OXYGEN ACQUISITION OF THE REEDFISH, *ERPETOICHTHYS CALABARICUS*

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SUMMARY

In normoxic water at 25 °C, the reedfish (*Erpetoichthys calabaricus*) can breathe in both air and water, although water is the primary source of oxygen. This pattern of oxygen partitioning is altered by a number of factors. Increases in metabolic costs (e.g. locomotor activity) and decreases in aquatic oxygen concentration are met by increases in aerial oxygen uptake.

Reedfish acclimated to 33 °C have increased lung breathing compared with fish acclimated to 25 °C. Also, activity stimulates lung breathing more at 33 °C than at 25 °C. Unexpectedly, weight-specific oxygen uptake rates of reedfish at these two temperatures were not significantly different. Increased lung-breathing compensates for changes in locomotor activity, dissolved oxygen and temperature, allowing reedfish to meet their overall oxygen requirements.

Exposure of reedfish to six different combinations of oxygen and carbon dioxide indicated that lung ventilation frequencies were more labile than gill ventilation frequencies. The maximum change in lung ventilation rates was 849 % above controls whereas the largest change in gill ventilation frequency was 27 %. Exposure of reedfish to hyperoxia significantly depressed both gill and lung ventilation. Hypercapnic gases stimulated gill ventilation at both low (0·5 %) and high (5 %) concentrations; however, lung ventilation was only stimulated at the higher carbon dioxide concentration. The observed depression of gill ventilation frequencies in reedfish exposed to combinations of hypercapnic and hypoxic gases indicates that the inhibitory effect of low oxygen on gill ventilation dominates the stimulatory effect of hypercapnia. Overall, these data suggest that oxygen exerts a stronger influence in the control of respiration than carbon dioxide in this air- and water-breathing fish.

Reedfish survived out of water for 6 and 8 h at 25 °C without obvious ill effects. Oxygen consumption rates of these fish were significantly less than those of similar sized reedfish measured in water at either 25 or 33 °C. The respiratory physiology of the reedfish frees it from aquatic oxygen constraints and allows it to tolerate at least short-term terrestrial exposures.

INTRODUCTION

Despite the large amount of research on air-breathing fishes, comparatively few investigations have examined the functional role of the lungs in fishes of the subclass Brachiopterygii (= arm finned). These fish possess paired lungs connected ventrally to the oesophagus. This anatomical arrangement is found in only one other extant group of fishes, the lungfishes. Contemporary Brachiopterygians are represented by one family, Polypteridae, which contains only two genera (*Polypterus* and *Erpetoichthys*). Members of these two genera are commonly known as the bichirs and reedfish, respectively.

All modern polypterids are distributed solely in tropical Africa (Sterba, 1960) where they occupy habitats ranging from flowing rivers to fringing flood plains and internal river deltas (Welcomme, 1979 and personal communication). The availability of oxygen can vary across this spectrum of habitats from anoxic to saturated concentrations. Aquatic hypoxia may occur simultaneously with severe hypercapnia (Dehadrai & Tripathi, 1976; Welcomme, 1979). Similarly, temperatures may fluctuate from moderate to extremes of 40°C (Welcomme, 1979).

Studies by Abdel Magid (1966), Abdel Magid, Vokac & Nasr el Din Ahmed (1970) and Abdel Magid & Babiker (1975) have demonstrated the importance of lungs in aerial respiration of *Polypterus senegalus*. In view of its uncommon lung anatomy, the objective of our research was to elucidate further the role that lungs play in the respiratory physiology of the reedfish, *Erpetoichthys calabaricus* (Smith), formerly *Calamoichthys*, the only member of this genus. Previous research has demonstrated that *E. calabaricus* acquires oxygen from both air and water, possesses three respiratory sites (trimodal breather, Sacca & Burggren, 1982), can withstand prolonged aquatic hypoxia by aerial breathing (Pettit & Beitinger, 1981) and does not strongly avoid hypoxic water (Beitinger & Pettit, 1984). Our specific objectives were to determine the effects of oxygen concentration, oxygen and carbon dioxide mixtures, temperature and terrestrial exposure on oxygen acquisition in this species.

MATERIALS AND METHODS

Reedfish, *Erpetoichthys calabaricus*, were obtained from local suppliers and held in the laboratory in oxygen saturated water at 25 ± 0.1°C for at least 2 weeks prior to experimentation. Temperature control was provided by circulating thermoregulators. Temperatures were measured to the nearest 0.1°C by a Digitec digital thermometer. During the holding period, reedfish were fed daily.

Oxygen partitioning from air and water

Aerial and aquatic respiration were measured with a respirometer modified from Guimond & Hutchison (1972). The aquatic phase was adjusted to 1.01 and an air-water diffusion barrier was provided by a fine layer (approx. 3–4 mm) of styrofoam chips. The styrofoam barrier was chosen instead of mineral oil because it did not

leave a residue on the gills (Pettit & Beitinger, 1981) and did not interfere with surfacing for aerial breathing. Experiments confirmed that oxygen diffusion through the styrofoam chip layer into water was less than detection limits over our measurement intervals. Aerial respiration was determined manometrically. Aquatic oxygen uptake was measured using a Weston & Stack BOD probe and meter. The probe was mounted on the respirometer with the tip in the aquatic chamber. A peristaltic pump supplied a stream of water across the probe tip. The pump was operated only during $\dot{V}_{O_2}$ measurements.

Respirometry was done in water from each fish's aquarium to minimize stress. Before placing a fish in the apparatus, air was bubbled through the respirometer water to produce saturated oxygen concentrations ($7 \cdot 0$–$8 \cdot 0$ mg l^{-1}). The fish was placed in the respirometer and the diffusion barrier was added. Fish were left in the respirometer for at least 1 h prior to initial measurements. Experiments were initiated from 09.00 to 13.00 Central Daylight Time (U.S.A.) and lasted approximately 2–3 h. Oxygen consumption measurements of individual fish were taken at 10- to 20-min intervals. After respirometry at 25 °C, fish were slowly acclimated to 33 °C over a period of 1 week. Fish were maintained at $33 \pm 0 \cdot 1$ °C for 2–3 weeks prior to determinations at 33 °C. During acclimation fish were fed daily. Eleven trials were performed at 25 °C and 12 trials at 33 °C. Oxygen consumption values are reported as weight-specific rates at STP (ml O$_2$ g^{-1} h^{-1}). All fish had gone 2–3 days without food prior to respirometry.

In oxygen partitioning trials, the aerial and aquatic oxygen uptake rates for individuals were summed to determine total weight-specific routine metabolic rates. The range of aquatic oxygen concentrations over which respiration was measured was greater in the 25 °C group than in the 33 °C group. Consequently, statistical comparisons included (a) data collected over the differing ranges of dissolved oxygen and (b) data which covered only coincident ranges of aquatic oxygen concentration. This allowed us to discriminate between possible artificial effects of differences in the ranges of aquatic oxygen concentration experienced by the two temperature groups and effects which resulted from the difference in acclimation temperature.

Pearson correlation coefficients were calculated for aquatic oxygen concentration, total metabolic rate and percentage aerial (arcsine transformed) rate for both temperature acclimation groups. Total metabolic rate and arcsine percentage aerial rate were individually regressed against dissolved oxygen for both temperature groups. Student's t-tests were employed to compare both the arcsine transformed percentage aerial rates between the two temperature groups, and the ranges of dissolved oxygen concentration over which data were collected.

Respiration in air

Respiration in air was studied in 10 reedfish ($12 \cdot 4$–$21 \cdot 6$ g) held at 25 °C for several weeks in laboratory aquaria. Aerial respiration was measured manometrically in a respirometer partially filled with wet pea gravel. The gravel was covered with damp paper towelling to minimize dessication during the trials. Fish were placed in the respirometer 2 h prior to the first measurement. $\dot{V}_{O_2}$ measurements of individual fish were taken at 20- or 30-min intervals for a total experimental time of 6–8 h.

Trials were run at 25 °C and fish were denied food for 2–3 days prior to trials.

All respiration rates were converted to weight-specific rates at STP $(ml O_2 g^{-1} h^{-1})$. The weight-specific aerial rate for each reedfish exposed to air was regressed against time by least squares. Student's t-tests were used to determine if individual regression slopes differed significantly from zero ($\alpha = 0.05$). A significant negative slope indicates a decrease in $\dot{V}_{O_2}$ with time, a significant positive slope indicates an increase in $\dot{V}_{O_2}$ with time, and a zero slope suggests no significant trend in respiration rate during the trial. Comparisons of weights and metabolic rates between the two oxygen-partitioning groups and the air-exposed group were accomplished by parametric analysis of variance.

Respiratory responses to various combinations of oxygen and carbon dioxide

In order to assess the roles of oxygen and carbon dioxide in the control of respiration, reedfish were exposed to the following six certified gas concentrations: $100\% O_2$; $3\% O_2$; $0.5\% CO_2$; $5.0\% CO_2$ and two mixtures: $3\% O_2$, $5\% CO_2$ and $8\% O_2$, $5\% CO_2$.

The experimental apparatus consisted of a glass tube (length = 40 cm, i.d. = 27 mm) fitted with rubber stoppers. The tube was tilted (angle 10°) so that after it was filled with water a space remained at the top. Gases were bubbled through the tube via an air diffuser in the bottom stopper. A narrow glass tube mounted in the top stopper vented all the gases and prevented back flow of ambient air into the tube.

A fish was placed in the tube and air was gently bubbled through. After 2 h, the ventilatory responses of the fish under these conditions (controls) was recorded for 15–30 min. Then a gas mixture was bubbled through the tube. Ten minutes after the introduction of the gas mixture, ventilatory data were taken for 20–30 min.

A magnifying mirror placed directly under the opercular region allowed visual monitoring of gill ventilation movements. Both frequency and depth of gill beats were observed. Gill- and lung-breathing rates were recorded on separate channels of a Narco Physiograph. The recording pens were actuated by the observer.

Experiments were carried out in water from each fish's aquarium. Temperatures were recorded but were not controlled, and ranged from 22–24 °C for all experiments. During any trial, temperature did not vary by more than 0.5 °C. Water pH was recorded during the hypercapnic gas exposures, and did not decrease by more than 0.3 of a pH unit. Experiments were conducted in a darkened room with illumination provided by two lamps equipped with low wattage red lights. These measures minimized the effect of the observer upon fish ventilation. Under these conditions reedfish did not exhibit fright responses.

Seven or eight reedfish were individually exposed to each gas mixture. The mean lung ventilation frequency for a fish in an experiment was determined as the number of air breaths divided by the total time (in min) of exposure. The gill ventilation frequency was determined as the mean of the gill beats for ten randomly chosen 1-min intervals. Pearson correlation coefficients were computed to determine if either control branchial or control lung ventilation frequencies were related to fish weight. Differences in fish weight, branchial and lung ventilation rates among the six control groups were tested by parametric one-way analysis of variance

($\alpha = 0.05$). Branchial and lung ventilation of the six experimental groups were compared with the same analysis. Wilcoxon matched pairs, signed rank tests were used to test for differences in ventilation rates between controls and experimentals for each test gas mixture.

<center>RESULTS</center>

<center>*Oxygen partitioning from air and water*</center>

Respirometry values were collected over a range of dissolved oxygen concentrations similar to those encountered by *E. calabaricus* in their natural habitat (Welcomme, 1979). Reedfish were relatively similar in their partitioning of oxygen from air and water at 25°C (Table 1). Overall these fish acquired $65.7 \pm 13.8\%$ ($\bar{X} \pm$ s.D.) of their oxygen from water. At 33°C, mean partitioning of oxygen from air and water by reedfish was nearly equal (Table 2). The mean contribution of water to total $\dot{V}_{O_2}$ was $53.6 \pm 12.3\%$. Aerial partitioning of the 33°C group was significantly higher than that of the 25°C group (independent t test, $P < 0.05$).

E. calabaricus breathed air over the entire range of aquatic oxygen concentrations at both 25°C (2.5–$8.3 \, \mathrm{mg \, l^{-1}}$) and 33°C ($3.7$–$6.1 \, \mathrm{mg \, l^{-1}}$). The two fish which did not breathe air during a measurement interval at 25°C only did so during a single 15-min interval at moderate oxygen concentrations (4.0–$4.8 \, \mathrm{mg \, l^{-1}}$). Both fish displayed little activity over the interval and had concomitant decreases in their metabolic rates. The three fish which did not breathe air at 33°C did so only during initial measurements when aquatic oxygen concentrations were relatively high ($6.0 \, \mathrm{mg \, l^{-1}}$). One fish did not breathe air for the first 15-min interval and its metabolic rate was not reduced. The other two fish did not breathe air for three consecutive 15-min intervals. Their metabolic rates were slightly reduced during the time. There were noticeable increases in the rate and depth of gill ventilation of both fish. Apparently, oxygen uptake from the water alone was not sufficient to meet the aerobic demand in these two fish. The 33°C-acclimated fish were more excitable to handling and visual disturbance than the 25°C group. It is possible that these fish were wary of surfacing during the initial stages of respirometry.

All combinations of logarithmically transformed and normal data produced significant inverse correlations (i.e. $P < 0.05$) between dissolved oxygen and the percentage of aerial oxygen acquisition; however, the combination of log aerial percentage *versus* dissolved oxygen yielded the highest correlations for both 25 and 33°C (at 25°C and untruncated dissolved oxygen range $r = -0.33$, $P < 0.01$; at 33°C, $r = -0.57$, $P < 0.001$). The slopes of the corresponding regressions for the two temperature groups were not statistically different. No correlation was found between the percentage of aerial partitioning and dissolved oxygen for the 25°C group tested over the truncated range of dissolved oxygen values ($P > 0.05$). A significant positive correlation was found between total oxygen uptake rate and percentage aerial partitioning for both temperature groups (at 25°C, $r = 0.31$, $P < 0.01$; at 33°C, $r = 0.33$, $P < 0.01$). A primary factor influencing the total metabolic rates in this study was routine activity; hence, the correlations indirectly demonstrate the relationship between activity and aerial partitioning. Not surprisingly, increases in activity corresponded to increases in air breathing.

Table 1. *Partitioning of oxygen uptake between air and water for 11 reedfish at 25°C*

Weight (g)	Mean $\dot{V}_{O_2}$ (ml O_2 g^{-1} h^{-1})			Percentage mean $\dot{V}_{O_2}$		$\dot{V}_{O_2}$ Percentage range	
	Aerial	Aquatic	Total	Aerial	Aquatic	Aerial	Aquatic
22·1	0·043	0·045	0·088	50	50	47–56	44–53
21·1	0·034	0·066	0·101	35	65	28–49	51–72
13·4	0·026	0·084	0·109	23	77	18–30	70–82
19·5	0·048	0·045	0·093	43	57	0–68	32–100
21·9	0·009	0·056	0·064	15	85	8–25	75–92
19·5	0·028	0·072	0·099	27	73	22–34	66–78
16·5	0·032	0·081	0·112	29	71	13–56	44–87
18·5	0·045	0·051	0·097	44	56	0–71	29–100
18·0	0·061	0·041	0·102	60	40	40–71	29–60
15·4	0·028	0·060	0·088	32	68	24–42	58–76
13·4	0·024	0·113	0·137	19	81	4–60	40–96
18·2	0·034	0·065	0·099	34	66	—	—

Values listed in the bottom row represent the arithmetic mean for each category.

Table 2. *Partitioning of oxygen uptake between air and water for 12 reedfish at 33°C*

Weight (g)	Mean $\dot{V}_{O_2}$ (ml O_2 g^{-1} h^{-1})			Percentage mean $\dot{V}_{O_2}$		$\dot{V}_{O_2}$ Percentage range	
	Aerial	Aquatic	Total	Aerial	Aquatic	Aerial	Aquatic
22·1	0·020	0·051	0·071	29	71	0–68	32–100
21·1	0·044	0·036	0·081	51	49	25–76	24–75
19·5	0·035	0·043	0·078	44	56	21–67	33–79
21·9	0·051	0·049	0·099	51	49	41–61	39–59
19·5	0·035	0·051	0·086	38	62	0–63	37–100
16·5	0·027	0·073	0·099	24	76	0–54	46–100
18·5	0·043	0·054	0·097	43	57	26–60	40–74
18·9	0·053	0·057	0·110	51	49	26–62	38–74
15·4	0·055	0·046	0·101	55	45	38–75	25–62
21·8	0·075	0·037	0·112	68	32	59–75	25–41
22·1	0·042	0·049	0·090	44	56	26–65	75–35
13·2	0·055	0·039	0·094	59	41	44–77	23–56
19·2	0·045	0·049	0·093	46	54	—	—

Values listed in the bottom row represent the arithmetic mean for each category.

Significant correlations between total oxygen uptake rate and dissolved oxygen did not occur in either group. However, a significant correlation was found between routine metabolic rate and log weight for fish at 25 °C ($r = 0.67$, $P < 0.05$), but not at 33 °C.

Respiration in air

All 10 reedfish survived 6–8 h exposures to air with no apparent ill effects. During these trials, fish were occasionally active for periods which usually lasted from one to several minutes after which the fish became quiescent. While in the respirometer fish were observed to gulp air regularly; however, they did not demonstrate opercular gasping or other signs of distress usually exhibited by teleosts removed from water. Opercular movements were quite rare and occurred only immediately before or immediately after gulping air. In these cases, the movements appeared to be coughs. When removed from the respirometer and returned to their aquaria at the end of both 6- and 8-h trials, all 10 individuals were moderately active within a few minutes. These fish ventilated deeply when returned to water; the depth of ventilation gradually decreased to normal levels within 15 min.

E. calabaricus demonstrated no consistent increase or decrease in $\dot{V}_{O_2}$ over time in air-exposure experiments. In the 10 trials, seven of the regression slopes were not significantly different from zero, while three were negative. Routine weight-specific oxygen consumption rates of these fish ranged from 0.052–$0.119\,\mathrm{ml}\,O_2\,g^{-1}\,h^{-1}$ (Table 3).

Analysis of variance indicated that oxygen uptake rates for the 25 °C and 33 °C groups were not significantly different from each other; however, the mean oxygen uptake rate for the air exposure group was significantly lower than those of the two aquatic groups ($P < 0.01$).

Responses to various combinations of respiratory gases

Upon introduction into the test chamber, the fish were often active; however, by the end of 2 h most were quiescent. An obvious increase in the rates of both gill and lung breathing with increased activity occurred; however, no attempt was made to

Table 3. *Weight-specific $\dot{V}_{O_2}$ of 10 reedfish during 6–8 h of air exposure*

Weight (g)	$\dot{V}_{O_2}$ (ml O_2 g^{-1} h^{-1})
12·4	0·066
12·9	0·094
15·2	0·060
15·5	0·089
15·6	0·062
16·7	0·055
18·2	0·075
18·3	0·119
20·5	0·062
21·6	0·052

quantify these processes. During vigorous bouts of activity, measurements were not taken.

No correlations were found between weight and either gill or lung breathing ($P > 0.05$ and $P > 0.50$, respectively). The weights of the six groups were all statistically similar ($P > 0.99$) and individual fish ranged from 12·4–22·4 g. Interestingly, the six control groups did not have different frequencies of lung and gill breathing ($P > 0.95$ and $P > 0.84$, respectively). Mean gill and lung ventilation frequencies of the six control groups ranged from 22–25 beats min^{-1} (Fig. 1) and 0·10–0·15 breaths min^{-1} (Fig. 2), respectively. Hence, comparisons were made not only between an experimental group and its control, but also between the six different experimental groups.

Exposure to hyperoxic gas (100 % O_2) produced significant decreases in both gill and lung breathing relative to controls. Mean branchial and lung breathing frequencies fell to 78 % and 51 % of their respective control values, respectively. These rates were the lowest among all groups. In addition, a notable decrease in the apparent branchial stroke volume occurred in all individuals exposed to this test gas.

Exposure to the 3 % O_2 concentrations produced different responses in frequencies of gill and lung ventilation. While the mean branchial rate decreased slightly (88 % of control), this change was not significant ($P > 0.10$). Conversely, the frequency of lung breathing increased seven times above that of the control group.

Exposure to the 0·5 % CO_2 gas resulted in a significant increase ($P < 0.02$) in gill ventilation frequencies relative to those of the controls. Also, an increase was noted in the apparent branchial stroke volume. However, no appreciable change was observed in the air-breathing frequency of these fish ($P > 0.25$).

Branchial rates increased significantly ($P < 0.05$) for the 5 % CO_2 exposure group (25 % above control mean). Furthermore, increases in the apparent branchial stroke volume were observed in all seven subjects. A significant increase ($P < 0.02$) in mean lung breathing of 229 % above the control mean was noted.

Hypercarbic and hypoxic gas mixtures resulted in different responses between the gill and lung ventilation rates. The 3 % O_2, 5 % CO_2 mixture resulted in a decrease in branchial rate to 84 % of the control mean, but this decrease was not statistically significant ($P > 0.05$). The rate of air breathing increased significantly ($P < 0.01$) to 846 % above the control mean, but the response was highly variable (coefficient of variation = 43 %).

A non-significant increase ($P > 0.10$) was seen in gill ventilation frequencies of fish exposed to the 8 % O_2, 5 % CO_2 gas mixture (15 % above control mean). Mean lung ventilation in these fish was 459 % larger than the control mean ($P < 0.01$).

DISCUSSION

Oxygen partitioning data demonstrate that the lungs of *Erpetoichthys calabaricus* participate significantly in oxygen uptake during exposure not only to hypoxic water but also to normoxic water. Aerial breathing accounted for fully 34 and 46 % of total $\dot{V}_{O_2}$ for reedfish at 25 and 33°C, respectively. Sacca & Burggren, (1982) reported
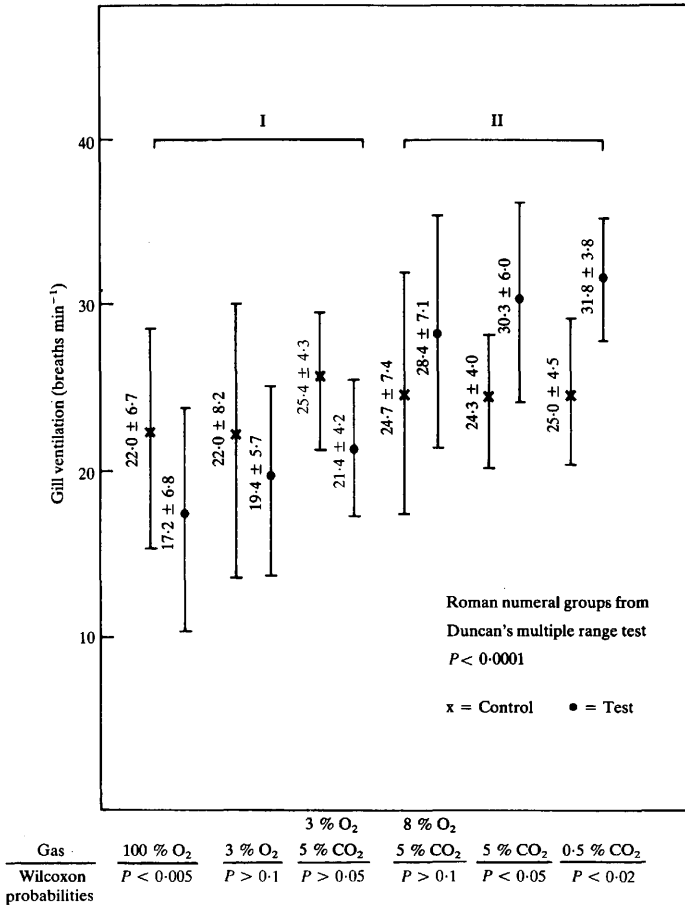
Fig. 1. Influence of various concentrations of oxygen and carbon dioxide on gill ventilation frequencies of reedfish. Mean and ± one standard deviation are both plotted and given numerically. Probabilities listed below the abscissa are from Wilcoxon matched pairs, signed ranks comparisons between control and experimental frequencies measured at each gas exposure. The Roman numerals indicate statistically distinct experimental groups following Duncan's multiple range test with $\alpha = 0.001$. Sample size was 7 or 8 for each group.
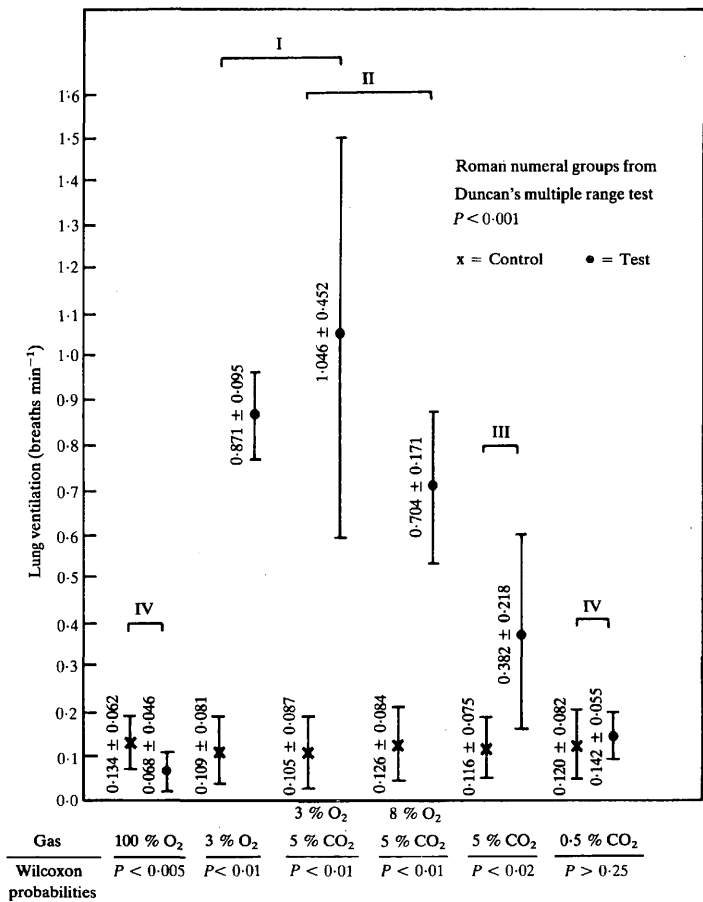
Fig. 2. Influence of various concentrations of oxygen and carbon dioxide on lung ventilation frequencies of reedfish. Mean and ± one standard deviation are both plotted and given numerically. Probabilities given below the abscissa are from Wilcoxon matched pairs, signed ranks comparisons between control and experimental frequencies measured at each gas exposure. The Roman numerals indicate statistically distinct experimental groups obtained from Duncan's multiple range test with $\alpha = 0.001$. Sample size was 7 or 8 for each group.

that *E. calabaricus* is a trimodal breather with total $\dot{V}_{O_2}$ being apportioned among skin (32 %), gills (28 %) and lungs (40 %) at 27 °C. These values for aquatic (60 % total) and aerial (40 % total) oxygen partitioning fall between those determined at 25 and 33 °C in our study (Tables 1, 2) and corroborate our observation that increased acclimation temperature results in an increased proportion of aerial oxygen acquisition. The proportion of aerial respiration appears to be variable (see Tables 1, 2) and is influenced by several environmental and physiological factors such as aquatic oxygen concentration, activity and temperature. The importance of these factors in relation to aerial partitioning has been demonstrated for the closely related species *Polyterus senegalus* (Abdel Magid, 1966; Abdel Magid & Babiker, 1975) and other species of air- and water-breathing fish (Stevens & Holeton, 1978a,b; Kramer & McClure, 1980; Burggren, 1979; Gee & Graham, 1978).

Although many investigators have measured air/water oxygen partitioning in fishes (e.g. Rahn *et al.* 1971; Stevens & Holton, 1978a,b; Burggren, 1979; Natarajan, 1980), surprisingly few studies have addressed the role of temperature upon this process. In the bowfish, *Amia calva*, the contribution of aerial respiration to total oxygen uptake increased dramatically at higher temperatures (Johansen, Hanson & Lenfant, 1970). At 10 °C, air did not contribute significantly to oxygen uptake; however, the ratio between aerial and aquatic oxygen acquisition approached 3:1 at 30 °C. The bowfish is a North American species which encounters a wide range of temperatures. We observed a similar pattern between temperature and aerial oxygen partitioning in the reedfish. Aerial contributions of oxygen at 33 °C were significantly greater than those at 25 °C. In contrast to the situation in *A. calva*, the shift in oxygen acquisition between 25 and 33 °C in reedfish represents the response of different temperature-acclimation groups. The increase in the percentage of aerial partitioning by reedfish is a temperature acclimatory adjustment rather than an acute response. Nevertheless, both of these species respond to high temperatures by shifting towards increased aerial oxygen acquisition. A similar pattern probably occurs in the long-nosed gar, *Lepisosteus osseus*. Rahn *et al.* (1971) reported that this species removed about 75 % of its oxygen from air at 22 °C. Although these investigators did not measure oxygen partitioning in gar at any other temperatures, it is quite likely that aquatic oxygen sources would play a larger role at lower temperatures. A preliminary pattern emerging from these limited data for these fish suggest that water is the predominant source of oxygen at low temperatures; however, aerial oxygen becomes increasingly important at elevated temperatures.

Similar studies concerning aquatic amphibians have produced inconsistent results. Some species such as *Ambystoma mexicanum, Amphiuma means* and *Siren lacertina* (Lenfant, Johansen & Hanson, 1970; Guimond & Hutchison, 1972, 1976) generate temperature-oxygen partitioning trends consistent with those of reedfish and bowfin. Nevertheless, several other species exhibit relatively constant ratios of aerial and aquatic oxygen uptake over wide temperature ranges. Included in this latter group are the caecilian, *Typhlonectes compressicanda* (Sawya, 1947), *Ambystoma tigrinum* (Whitford & Sherman, 1968), *Necturus maculosus* (Miller & Hutchison, 1979) and tadpoles of *Rana berlandieri* and *R. catesbeiana* (Burggren, Feder & Pinder, 1983).

Burggren *et al.* (1983) discuss possible advantages and disadvantages of increasing aerial respiration in the face of elevated temperatures. One proposed advantage is the conservation of energy by switching to aerial breathing instead of increasing gill ventilation. Alternatively, it has been suggested that the increased oxygen demand and decreased oxygen availability at higher temperatures converge to prevent aquatic respiration from offsetting an increased metabolic requirement.

Potential disadvantages of greater aerial breathing include increased energetic and temporal costs of transport to and from the surface, negative effects on buoyancy and locomotory control imposed by carrying gas under water, and increased risk of predation at the surface.

The findings that *E. calabaricus* generally breathed air at both 25 and 33°C and that increases in the metabolic rate produced increases in the proportion of aerial oxygen uptake suggest that aquatic oxygen uptake is insufficient to meet the respiratory requirements of reedfish, except for relatively brief periods during which activity was minimal, and/or aquatic oxygen concentrations were high. Abdel Magid & Babiker (1975) demonstrated that adult *Polypterus senegalus* are obligate air breathers even at high aquatic oxygen levels. Randall, Burggren, Farrel & Haswell (1981a) and Randall, Cameron, Daxboeck & Smatresk, (1981b) report that the gills of many air-breathing fish are reduced. A reduction of functional gill surface in the reedfish may provide an answer; however, this is an untested hypothesis at this time. The explanation accounting for increased aerial respiration in the reedfish at elevated temperatures is not clear.

Routine metabolic rates, indirectly measured as the sum of aquatic and aerial oxygen acquisitions, obtained for reedfish (Tables 1, 2) are approximately similar to those of other air-breathing fish of similar size measured at comparable temperatures (Singh, 1976; Burggren, 1979; Stevens & Holeton, 1978a). Also, the values in our study measured at 25 and 33°C agree closely with a previously reported metabolic rate for reedfish of $0.088 \, ml \, O_2 g^{-1} h^{-1}$ at 27°C (Sacca & Burggren, 1982). Stevens & Holeton (1978a) reported an exponent of 0.76 relating weight and metabolic rate for the tropical air breather *Hoplerythrinus unitaeniatus*. The corresponding value for reedfish at 25°C is 0.70. Considering the small weight range of fish in our study, this result is surprisingly close to the generally reported value of 0.8 associated with routine metabolism (Fry, 1971). This result was possibly fortuitous, since $\dot{V}_{O_2}$ and weight were not significantly related in the 33°C group. The limited range in weight ($13.2-22.1 \, g$) of reedfish in this study makes our assessment of weight-metabolic rate relationships tenuous.

The observation that routine metabolic rate was temperature insensitive (i.e. $Q_{10} = 1.0$) indicates that the reedfish is able to compensate over the tested temperature range. The metabolic rates of ectotherms often follow the van't Hoff rule (i.e. Q_{10} approaches 2.0); however, many species show metabolic compensation over a temperature range where various physiological processes are optimized (Beitinger & Fitzpatrick, 1979). Interestingly, this range includes the behaviourally selected temperatures. In any case, the similar routine respiration rates indicate that reedfish possess effective physiological mechanisms for dealing with moderately high temperatures.

Reedfish are able to exist out of water for at least 6–8 h at a time. The reduction

in $\dot{V}_{O_2}$ of air-exposed fish by approximately 25 % contradicts the results of Sacca & Burggren (1982) who reported a doubling in the $\dot{V}_{O_2}$ of reedfish during a 2-h air exposure. The discrepancy between these findings may be related to the time at which the two sets of data were collected. Sacca & Burggren (1982) measured $\dot{V}_{O_2}$ during the initial 2 h of air exposure. Measurements of $\dot{V}_{O_2}$ in our study were begun after fish had been exposed to air for 2 h. Exposure to air produces an initial increase in the oxygen uptake of the bimodial breathers *Clarias batrachus, Anabas testudineus* and *Heteropneustes fossilis* (Singh, 1976). In general, it appears that few species of freshwater, air-breathing fish can sustain aquatic levels of oxygen consumption during air exposure (Singh, 1976; Burggren & Haswell, 1979). Graham (1976) found that a majority of amphibious marine teleosts (e.g. *Periophthalamus sobrinus* and *Tomicodon humeralis*) are able to maintain similar metabolic rates when removed from water and placed in air, while most amphibious freshwater teleosts have reduced metabolism in air. Our data for the reedfish support this trend.

The increased frequency and depth of gill breathing observed in reedfish upon return to their aquaria following 6–8 h of terrestrial exposure probably served to assist the excretion of carbon dioxide accumulated during air exposure. Burggren & Haswell (1979) examined the role of carbonic anhydrase in aerial carbon dioxide excretion in the air-breathing anabantid, *Trichogaster trichopterus*. A correlation between the presence of this enzyme and the ability to maintain a high respiratory quotient during air exposure was established. This same study demonstrated that the lungs of the reedfish contained almost none of this enzyme. This suggests that reedfish have a limited capacity for carbon dioxide excretion *via* the lungs. Skin may serve as an alternative site for carbon dioxide excretion during air exposure. Sacca & Burggren (1982) found that aquatic oxygen uptake *via* skin accounted for one-third of the reedfish's total oxygen uptake.

In addition to possessing a respiratory system that can sustain terrestrial existence, the reedfish seems to be adapted for terrestrial movement. We observed individuals spontaneously leave their aquaria. This behaviour has been reported by Sacca & Burggren (1982) who also observed that *E. calabaricus* will catch insects on land. We videotaped several individual reedfish within a chamber containing plants and a moist substrate. In the chamber, reedfish displayed a vigorous righting response and travelled easily both forward and backward. During terrestrial movement, reedfish place loops of their bodies against objects and form standing waves as the body passes through each loop. This pattern is identical to the lateral undulation movement demonstrated in some snakes and other limbless terrestrial vertebrates (Gans, 1974). Individuals were also observed to elevate their heads and pass over low vertical barriers. Head lifting requires the development of musculature not necessary for aquatic environments where body mass is neutrally buoyant. In summary, the reedfish appears to possess a suite of respiratory and locomotory adaptions which support temporary existence and locomotion on land.

Exposure of reedfish to various combinations of oxygen and carbon dioxide concentrations resulted in three major findings. First, variation in ambient oxygen concentration exerts a far greater influence on ventilation rates than variation in carbon dioxide. Second, the most dramatic ventilatory adjustment is the inhibition

of gill ventilation produced by aquatic hypoxia. Finally, lung ventilation frequencies were far more labile than gill ventilation frequencies in our trials.

Although exposure to hypoxic gas (3 % O_2) did not produce a significant reduction in the frequency of gill ventilation, we believe this result was primarily an artifact produced by the experimental design. In all of these experiments, fish were simultaneously exposed to the same aquatic and aerial gas concentrations. As pointed out by Johansen & Lenfant (1968), this type of design does not allow a distinction between ventilatory responses owing to the separate stimuli of aquatic and aerial gas compositions. Reedfish in hypoxic water with access to normoxic air demonstrated an apparent total inhibition of gill ventilation (Pettit & Beitinger, 1981). Conversely, individuals in normoxic water which did not surface for an extended period of time had a marked increase in both the depth and rate of branchial movements. These observations indicate that combined aquatic and aerial hypoxia produce antagonistic gill ventilatory responses; hence, the gill ventilatory response in our experiments could have been masked by competing stimuli. Nevertheless, it is noteworthy that no increase in gill ventilation occurred. This suggests that the inhibition of branchial rate by hypoxic water overrides any stimulation in branchial rate owing to possible low internal oxygen tensions.

Since the lungs of the reedfish have little ability to excrete carbon dioxide (Burggren & Haswell, 1979), decreases in gill ventilation would be expected to produce respiratory acidosis. However, the skin of the reedfish is important in respiration (Sacca & Burggren, 1982), and carbon dioxide excretion *via* this site probably serves to ameliorate acidosis. The air breather, *Hypostomus* sp., is able to compensate for increased blood carbon dioxide levels resulting from reduced aquatic gas exchange during aquatic hypoxia by increases in plasma bicarbonate (Wood, Weber & Powers, 1979).

A significant increase occurred in the gill ventilation of juvenile *P. aethiopicus* exposed to hypoxic gases under experimental conditions similar to ours (Jesse, Shub & Shelton, 1967). Apart from the data for *Hypostomus* (Wood *et al.* 1979), decreases in gill ventilation in response to hypoxic water have been reported for only two other air-breathing fish species, *Anabas testudineus* (Singh & Hughes, 1973) and *Lepisosteus oculatus* (Smatresk & Cameron, 1982a). This moderate decrease in gill ventilation was thought to have only a slight influence in reducing oxygen loss at the gills *via* reverse diffusion. In general, air- and water-breathing fish show increases in both gill and lung ventilation rates when exposed to hypoxic water (Abdel Magid, 1966; Johansen, Lenfant & Grigg, 1967; Singh, 1976; Burggren, 1979; Randall *et al.* 1981a,b). Adult *P. aethiopicus* are primarily aerial oxygen breathers and have lung and gill ventilatory reflexes which are unresponsive to aquatic hypoxia (Johansen & Lenfant, 1968). Aerial hypoxia stimulates air breathing in all air- and water-breathing fish studied (Abdel Magid, 1966; Johansen *et al.* 1967; Johansen & Lenfant, 1968; Johansen, Lenfant & Hanson, 1968; Burggren, 1979). Thus the reedfish's ventilatory responses to hypoxia are typical with regard to air-breathing and unusual with regard to gill-breathing fishes. The exact controls of respiration in air- and water-breathing fishes are not known. This is not surprising since Randall (1982) concluded that even in typical gill-breathing fish, information on mechanisms of respiratory integration is sparse and patchy.

The experiments with hypercapnic gas mixtures demonstrate that carbon dioxide stimulates gill ventilation similarly at low (0.5% CO_2) and high (5% CO_2) concentrations (Fig. 1), while lung ventilation was only stimulated at the higher carbon dioxide concentration (Fig. 2). Numerous studies with air- and water-breathing fish have demonstrated that aquatic hypercapnia stimulates lung breathing and/or inhibits gill breathing (Johansen, 1966; Johansen *et al.* 1967; Johansen & Lenfant, 1968; Hughes & Singh, 1971; Singh, 1976; Graham, Kramer & Pineda, 1977; Burggren, 1978; Graham & Baird, 1982). Such responses comprise a mechanism by which these fish may regulate their blood acid-base balance when experiencing aquatic hypercapnia (Johansen, 1971). However, recent studies have shown that the effects of hypercapnia upon ventilation may be transient in some species. The loricardis, *Ancistrus* and *Hypostomus*, are stimulated to breathe air during initial exposure to aquatic hypercapnia, but this response disappears after 2-6 h of hypercapnic exposure (Graham & Baird, 1982). The spotted gar, *L. oculatus*, demonstrates a slight increase in gill ventilation along with a possible increase in aerial ventilation upon exposure to moderate aquatic hypercapnia. After 8 h of hypercapnia, gill ventilation frequency decreases and acidosis is compensated for by an elevation in blood bicarbonate (Smatresk & Cameron, 1982*c*). Hence, the reedfish's ventilatory responses are consistent with these recent data.

Gill ventilation responses of reedfish to combinations of hypercapnic and hypoxic gases demonstrate that the hypoxic inhibition dominates the stimulation due to hypercapnia (Fig. 1). Under these twin influences, increased gill ventilation by a fish to compensate for hypoxia could produce respiratory acidosis as a result of increased carbon dioxide uptake at the gills. In some air-breathing fishes, aquatic hypercapnia inhibits branchial respiration while simultaneously stimulating aerial respiration, a combination of responses which would avoid respiratory acidosis (Johansen, 1971; Singh, 1976). However, other studies have indicated that some species of fish may regulate their blood pH with other mechanisms (Smatresk & Cameron, 1982*a,b,c*; Graham & Baird, 1982; Dejours, Toulmond & Truchot, 1977). The rates of gill ventilation for the 3% O_2 and the 3% O_2, 5% CO_2 exposures were not significantly different; however, the gill ventilation frequencies were significantly elevated in fish exposed to the 8% O_2, 5% CO_2 gas mixture. Similarly, while the lung ventilation frequency was most stimulated by the 3% O_2, 5% CO_2 gas mixture, the frequency of lung ventilation was significantly greater for the 3% O_2 group than for the 8% O_2, 5% CO_2 group (Duncan's multiple range test, $P < 0.001$). These data indicate that oxygen exerts a stronger influence upon the control of respiration than carbon dioxide in reedfish. Smatresk & Cameron (1982*c*) suggested that this is the case for spotted gar. Additionally, growing evidence indicates that oxygen is predominant in controlling the ventilation of water breathers (Dejours *et al.* 1977). In contrast, breathing depth and frequency in lung breathers are controlled by blood CO_2 levels.

The observation that lung ventilation rates responded much more noticeably to gas changes than did the gill ventilatory frequencies may be due to several reasons. In nature it is probable that the lungs are of primary importance for maintaining blood oxygen homeostasis in the face of large and often rapid changes in aquatic oxygen levels. If lung P_{O_2} and perfusion increase, a small change in lung ventilation

rate could have a much greater influence on internal oxygen concentration owing to differences in oxygen availability between air and water. Consequently it is advantageous for the reedfish to have a labile lung ventilatory response.

In summary, this study in combination with previous research (Pettit & Beitinger, 1981; Sacca & Burggren, 1982; Beitinger & Pettit, 1984) clearly indicates that lung breathing frees *Erpetoichthys calabaricus* from the typical oxygen related constraints of aquatic environments. The observation that decreases in dissolved oxygen concentrations produced increases in the proportion of aerial respiration without affecting the total metabolic rate indicates that adjustments in lung respiration rate serve as the primary mechanism for maintaining oxygen homeostasis over the range of aquatic oxygen concentrations this species may encounter. The respiratory responses to increased temperature and increased activity also suggest the predominance of lung respiration with regard to oxygen homeostasis. One apparent trade-off of this 'strategy' is that the gills alone are generally unable to meet total oxygen demand.

Our results indicate that oxygen concentration exerts a greater influence on ventilation frequencies than carbon dioxide. The most dramatic ventilatory adjustment is the inhibition of gill ventilation frequency produced by aquatic hypoxia. This consistent response to hypoxia, regardless of aquatic carbon dioxide concentration, suggests that this may be an important adaptation permitting the reedfish to occupy habitats with limited amounts of oxygen. Pulmonary function of the reedfish is able to support both terrestrial existence and active locomotion over land. Further insight into understanding the role of the lungs could be provided by experiments where reedfish are denied access to the surface. It is possible that the reedfish is an obligate air breather.

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