were made until 30 min after the fish had been placed in the respirometer. The respirometer was immersed in a water bath and maintained at 25 °C. Air samples were collected at half-hour intervals and equal volumes of water injected into the respirometer. About 2 min before samples were

taken the respirometer was turned to one side and movement of the fish ensured that the air was mixed before sampling.

All experiments were carried out at 25 ± 0.5 °C. The fish was fed immediately the experiment was over. All the fish were intact, unrestrained and unanaesthetized, and were being subjected to gradually increasing environmental hypoxia by the use of closed respirometers.

(4) O_2 uptake from water was also studied in three specimens which were kept in a respirometer with a continuous flow of air-saturated water and so were not subjected to hypoxia. The fish were prevented from taking air breaths, and measurements were made while they remained in a resting condition.

RESULTS

1. Respiration in water with free access to air

Under these conditions the opercular movements of *Anabas* are intermittent. The mouth and operculum remain closed for relatively long periods but the fish often shows ventilation movements just before and after an air breath. Opercular frequencies varied in different specimens usually in the range 30–50/min. There was little change in amplitude during these experiments. *Anabas* usually surfaces for air at intervals of 3–15 min. but the intervals may be as short as 30 sec.

Results obtained for the routine O_2 consumption and CO_2 elimination by *Anabas* respiring in still water and in air are summarized in Table 1. The mean rate of O_2 consumption was 113·42 c.c./kg./h at 25 °C, but individual fish varied from 100·8 to 122·6. In most cases the fish obtains more oxygen from the air (mean = 53·6%) than from the water (46·4%: Table 1, Fig. 2). However, it was found that one specimen (34·5 g) obtained nearly equal volumes of oxygen from water and air. Another (51 g) normally took more oxygen from the air, but as the O_2 content of the air gradually fell to 3–10 vols. % ($P_{O_2} = 22$–73 mmHg) during the experiment, it was found that the O_2 uptake from the water exceeded that from the air. Such behaviour was not observed in the other specimens, and in these experiments the O_2 tension never fell below 100 mmHg, i.e. 14 vols. %. It can also be seen (Figs. 2, 3) that more oxygen is consumed when, at the beginning of the experiment, the P_{O_2} in both water and air is nearly 150 mmHg, but total O_2 uptake gradually falls to 90–100 c.c./kg/h when the P_{O_2} in the water falls to 100 mmHg. A levelling of the O_2 consumption from water is clearly shown in Fig. 2.

The O_2 uptake from the air gradually falls as the O_2 tension falls to about 100 mmHg (Fig. 5). There was a very rapid reduction in O_2 consumption from the air in the case of the 51 g specimen, and in this case the P_{O_2} of the air fell to 54–23 mmHg. It is of interest that this fish maintained a high rate of O_2 consumption even when the P_{O_2} was as low as 75–45 mmHg; the rapid decline only came below 40 mmHg.

Elimination of carbon dioxide. Table 1 shows that a greater amount of carbon dioxide is eliminated into the water through the gills than to the air via the accessory organs. The proportion is about 10:1, but it must be borne in mind that because of its high solubility in water some of the carbon dioxide eliminated to the air might have diffused into the water during the course of an experiment. The overall respiratory quotient of climbing perch respiring from water and from air is about 1 or greater.

Table 1. *Summary of gas exchange of Anabas testudineus under the five different experimental conditions*

(Figures in brackets give the ranges of the measurements)

Body weight (g.)	Experimental conditions	O_2 consumption (c.c./kg/hr)			CO_2 release (c.c./kg/hr)			Respiratory quotient (mean CO_2/mean O_2)		
		From water	From air	Total	To water	To air	Total	Water	Air	Total
40·2 (29–51)	In water with access to air	52·62 (45·44–61·88) 46·4%	60·80 (52·23–72·59) 53·6%	113·42 (100·82–122·58)	120·44 (99·66–136·84) 90·7%	12·26 (5·66–15·99) 9·3%	132·70	2·29	0·20	1·17
34·0 (28·0–39·5)	In air	—	104·78 (100·0–107·5) 100%	104·78	—	74·62 (71·90–76·01) 100%	74·62	—	0·71	0·71
32·0 (29–35)	In de-oxygenated water with access to air	—	132·83 (118·43–147·22)	132·83	113·66* 89·7%*	19·17 (17·04–21·29) 10·27%	132·83*	—	0·15	?
34·0 (28·0–39·5)	In water with access to nitrogen	127·48 (122·22–135·34)	?	127·48	137·56 (129·53–142·57)	—	137·56	1·08	—	1·08
36·0 (32–40)	In water without access to air	75·5 (70·7–85·0) 100%	—	75·5						

* Assuming overall RQ = 1.

2. Respiration when exposed to air alone

When *Anabas* is kept in a respirometer and allowed to breathe air only, the frequency of air-breathing movements was not very regular, but it was greater than the frequency of surfacing when the fish was in air-saturated water. The mean interval between air-breathing movements was 2–5 min in different specimens with a range from 5 sec to 7 min. Occasionally longer intervals between air breaths of 10–14 min were observed when gas samples had been collected from the respirometer chamber.
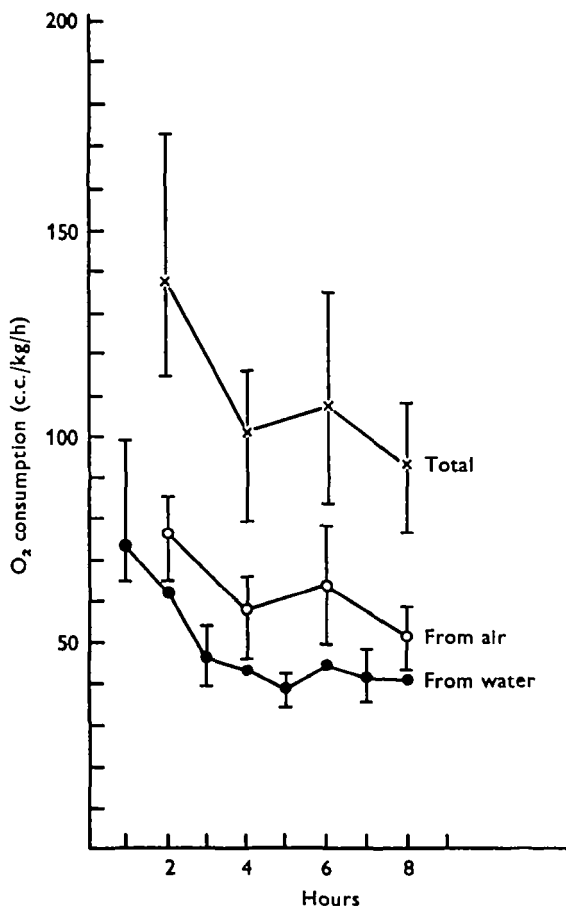

Fig. 2. Changes in O_2 consumption when the fish is maintained in a closed respirometer with free access to air. The portions of the O_2 consumption obtained from the air and from the water are plotted separately. Mean values and total range are plotted for each period after the closure of the respirometer in Figs. 2, 3, 4, 5, and 7.

The overall O_2 uptake of fish under these conditions was reduced. In three of the specimens exposed to air for a period of 3–4 h, the routine O_2 consumption varied between 100·1 and 107·5 c.c./kg/h as compared with routine O_2 consumption from water and from air of 116·2 and 122·5 c.c./kg/h (Table 2).

Measurements of the amount of carbon dioxide liberated during exposure to air indicate that relatively more carbon dioxide is released to the air than when the fish

is in water (Tables 1, 2). However, the total carbon dioxide eliminated into the air is less than that released into both air and water under the conditions of the previous experiment. The RQ of air-exposed fish is consequently lower and is about 0·7. Whether any of the CO_2 loss from these fish occurs from the gills has not been established.
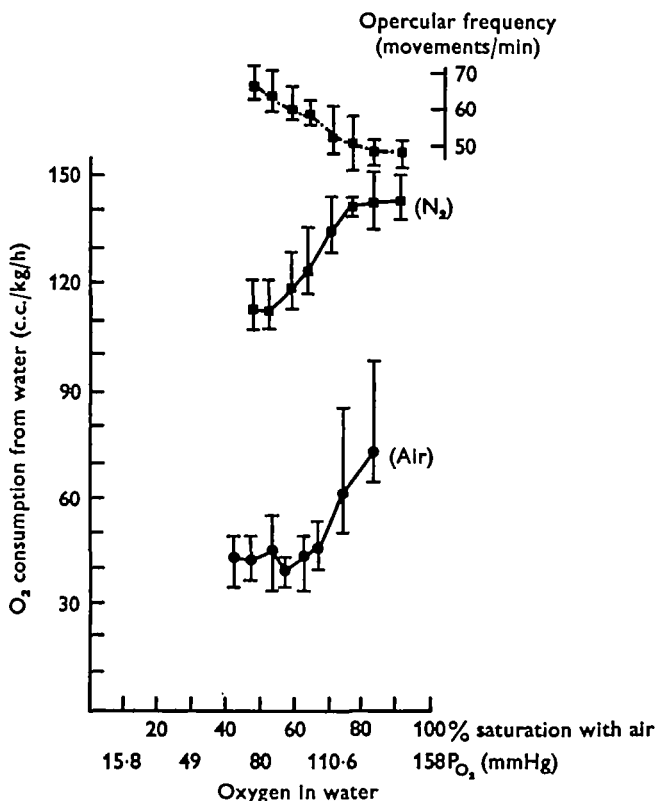

Fig. 3. Relationship between oxygen consumed from water and P_{O_2} in experiments in which the fish was allowed (a) free access to air (●) and (b) when the gas chamber contained nitrogen (■). The increase in opercular frequency during the latter experiment is also shown.

3. *Oxygen consumption from air when maintained in de-oxygenated water*

Under these conditions the fish tend to swim more actively and this no doubt affects their total O_2 uptake. It was certainly higher in two specimens (29, 35 g) as compared to the same specimens when exposed to air (Table 2). The O_2 uptake in these experiments remained higher than that from air alone as the P_{O_2} became lower (Fig. 5). Some diffusion of oxygen from the air into the de-oxygenated water must occur, but so far it has not been possible to determine the amount. Any transfer of this kind would tend to increase the values obtained for O_2 consumption (118·4–147·2 c.c./ kg/h).

Carbon dioxide eliminated through the accessory organs into the air is again very low. It was observed that for most of the time the mouth and opercula remained closed and only moved periodically at frequencies of 8–45/min and with very low amplitude. This pattern of respiratory behaviour suggests that under these conditions *Anabas* is

taking up oxygen mainly from the air and eliminating most of the carbon dioxide through the gills. It was not possible to determine the change in CO_2 content during the continuous flow of de-oxygenated water because it was very small.

The O_2 content of the de-oxygenated water varied from 0·1 to 0·28 c.c./l. One fish (29 g) was very active during the experiments but the other (35 g), although active at the beginning of the experiment, later came to rest at the bottom or top of the water chamber.
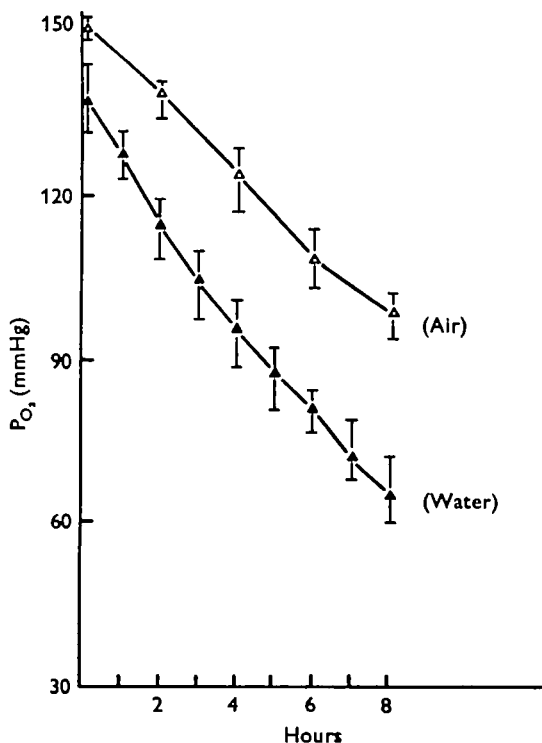

Fig. 4. Changes in P_{O_2} in the air and water breathed by the fish over a period of 8 h. The air and water are in contact with each other but are not completely in equilibrium. The fish in this experiment had free access to the air (as in Fig. 2).

4. Respiration from water alone with access to nitrogen

Under these conditions *Anabas* could only obtain oxygen from the water because the air chamber of the respirometer contained pure nitrogen. The metabolic rate of the fish remained normal or slightly elevated (Tables 1, 2). There appears to be no significant change in the overall O_2 uptake as compared with specimens which were allowed free access to the air. On the other hand, the O_2 uptake was greatly reduced when the P_{O_2} of the water fell below 100 mmHg (Fig. 3). *Anabas* shows continuous opercular movements when respiring under these conditions. Their frequency and amplitude increase as the P_{O_2} is lowered (Fig. 3). The minimum and maximum frequencies recorded in three specimens were very variable. The amplitude of the respiratory movements also increased very noticeably when the P_{O_2} fell below 110 mmHg.

It is of interest that the fish did not discontinue surfacing even though they were

Table 2. O_2 *consumption and* CO_2 *release in three specimens of Anabas testudineus studied individually under five different conditions*

(Mean values are given in each case.)

Weight of fish (g)		In water without access to air	In water with access to nitrogen	In water with access to air			In de-oxygenated water with access to air		In air
				Water	Air	Total	Water	Air	
29	O_2 consumption c.c./kg/h	85·05	135·34	47·01	72·60	119·76	—	147·22	106·73
—	CO_2 release c.c./kg/h	—	140·57	136·84	15·15	151·99	~	21·29	76·01
—	RQ	—	1·04	2·91	0·21	1·27	—	0·15	0·71
34·5	O_2 consumption c.c./kg/h	70·73	122·22	61·88	60·70	122·58	—	118·43	107·50
—	CO_2 release c.c./kg/h	—	142·57	128·31	13·21	141·52	~	17·04	71·90
—	RQ	—	1·17	2·07	0·22	1·15	—	0·14	0·69
39·5	O_2 consumption c.c./kg/h	70·70	124·32	53·15	63·02	116·17	—	—	100·10
—	CO_2 release c.c./kg/h	—	129·53	99·66	15·99	115·65	—	—	75·94
—	RQ	—	1·04	1·88	0·25	1·00	—	—	0·76

only able to take nitrogen into their suprabranchial chambers. These 'nitrogen breaths' occurred at intervals of 2–15 min. The pattern was quite different from normal breathing, as the fish often took 4–6 breaths repeatedly during a 2 min period. This tendency to take repeated breaths might well be due to the extremely low levels of oxygen in the 'air' chambers. The opercular movements did not stop or change in amplitude following the nitrogen breaths. The tendency to come to the surface became
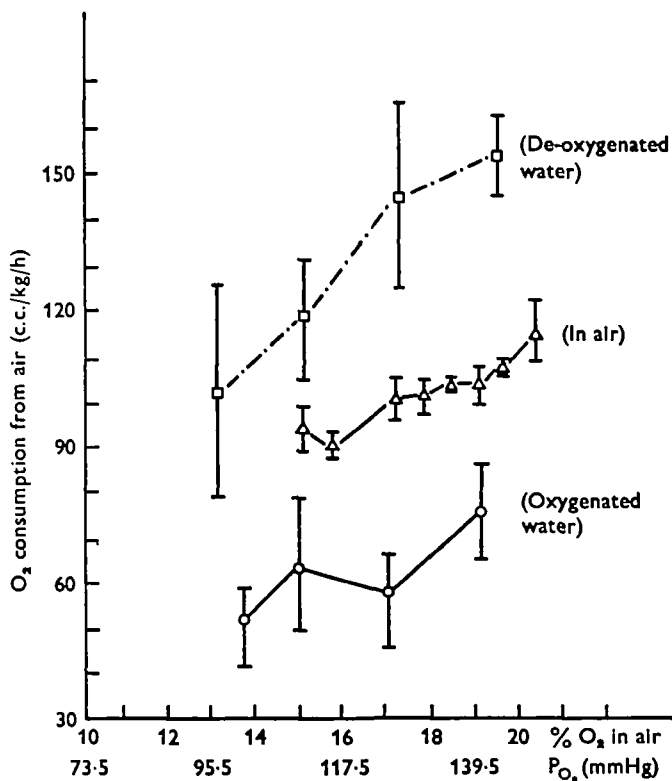

Fig. 5. Relationship between O_2 uptake from air and P_{O_2} of the air, in experiments in which the fish was maintained (a) in de-oxygenated water with access to air, (b) in air alone, or (c) in oxygenated water with free access to air. In all three cases there is a progressive fall in O_2 consumption with decline in P_{O_2}. The greater O_2 consumption of the fish maintained in de-oxygenated water relative to that in air alone is clearly shown.

more frequent with decreasing levels of oxygen in the water. When the O_2 content of the water was reduced below 60% saturation, a fish took 10–14 breaths of nitrogen within a 2–3 min period. But after these breaths it neither stopped its opercular movements nor reduced their amplitude.

5. Respiration from water in a continuous flow system with surfacing prevented

Under these conditions the fish could obtain oxygen from water only. They rest quietly in air-saturated water for comparatively longer periods, i.e. about 6–8 h. Air-breathing is rarely attempted once the fish has found that surfacing is prevented. Rhythmic opercular movements are continuous, slightly higher in frequency (40–46/min), but no significant change in amplitude has been observed.

The mean O_2 consumption was 75·5 c.c./kg/h (Tables 1, 2). If the experiment was continued beyond 6–8 h the fish began struggling movements, probably as an attempt to search for air. During this period the O_2 consumption rises slightly higher. One specimen, which started such a search for air after 6 h, showed a rise in O_2 consumption to 84·0 c.c./kg/h from the resting rate of 70·7 c.c./kg/h. This was also accompanied by an increase in opercular frequencies from 48 to 50/min.
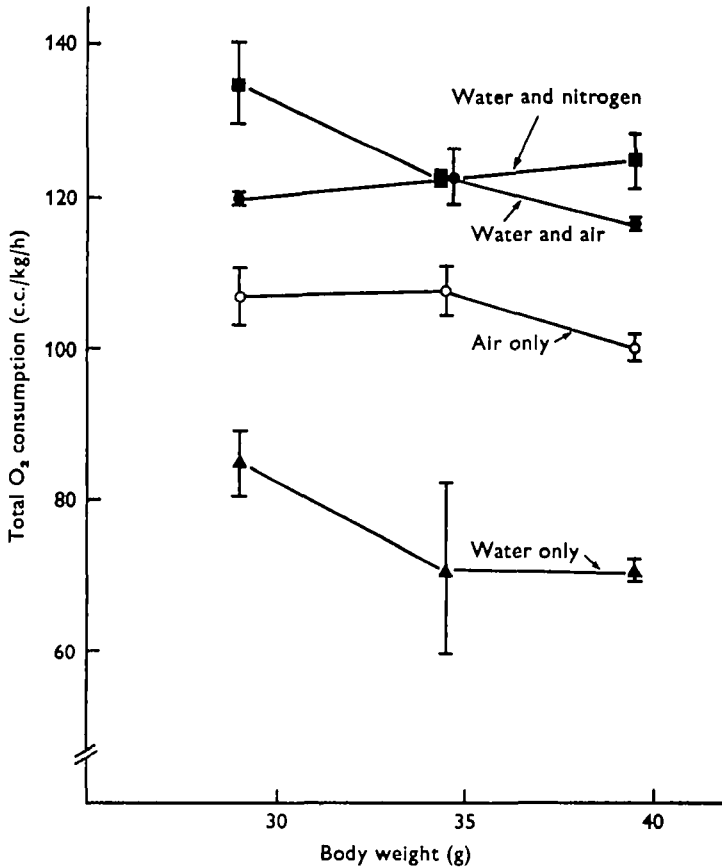

Fig. 6. O_2 consumption of three fish under four different experimental conditions. Mean figures are plotted for each fish together with ± two standard errors of the mean. The results indicate statistically significant differences in O_2 consumption under these conditions, except in the case of the 34·5 g fish when allowed access to either air or nitrogen.

DISCUSSION

1. *Levels of O_2 consumption under different conditions*

Table 1 summarizes the results and sets out the mean levels of O_2 uptake under the five different conditions that were investigated. The routine O_2 consumption when the fish was allowed free access to air was about 113 c.c./kg/h at 25 °C. Of the oxygen consumed, a little more than half (53·6%) was removed from the air on average, but in some individuals this proportion was greater, and in one case the fish used more oxygen from the water. As had been suggested (Hughes, 1966), the release of carbon dioxide to the water is far greater (90·7%) than into the air.

The levels of O_2 consumption under the other conditions are of the same order of magnitude, but the differences in mean levels are related to the particular experimental conditions (Fig. 6). The lowest O_2 consumption was found in fish which could only breathe water (mean: 75·5 c.c./kg/h). Such a level was only found if the fish was completely at rest in a continuous flow of air-saturated water and it was not maintained for longer than 6–8 h. A relatively low O_2 consumption was also found in fish which could only breathe air. It has been assumed that under these conditions all the oxygen entered through the accessory organs but there is a possibility that some entered via the gills. Release of carbon dioxide was reduced when the fish could only breathe air, and the overall RQ was about 0·7.
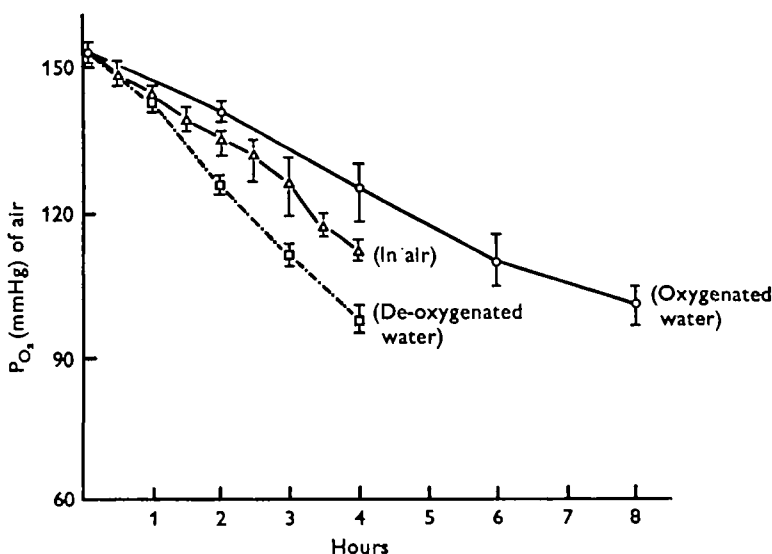

Fig. 7. Changes in P_{O_2} of the air in the respirometer chamber with time during respiration of a fish with free access to the air. The conditions of the fish are (a) in oxygenated water, (b) in air alone and (c) in de-oxygenated water.

The highest levels of O_2 consumption were found in experiments where the fish was maintained in a continuous flow of almost completely de-oxygenated water, so that oxygen could only be obtained from the air. These fish were much more active than in the other conditions, which probably accounts for their higher O_2 uptake (Fig. 7). There was also the danger of oxygen being lost to the water from the gills. For, although the fish responded by closing the opercular cavity, there must have been some replenishment of the water because presumably much carbon dioxide was lost by this route. Assuming an overall RQ of about 1, there would be about 89 % of carbon dioxide lost to the water which is very close to that in air-saturated water. It is possible that some modification of the circulation through the gills effectively reduces its importance for O_2 transport. The more rapid diffusion of carbon dioxide and its high solubility would ensure that CO_2 loss is not impeded to such a great extent. When the fish was in well-aerated water, the gaseous phase containing nitrogen only, the O_2 consumption was above that of a fish allowed access to air and greatly exceeded that of a fish confined to water alone. The increased uptake is presumably related to the greater activity, for

the fish continued to surface and to take nitrogen breaths. Although it is assumed that all this oxygen entered via the gills, it is conceivable that some oxygen diffused into the bubble of nitrogen contained in the suprabranchial chamber. If oxygen entered via this route, it would be analagous to the air gill of aquatic insects (Ege, 1915).

When *Anabas* is confined to still water, it rests quietly for about 1 h but then shows struggling movements. Apparently the fish is unable to obtain all its O_2 requirements from the water. However, the experiments with a continuous flow of air-saturated water showed that *Anabas* can breathe for relatively long periods without showing much sign of struggling even when prevented from surfacing. All three fish studied in this condition remained at rest, continuously pumping water over the gills for periods of 6–8 h. After such periods fish often shows signs of restlessness and an increase in opercular frequency. This is followed by vigorous movements, presumably attempts to take air breaths. One of the fish (40·0 g) showed restlessness after 6 h of water breathing which was followed by an increase in activity and opercular frequencies. The resting O_2 consumption rose from 70·7 to 84·0 c.c./kg/h. After being allowed to take air breaths the opercular frequency of this fish was reduced and the normal pattern of water and air breathing was restored after several air breaths. These experiments indicate that although *Anabas* can live in air-saturated water for several hours, breathing via gills only, it cannot maintain normal rates of resting O_2 consumption for longer periods.

One difficulty in interpreting the results of all except these latter experiments is inherent in method using a closed respirometer, for the O_2 tensions in the water and air phases are changing throughout the experiment (Figs. 4, 7). With an air-breathing fish, the situation is further complicated because of the possible diffusion of gases between air and water. Monitoring gas tensions in both phases throughout the experiment gave some indication of the changing conditions (Fig. 4).

2. *Comparison with metabolic levels of other fish at 25 °C*

Since studies on the routine and standard rates of O_2 consumption in most teleosts have been carried out at temperatures of 10–20 °C it is difficult to compare the values obtained for *Anabas* directly with those for other fish. However, the well-known increase in metabolic rate of animals with increasing temperature also applies to fish. Fry (1947) and Fry & Hart (1948) studied the rate of O_2 consumption of goldfish under active and standard conditions at various temperatures. Their value for O_2 consumption at 20 °C was about 120 c.c./kg/h which is very close to the overall value obtained here for *Anabas*. Marvin & Heath (1968) found that the O_2 consumption in three species of freshwater fish at 25° C was about 70 c.c./kg/h which is in good agreement with the mean value (75·5 c.c./kg/h) obtained for *Anabas* when respiring from air-saturated water only.

There are a number of cases where fish, when brought out of water, show a lowering of their O_2 consumption, as for example in the eel and the South American lungfish. Berg & Steen (1965) found nearly 50% reduction in O_2 consumption when the eel (*Anguilla vulgaris*) was exposed to air. Johansen & Lenfant (1967) also found a significant lowering of O_2 uptake in air-exposed South American lungfish (*Lepidosiren paradoxa*). However, Lenfant & Johansen (1968) did not find any reduction in overall O_2 uptake of *Protopterus aethiopicus* until O_2 and CO_2 tensions in air became 85 and

35 mmHg respectively. Gordon *et al.* (1969) found no significant change in the metabolic rates of air-exposed mudskippers (*Periophthalmus sobrinus*).We have also found significant reduction in overall O_2 uptake in air-exposed *Clarias batrachus* (unpublished). Thus it appears that the effect of air exposure varies a great deal in different air-breathing fishes. This variation might be related to the type of respiratory organs possessed by the fish.

3. *The effect of hypoxia on* Anabas

With fish under conditions where they could exchange gases with both water and air, the simultaneous decline in O_2 tension in both media were plotted (Fig. 4). Mixing of the water being inadequate, tensions in air exceeded those in the water. The changes in O_2 consumption from the air and from water follow similar general trends with a fairly rapid fall during the first 4 h (Fig. 2). It must be emphasized that this is not a settling-down period, for the fish had already been acclimated for at least 1 h to the experimental conditions. Furthermore the same result was obtained when the same fish was used under the same experimental procedure after it had been in the respirometer for a day or more. The fall in O_2 consumption is presumably related to the falling P_{O_2} in the media and suggests a dependent form of respiration. However, when the P_{O_2} reaches levels of about 100 mmHg the O_2 uptake appears to become less dependent. This is especially true of O_2 uptake from the water, for which the mean figures show a remarkably constant level (Fig. 3) in fish with free access to air. From air there is a continual fall in O_2 consumption over the range studied. The experiments were not continued to lower P_{O_2} except in one case in which oxygen consumption from air became far more dependent once more at P_{O_2} below about 60–70 mmHg.

The behaviour of *Anabas* in this respect is perhaps adapted to the P_{O_2} levels of its normal environment. The increased O_2 uptake at P_{O_2} above 100 mm is presumably due to an increase in the gradient across all the respiratory surfaces; similar rising levels of uptake are found in typical fish at P_{O_2} above 160 mm. (Hughes & Umezawa, 1968). It is as though in *Anabas* the whole curve relating O_2 uptake to environmental P_{O_2} has been shifted to the left.

In fish which were forced to obtain all their oxygen from the water, because the aerial phase contained nitrogen only, there was a decline in O_2 consumption with P_{O_2} over the whole range from 120 to about 60 mmHg (Fig. 3). This shows a more typical dependent type of respiration.

4. *Comparison with the effects of hypoxia on typical aquatic fish*

The effect of hypoxia on most fish is a slight increase in O_2 consumption as the ventilation rate increases before the respiration becomes dependent at a tension usually of about 60 mmHg. The results obtained in such experiments are affected by the rate at which the O_2 level in the water is lowered; the increased O_2 uptake associated with the augmented ventilation is found particularly in shorter-term experiments (Hughes & Saunders, 1970). Where the rate of lowering takes place over a period of up to 8 h, the rise in O_2 uptake is relatively slight (Marvin & Heath, 1968). In other fish, such as the toadfish, respiration is dependent over a whole range of P_{O_2} down to about 10 mm-Hg. It seems that *Anabas* when restricted to water-breathing is of this latter type, but given free access to the air it is similar to most fish in having a range of P_{O_2} (which are

those most usually met with in the environment) over which O_2 uptake from the water is independent of P_{O_2}.

Such a relationship is clearly advantageous to the fish and provides a homeostatic zone where its activity is not closely determined by the environmental gas tensions (Hughes, 1964).

SUMMARY

1. Respiration of the climbing perch *Anabas* has been studied under five different experimental conditions. (a) The mean O_2 consumption of a fish allowed free access to air, is about 113 c.c./kg/h at 25 °C. The fish obtain nearly equal amounts of oxygen through the gills and through the accessory organs. (b) The overall O_2 consumption from water of a fish allowed free access to nitrogen is nearly the same as during normal respiration from water with access to air. (c) The O_2 consumption is reduced when the fish is out of water and obtains all its oxygen from air. (d) The O_2 consumption from air increases considerably when the fish is maintained in de-oxygenated water and depends upon surfacing for its oxygen supply. (e) The O_2 consumption of a fish kept in aerated water and prevented from surfacing remains at a minimum level relative to the other four conditions.

2. Much more carbon dioxide is released through the gills than through the accessory organs (10:1) when the fish respires from aerated water with access to air. The accessory organs are much more important for O_2 uptake.

3. The respiratory quotient is approximately 1 when the fish is in aerated water, with or without access to air, but is only 0·7 when the fish is out of water.

4. *Anabas* can live out of water for 6–10 h if protected from dehydration. It continues to breathe quietly in air-saturated water using its gills alone for shorter periods (6–8 h) when denied free access to air.

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