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

II. RESPIRATORY PATTERNS AND THE CONTROL OF BREATHING

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INTRODUCTION

Some of the adaptations shown by air-breathing fish give suggestive indications of physiological changes that may have occurred during the transition of aquatic vertebrates to aerial respiration. The patterns and relative importance of water and air-breathing among these fish have been studied by a number of workers, including Willmer (1934), Berg & Steen (1966), Johansen, Lenfant & Grigg (1967), Johansen & Lenfant (1967, 1968) and McMahon (1969). These studies have indicated that the patterns of respiration shown by different species varies when changes are made in the concentrations of oxygen and carbon dioxide of the air and water relative to their normal concentrations. Whereas aquatic respiration is mainly sensitive to changes in oxygen (Hughes & Shelton, 1962), there are a number of air-breathing fish where sensitivity to carbon dioxide seems to play an increasingly important role in the control of water-breathing and air-breathing. In view of a general lack of knowledge about these control mechanisms, studies with a wider range of air-breathing forms might well prove of value.

The climbing perch inhabits water of low O_2 content in tropical countries; the O_2 content of this water becomes extremely low and the CO_2 content increases markedly, especially during the summer, but no measurements are available. Climbing perch have also been known to come out of water and spend some time in air if their pools dry up. During such excursions gas exchange occurs through the aerial respiratory organs contained in the suprabranchial cavities. When the fish is in water, exchange occurs both through the gills and through the accessory organs (Hughes & Singh, 1970).

The present paper describes the way in which the normal pattern of aerial and aquatic respiration is affected when the CO_2 and O_2 content of water and/or air are changed. This indicates the limits of hypercarbic and hypoxic water that such fish can withstand for certain periods of time.

MATERIALS AND METHODS

Living specimens were transported and kept in the laboratory as described previously (Hughes & Singh, 1970). Eight specimens were available for use in the present study; three of which were subjected to the complete series of experiments. These fish were kept in waters of known O_2 content and CO_2 content obtained by bubbling known gas mixtures of oxygen, carbon dioxide and nitrogen through the waters in an exchange column. Gas mixtures were obtained by use of gas-mixing pumps and stored in tyre inner tubes.

After about 1 h of bubbling the gas content of the water in the fish tank became

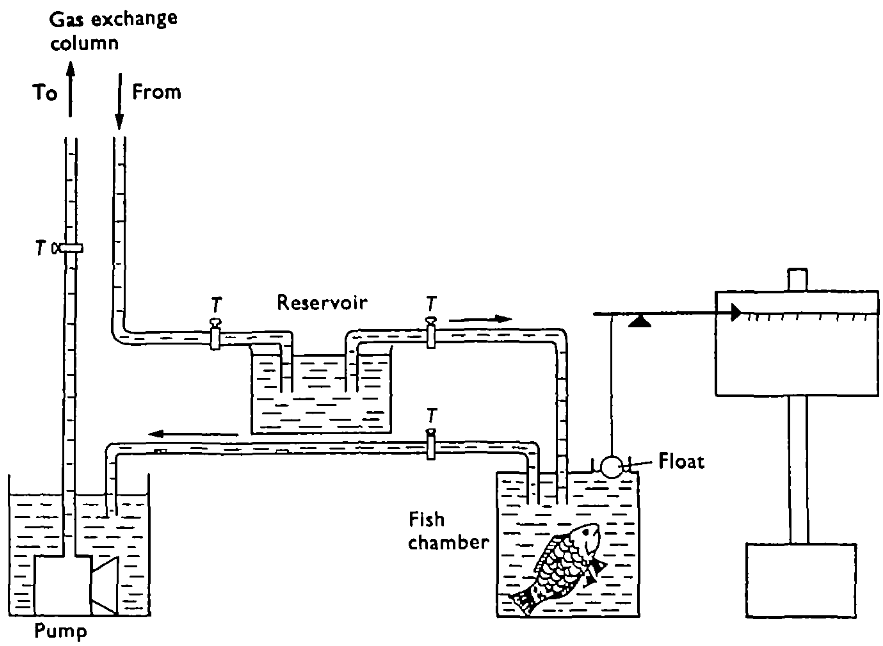

Fig. 1. Diagram to show the method used to record the air-breathing frequencies of *Anabas* when subjected to water of different O_2 and CO_2 content in a continuous flow system. The water was equilibrated with known gas mixtures in an exchange column. The flow of water was adjusted by means of the taps so as to maintain a constant water level in the fish chamber.

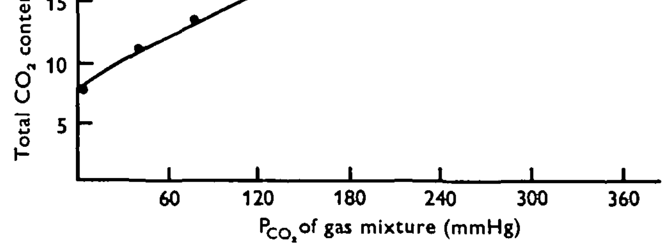

Fig. 2. CO_2 content of water in fish chamber (Fig. 1) following equilibration with gas mixtures containing 5–50% CO_2 with oxygen and nitrogen. Each point is the mean of at least six determinations. After equilibration some carbon dioxide was lost from the water in the reservoir tank before being circulated into the fish chamber.

constant, and a continuous flow of water was maintained between the fish tank and the reservoir tank (Fig. 1). Measurements showed that the O_2 and CO_2 content of water in the fish tank differed from that expected from the gas-mixture used (Fig. 2). The water levels were maintained constant by regulating the water flow of the outlet and inlet from the reservoir and fish tanks. The fish tank was always filled completely with water so that no air was trapped inside the cover. Thus the fish could only obtain air from the tube open to the air where it was forced to disturb a table tennis ball whose movements were registered by means of a lever and a kymograph drum. Time intervals between air breaths were measured from these recordings. Opercular frequencies were counted at $\frac{1}{2}$ h intervals and the depth of breathing was assessed by visual observation alone.

The fish was introduced into the tank 1 or 2 days before the start of an experiment. On the completion of an experiment the water was changed to normal tap water and the fish was kept in the tank until the start of another experiment on the following day. The fish was fed at intervals of 3 or 4 days. Most observations were made at a temperature of 25 °C ± 1 °C. Total period of observation in a given experiment ranged from 5 to 11 h.

Different types of water were obtained as follows:

Hyperoxic water (9–25 c.c. O_2/l) by bubbling mixtures of 40–90% oxygen in varying concentrations of nitrogen and carbon dioxide; sometimes pure 100% oxygen was bubbled in the exchange column.

'Air' saturated water (O_2 content 5–7·5 c.c./l) obtained by bubbling gas mixtures containing 20–32% oxygen in nitrogen and carbon dioxide.

Hypoxic water (O_2 content 0·68–2·5 c.c./l) was obtained by bubbling gas mixtures with nitrogen and varying concentrations of carbon dioxide, and also by bubbling pure nitrogen into water.

Hypercarbic water varied over a wide range of CO_2 content (13–33 vols. %). CO_2 content in the gas mixture used to obtain hypercarbic water varied from 5 to 50% in different combinations of gases containing oxygen and carbon dioxide, and mixtures of oxygen and carbon dioxide and nitrogen. Normal 'air-saturated' water had a CO_2 content of 8–10 vols. %.

O_2 content of the water was determined by titration using the Alsterberg (azide) modification of the Winkler method (Standard Methods: American Public Health Association, 1961). Total CO_2 content of the water was determined by methods following Van Slyke & Neill (1924). Free CO_2 content of the water was determined at 2–3 h intervals using 100 ml samples collected from the outflow and titrated against 0·02 N-NaOH using phenolphthalein as indicator. Titration was to an end-point at pH 3. The pH of the water was measured with a Pye pH meter model 290.

RESULTS

Different patterns of respiration, as indicated by the frequencies of air-breathing and opercular movements, are given for two specimens in Tables 2 and 3. Marked differences were observed when the same fish were kept in normal, hyperoxic, hypoxic and hypercarbic waters. Table 1 mainly summarizes changes in the interval between air breaths in relation to different concentrations of oxygen and carbon dioxide for another specimen. The maximum and minimum intervals given in these Tables indicate values obtained at least twice during the period of observation.

The effects of changes in the O_2 and CO_2 content of the water on the frequencies of gill ventilation and air-breathing are summarized in Fig. 3 which is based on the data given in Tables 1–3.

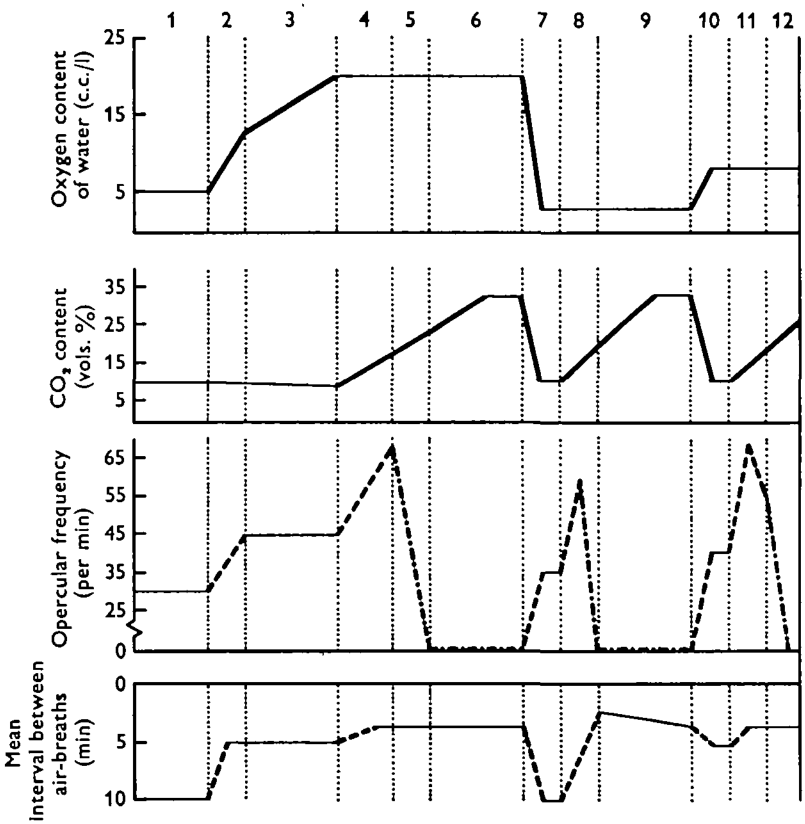

Fig. 3. Diagram to show the effect of changes (thick lines) in the O_2 and CO_2 content of water on the frequencies (dashed lines) of aquatic and aerial breathing in climbing perch. A typical pattern of respiration occurs in water containing 0·7–5 ml O_2/l when the CO_2 content of water is normal (Sections 1, 7). The opercular and air-breathing frequencies increased when the O_2 content of water was raised above 7 ml/l, the total CO_2 content was slightly reduced but free CO_2 was completely absent (Sections 2, 3, 10). The opercular and air-breathing frequencies increase further with a rise in CO_2 content (Sections 4, 8, 11). When the CO_2 content of water is between 18 and 22 vols. % (Sections 5, 8, 12), the fish shows a 'test water zone' (–·–·–) during which periods it shows irregular opercular movements. If the CO_2 content is above 22 vols. %, the mouth and opercula close tightly but aerial respiration takes place very frequently (Sections 6, 9, 12).

1. *Normal and hyperoxic water with normal CO_2 content*

In water containing 5–25 c.c. O_2/l and a total CO_2 content of 8–11 vols. %, *Anabas* uses both aquatic and aerial respiration. Gill ventilation is intermittent; the fish show opercular movements for certain periods but they then cease and re-appear later. The interval between periods of ventilatory movements is related to the frequency with which the fish takes air breaths. For example, if an air breath is taken after a period of about 35 min the fish will tend to show opercular movements continuously for more

Table 1. *Patterns of respiration shown by three climbing perch in ordinary tap water and in tap water following equilibration with different gas mixtures*

(The degree of dependence on air-breathing is shown as follows: +, normal air-breathing or water-breathing; + +, air-breathing predominant; + + +, only air-breathing.)

Weight of fish (g)	Water temp. (°C)	Gas mixture	O_2 content of water (c.c./l)	Free CO_2 content of water (mg/l)	pH of water	Type of respiration: Air	Type of respiration: Water	Interval between air breaths (min): Mean	Max.	Min.	Recording time: h	min
50.0	24	None	1·02	*a*	*a*	+	+	7·95	15·40	3·34	5	26
50·0	25	None	1·19	*a*	*a*	+	+	9·64	16·10	3·70	8	28
50·0	25	None	2·22	*a*	*a*	+	+	8·60	14·85	2·96	7	12
50·0	22	20% O_2+80% N_2	5·49	*a*	*a*	+	+	5·84	16·65	1·85	3	58
50·0	23	50% O_2+50% N_2	13·80	*a*	*a*	+	+	4·5	14·40	0·74	3	44
50·0	22	100% O_2	24·31	*a*	*a*	+	+	11·87	26·50	2·02	7	16
76·5	22·5	100% O_2	27·76	*a*	*a*	+	+	9·00	30·00	2·60	3	28
46·0	25	5% CO_2+95% O_2	20·71	17·82	7·27	+	+	7·7	21·0	1·72	4	31
46·0	25	10% CO_2+90% O_2	21·75	46·64	6·84	+	+	6·6	22·0	0·73	8	18
46·0	25·5	20% CO_2+80% O_2	18·00	77·0	6·36	+ +	+	4·9	12·7	0·59	8	52
46·0	25	50% CO_2+50% O_2	13·98	320·32	6·04	+ + +	*a*	2·0	5·8	0·30	7	1
46·0	23	5% CO_2+95% N_2	3·24	14·96	7·70	+	*a*	2·72	11·1	0·18	2	49
46·0	25	10% CO_2+90% N_2	3·07	30·8	7·25	+ +	+	4·39	9·2	1·72	7	12
46·0	25	20% CO_2+80% N_2	2·51	72·7	6·4	+ +	+	2·41	7·46	0·74	7	2
46·0	25·5	40% CO_2+60% N_2	2·56	232·0	6·2	+ + +	*a*	2·4	7·03	0·70	7	6
46·0	25	50% CO_2+50% N_2	1·88	322·08	5·88	+ + +	*a*	3·12	9·16	0·43	5	52

a = no measurement.

Table 2. *Patterns of respiration shown by a climbing perch (30 g) in water following equilibration with different gas mixtures*

(The degree of dependence of air-breathing is indicated as follows: +, normal water-breathing or air-breathing; + +, air-breathing predominant; + + +, only air-breathing.)

Gas mixture	O_2 content (c.c./l)	CO_2 content of water in fish chamber: Free CO_2 (mg/l)	Total (vols. %)	pH of water	Type of respiration: Air	Water	Opercular frequency /min	Interval between air breaths (min): Mean	Max.	Min.	Recording time: h	min
None	1·47	5·28	9·50	7·78	+	+	30	9·3	27·05	2·6	7	
None	4·87	0·88	9·64	8·07	+	+	31	12·9	18·5	8·0	3	34
10% O_2+90% N_2	3·55	0	9·34	*a*	+	+		4·9	18·9	0·68	5	29
20% O_2+80% N_2	5·57	0	9·29	*a*	+	+	38·5	3·72	10·6	1·03	8	23
40% O_2+60% N_2	9·37	0	8·78	*a*	+	+	46	4·8	13·06	2·28	5	28
50% O_2+50% N_2	10·09	0	10·37	*a*	+	+	45	2·68	8·03	0·55	7	56
100% O_2	22·25	0	10·90	*a*	+	+	47	2·8	9·1	0·46	7	17
5% CO_2+95% O_2	16·91	24·41	12·97	7·27	+	+	67	6·7	14·5	1·53	8	5
10% CO_2+90% O_2	21·5	44·0	14·01	6·84	+	+	72	3·4	10·4	0·46	4	49
20% CO_2+80% O_2	14·1	67·32	16·60	6·36	+ +	+	*	4·3	13·4	1·6	8	14
40% CO_2+60% O_2	14·27	114·4	24·13	6·15	+ +	+	*	5·0	12·1	1·5	7	3
50% CO_2+50% O_2	12·15	185·68	33·19	6·04	+ + +	−	0	4·5	11·3	1·7	8	10
5% CO_2+95% N_2	1·62	25·23	11·41	7·65	+	+	60	2·7	7·8	0·69	9	24
10% CO_2+90% N_2	1·75	47·08	12·71	7·20	+ +	+	*	1·7	4·2	0·64	10	41
20% CO_2+80% N_2	1·62	92·16	16·61	6·50	+ + +	−	0	1·6	3·8	0·41	10	16
40% CO_2+60% N_2	1·58	136·24	24·13	6·18	+ + +	−	0	2·2	6·4	0·36	11	18
50% CO_2+50% N_2	1·15	233·80	32·44	6·01	+ + +	−	0	3·1	11·4	0·51	10	19
10% CO_2+31·5% O_2 +58·5% N_2	7·56	51·21	13·55	6·85	+	+	54	3·1	10·6	0·73	6	30
20% CO_2+32% O_2 +48% N_2	7·42	72·10	15·74	6·43	+ +	+	56	5·8	10·75	1·27	6	18
30% CO_2+31·5% O_2 +38·5% N_2	7·45	136·40	21·94	6·11	+ + +	−	0	3·4	9·3	0·58	7	53
40% CO_2+30% O_2 +30% N_2	7·07	146·36	25·73	5·89	+ + +	−	0	3·3	10·3	0·58	6	0

* Occasional opercular movements.

a = no measurement.

Table 3. *Patterns of respiration shown by a climbing perch (mean weight 22 g) in tap water, and tap water following equilibration with different gas mixtures*

(The degree of dependence on air-breathing is shown as follows: +, normal water-breathing or air-breathing; + +, air-breathing predominates; + + +, only air-breathing.)

Gas mixtures	O_2 content of water (c.c./l)	CO_2 content of water in fish chamber: Free CO_2 (mg/l)	Total (vols. %)	Type of respiration: Air	Water	Opercular frequency/min	Interval between air breaths (min): Mean	Max.	Min.	Recording time: h	min
None	3·76	6·6	*a*	+	+	29	14·1	17·6	6·6	6	8
None	5·68	3·08	8·30	+	+	29·5	12·2	25·3	2·9	7	33
None	3·29	7·7	9·60	+	+	30	15·8	34·7	4·5	7	44
None	0·69	11·44	10·12	+	+	32·5	11·6	35·0	1·3	2	31
None	4·15	5·94	10·66	+	+	31	9·8	19·6	4·2	6	53
5% O_2+95% N_2	2·65	0	9·10	+	+	35	5·2	11·4	1·12	9	56
10% O_2+90% N_2	4·48	0	8·58	+	+	41·5	5·2	14·9	1·18	7	39
20% O_2+80% N_2	6·40	0	8·77	+	+	41	4·2	10·8	1·05	6	34
40% O_2+60% N_2	10·01	0	8·58	+	+	39	7·4	21·4	1·62	8	0
50% O_2+50% N_2	10·6	0	9·34	+	+	40	4·9	13·8	0·55	11	19
100% O_2	24·85	0	8 58	+	+	45	2·3	8·6	0·62	8	35
5% CO_2+95% O_2	19·00	13·64	10·79	+	+	54	5·1	19·1	0·63	7	29
10% CO_2+90% O_2	20·70	43·5	13·78	+	+	49	3·7	10·1	0·75	11	2
20% CO_2+80% O_2	20·15	92·18	17·40	+ +	–	*	2·3	10·5	0·22	10	20
40% CO_2+60% O_2	16·40	199·52	29·95	+ + +	–	0	2·8	8·7	0·27	10	1
50% CO_2+50% O_2	14·16	217·9	30·12	+ + +	–	0	3·3	8·3	0·24	7	15
5% CO_2+95% N_2	3·28	21·34	10·93	+	+	53	3·96	9·7	0·43	10	56
10% CO_2+90% N_2	2·18	42·24	13·58	+	+	57	1·9	6·45	0·22	9	3
20% CO_2+80% N_2	2·14	75·68	17·76	+ + +	–	*	3·75	9·0	0·25	8	48
40% CO_2+60% N_2	1·80	177·54	30·24	+ + +	–	0	3·8	8·1	0·18	9	34
50% CO_2+50% N_2	1·92	239·8	32·68	+ + +	–	0	2·9	6·3	0·18	7	0
5% CO_2+33% O_2+62% N_2	7·7	23·22	10·75	+	+	62	3·6	12·6	0·27	8	59
10% CO_2+31·5% O_2+58·5% N_2	7·77	45·10	12·13	+	+	55	2·4	12·1	0·27	8	0
20% CO_2+32% O_2+48% N_2	7·42	72·10	15·74	+ +	+	50	1·59	7·7	0·18	7	0
30% CO_2+31·5% O_2+38·5% N_2	7·45	136·90	21·94	+ + +	–	0	1·89	8·46	0·18	6	55
40% CO_2+30% O_2+30% N_2	7·07	146·36	25·73	+ + +	–	0	2·5	11·5	0·18	4	57

* Occasional opercular movements.

a = no measurement.

than 30 min. However, if the interval between air breaths is much shorter, then the period of regular opercular movements also occurs for shorter intervals during this period. The ventilatory movements occur for a few minutes prior to an air breath and for a variable period after the fish has been to the surface.

The mean time interval between air breaths in such waters varies between 2·5 and 15 min. The maximum and minimum intervals recorded in any given experimental series ranged from 8 to 35 min and from 30 sec to 8 min respectively (Tables 1–3). Opercular frequencies vary between 29/min and 47/min, the highest frequencies generally occurring in hyperoxic water. As far as can be judged from direct visual observation, the depth of breathing does not change appreciably, but under certain circumstances the opercular movements are difficult to make out because of their low amplitude.

2. *Hyperoxic and hypercarbic water*

The O_2 content of these waters varied between 12 and 20·5 c.c./l and the total CO_2 content was between 12 and 33 vols. %.

The climbing perch shows three different patterns of respiration in hyperoxic and hypercarbic water depending on the concentrations of oxygen and carbon dioxide. At high levels of oxygen and a total CO_2 content of up to 16 vols. % the fish shows both aquatic and aerial respiration. At CO_2 contents above about 16 vols. % aerial respiration becomes predominant and gill ventilation is reduced. When the CO_2 content exceeds 20 vols. % only aerial respiration occurs (Tables 1–3; Figs. 3, 5). Cessation of aquatic respiration occurs even though the O_2 content in the water is as high as 15 ml/l. Under these circumstances the mouth and opercula remain closed even when the fish swims to the surface for air. The mean time interval between air breaths in this type of water varied from 2 to 7·5 min with a maximum in the range 6–22 min. The minimum intervals recorded are more markedly reduced and ranged from 15 to 100 sec. The frequency of the opercular movements increase to as high as 72/min in waters where aquatic respiration continues with frequent visits to the surface. The amplitude of the movements is either normal or slightly increased.

Air-breathing becomes predominant and the opercular movements finally stop as the total CO_2 content of the water rises above 20 vols. % (Fig. 3). After the fish has stopped aquatic respiration and the mouth and opercula are closed, intermittent movements of very low amplitude are sometimes observed. These may be 10–15 opercular movements during a very short period (a few seconds only) and such periods of breathing are separated by very long intervals during which the opercula remain tightly closed. Such behaviour might enable the fish to 'test' the suitability of the water for gill ventilation.

3. *Hypoxic and hypercarbic water*

In this water the O_2 content was only 0·68–3·3 c.c./l and the total CO_2 content was 11–33·6 vols. %. Both aquatic and aerial respiration occur in such hypoxic waters so long as the CO_2 content only rises a small amount (up to 14 vols. %). Above this level aerial respiration predominates and ventilation of the gills is reduced. The fish switches to a fully aerial mode of respiration when the CO_2 content exceeds about 16 vols. % (Tables 2, 3; Figs. 3, 5). The mean time interval between air breaths varies between 1·6 and 4·5 min. The minimum time interval ranging from 15 to 80 sec.

Opercular frequencies are very high (53–60/min) in this hypoxic and hypercarbic water, but at higher CO_2 contents (above 13 vols. %) the opercular movements slowly reduce in frequency and/or amplitude as the fish comes to depend more upon aerial respiration. Opercular movements finally stop at CO_2 contents above 16 vols. %.

4. *Fully oxygenated water with different concentrations of carbon dioxide*

The climbing perch shows both aquatic and aerial respiration as long as the total CO_2 content remains below 15 vols. %. Opercular frequencies vary from 50/min to 62/min. However, when the CO_2 content is in excess of 18 vols. %, gas exchange mainly seems to take place with the air, and gill ventilatory movements occur at long intervals. The rhythm of the opercular movements becomes irregular and aerial respiration becomes predominant (Tables 2, 3; Fig. 3). This dependence upon surfacing becomes very clear at CO_2 contents of 20 vols. % and finally at 25 vols. % CO_2 in the water the fish becomes completely dependent upon aerial respiration.

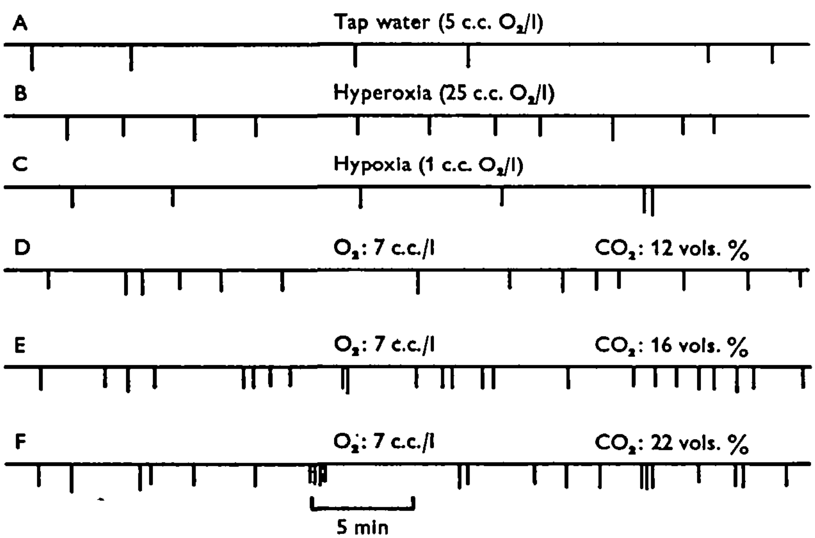

Fig. 4. *Anabas testudineus*. Tracings from kymograph recordings showing air-breathing excursions in normal tap water and following equilibration with different gas mixtures. (A) Normal tap water; (B) water equilibrated with 100% O_2; (C) tap water with low O_2 content. The total CO_2 content was at the normal level of about 9–10 vols. % in (A), (B) and (C), but free CO_2 was absent in (B). (D, E, F) Water equilibrated with gas mixtures of (D) 10% CO_2 + 31·5% O_2 + 58·5% N_2; (E) 20% CO_2 + 32% O_2 + 48% N_2; (F) 30% CO_2 + 31·5% CO_2 + 38·5% N_2. The first three recordings show the increase in frequency of air-breathing when the O_2 content is above normal. Recordings (D–F) show the predominance of air-breathing at high levels of CO_2 content and the tendency for repeated air-breaths. Measurements of the O_2 and CO_2 content of water from the fish chamber are given.

The mean time intervals between air breaths varied from 2 to 6 min with maximum recorded interval of 7·5–13 min. The minimum time intervals varied from 20 to 60 sec (Tables 2, 3). It is clear from these results that the mean and minimum intervals between air breaths gradually reduced with increasing CO_2 content of the water, even when the water was saturated with oxygen (Fig. 4D, E & F). However, the maximum intervals during the period of these observations does not show any regular change.

The relationship between the pH of the water and the pattern of respiration shown by the climbing perch can be seen in Fig. 6. The fish carries out both aquatic and aerial respiration in the pH range 8·07–6·85. It shows a predominance of aerial respiration and depressed aquatic respiration (intermediate conditions) at pH of 6·5–6·25. The change to fully aerial respiration occurs when the pH is below 6·25.

5. *Respiration in air-exposed climbing perch*

When exposed to air these fish breathe quietly for about 3–4 h. To begin with they show some restlessness but after about 10 min these movements subside. Air-breathing is not very regular and usually takes place at intervals of between 2 and 8 min. Intervals as short as 5 sec and longer than 15 min were also observed. The frequency of the air-breathing movements generally increases when the air-exposed fish becomes more active.

When such a fish is returned to oxygenated water, it starts gill ventilation movements with a frequency of about 30–40/min. The amplitude of the movements is lower and their rhythm is not very regular. The fish does not come to the surface for air until at least 15 min after its return to the water. The regular aquatic and aerial breathing patterns become re-established about 15 min later.

6. *Respiration of* Anabas *when aerial phase contains nitrogen or hypoxic air* (O_2 content less than 1 vol. %)

It has been observed previously that climbing perch exchanging gases with water but allowed to come up to a chamber containing nitrogen or hypoxic gas continues to show both aquatic and aerial respiratory movements. It is particularly interesting that the fish continues its repeated visits to the surface even after taking the nitrogen breaths (Hughes & Singh, 1970). Under these conditions the fish shows continuous and regular ventilatory movements and the opercular frequency rises from 30 to 70/min as the O_2 content of the water falls. The amplitude of the movements also increases. With the water only 50% saturated the breathing frequencies were as high as 60–70/min and their amplitude very significantly increased.

When a fish, which had been respiring in water nearly 50% saturated with oxygen, and taking nitrogen breaths every 5–10 min, was allowed to take a breath after the nitrogen had been replaced by fresh air, the first changes to be seen were in the pattern of the gill ventilatory movements and especially a reduction in amplitude to nearly normal levels. After a fish had taken about four to eight breaths of fresh air, the opercular frequency fell from 60 to about 50/min. After further air breaths the opercular movements stop for a period before the normal pattern of water-breathing and air-breathing becomes re-established.

When a climbing perch in oxygenated water surfaces for a breath of nitrogen or hypoxic gas, it often repeats the movements two to six times in rapid succession in about 2 min. The opercular movements never stop after breaths of nitrogen or of hypoxic air. The frequency of air-breathing increases as the O_2 tension in the water falls. In water of P_{O_2} below 90 mmHg the fish takes repeated air breaths (10–14) at very short intervals (10–40 sec) within 2–3 min periods.

As reported earlier (Hughes & Singh, 1970), a fish submerged in air-saturated water and not allowed to come into contact with air often swims towards the surface as though in search of air. The fish becomes excited and often makes quite vigorous efforts (over a period of 7 h). In nearly deoxygenated water (0·20–0·30 c.c. O_2/l) the fish shows only aerial respiration. However, under these conditions the mouth and opercula are not closed tightly as they are when the CO_2 content of the water is high. The fish shows occasional opercular movements of very low amplitude. They occupy brief periods of a few sec to 1 min during which 8–45 opercular movements are observed. It seems possible that in de-oxygenated water these movements may serve to eliminate carbon dioxide from the gill chambers and to sample water whose suitability for ventilation can be tested.

DISCUSSION

From this study it is clear that the degree to which climbing perch depend upon air and water for their oxygen supply varies according to the O_2 and CO_2 content of the water. The gills are inadequate to supply the respiratory needs entirely from the water under any conditions, and the fish must surface for air breaths. Intervals between successive air breaths are irregular, and the mean, maximum and minimum values vary a great deal, even under a particular set of conditions during a given period of observation. Ventilation of the gills is not continuous and aquatic breathing is interrupted at varying intervals as the fish surfaces for air. Thus long intervals between air breaths are associated with long periods of water breathing, and short intervals with short periods of water breathing. Differences in interval between successive air breaths appear to be related to the frequency and amplitude of the ventilation movements. In all specimens examined in air-saturated water, the ventilatory frequency was fairly constant (30–32/min) prior to and immediately following an air breath.

It is concluded that the pattern and type of respiration shown by *Anabas* is controlled by three main factors, CO_2 content, pH, and O_2 content of the water (Figs. 5, 6). Of these, carbon dioxide seems to be the most important in determining the type of respiration. Fig. 5 shows that both aquatic and aerial respiration is used in water with an O_2 content between 0·68 and 25 ml/l (equivalent to P_{O_2} of 18·93–675 mmHg) provided that the CO_2 content of the water is within the range 8–15 vols. %. If the CO_2 content of the water increases above this level, aerial respiration becomes more common. Changes produced in the pattern of respiration vary with respect to both the CO_2 and O_2 content of the water. Figs. 5 and 7 show that when the O_2 content is high (above 7 ml/l), aquatic ventilation is found together with more frequent air breathing until the CO_2 content reaches levels of about 18 vols. %. Above this level e.g. at about 22 vols. %, *Anabas* shows occasional opercular movements of short duration until finally at still higher CO_2 contents the opercula and mouth remain closed. However, when the O_2 content of the water is low (1·5–2·14 ml/l; P_{O_2} 31·17–57·85 mmHg) the higher frequencies of air-breathing occur at a relatively lower level of carbon dioxide (15 vols. % as compared to 18 vols. %) and ceases completely when the CO_2 content is only 17 vols. %, as against 22 vols. % under the previous conditions (Figs. 5, 7). These findings indicate that when the O_2 content of the water is high, some exchange of oxygen and carbon dioxide occurs through the gills even when the CO_2 content is high, but *Anabas* does not seem able to continue such exchange when the O_2 content of the water is very low. From a functional point of view, this has the advantage that in waters of low O_2 content and high CO_2 content, the danger of oxygen being lost through the gills will be averted.

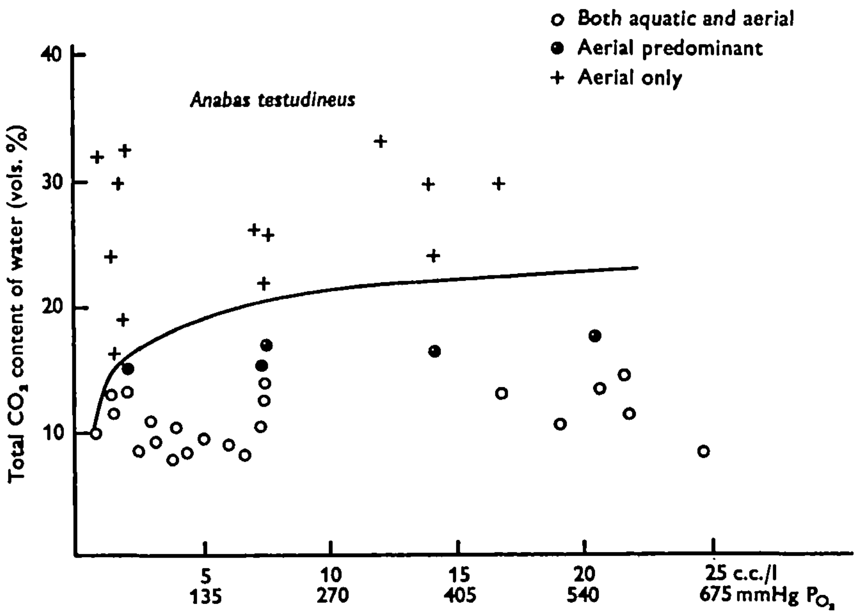

Fig. 5. Diagram showing the patterns of respiration used by *Anabas* in waters containing different concentrations of oxygen and carbon dioxide. Both aquatic and aerial respiration occur in waters of 0·7–25 ml O_2/l with a total CO_2 content below 15 vols. %. Above this level of CO_2 content a line has been drawn where aerial respiration becomes predominant and the fish depends on air for most of its O_2 supply. When the CO_2 content of the water is above 20 vols. %, only aerial respiration is present.

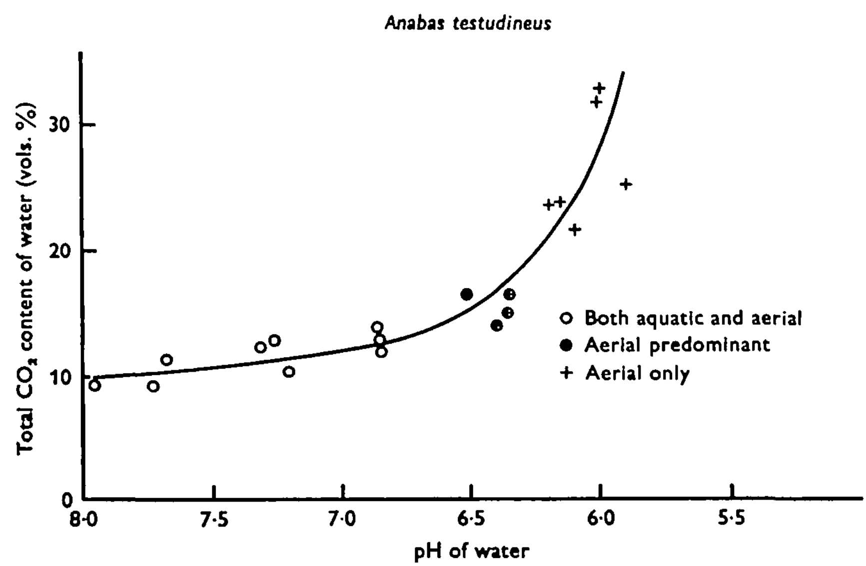

Fig. 6. Graph to show the relationship between CO_2 content of the water and its pH. The mode of respiration used by *Anabas* is indicated.

A striking feature shown by *Anabas* is the increased ventilatory frequency when the CO_2 content of the water is slowly increased above 10 vols. % by bubbling gas mixtures of 5 and 10% CO_2, but the opercular movements cease if the gas mixture contains more than 20% CO_2. The increased opercular frequency is often accompanied by a normal or slightly increased depth of breathing to begin with, but returns to normal or slightly lower amplitude at higher CO_2 contents. Such increases in opercular frequency are also correlated with an increase in air-breathing frequencies. This indicates that the climbing perch, in water of high CO_2 content, is unable to obtain enough oxygen

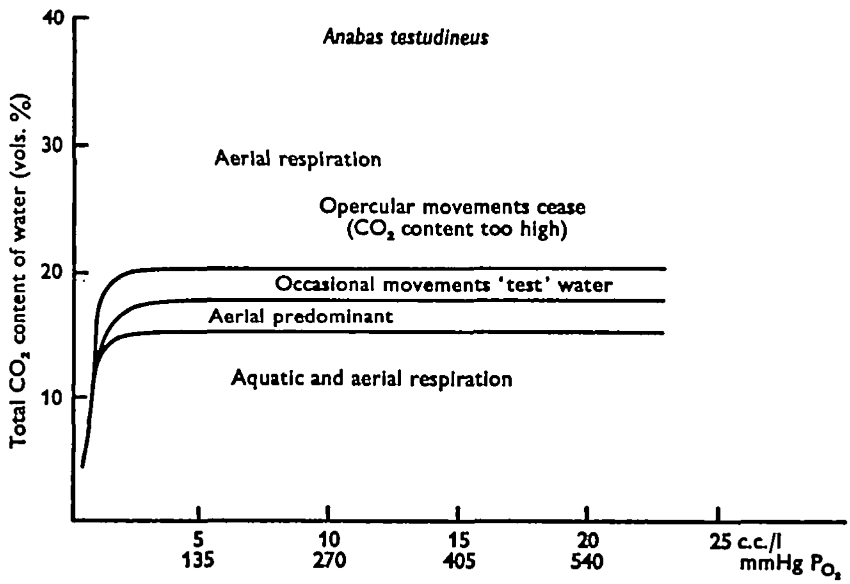

Fig. 7. Diagram summarizing the effect of different concentrations of carbon dioxide and oxygen in water on the mode of respiration shown by *Anabas testudineus*.

through the gills and therefore shows increased frequencies of surfacing (it may also be related to increased difficulties of CO_2 loss with higher carbon dioxide in the water). Increases in opercular frequency and ventilation volume at low O_2 tensions and high CO_2 tensions have been reported by Hughes & Shelton (1962) and Saunders (1962) for typical freshwater fish. In this situation it was found that the percentage utilization of oxygen was reduced. Similar changes in gill ventilation of *Anabas* might result from similar effects with increasing CO_2 content, but the accompanying air-breathing frequencies suggest that this fish might be able to maintain its standard rate of O_2 consumption even in this situation by exchanging gases efficiently through the accessory organs, as in fact seems to be the case (Hughes & Singh, 1970).

Johansen (1966), on the contrary, found a decrease in the frequency of gill ventilation in the air-breathing fish *Symbranchus marmoratus* and also in the lung-fishes when gas mixtures containing 2–5% CO_2 were bubbled into the water (Johansen *et al.* 1967; Johansen & Lenfant, 1968). In these fishes the decrease in opercular frequency was always associated with an increase in air-breathing. Their data also revealed that if the external carbon dioxide was raised to high levels in the water a substantial amount of carbon dioxide leaked into the blood stream in spite of the reduced gill ventilation. It seems likely that some carbon dioxide might enter the blood across the gills of climbing perch which continue branchial respiration in CO_2-rich water. Since in *Anabas* the opercular frequency is reduced, even to zero, only when gas mixtures containing more than 20% CO_2 are bubbled into the water, it appears very likely that this fish manages to respire in water of very high CO_2 content. From the results obtained, it is clear that the climbing perch is certainly able to tolerate high levels of carbon dioxide in the water.

Willmer (1934) found that air-breathing of the electric eel (*Electrophorus electricus*) was little affected by the presence of carbon dioxide but the O_2 dissociation curve of the blood of another air-breathing fish, *Myleus setiger*, was markedly shifted to the right in the presence of carbon dioxide. As yet the blood of *Anabas testudineus* has not been studied, but it may well be similar in type to that of the electric eel, because of their similar mode of life. The insensitivity of *Anabas* to the bubbling of gas mixtures containing 10% CO_2 also supports this view.

From the foregoing account it can be concluded that the chemoreceptors controlling respiration in this fish are located either on the lining of the mouth and gill surface or somewhere in the vascular system. So far receptors have not been recognized on the gills of *Anabas* in electron-microscope studies, but chemoreceptors are definitely present on the labyrinthine plates (G. M. Hughes, J. S. Datta Munshi, unpublished).

When water of high CO_2 content was continuously circulated through the fish chamber, opercular movements were sometimes seen at the beginning of the experiment but stopped after some time. Closure of the mouth and opercula and inhibition of branchial respiration might result from the fish testing the hypercarbic water and hence some carbon dioxide could diffuse into the blood across the gill surface. The rise in blood P_{CO_2} to a critical level might act as a strong stimulus to close the mouth and opercula and thus bring gill ventilation to an end. Sampling of the blood and measurement of its gas content would be needed to confirm this view.

Willmer (1934) studied the effect of water with different O_2 and CO_2 contents on the pattern and control of breathing in the yarrow (*Erythrinus erythrinus*). He found that three factors, pH, O_2 content and CO_2 content of the water are concerned in the control of breathing. Of the three, he concluded that the CO_2 content is most often the limiting factor. However, the yarrow differs from the climbing perch in the important aspect that its gills are adequate for the transfer of sufficient oxygen to the blood when the O_2 content of the water is between 1·5 and 4 c.c./l, and CO_2 content is between 7 and 27 c.c./l. Under these conditions *Erythrinus* does not normally come to the surface for air breaths. The mouth and opercula close and the fish shows aerial respiration alone at CO_2 contents above 31 c.c./l or below 5 c.c./l. This suggests that the very high CO_2 content results in acidaemia because this gas passes easily through the gill membranes and thus affects the respiratory control mechanism. The absence of gill ventilation at very low CO_2 content was because there was insufficient carbon dioxide to stimulate the respiratory centres. In the climbing perch the tolerance to carbon dioxide seems very much greater than in the yarrow. *Anabas* shows normal patterns of aquatic and aerial respiration even at CO_2 contents of 8–15 vols. % whereas the yarrow breathes air entirely at much lower CO_2 levels (C_{CO_2} of 0·3 vols. % or 3 mmHg).

Anabas also shows greater tolerance to higher levels of carbon dioxide as the opercular movements continue until the CO_2 content is 18 vols. % though aerial respiration becomes predominant. Willmer (1934) also studied the control of breathing in *Hoplosternum littorale*, which like *Anabas* never stops breathing air while it is living in water. Unlike the yarrow, *Hoplosternum* was found with closed mouth and opercula at much higher CO_2 contents of the ambient water. It seems clear that the climbing perch resembles much more closely this fish than the yarrow, and that in all of them carbon dioxide is the most important factor controlling their respiratory pattern when in water.

The effect of pH on the control of breathing is very marked in *Anabas* (Fig. 6). As the pH is lowered by bubbling gas mixtures with high percentages of carbon dioxide, the fish shows a progressively increased frequency of air-breathing, until finally it is the only form of respiration. It is difficult to draw a clear conclusion from the present data as to whether H^+ ions alone or together with CO_2 content play an important role in eliciting closure of the operculum, as the pH changes were always associated with an increase in CO_2 content. Willmer (1934) suggested that the acidity of the water and the presence of carbonic anhydrase in the blood may assist the passage of carbon dioxide across the gill membranes. It seems safe to conclude that acidity of the water aids the transfer of carbon dioxide into the blood and therefore low pH at least assists in the control of breathing by acting together with carbon dioxide.

It is of interest that an increase in CO_2 content of the water always results in an increased frequency of air breathing in *Anabas*. A similar effect was found in lung-fishes by Johansen *et al.* (1967) and by Johansen & Lenfant (1968). The mean, maximum and minimum intervals between air breaths diminish slowly with increasing CO_2 content.

When climbing perch are in saturated tap water the mean time interval between air breaths is about 10 min. It was found that when gas mixtures of oxygen and nitrogen were bubbled into the water, air-breathing increased to higher levels (Tables 1–3). This increase in air-breathing occurred even though the fish had a raised branchial ventilatory frequency. It can be seen from Tables 2 and 3 that though the total CO_2 content of the water was about 9–10 vols. % there was an absence of free carbon dioxide in such water brought about by bubbling of the nitrogen gas mixture.

These results indicate that despite the high frequency of opercular movements (38–47/min), gas exchange across the gills is inadequate and the fish depends more on aerial respiration. It appears that a certain amount of free carbon dioxide is necessary to produce full ventilation of the gills. It is also observed that in water without free carbon dioxide the fish was often restless and sometimes even jumped out of the fish chamber. Willmer (1934) found that the yarrow showed aerial respiration when the CO_2 content was below 5 ml/l. However, he did not determine the free CO_2 content of these waters and concludes that CO_2 content was too low to stimulate gill ventilation. In the present study it was also observed that the opercular movements did not completely stop in hypocarbic water having a total CO_2 content as low as 2·3–2·7 vols % (free CO_2 content 1·76–4·4 mg/l., PO_2 122–142 mmHg). The observations of Willmer (1934), together with the present study, suggest that a certain amount of carbon dioxide must be present in the water to stimulate full ventilatory movements of the gills and that in the absence of free carbon dioxide air-breathing is increased.

A particularly interesting feature brought to light in the present work is the ability of *Anabas* to live in waters containing large quantities of carbon dioxide for long periods (6–12 h) without any significant vigorous restlessness. It was noticed, however, that when first exposed to hypercarbic water by bubbling in 5–10% CO_2, each fish was restless to begin with and sometimes moved violently in the chamber. As the fish was exposed to still higher concentrations of carbon dioxide during successive experiments, it did not show such restlessness. Thus it appears that during the course of experimentation, *Anabas* slowly adapts to life in CO_2-rich water. Under these conditions, climbing perch shift entirely to aerial respiration.

In the study of O_2 consumption by *Anabas* under these various conditions (Hughes & Singh, 1970), it was found that the fish consumed about equal volumes of oxygen from the water and from the air when placed in air-saturated water, although most of the carbon dioxide was eliminated through the gills. When the fish was exposed to air alone, it was able to eliminate relatively higher amounts of carbon dioxide through the accessory organs and thus exchange of both carbon dioxide and oxygen occurred efficiently through these organs under these conditions. This finding throws light on the mechanism of adaptation of *Anabas* to life in CO_2-rich waters. Thus in water of very high CO_2 content the fish exchanges both carbon dioxide and oxygen through the accessory organs and presumably maintains almost normal CO_2 and O_2 levels in the blood. The mouth and opercula remain closed in such waters and the skin is thick and scaly, and hence carbon dioxide is prevented from entering the blood. However, some carbon dioxide may enter through the fins which are thin and smooth, but the quantities do not seem to have such a marked effect on the metabolism of the fish. Moreover, Eddy & Morgan (1969) found that the O_2 dissociation curve of trout blood was less sensitive to carbon dioxide in fish kept in water of high CO_2 content. However, until the gas content of blood samples from *Anabas* are analysed, such possibilities cannot be explored.

Berg & Steen (1965) found that hypoxia in the air acts as a factor controlling air-breathing in the eel. Johansen *et al.* (1967) and Johansen & Lenfant (1967), also found that hypoxia acts as a strong stimulus in the control of both aquatic and aerial respiration of *Neoceratodus* and *Lepidosiren*. However, Johansen & Lenfant (1968) found that hypoxic water has no significant effect on respiratory control of *Protopterus* but that hypoxic gas did stimulate air-breathing. Thus it appears from the existing literature that the effect of hypoxia in water on air-breathing fishes is related to the function of their aquatic and aerial respiratory organs and the habitat of the fish. For example, *Protopterus* is primarily an air-breather and shows no significant effect of hypoxic water whereas *Neoceratodus* is primarily a water-breathing lungfish and shows a strong effect of lowering the oxygen in the water (Johansen *et al.* 1967; Johansen & Lenfant, 1968). However, in the climbing perch where the gills and air-breathing organs are nearly equally important in O_2 exchange (Hughes & Singh, 1970), there is much less effect of hypoxic water when the fish is breathing both water and air. The effect of hypoxia becomes more apparent when *Anabas* breathes mainly water. Thus, when *Anabas* living in hypoxic water was forced to inspire either hypoxic air or pure nitrogen it showed increased air-breathing. This effect resembles the increased air-breathing of *Protopterus* when hypoxic gas or pure nitrogen was introduced into its lungs (Johansen & Lenfant, 1968).

SUMMARY

1. A study has been made of the patterns of respiration in climbing perch (*Anabas testudineus*) living in water containing different concentrations of oxygen and carbon dioxide and also in air-exposed fish.

2. The fish breathes both water and air in normal tap water. The intervals between air-breaths are irregular and vary within the range 8–15 min.

3. Air-breathing increases in water of high CO_2 content. The time interval between air breaths falls with increasing CO_2 content. Gill ventilatory frequency increases when 5–10% CO_2 is bubbled into the water. Aquatic respiration stops and only aerial respiration occurs if more than 20% CO_2 is bubbled into the water.

4. Three factors, CO_2 content, pH and O_2 content of water, control the respiratory patterns in climbing perch. Of these CO_2 content appears to be most important for fish living in water.

5. The climbing perch can live for long periods (6–12 h during observations made) in water of very high CO_2 content (20–33 vols. %). In such hypercarbic water gases are only exchanged through the air-breathing organs. The mouth and opercula are closed tightly and gill ventilation stops completely.

6. Exposure to air increases air-breathing but the frequencies are irregular. Inhalation of hypoxic gas or pure nitrogen also evokes air-breathing.

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