

OXYGEN UPTAKE IN AIR
AND WATER IN THE AIR-BREATHING REEDFISH
CALAMOICHTHYS CALABARICUS: ROLE OF
SKIN, GILLS AND LUNGS

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SUMMARY

The reedfish *Calamoichthys calabaricus* (Smith) is amphibious, making voluntary excursions on to land (in a simulated natural environment) an average of 6 ± 4 times/day for an average duration of 2.3 ± 1.3 min.

Oxygen uptake is achieved by the gills, skin and large, paired lungs. In water at 27 °C, total oxygen uptake is 0.088 ml O₂/g.h. The lungs account for 40%, the gills 28%, and the skin 32% of total \dot{V}_{O_2} .

Total oxygen uptake during 2 h of air exposure increases from 0.117 ml O₂/g.h to 0.286 ml O₂/g.h, due largely to an enhanced lung \dot{V}_{O_2} , and a small increase in skin \dot{V}_{O_2} .

Calamoichthys is both capable of aerial gas exchange and adapted to maintain O₂ uptake during brief terrestrial excursions.

INTRODUCTION

The partitioning of oxygen uptake between the aerial and aquatic gas exchange organs has been extensively investigated in air-breathing fish (Johansen, 1970; Singh, 1976; Hughes & Singh, 1971; Singh & Hughes, 1971; Burggren, 1979). However, most studies have simply attributed aquatic oxygen uptake either entirely to the gills, or to an unspecified combined effect of the gills plus the skin, without directly quantifying the relative contributions of these structures when both air and water breathing are occurring. Yet, the potential significance of cutaneous respiration to total oxygen uptake in air-breathing fish has been suggested (Johansen *et al.* 1968; Hughes & Singh, 1971; Burggren & Haswell, 1979). It is thus surprising that the simultaneous three-way partitioning of O₂ uptake, i.e. between air-breathing organ, gills and skin, has not been determined, especially since many air-breathing species (*Electrophorus*, *Symbranchus*, *Lepisosteus*, *Saccobranchus*, *Polypterus*, *Calamoichthys*) are very elongate and probably have a high skin-surface-area to body-mass ratio.

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The intent of the present study was to measure simultaneously oxygen uptake by the gills, skin and aerial exchange organ of an air-breathing fish likely to exploit cutaneous respiration, and to examine the effects of short-term air exposure on this partitioning. The reedfish *Calamoichthys calabaricus* was chosen because of its elongate, eel-like body shape, and its habit of making brief terrestrial excursions. *Calamoichthys*, which along with *Polypterus* constitutes the survivors of the family Polypteridae, inhabits swamps and rivers in Western Africa. *Calamoichthys* has large paired lungs connected ventrally with the oesophagus, but retains gills which are heavily vascularized and apparently well developed (Purser, 1926).

METHODS

All experiments were conducted at 27 °C on a total of ten fish, ranging from 20–29 g in mass and 26–32 cm in length. The fish were obtained from a local supplier (although of African provenance) and were maintained in aquaria at 27 °C for at least 4 weeks prior to experimentation.

(1) *Voluntary emersions from water*

An experimental apparatus was devised to quantify the voluntary land excursion of *Calamoichthys* in a simulated natural environment. Two glass chambers (vol. 10 l) were interconnected by a plexiglass tunnel (19 cm in length, 4 cm width, 4 cm height). One chamber contained a 1–4 cm layer of rocks and gravel on its floor and was filled to a depth of 15 cm with water maintained at 27 °C. The other compartment contained a 15 cm layer of moist soil covered with short grass. Water and soil levels were adjusted to the level of the floor of the plexiglass tunnel. Two photocells with pinpoint light sources were arranged at each opening of the connecting tunnel so that any object moving from one chamber to the other interrupted the light beam and produced a change in output from the photocell. This was continuously recorded on a Fisher 2-channel Recordall strip chart recorder. Individual fish were placed in the apparatus, which was kept in subdued light, and their movement between the aquatic and terrestrial chamber monitored continuously for 2–8 days.

(2) *Oxygen uptake partitioning in water*

The respirometer used to measure simultaneous oxygen-uptake partitioning between gills, skin and aerial exchange organ, is depicted in Fig. 1. The respirometer, after van Dam (1938) and Berg & Steen (1965), was highly modified to allow for air breathing as well as gill and skin ventilation.

It consisted of an anterior plexiglass chamber (4.5 cm square, 23 ml volume), connected to a posterior glass tube (33 cm in length, 2.5 cm diameter, 130 ml volume). The two chambers were clamped together, with a slightly unrolled condom serving both as a highly flexible membrane separating water flow between anterior and posterior chamber, and as an O-ring preventing external water leakage. A 1 cm hole was made in the centre of the condom, through which the anterior portion of the fish's body was inserted. When the respirometer was assembled with a reedfish within, the anterior 3 cm of the fish, including the mouth, eyes, opercular slits, and an add

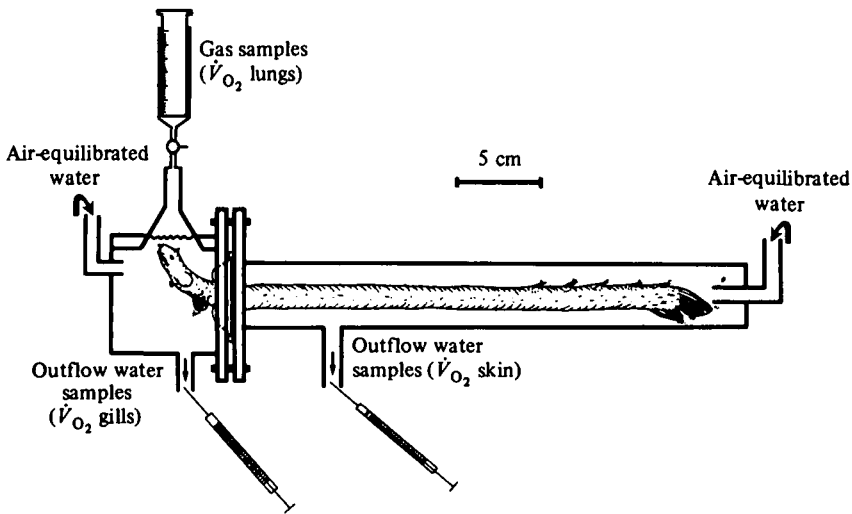


Fig. 1. Schematic diagram of apparatus used for measurement of oxygen uptake partitioning in *Calamoichthys calabaricus*.

tional 1.5 cm of body, thus rested within the anterior chamber. The remainder of the body (i.e. approximately 90% of the total body length) lay within the glass tube. The opening in the condom tip was totally occluded by the fish's body, yet great care was taken to prevent constriction of the body wall. The condom was sufficiently flexible to allow a 2 cm forwards or backward motion of the fish without disrupting the actual membrane positioning around the body wall. This constituted an improvement over the membrane system and severe restraining devices of the respirometer described by Kirsch & Nonnotte (1977) for determination of cutaneous respiration in trout, eel and tench; the present design for an air-breathing fish allowed the movements necessary for lung ventilation.

The positioning of the membrane 1.5 cm posterior to the gill slits ensured that no impairment of the buccal or opercular movements could occur. Methylene blue injected into the water beside the rubber membrane during movements of the fish revealed no leakage of water between chambers.

An inverted funnel (rim 3 cm in diameter) was attached to the roof of the anterior chamber immediately above the head of the reedfish. A syringe was attached to this funnel, and allowed air to be injected down into the funnel. The reedfish could thus voluntarily ventilate its lungs simply by raising its head approximately 1 cm up into the funnel, and breathing from the air bubble. Throughout all experiments the movements of the experimenters were shielded from the fish.

Constant but separate flows of air-equilibrated water at 27 °C through both the anterior and posterior chambers were maintained throughout the experiments. Flow rates were different between the two chambers, because water flow was adjusted to the oxygen uptake from each chamber. The P_{O_2} of water exiting from each chamber was approximately 10–20 mmHg lower than the water entering. Water and gas partial pressures were measured by a Radiometer P_{O_2} electrode thermostated to 27 °C in a Radiometer BMS-2 blood gas analyser, and connected to a pH M72 meter.

Calibrations of the electrode were performed with water-saturated N_2 and air. The measured P_{O_2} of air-saturated water deviated from that of water-saturated air by less than 1 mmHg.

The uptake of O_2 from the water flowing through each chamber was calculated in ml $O_2/g \cdot h$ (BTPS), in the conventional manner, from the water P_{O_2} decrease, the rate of water flow and the O_2 solubility at 27 °C. O_2 uptake from the anterior chamber was attributed entirely to branchial exchange, with any contribution from the very small amount of skin on the head assumed to be negligible. O_2 uptake from the posterior chamber was attributed entirely to cutaneous uptake.

Oxygen uptake from the lungs was calculated by injecting 5.0 ml of water-saturated air at 27 °C into the funnel, and allowing the reedfish to breathe from this bubble. After 30 min, the P_{O_2} and new volume of this gas bubble was determined, and the \dot{V}_{O_2} of the lungs calculated from the rate of O_2 depletion. Pressure in the gas bubble was kept constant by the actions of water-filled calibrated manometers open to the atmosphere and connected to the water inlet tubes to the head and body chambers. A small fall in gas volume consistently occurred during the measurement period, presumably due to lung gas exchange ratios less than 1.

Although the air-water interface was only approximately 1.5 cm², the possibility of O_2 transfer between gas and water was tested. Pure N_2 was injected into the funnel of an irrigated respirometer without a fish. After a 30 min period, during which a P_{O_2} gradient from water to air of approximately 150 mmHg existed, the P_{O_2} of the funnel gas rose from 0 mmHg to less than 6 mmHg. In the presence of P_{O_2} gradients normally 1/3-1/5 this magnitude when a reedfish was breathing from the funnel, we assumed that there was no significant O_2 transfer across the air-water interface in the funnel.

Determination of water flow rates and collection of water and gas samples were completed within 1 min of each other, at intervals throughout at least 3 successive days. All fish, which were handled minimally, were allowed to acclimate to the respirometer for 24 h prior to collection of any data. During acclimation there was constant flow of air-equilibrated water through anterior and posterior chambers, and a constant flow of moist air through the funnel.

(3) *Air exposure*

Experiments were conducted on 4 fish acclimated to the respirometer. Water and air samples for oxygen uptake partitioning were collected and analysed (as described above) over a 180 min period before air exposure. Water in the respirometer was then slowly drained, replaced by humidified air, and all connexions were sealed. A film of water remained at the bottom of the respirometer resulting in water-saturation of the gas within. The manometers remained attached to the head and body chambers to measure volume changes and ensure that no pressure differences developed.

Each fish remained air-exposed for 120 min, while gas samples from the head and body chambers were collected and analysed every 30 min. Oxygen uptake was calculated from the rate of oxygen depletion and from the volume of gas present in each chamber at the time of measurement. After 120 min, the respirometer was refilled with water, and measurements continued for a further 180 min.

RESULTS AND DISCUSSION

(1) Voluntary emersion from water

Frequency and length of voluntary emersion varied greatly between individuals (Table 1), but *Calamoichthys* normally made several daily excursions on to land of about 2 min each. At the extreme, one fish emerged 17 times in one day, while another voluntarily remained out of water for 74 min. In every case return to water was followed by typical 'aquatic behaviour'.

Although many fishes use aerial respiration, a relatively small number of these are adapted to survive complete air exposure for substantial periods of time on a daily basis, e.g. *Clarias* (Singh & Hughes, 1971), *Periophthalmus* (Gordon *et al.* 1968) and *Anabas* (Hughes & Singh, 1970). Evaporative water loss may be as problematic as gas exchange with longer exposure times.

(2) Oxygen uptake partitioning in water

Values for oxygen uptake and its partitioning between lungs, gills and skin are presented for six *Calamoichthys calabaricus* in Table 2. Neither oxygen uptake partitioning nor total \dot{V}_{O_2} showed any significant difference ($P > 0.10$, Student's *t* test) when comparing values measured 1, 2 or 3 days after the initial acclimation period. Thus, the values used to calculate \dot{V}_{O_2} for each fish include data from every test day. Total oxygen uptake in *C. calabaricus* was approximately 0.088 ml O₂/g.h at 27 °C, which is comparable with many other air-breathing fish at this temperature (see Singh, 1976). Aerial oxygen uptake in *Calamoichthys* only accounted for about 40% of this \dot{V}_{O_2} . A similar aerial oxygen uptake at 25–27 °C has been reported for *Saccobranchus* (Hughes & Singh, 1971), *Amia* (Johansen, Hanson & Lenfant, 1970) and *Trichogaster* (Burrgrén, 1979), although many other air-breathing fishes show a larger dependence upon aerial respiration for O₂ uptake.

With respect to aquatic O₂ uptake, the skin of *C. calabaricus* is equally important as the gills (approximately 30% of total \dot{V}_{O_2}) in obtaining O₂ from water (Table 2). The large scales on the skin apparently interfere little with cutaneous exchange, and the rate of perfusion of the cutaneous epithelium must be substantial. The skin is an equally important gas exchange organ in *Electrophorus* (Farber & Rahn, 1970), *Protopterus* (Lenfant & Johansen, 1968) and *Saccobranchus* (Singh & Hughes, 1971).

Table 1. Frequency of voluntary emersion from water and emersion length in *Calamoichthys calabaricus*.

Fish	No. of days tested	Number of emersions/24 h	Emersion length (min)
1	5	3.5 ± 2.7 (0-5)	2.0 ± 1.2 (1.2-5.4)
2	8	3.3 ± 2.8 (0-11)	4.9 ± 14.4 (0.3-74.4)
3	5	3.4 ± 2.3 (1-7)	1.2 ± 0.2 (1.2-1.8)
4	5	5.2 ± 2.8 (3-10)	2.2 ± 2.0 (1.2-8.4)
5	2	12.5 ± 6.4 (8-17)	1.9 ± 1.2 (1.2-8.4)
6	6	6.5 ± 4.9 (1-13)	1.8 ± 1.3 (1.2-8.4)
\bar{X}	5	5.7 ± 3.6 (3-13)	2.3 ± 1.3 (1.2-4.9)

(Means ± 1 standard deviation.)

Table 2. Total oxygen consumption and partitioning in *Calamoichthys calabaricus* at 27 °C (body weight 27.4 ± 1.5 g)
(Means ± 1 standard deviation ($n = 6$)).

	ml O ₂ /g.h (BTPS)	% of total \dot{V}_{O_2}
Skin	0.029 ± 0.006	32 ± 8
Gills	0.024 ± 0.006	28 ± 8
Lungs	0.035 ± 0.009	40 ± 9
Total	0.088 ± 0.008	100

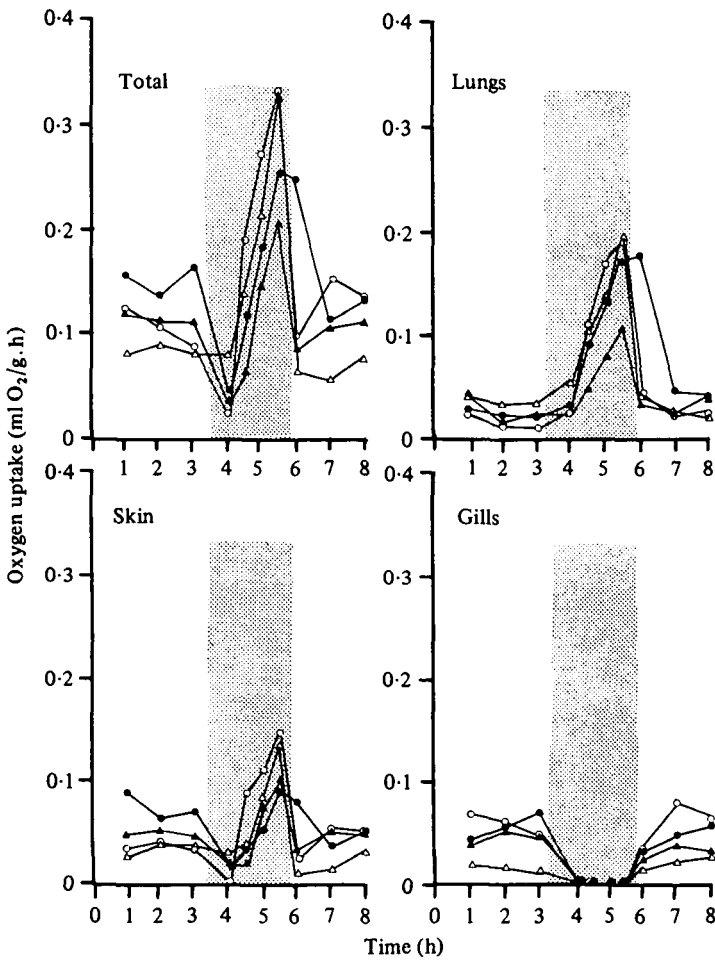


Fig. 2. Oxygen uptake partitioning before, during and after air exposure, in four *Calamoichthys calabaricus*.

(3) Air exposure and oxygen uptake

Calamoichthys was extremely consistent in its respiratory responses to brief air exposure (Fig. 2). Total O₂ consumption increased significantly ($P < 0.05$) from 0.117 ± 0.006 ml O₂/g.h (mean \pm 1 S.D., $n = 9$) in water to 0.286 ± 0.060 ml O₂/g.h after 2 h of air exposure. Total O₂ consumption returned to pre-exposure levels within 30 min after the return to water, and there was no significant difference ($P > 0.10$) between the values obtained before air exposure and those obtained immediately after return to water. Increases in total O₂ consumption during air exposure have also been reported in the air-breathing fishes *Propterus* (Lenfant & Johansen, 1968), *Amphipnous* (Lomholt & Johansen, 1974) and *Trichogaster* (Burggren & Haswell, 1979), but *Neoceratodus* (Lenfant, Johansen & Grigg, 1967) *Saccobranchus* (Singh & Hughes, 1971) and *Anabas* (Hughes & Singh, 1970) all showed decreased O₂ uptake during air exposure. While air exposure may be a rare event for some of these fish, there is clearly an adaptive advantage for *Calamoichthys* to be able to maintain or even increase O₂ uptake during air exposure, since emersion from water is part of the normal behaviour of the reedfish (Table 1). Moreover, in the laboratory we have observed *Calamoichthys* to leave water regularly and consume terrestrial insects. Presumably, such terrestrial activities might be limited if oxygen uptake could not be maintained when air exposed.

Oxygen partitioning between the gas exchange organs changed substantially during air exposure. It was assumed that the gills make no significant contribution to oxygen uptake during air exposure, since the gills outwardly appear to have no specializations for preventing collapse in air, and the operculae are characteristically held tightly closed throughout air exposure. The lungs are primarily responsible for the overall increase in O₂ uptake, indicating that an increase in pulmonary ventilation and/or perfusion may be occurring. The absolute increase in cutaneous O₂ uptake during air exposure is much smaller, but is significant ($P > 0.05$) after 2 h. Berg & Steen (1965) reported a substantial cutaneous O₂ uptake in air-exposed *Anguilla vulgaris*. Hughes & Singh (1971) also stress the possible role of cutaneous respiration during air exposure in *Saccobranchus*. Increased skin perfusion could account for the doubling of cutaneous O₂ uptake in *Calamoichthys* during air exposure, but might also hasten the transcutaneous loss of water if air exposure were prolonged. Control over skin perfusion, though not yet substantiated, would increase the effectiveness of the skin as a gas exchange organ under conditions of variable humidity.

Calamoichthys, as one of the phylogenetically most primitive extant air-breathing fishes, represents an animal successfully adapted not only for air breathing but also for making short-term use of terrestrial environments. Further experiments are in progress to elucidate the physiology of air exposure in this fish.

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