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Respiratory Physiology of the Lake Magadi Tilapia (*Oreochromis alcalicus grahami*), a Fish Adapted to a Hot, Alkaline, and Frequently Hypoxic Environment

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Abstract

The tilapia Oreochromis alcalicus grahami *is a unique ureotelic teleost, the only fish that lives in the alkaline hotsprings of Lake Magadi, Kenya. Physical conditions and fish behavior were monitored in the Fish Springs Lagoon area, a site where the tilapia were particularly abundant. Water Po_2 and temperature fluctuated more or less in parallel in a diurnal cycle from less than 20 Torr and less than $25°C$ at night to greater than 400 Torr and $38°C$ during the day, whereas pH remained constant at approximately 9.8. Field laboratory tests demonstrated that routine $\dot{M}o_2$ (under normoxia) increased greatly from $27°C$ to $36°C$ (Q_{10} = 6.2) but then stabilized at a very high level ($\sim34.5\ \mu mol\ g^{-1}\ h^{-1}$) up to the lethal temperature ($\sim42.5°C$), a pattern that was adaptive to the natural diurnal regime. The Po_2 threshold for survival during acute exposure ($\leq1\ h$) was approximately 16 Torr. $\dot{M}o_2$ from water was well maintained down to a Po_2 of 60 Torr, below which it declined. Under such hypoxic conditions, the fish performed supplementary surface breathing when allowed access to air. Both the better oxygenated surface layer and air bubbles were inspired, resulting in significant uptake of O_2. The Po_2 threshold for surface breathing was 1.8-fold higher at $37.5°C$ than*

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at 31°C. Surface breathing and voluntary entry of fish into air were observed in the field. The blood O_2 dissociation curve at 30°–32°C was hyperbolic, with a high affinity (P_{50} = 6 Torr), low cooperativity (Hill coefficient = 1.18), and no Bohr effect over the extracellular pH range 8.2–8.6.

Introduction

The tilapia *Oreochromis alcalicus grahami* is the only fish species known to exist in the highly alkaline pools fed by geothermal springs at Lake Magadi in the Rift Valley of Kenya (Coe 1966). This fish thrives in a hostile environment (water of pH 9.6–10.4, osmolality = 525 mOsmol kg^{-1}, Cco_2 = 180 mmol L^{-1}, temperature ≤ 42°C), with intense predation by birds. Coe (1966) and Trewevas (1983) have described the physical properties of the lake and much of the basic biology of *O. alcalicus grahami,* while Reite et al. (1974) have documented its resistance to high pH and high temperature. Earlier studies have investigated various aspects of its ionoregulatory physiology (Leatherland et al. 1974; Johansen et al. 1975; Maetz and De Renzis 1978; Maloiy et al. 1978; Skadhauge et al. 1980; Eddy et al. 1981; Eddy and Maloiy 1984). More recent studies have concentrated on its unique ability to excrete all nitrogenous waste as urea rather than ammonia through expression of a full complement of ornithine-urea cycle enzymes in the liver and the possible relationship of this trait with acid-base balance (Randall et al. 1989; Wood et al. 1989, 1994; Wright et al. 1990; Walsh et al. 1993; Laurent et al. 1995).

Rather less is known about the respiratory physiology of *O. alcalicus grahami.* Blood pH's, both extracellular and intracellular, are high relative to the predictions of standard pH versus temperature relations in fish (Cameron 1984), reflecting low Pco_2 and high HCO_3^- levels (Johansen et al. 1975; Johnston et al. 1983; Wood et al. 1989, 1994). The only study on O_2 transport in this species used a dialyzed hemolysate of whole blood rather than intact red cells (Lykkeboe et al. 1975). At 35°C, O_2 affinity of this stripped hemoglobin was high (P_{50} = 4 Torr) and there was no Bohr effect over what is the probable physiological pH range (7.5–8.5). Despite the high environmental temperature, the hemoglobin does not exhibit unusual thermostability (Franklin et al. 1994). Maina (1990) reported that respiratory surface area of the gills was unusually high and diffusion distance low, presumably adaptations to hypoxia and/or high metabolic rates.

The present study investigates several aspects of the respiratory physiology of this unique species. Our first objective was to characterize the temperature and O_2 conditions faced by *O. alcalicus grahami* in its natural environment and to relate this to the fish's behavior. The Fish Springs Lagoon area (see

Coe 1966), employed as the collection site in this and virtually all previous studies on the Magadi tilapia, was chosen as the study site. We suspected that large diurnal fluctuations in dissolved O_2 might occur because the lagoon supports an abundant growth of cyanobacteria (the principal food source for the fish). Cyanobacteria perform photosynthesis and respiration during the day but only the latter at night. The second objective was to determine the effect of environmental temperature and P_{O_2} on the oxygen consumption rate ($\dot{M}_{O_2}$) of these fish and to assess their resistance to hypoxia. A third, and related, objective was to investigate the possible respiratory significance of apparent air and/or surface breathing that we had observed in fish in the lagoon during our first several days at Lake Magadi and the effects of water P_{O_2} and temperature on this behavior. The final objective was to determine the blood O_2 transport characteristics (P_{50}, pH sensitivity) of intact, freshly collected whole blood of *O. alcalicus grahami* for comparison with the earlier observations of Lykkeboe et al. (1975) on stripped hemoglobin. All experiments were performed in a makeshift outdoor laboratory on fish freshly collected from the wild.

Material and Methods

Experimental Animals

Small adult *Oreochromis alcalicus grahami* (1–4 g) used in most experiments were collected with a seine in January and February, 1992, from the Fish Springs Lagoon site on Lake Magadi, Kenya, described by Coe (1966). Much larger adult fish (10–40 g) used as blood donors for the O_2 dissociation curve determinations were seined from a cement holding tank supplied with a continuous flow of lagoon water. An incidental population of *O. alcalicus grahami* lives in the tank, the initial stock having been pumped in with the water. Because this population is protected from intense avian predation by a chain-link fence and by the water depth in the tank, average fish size is much larger than in the lagoon population. Fish were immediately transported to an outdoor laboratory set up on the porch of the chemistry building of the Magadi Soda Company and transferred to fresh aerated lagoon water in 20-L buckets kept in the shade. Temperature ranged from 30° to 36°C diurnally, similar to that at the fish collection site (see Results). These fish are extremely territorial and aggressive; we found higher survival rates when fish density was less than 10 fish per 20 L and when the bucket was covered. Fish were deprived of food after capture and used within 24 h for experiments; survivors were returned to the wild.

Diurnal Dissolved O_2 Concentrations

Figure 1 is a map of the Fish Springs Lagoon field site, with individual data collection points depicted for Po_2, pH, and temperature measurements. The site has been modified by a retaining wall and an overflow dam of many years' standing (Coe 1966); the barrier serves to collect water for the carbonate trona processing operations of Magadi Soda Company. Some of this water is pumped elsewhere, and overflow from the pump house collects in a large pond. Temperature, pH, and Po_2 of the Fish Springs Lagoon sites (typical depth = 1 m) were determined by a mercury thermometer and a YSI Model 54 pH and O_2 meter; the standard polarographic membrane O_2 probe was calibrated with air, with appropriate corrections for ambient temperature and the 700-m elevation of the site. The dissolved O_2 (DO), pH, and temperature readings were collected on most days in January, and systematic measurements over discrete intervals and depths were made January 22 and 29–30. Measurements were made at 14 sites at the surface (5–10 cm below surface) and bottom (2–3 cm from bottom). DO values were converted to Po_2 with appropriate salinity-corrected solubility coefficients from Boutilier et al. (1984).

Effect of Temperature on $\dot{M}o_2$

Closed-system respirometry in 530-mL Tusker chambers (amber Tusker beer bottles fitted with aeration tubes and ports for drawing 1-mL syringe samples anaerobically) kept in the shade was used to determine the effect of temperature on routine $\dot{M}o_2$ of *O. alcalicus grahami*. We use the term "routine" as defined by Fry (1957) to refer to $\dot{M}o_2$ in unfed fish with spontaneous rather than directed movements. Small adult fish (1–4 g) were loaded into Tusker chambers filled with continuously aerated lagoon water at approximately 27°C and then allowed to settle for 30 min. Aeration was then stopped, and the chambers were sealed, with care taken not to trap any air bubbles, thereby eliminating any attempts of the fish to obtain O_2 from the air or the air-water interface. Subsequent runs were done on the same fish with fresh lagoon water at higher temperatures (up to 42°C, water preheated by exposure to direct sunlight) as ambient temperature increased over the day in the outdoor laboratory. The decrease in water Po_2 over 10–60-min intervals was monitored with a Radiometer E5046 O_2 electrode attached to a pHM 71 meter thermostated to the experimental temperature. The Po_2 calibration at time zero was based on the current experimental temperature, with a correction factor of 1.03 for measuring the Po_2 of water when calibrating on air. A chamber containing water but no fish was included in each series to serve as a blank.
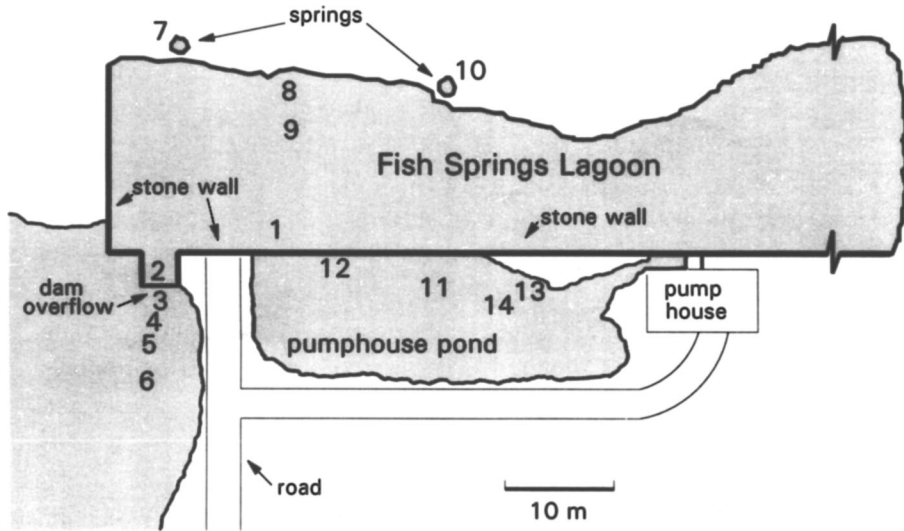
Fig. 1. A diagrammatic map of the Fish Springs Lagoon area on the edge of Lake Magadi, Kenya, indicating the 14 monitoring sites for water temperature and partial pressure of oxygen (Po_2). See Table 1 for the range of values for each site.

Effect of Temperature on Threshold Po_2 for Surface Breathing

Small adult fish (1–4 g) were placed into aerated Tusker chambers at either 31°C ($N = 12$) or 37.5°C ($N = 11$) and allowed to settle for 1 h. Aeration was then stopped, and an air bubble introduced into the neck of the chamber. The fish were then observed continuously as they depleted the O_2 from the water. Pco_2 did not increase, because of the very high absorptive capacity of the water for CO_2 at pH 10. As soon as the fish was observed to first breathe from the air or air-water interface, a water sample was drawn for measurement (as above) of the threshold Po_2 for this behavior.

Effect of Water Po_2 on $\dot{M}o_2$

Flow-through respirometry was used to measure changes in $\dot{M}o_2$ in response to changes in Po_2. Respirometers were darkened 50-mL centrifuge tubes cut off at one end and sealed with rubber stoppers; three-way valves at both ends facilitated anaerobic sampling of inflow and outflow water into 1-mL syringes. A 20-L bucket filled with aerated lagoon water at 31°–32°C served as a head tank. Flow rates were adjusted by volumetric measurement to 9–11 mL min^{-1}. Small adult fish (2–4 g; $N = 12$) were allowed to settle for about 1.5 h as the respirometers were flushed with near-saturated water ($Po_2 > 130$ Torr, where 1 Torr = 133.322 Pa) before control $\dot{M}o_2$ measurements

were made in duplicate. The P_{O_2} was then lowered to a new constant level by gassing the head tank with N_2; the fish was allowed to adjust to the new P_{O_2} for about 30 min, and then $\dot{M}_{O_2}$ was measured again in duplicate. Each fish was exposed to seven or eight levels of P_{O_2} between 135 and 25 Torr. In each case, the exposure P_{O_2} was taken as the mean of the measured inflow and outflow P_{O_2} values.

Critical P_{O_2} for Survival

In the course of a separate study comparing urea excretion and $\dot{M}_{O_2}$ (Wood et al. 1994), $\dot{M}_{O_2}$ measurements were made by closed-system respirometry in 530-mL Tusker chambers on several hundred small adult fish (1–5 g) at 30°–33°C. Seven of these fish died because the water P_{O_2} was accidentally permitted to fall too low. Water P_{O_2} measurements were made within 5 min of death for these fish and recorded as the critical P_{O_2} for survival. In addition, records were kept of unusually low P_{O_2}'s at which fish survived and of P_{O_2}'s associated with death of some individuals in the preceding flow-through respirometry experiments.

Behavioral Observations of Respiration

A 20-L glass aquarium was built for behavioral observations of *O. alcalicus grahami* in aerated and nonaerated Fish Springs water, which was changed daily. The temperature was allowed to fluctuate diurnally with ambient air temperature. The sides were masked to reduce the effects of outside stimuli on behavior, and the top was left open for natural light cycles. One to three fish were held in the aquarium at any one time. We noted periods of air or surface breathing and measured the incidental water temperature and P_{O_2}. We also noted any other unusual behaviors by the fish.

$\dot{M}_{O_2}$ from Water and Air during Hypoxic Conditions

In the laboratory, air breathing was observed only at times of low water P_{O_2}. Therefore, the objective of this series of experiments was to partition $\dot{M}_{O_2}$ from air versus $\dot{M}_{O_2}$ from water during aquatic hypoxia and to compare the total $\dot{M}_{O_2}$ with that seen under normoxic control conditions. Flow-through respirometry was used for $\dot{M}_{O_2}$ from water (to maintain near-constant water P_{O_2}) and closed-system respirometry for $\dot{M}_{O_2}$ from air. Smaller, 350-mL Tusker chambers were set up on a flow-through basis (9–11 mL min^{-1}), with water entering at the bottom and leaving by siphon close to the top. In addition to sampling ports for water inflow and outflow, an additional port allowed sampling of a 10-mL air bubble placed in the neck of the

chamber. The placement of the bubble was designed to minimize contact between water and air yet still allow the fish to access it.

Small adult fish (2–5 g; $N = 7$) were allowed to settle for at least 1 h as the respirometers were flushed with water of Po_2 greater than 120 Torr at 31°–32°C from the head tank (as described above). This produced a mean water Po_2 of about 110 Torr in the respirometers. $\dot{M}o_2$ was measured in duplicate, and then the inflowing Po_2 was lowered to 50 Torr, which produced a mean water Po_2 of about 40 Torr in the respirometers. A 10-mL air bubble was introduced into the neck of the chamber. After a 1-h adjustment period, $\dot{M}o_2$ from air was measured by monitoring the depletion of O_2 from the air bubble over the next hour, and $\dot{M}o_2$ from water was measured by flow-through respirometry twice during the period. Appropriate blank chambers were run to correct for the very slight transfer of O_2 from the air phase to the water phase. Measurements of both air and water Po_2's were made with the Radiometer O_2 electrode, as described above.

Blood O_2 Dissociation Curves

A blood pool of 5.5 mL was obtained from 16 larger adult fish (10–40 g). Fish were first anesthetized in the anesthetic Transmore as described by Wood et al. (1994), and blood was drawn from the hemal arch into syringes prerinsed with 1,000 i.u. mL^{-1} sodium heparin (Richter) in 180 mmol L^{-1} NaCl. The blood was diluted about 15% by this heparinization. The blood was then divided into two 2.75-mL pools and equilibrated with either air or N_2 in a homemade tonometry setup consisting of two 10-mL volumetric flasks suspended in a water bath at 30°–32°C and mounted on a shaker table. Tonometry gas was bubbled first through 180 mmol L^{-1} NaCl at the experimental temperature so as to match vapor pressure with that of the blood. A preliminary experiment demonstrated that there was no loss or gain of volume over 4 h of equilibration. Blood O_2 dissociation curves were constructed through the mixing technique of Scheid and Meyer (1978). Various aliquots of oxygenated and deoxygenated blood were mixed in a modified gastight 250-μL Hamilton syringe by using a small bead of mercury; 10 mixtures in total were prepared in this way. The Po_2 of each mixture was measured with a Radiometer E5046 O_2 electrode thermostated to the experimental temperature, and the percentage saturation was calculated. The procedure was then repeated after adding 6 μmol HCl (in 3 μL of 180 mmol L^{-1} NaCl) per milliliter of whole blood to each tonometer flask, so as to assess the extent of the Bohr effect.

In both control and acidified runs, extracellular pH and red blood cell intracellular pH (freeze-thaw method of Zeidler and Kim [1977]) were determined on the 0%, 50%, and 100% saturation mixtures, and hematocrit

was determined gravimetrically after spinning at 13,000 g for 2 min. The plasma from the hematocrit determinations was decanted and used to measure plasma total CO_2 concentrations by the method of Cameron (1971), and plasma protein concentration by refractometry (Alexander and Ingram 1980). Radiometer pH (E5021) and CO_2 electrodes (E5036) were used for these determinations and displayed on the same Radiometer pHM 71 meter as the O_2 electrode.

Results

Diurnal Temperature and Po_2

The Po_2 levels varied in approximate synchrony with temperature at all 14 sites in Fish Springs Lagoon (Fig. 1). Very probably, this was a result of net photosynthetic production of O_2 during the day and consumption at night by cyanobacteria. The pH of this extremely well buffered water did not significantly vary spatially or temporally; mean pH for all 14 sites was 9.84 (range = 9.76–9.97). *Oreochromis alcalicus grahami* were present at all 14 sites, sometimes in such quantity that the water appeared dark in areas where the fish congregated in large masses. In Table 1 we present the absolute range of surface data for temperature and Po_2 at each site, although we did obtain even lower values for Po_2 in a few bottom samples. In general, there was little variation with depth, as the water was well mixed by the high inflow from the springs and by frequent high winds combined with the shallow depth of the lagoon (<1 m). Temperatures in 12 of 14 sites fluctuated diurnally, ranging from a low of 22°C recorded just before dawn to a high of 38°C recorded in midafternoon. The temperature in the two other sites (small warm springs feeding the main lagoon) remained fairly constant (35°–37°C), but Po_2 did fluctuate diurnally at these sites from 0.30 mg L^{-1} (7.2 Torr) during the night to 3.16 mg L^{-1} (77.1 Torr) at midmorning. Figure 2 shows surface temperature and Po_2 data for one site (no. 11) where fish were particularly abundant and that is representative of all 12 sites in which temperature fluctuated over a 24-h period. Po_2 fluctuated diurnally with temperature at this site; the lowest Po_2 was 18.9 Torr at 25°C at midnight, and the highest Po_2 was 402.4 Torr at 35.5°C in midafternoon.

Behavioral Observations

Many different fish behaviors observed in both the field and the lab appeared to be related to respiration. Fish congregated in dense numbers in the more oxygenated water just below the dam overflow (sites 2 and

3; Fig. 1) during both day and night, even though piscivorous birds used this site for feeding during daylight hours. Other areas in and adjacent to the lagoon (e.g., pump house pond sites 11–14) were supersaturated (hyperoxic) during the day and almost anoxic at night (Table 1, Fig. 2) but still contained abundant fish. In general, fish were most numerous in the shallow, warmest areas during the hottest part of the day and re-treated to deeper areas at night.

We collected fish by seining in the early morning (0700–0800) when temperatures were lower than those recorded at any diurnal peak (Fig. 2). While surface breathing was seen only occasionally before seining, many fish that had escaped the seine were observed breathing at the surface for up to 30 min after seining. At times, the mouths protruded above the water surface and the fish seemed to be gulping. This did not appear to be a feeding behavior. *Oreochromis alcalicus grahami* were normally observed grazing on periphytic cyanobacteria; we are not aware of filter feeding in these fish. It is interesting that fish in the pump house pond (sites 11–14) were observed during the day repeatedly jumping

TABLE 1

Range of values for P_{O_2} and temperature at 14 sites in the Fish Springs Lagoon area of Lake Magadi, Kenya in January 1992

Site	P_{O_2} Range (Torr)	Temperature Range (°C)
1	67.7–242.9	31.0–36.5
2	58.8–192.5	31.0–37.0
3	91.5–175.6	30.0–36.0
4	51.5–144.6	25.0–36.0
5	15.1–130.8	25.0–36.0
6	8.3–127.5	24.0–36.0
7	26.5–76.5	34.5–37.0
8	62.6–223.5	31.0–38.0
9	59.5–202.5	31.0–37.0
10	7.2–77.1	35.0–37.0
11	18.9–402.4	23.0–38.0
12	31.1–220.6	28.0–35.0
13	19.8–390.5	24.0–38.0
14	12.7–378.5	23.0–35.0

Note. Depth, 5–10 cm below surface. See Fig. 1 for site locations.
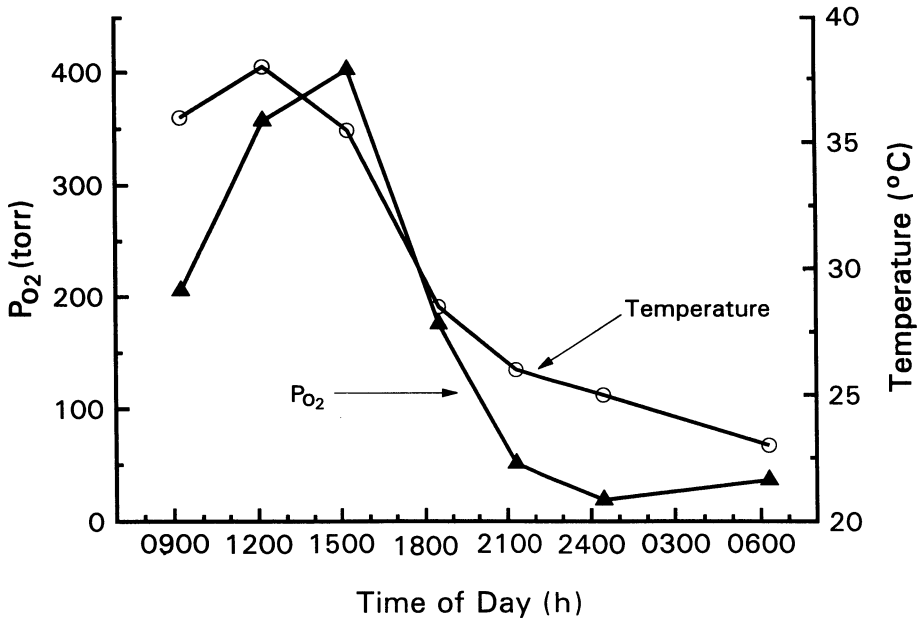
Fig. 2. Fluctuations in water temperature and P_{O_2} (depth = 5–10 cm be-
low surface) over a 24-h cycle January 29–30, 1992, at site 11 (see Fig.
1), a region with an abundant fish population, at Fish Springs Lagoon
area on the edge of Lake Magadi, Kenya.

entirely out of the water to bite off pieces of cyanobacteria growing in
mats down the side of the damp stone retaining wall that separated the
higher water level in the Fish Springs Lagoon from that in the pump
house pond (see Fig. 1). A graze line on the cyanobacterial mat could
be seen above the water level.

In the lab, we observed fish gulping at the surface in the 20-L holding
buckets when densities were too high or aeration inadequate. This be-
havior became more common in the late afternoon as water warmed up
over the day in the outdoor laboratory. The glass aquarium allowed closer
observation of the surface breathing events. While at times the fish ap-
peared to be skimming the better oxygenated surface water, we clearly
observed many events where the entire mouth was out of the water and
in which air bubbles were later released from the mouth and operculi
underwater. We conclude that these fish are not just surface skimming
but are actually breathing air as well (though perhaps mixed with water).
When males in breeding coloration were placed in the aquarium, they
were extremely territorial and spent much of their time in high-energy
activities such as chasing, charging, and locking mouths. Under normoxic

conditions, these behaviors were not followed by surface breathing. However, territorial behavior ceased when hypoxic conditions forced fish to surface breathe.

Temperature Effects on $\dot{M}_{O_2}$

$\dot{M}_{O_2}$ was extremely sensitive to temperature over the naturally occurring range. In a single batch of fish observed during the day as ambient temperature increased, routine $\dot{M}_{O_2}$ under normoxic conditions increased progressively from about 6.6 μmol g^{-1} h^{-1} at 27°C to about 34.5 μmol g^{-1} h^{-1} at 36°C (Fig. 3). Above 36°C, $\dot{M}_{O_2}$ leveled off abruptly; temperature had no significant effect between 36° and 41°C. We were able to measure routine $\dot{M}_{O_2}$ at a mean temperature of 41.2° $\pm$ 0.3°C, but at 42.5°C, fish begin dying. The effective Q_{10} for the temperature range of 27°–36°C was 6.2, a very high value.

Temperature Effects on Threshold P_{O_2} for Surface Breathing

Temperature also had a pronounced effect on the P_{O_2} threshold for surface breathing, which increased significantly from 29.6 $\pm$ 1.9 ($N = 12$) Torr at 31°C to 54.7 $\pm$ 9.5 ($N = 11$) Torr at 37.5°C.

Critical P_{O_2} for Survival

In the closed-system respirometry experiments (no access to air) at 30°–33°C, seven fish died when P_{O_2} was allowed to drop below the required O_2 level for survival. The mean P_{O_2} measured at the time of death was 12.6 $\pm$ 0.9 (range = 8.0–15.5) Torr. The three lowest P_{O_2}'s recorded that did not cause death were 16.5, 17.5, and 17.5 Torr, so the threshold was well defined at about 16 Torr.

Effect of Water P_{O_2} on $\dot{M}_{O_2}$

$\dot{M}_{O_2}$, measured at 31°–32°C by flow-through respirometry, increased slightly as the P_{O_2} of the water was initially decreased from fully saturated down to about 110 Torr, but metabolic rate did not change significantly between 140 and 60 Torr (Fig. 4). $\dot{M}_{O_2}$ then rapidly declined as P_{O_2} was decreased in stages below this level. At a P_{O_2} of less than 50 Torr, some fish began to show signs of respiratory stress, and five of the 12 fish died at a mean P_{O_2} of 31.6 $\pm$ 4.6 (range = 20–47.5) Torr, while the other seven remained alive at a P_{O_2} of about 25 Torr, the lowest level tested. These results indicate a somewhat higher critical P_{O_2} for survival than in the closed-system respi-
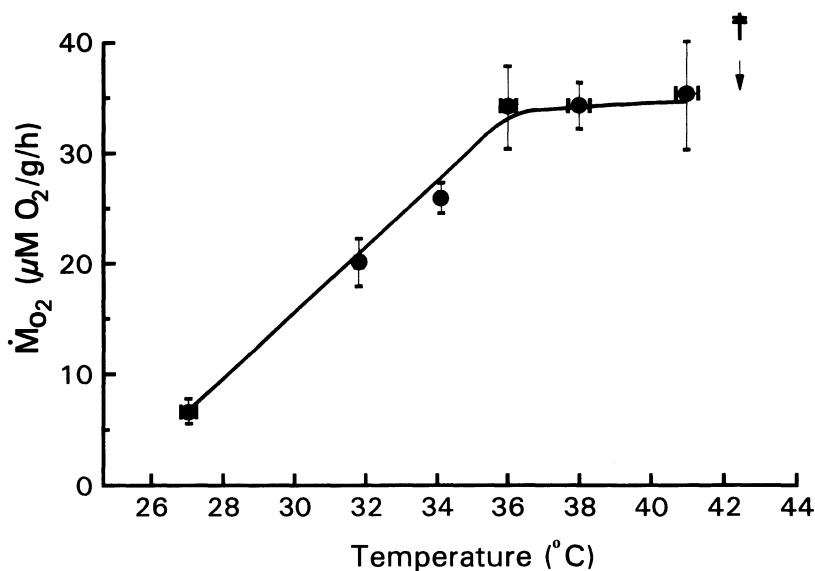
Fig. 3. The influence of environmental temperature on $\dot{M}_{O_2}$ *in individual* Oreochromis alcalicus grahami, *as measured by closed-system respirometry. The same batch of fish (N = 11) was followed at progressively higher temperatures as ambient temperature increased over the day in the outdoor laboratory. Error bars show means* ± *1 SEM for both* $\dot{M}_{O_2}$ *and temperature. Arrow indicates that fish started to die at 42.5° C. The outcome of ANOVA followed by Duncan's multiple range test on temperature values of ~27° C, ~32° C, ~34.5° C, ~36° C, ~38° C, and ~41.5° C indicated that the following values are not significantly different from one another at* P = 0.05: *the ~32° C and the ~34.5° C values; the ~34.5° C and the ~36° C values; the ~36° C, the ~38° C, and the ~41.5° C values; and the ~34.5° C and the ~41.5° C values.*

rometry experiment, but the protocol was different, involving exposure to progressive hypoxia over 5–7 h rather than in 1 h or less.

Po₂ from Water and Air during Hypoxic Conditions

When mean water P_{O_2} was reduced from about 110 Torr to about 40 Torr at 31°–32°C in the flow-through respirometry system, five of the seven fish tested used the air bubble for surface breathing. Total $\dot{M}_{O_2}$ fell significantly by about a third relative to the normoxic values; of the remaining $\dot{M}_{O_2}$, 12.5% was taken from air and 87.5% from water (Fig. 5).
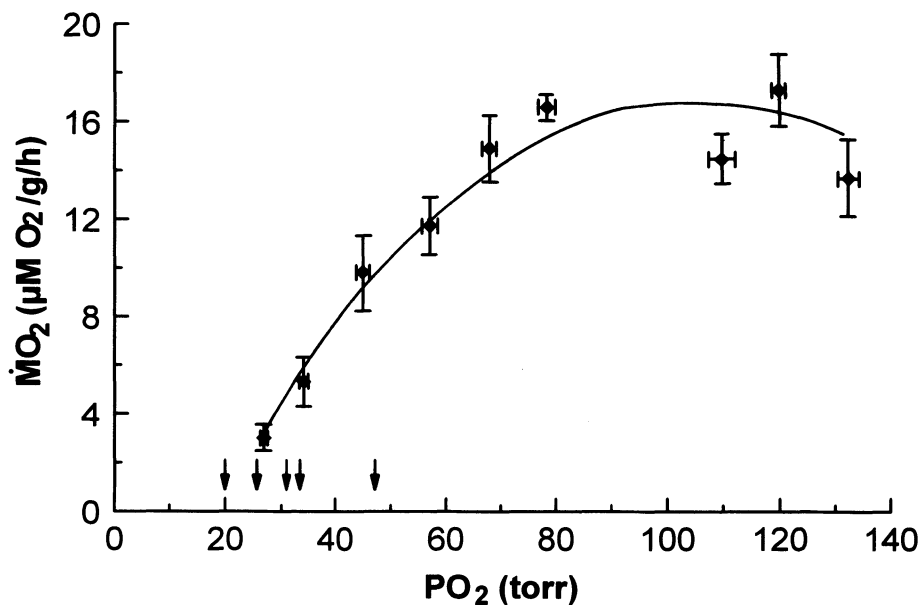
Fig. 4. The influence of environmental Po_2 on $\dot{M}o_2$ at $31°-32°C$ in indi-
vidual Oreochromis alcalicus grahami as measured by flow-through respi-
rometry. Po_2 was taken as the mean of the inflow and outflow values of
the respirometer. The same batch of fish (N = 12) was exposed at approx-
imately hourly intervals to seven or eight progressively lower levels of Po_2
between about 135 and 25 Torr. Error bars show means ± 1 SEM for both
$\dot{M}o_2$ and Po_2. Arrows indicate Po_2 levels at which individual fish died.
The outcome of ANOVA followed by Duncan's multiple range test on $\dot{M}o_2$
values of ~3.0, ~5.3, ~9.8, ~11.7, ~13.7, ~14.4, ~14.8, ~16.5, and
~17.3 indicated that the following values are not significantly different
from one another at P = 0.05: the ~3.0 and ~5.3 values; the ~9.8 and
~11.7 values; the ~11.7, ~13.7, ~14.4, and ~14.8 values; and the
~13.7, ~14.4, ~14.8, ~16.5, and ~17.3 values.

Blood O_2 Dissociation Curves

The blood O_2 dissociation curve of O. alcalicus grahami at 30°–32°C was
hyperbolic, with very low cooperativity (Hill coefficient = 1.18) and a high
O_2 affinity (P_{50} = 6 Torr at mean extracellular pH 8.58) (Fig. 6). When the
mean extracellular pH was experimentally lowered to 8.19 with HCl, there
was no Bohr shift: P_{50} remained at 6 Torr. There was no detectable red cell
swelling associated with acidification or oxygenation and deoxygenation;
hematocrit averaged 18.4% and plasma protein 2.25 g $(100 \text{ mL})^{-1}$. There
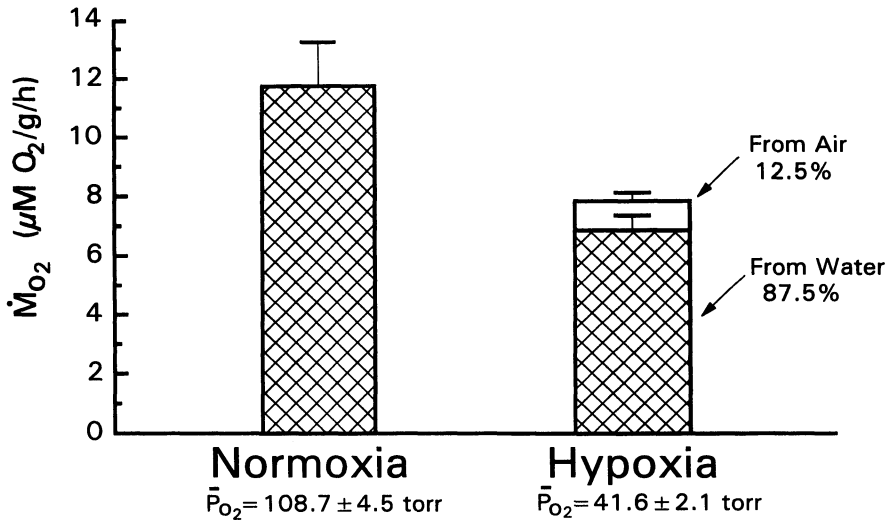was also no detectable effect of oxygenation and deoxygenation on plasma

Fig. 5. *The partitioning of routine* $\dot{M}_{O_2}$ *at 31°–32° C from air and water in* Oreochromis alcalicus grahami *under normoxic and hypoxic conditions (mean* P_{O_2} *levels shown). See text for experimental details. At both* P_{O_2}'s, *means* ± *1 SEM are reported for the five fish (of seven tested) that exhibited surface breathing at the lower* P_{O_2}.

C_{CO_2} in either control (mean = 8.20 mmol L^{-1}) or acidified runs (1.91 mmol L^{-1}). In contrast, the pH measurements (Table 2) indicated the presence of a Haldane effect, with deoxygenation causing increases in red blood cell intracellular pH of 0.2 to 0.4 units; effects on extracellular pH were not clear-cut.

Discussion

To our knowledge, no other fish is able to live in this particular combination of very high pH, very high alkalinity, and extreme diurnal fluctuations in DO and temperature. The present results cast some light on the respiratory adaptations that allow *Oreochromis alcalicus grahami* to thrive in this hostile environment. These include (1) a high sensitivity of routine metabolism to temperature over part of the range and insensitivity over another part, which is matched to the natural diurnal temperature and O_2 regime; (2) an ability to regulate routine $\dot{M}_{O_2}$ over a fairly wide range of environmental P_{O_2}; (3) short-term tolerance of extremely low environmental P_{O_2}; (4) supplementary air and water surface breathing at times of low O_2 availability and/or
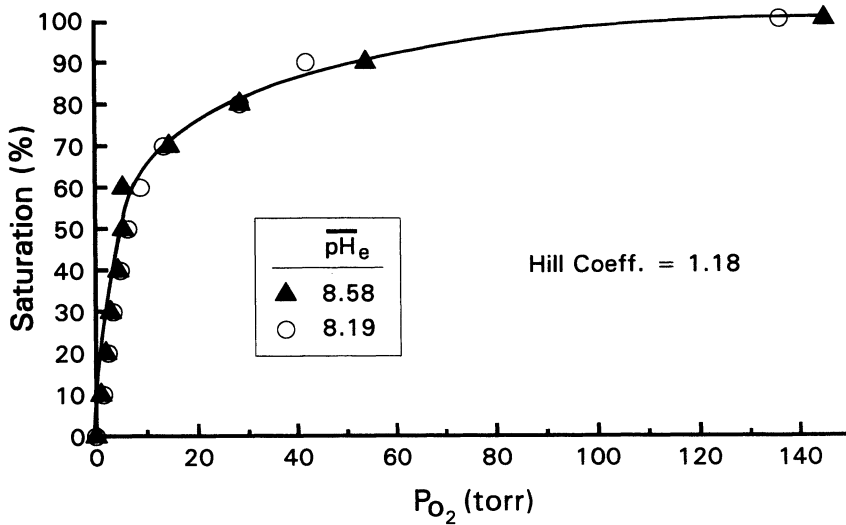
Fig. 6. The oxygen dissociation curve of the blood of Oreochromis alcali-
cus grahami *(pooled from 16 fish) at 30°–32° C, equilibrated with air.
There was no detectable Bohr shift (P_{50} remained at 6 Torr) or other al-
teration in the curve when the blood was acidified from a mean extra-
cellular pH (pHe) of 8.58 (triangles) to 8.19 (circles) with HCl.*

high O_2 demand; and (5) a blood O_2 dissociation curve with high O_2 affinity
and low pH sensitivity.

Routine $\dot{M}O_2$ and the Effects of Temperature and Po_2 in Relation to the Natural Regime

Routine $\dot{M}O_2$ has been studied extensively in other species of *Oreochromis*
(e.g., *O. nilotica(us):* Farmer and Beamish [1969]; Magid and Babiker [1975];
Verheyen et al. [1985]; Fernandes and Rantin [1989]; *O. mossambica(us):*
Job [1969*a*, 1969*b*]; Kutty [1972]; Caulton [1978]; *O. mossambicus* × *horno-
rum:* Febry and Lutz [1987]; *O. rendelli:* Caulton [1977]) and very recently
in *O. alcalicus grahami* by Franklin et al. (1995), who examined the size
dependence of routine metabolism at 37°C. The results of the present study
are rather different from those on other tilapia in several respects but in
relatively good agreement with those of Franklin et al. (1995) on *O. alcalicus
grahami.* For example, we recorded a routine $\dot{M}O_2$ of about 34.5 μmol g^{-1}
h^{-1} for 2-g fish at 36°–38°C (Fig. 3), while their reported allometric equation
yields a routine $\dot{M}O_2$ (after a longer settling period) of about 28 μmol g^{-1}
h^{-1} for 2-g fish at 37°C. Upon acutely raising temperature from 37° to 42°C,
they recorded a small increase (30%) in $\dot{M}O_2$, whereas we found no signif-

Table 2

The influence of oxygenation status on plasma pH (pHe) and red blood cell intracellular pH (pHi) in the blood of Oreochromis alcalicus grahami at 30°–32°C

% O_2 Saturation	0%	50%	100%
Control:			
pHe	8.56	8.60	8.59
pHi	7.91	7.96	7.69
Acidified:			
pHe	8.23	8.26	8.08
pHi	7.78	7.63	7.41

Note. Duplicate measurement was taken for pHe and single measurements were taken for pHi on a common blood pool from 16 fish.

icant change when temperature was gradually increased over this range (Fig. 3). Both studies indicate that routine $\dot{M}O_2$ in the Magadi tilapia at the normal peak daytime temperature in the Fish Springs Lagoon area (about 38°C; Table 1, Fig. 2) is extremely high and relatively temperature insensitive.

To our knowledge, these routine metabolic rates are the highest reported in poikilothermic teleosts. This is perhaps not surprising in view of the high activity level of Magadi tilapia (we almost never observed stationary fish under normoxia, either in the wild or in the respirometers) and the high environmental temperature that would be lethal to most teleosts. Several other tilapia do tolerate this high temperature range but exhibit 25%–60% lower $\dot{M}O_2$'s at comparable temperatures above 35°C (Job 1969a, 1969b; Caulton 1977, 1978; Fernandes and Rantin 1989). A more striking difference from other tilapia is the transition from temperature insensitivity of $\dot{M}O_2$ above 36°C to marked sensitivity below this temperature (Fig. 3). The Q_{10} from 27°C to 36°C was 6.2, in comparison to 1.0–3.0 over the same range in other tilapia. This may be suitable to the natural diurnal cycle of dissolved O_2 and temperature in the Fish Springs Lagoon area. Thus, at night, when O_2 availability in water is lowest (Fig. 2) due to cyanobacterial respiration, metabolic O_2 demand is only a fraction of that during the day because of the simultaneous decline of water temperature to the 23°–28°C range (Fig. 3). Birds at Lake Magadi do not feed at night, so the need for energy expenditure for predator avoidance at this time is likely reduced. In contrast,

during the heat of the day, the fish can sustain a high metabolic rate for feeding, social, and predator escape behaviors, and this rate is more or less independent of exact environmental temperature right up to the lethal limit (~42.5°C; Fig. 3). This upper lethal temperature is almost identical to that reported in other studies on *O. alcalicus grahami* (Coe 1966; Reite et al. 1974; Johnston et al. 1994).

In their study on *Blennius pholis,* Bridges et al. (1984) found a diurnal fluctuation in P_{O_2} in intertidal rock pool sites similar to the diurnal fluctuation in our study (Fig. 2) but with two noticeable differences—no corresponding fluctuation in temperature and a pH fluctuation similar in magnitude to P_{O_2}. In comparable studies on pupfish from saline waters in the southwest United States, Stuenkel and Hillyard (1981) found that the metabolic rates of fish acclimated to freshwater and half-strength seawater increased with increased acclimation temperatures. However, the plasma osmolality of fish acclimated to seawater decreased significantly, and the fish were able to osmoregulate efficiently at higher temperatures (25°–30°C).

At the intermediate temperature where $\dot{M}_{O_2}$ was measured (31°–32°C), *O. alcalicus grahami* proved to be a good O_2 regulator, maintaining $\dot{M}_{O_2}$ until a P_{O_2} of about 60 Torr (Fig. 4). Exact quantitative comparisons with other species are difficult because of methodological considerations, as pointed out by Fernandes and Rantin (1989). For example, some authors incorrectly plot $\dot{M}_{O_2}$ against inflowing P_{O_2} rather than against directly determined inspired or averaged P_{O_2}, and techniques for defining the inflection point vary. Nevertheless, tilapia in general appear to be good O_2 regulators, with P_{O_2} dependence starting in the P_{O_2} range of 30–90 Torr (Dusart 1963; Job 1969*b;* Kutty 1972; Magid and Babiker 1975; Verheyen et al. 1985; Fernandes and Rantin 1989). The Magadi tilapia is therefore similar to other tilapia species in this respect.

We observed movement of the fish into the shallowest, warmest water where food supply and O_2 availability (due to cyanobacterial growth and photosynthesis) were greatest during the day, and a retreat to deeper, cooler water and/or sites of aeration such as the dam outflow at night. This pattern was originally noted in the Magadi tilapia by Coe (1966) and has been reported in other tilapia as well (Caulton 1982). Thus a combination of metabolic and behavioral characteristics match this fish to the severe natural regime.

Short-Term Tolerance of Severe Hypoxia and the Importance of Surface Breathing

When the above-mentioned metabolic and behavioral adaptations prove insufficient, there appear to be two more that the animal can recruit. The

first fallback mechanism, which is essentially metabolic, is an ability to resist extremely low P_{O_2} for short periods. The second, essentially behavioral, is an ability to breathe at the water surface.

The survival threshold at $30°–33°C$ for short-term hypoxic exposure (i.e., < 1 h) was well-defined at about 16 Torr, and somewhat higher ($\sim$32 Torr) when hypoxia was imposed in progressive steps over 5–7 h. In the lagoon, we observed fish living in conditions as severe as approximately 7 Torr (0.3 mg L^{-1} at 36°C), although it is likely that they did not spend long periods in such hypoxic areas. This tolerance appears to be characteristic of tilapia in general; comparable figures for short-term tolerance are approximately 10 Torr at 23°C in *Oreochromis shirana chilwae* (Morgan 1972), approximately 5 Torr at 24°C in *Oreochromis macrochir* (Dusart 1963), and almost 0 Torr at 25 °C in *Oreochromis niloticus* (Verheyen et al. 1985). This characteristic may result from an ability to suppress metabolic rate and/or a capacity for anaerobic metabolism, both of which may prove fruitful areas for future research on the Magadi tilapia.

Surface breathing (aquatic surface respiration; reviewed by Kramer and McLure [1982]; Gee and Gee [1995]) is a common adaptation to aquatic hypoxia in tropical fish. The benefits of this strategy in improving arterial oxygenation under hypoxic conditions have been shown by Burggren (1982). In some species, the behavior involves only the extraction of O_2 from the upper several millimeters of water, which are better oxygenated, while in others additional benefits are achieved by mixing water with air in the mouth, by holding air bubbles next to a well-vascularized buccal epithelium, and even by taking air into the swim bladder. Based on our observations, both in the field and in the aquarium, and on our anatomical studies of the swim bladder and buccal epithelium (Maina et al. 1996; J. N. Maina, unpublished data), we suspect that *O. acalicus grahami* exploits all of these behaviors. Both the buccal epithelium and the physostomous swim bladder are very well vascularized; the latter has surface infoldings and can be inflated by the fish when held in air, and blood draining from the swim bladder joins with the branchial arches (Maina et al. 1996). Tilapia are not usually considered to be air or surface breathers, but we have found two anecdotal reports of apparent surface-breathing behavior in other tilapia species subjected to acute hypoxia (Dusart 1963; Morgan 1972). Our observations on fish chased by seining in the lagoon suggest that surface breathing may also be used to help pay off excess postexercise O_2 consumption (formerly "O_2 debt").

Experiments at $31°–32°C$ demonstrated that under moderate hypoxia, surface-breathing fish obtained a small but significant fraction (12.5%) of their $\dot{M}_{O_2}$ from the air phase, although this was not sufficient to sustain routine $\dot{M}_{O_2}$ at normoxic levels (Fig. 5). We predicted that this fraction

would become more important at a higher temperature, but our intention to repeat the experiment at 37.5°C was thwarted by logistic problems. Instead, we conducted the simple experiment of measuring the P_{O_2} threshold at 31°C versus 37.5°C. The threshold was significantly higher (1.8-fold) at the higher temperature, in accord with these ideas.

Blood O_2 Transport Characteristics

The O_2 dissociation characteristics of *O. alcalicus grahami* (determined at 30°–32°C) exhibit an extremely high O_2 affinity (P_{50} = 6 Torr), low cooperativity (Hill coefficient = 1.18), and an insensitivity to pH in the tested range (8.2–8.6; Fig. 6). It must be noted that these curves were determined only at a P_{CO_2} close to 0 Torr (i.e., with air/N_2 equilibration only), again a logistic consequence of research in an isolated location. However, inasmuch as this fish lives in a "P_{CO_2} vacuum" caused by an external pH close to 10 (Johansen et al. 1975; Wood et al. 1989), it is likely that arterial P_{CO_2} is extremely low in vivo anyway. On the basis of reviewing studies of other teleosts at high environmental pH, we have argued elsewhere (Wood et al. 1994) that true resting arterial extracellular pH in vivo may be as high as 8.7, and true arterial P_{CO_2} is well below 1 Torr. Therefore, the conditions of the equilibrations appear realistic, and the pH range tested probably represents the normal variation between rest (8.6) and exercise (8.2).

The present results agree with those of the only previous study on the blood of this species, by Lykkeboe et al. (1975). These workers reported a P_{50} of 4 Torr, a Hill coefficient of 1.2, and the absence of a Bohr effect (and Root effect) over the pH range 7.5–8.5 at 35°C. However, what is remarkable is that Lykkeboe et al. (1975) determined these properties using a dialysed hemolysate (stripped hemoglobin), whereas we found almost identical values using intact whole blood. Assuming that the two studies can be compared, this suggests that the intracellular milieu (red blood cell intracellular pH, ions, organic phosphates) has negligible effect on O_2 transport in the Magadi tilapia, a conclusion that is supported by the intracellular and extracellular pH insensitivity (Fig. 6, Table 2). In most other species, the intracellular milieu alters the binding properties of the hemoglobin such that whole blood behaves differently from stripped hemoglobin (Perry and McDonald 1993). For example, in *O. niloticus* at 30°C, Verheyen et al. (1985) determined that whole blood exhibited a P_{50} of 23 Torr, a Hill coefficient of 1.54, and a large Bohr effect, while stripped hemoglobin exhibited a P_{50} of 3 Torr, a Hill coefficient of 1.20, and a small Bohr effect. Bridges et al. (1984) found a Bohr shift in the hemoglobin of *B. pholis,* which, like *O. alcalicus grahami,* lives in an environment with an exaggerated diurnal fluctuation in P_{O_2} . However, unlike the pH in Lake Magadi thermal pools, the pH in the intertidal rock pools fluctuates from 7.6 to 9.6 within a 24-h period.

The absence of a Bohr effect in the Magadi tilapia is not due to a failure of extracellular pH changes to alter red blood cell intracellular pH. The measurements of Table 2 indicate a fairly standard relationship between extracellular and intracellular pH, explicable by normal Donnan distribution, such that the extracellular $[H^+]$/intracellular $[H^+]$ rises as extracellular pH falls; that is, changes in red blood cell intracellular pH reflect, but are smaller than, changes in extracellular pH. Surprisingly, these measurements also suggest that there is a definite Haldane effect manifested as a 0.2–0.4-unit decrease in red blood cell intracellular pH with oxygenation, despite the lack of Bohr effect. The Haldane effect is generally considered the reciprocal of the Bohr effect: oxygenation releases Bohr protons from the hemoglobin molecule so that oxygenated blood exhibits a lower pH and Cco_2 (Jensen 1989). In the present study, the effect was seen clearly only in red blood cell intracellular pH and not in extracellular pH or plasma CO_2 measurements. Unfortunately, erythrocytic Cco_2 was not measured in these experiments. In the absence of more data, speculation on mechanisms is unwarranted, but the possible uncoupling of the Haldane effect from the Bohr effect in this species deserves further investigation.

In conclusion, our blood O_2 results reinforce the arguments of Lykkeboe et al. (1975) that the high O_2 affinity and pH insensitivity of blood O_2 binding are present for an extremely active fish with a high metabolic rate living in a frequently hypoxic environment. O_2 uptake will be maximized regardless of exercise-induced pH changes or environmental Po_2 fluctuations over a wide range. In combination with the unusually high respiratory surface area and low diffusion distance of the gills (Maina 1990), the unusual temperature sensitivity of metabolic O_2 demand, numerous behavioral adaptations, and the ability to withstand acute hypoxia and perform supplementary surface and/or air breathing, these blood characteristics allow *O. alcalicus grahami* to thrive in one of the most hostile aquatic environments on earth.

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Literature Cited

ALEXANDER J.B. and G.A. INGRAM. 1980. A comparison of five of the methods commonly used to measure protein concentrations in fish sera. J. Fish Biol. 16:115–122.

BOUTILIER R.G., T.A. HEMING, and G.K. IWAMA. 1984. Physiochemical parameters for use in fish respiratory physiology. Pp. 403–430 in W.S. HOAR and D.J. RANDALL, eds. Fish Physiology. Vol. 10. Academic Press, New York.

BRIDGES C.R., A.C. TAYLOR, S.J. MORRIS, and M.K. GRIESHABER. 1984. Ecophysiological adaptations in *Blennius pholis* blood to intertidal rockpool environments. J. Exp. Mar. Biol. Ecol. 77:151–167.

BURGGREN W.W. 1982. "Air gulping" improves blood oxygen transport during aquatic hypoxia in the goldfish *Carassius auratus*. Physiol. Zool. 55:327–334.

CAMERON J.N. 1971. Rapid method for determination of total carbon dioxide in small blood samples. J. Appl. Physiol. 31:632–634.

———. 1984. Acid-base status of fish at different temperatures. Am. J. Physiol. 246: R452–R459.

CAULTON M.S. 1977. The effect of temperature on routine metabolism in *Tilapia rendalli* (Boulenger). J. Fish Biol. 11:549–553.

———. 1978. The effect of temperature and mass on routine metabolism in *Sarotherodon (Tilapia) mossambicus* (Peters). J. Fish Biol. 13:195–201.

———. 1982. Feeding, metabolism and growth of tilapias: some quantitative considerations. Pp. 157–180 in R.S.V. PULLIN and R.H. LOWE-McCONNELL, eds. The Biology and Culture of Tilapias. International Center for Living Aquatic Resources Management, Manila.

COE M.J. 1966. The biology of *Tilapia grahami* (Boulenger) in Lake Magadi, Kenya. Acta Trop. 23:146–176.

DUSART J. 1963. Contribution a l'étude de l'adaptation des tilapia (Pisces Cichlidae) a la vie en milieu mal oxygène. Hydrobiologia 21:328–341.

EDDY F.B., O.S. BAMFORD, and G.M.O. MALOIY. 1981. Na^+ and Cl^- effluxes and ionic regulation in *Tilapia grahami,* a fish living in conditions of extreme alkalinity. J. Exp. Biol. 91:349–353.

EDDY F.B. and G.M.O. MALOIY. 1984. Ionic content of body fluids and sodium efflux *Oreochromis alcalicus grahami,* a fish living at temperatures above 30°C and in conditions of extreme alkalinity. Comp. Biochem. Physiol. 78A:359–361.

FARMER G.J. and F.W.H. BEAMISH. 1969. Oxygen consumption of *Tilapia nilotica* in relation to swimming speed and salinity. J. Fisheries Res. Board Can. 26:2807–2821.

FEBRY R. and P. LUTZ. 1987. Energy partitioning in fish: the activity-related cost of osmoregulation in a euryhaline cichlid. J. Exp. Biol. 128:63–85.

FERNANDES M.N. and F.T. RANTIN. 1989. Respiratory responses of *Oreochromis niloticus* (Pisces, Cichlidae) to environmental hypoxia under different thermal conditions. J. Fish Biol. 25:509–519.

FRANKLIN C.E., T. CROCKFORD, I.A. JOHNSTON, and C. KAMUNDE. 1994. The thermo-stability of haemoglobins from the hot-spring fish, *Oreochromis alcalicus grahami:* comparisons with Antarctic and temperate species. J. Therm. Biol. 19:277–280.

FRANKLIN C.E., I.A. JOHNSTON, T. CROCKFORD, and C. KAMUNDE. 1995. Scaling of oxygen consumption in Lake Magadi tilapia, *Oreochromis alcalicus grahami:* a fish living at 37°C. J. Fish Biol. 46:829–834.

FRY F.E.J. 1957. The aquatic respiration of fish. Pp. 1–63 in M.E. BROWN, ed. The Physiology of Fishes. Vol. 1. Academic Press, New York.

GEE J.H. and P.A. GEE. 1995. Aquatic surface respiration, buoyancy control and the evolution of air-breathing in gobies (Gobiidae: Pisces). J. Exp. Biol. 198:79–89.

JENSEN F.B. 1989. Hydrogen ion equilibria in fish haemoglobins. J. Exp. Biology 143: 225–234.

JOB S.V. 1969*a*. The respiratory metabolism of *Tilapia mossambica* (Teleostei). I. The effect of size, temperature, and salinity. Mar. Biol. 2:121–126.

———. 1969*b*. The respiratory metabolism of *Tilapia mossambica* (Teleostei). II. The effect of size, temperature, salinity, and partial pressure of oxygen. Mar. Biol. 3:222–226.

JOHANSEN K., G.M.O. MALOIY, and G. LYKKEBOE. 1975. A fish in extreme alkalinity. Respir. Physiol. 24:159–162.

JOHNSTON I.A., F.B. EDDY, and G.M.O. MALOIY. 1983. The effects of temperature on muscle pH, adenylate and phosphagen concentrations in *Oreochromis alcalicus grahami,* a fish adapted to an alkaline hotspring. J. Fish Biol. 23:717–724.

JOHNSTON I.A., H. GUDERLY, C.E. FRANKLIN, T. CROCKFORD, and C. KAMUNDE. 1994. Are mitochondria subject to evolutionary temperature adaptation? J. Exp. Biol. 195:293–306.

KRAMER D.L. and M. McCLURE. 1982. Aquatic surface respiration, a widespread adaption to hypoxia in tropical freshwater fishes. Environ. Biol. Fishes 7:47–55.

KUTTY M.N. 1972. Respiratory quotient and ammonia excretion in *Tilapia mossambica*. Mar. Biol. 16:126–133.

LAURENT P., H.L. BERGMAN, A. NARAHARA, P.J. WALSH, J.N. MAINA, and C.M. WOOD. 1995. Gill structure of a fish from an alkaline lake: effect of exposure to pH 7. Can. J. Zool. 73:1170–1181.

LEATHERLAND J.F., M. HYDER, and D.M. ENSOR. 1974. Regulation of plasma Na^+ and K^+ concentrations in five African species of *Tilapia* fishes. Comp. Biochem. Physiol. 48A:699–710.

LYKKEBOE G., K. JOHANSEN, and G.M.O. MALOIY. 1975. Functional properties of he-moglobins in the teleost *Tilapia grahami*. J. Comp. Physiol. 104:1–11.

MAETZ J. and G. DE RENZIS. 1978. Aspects of the adaptation of fish to high external alkalinity; comparison of *Tilapia grahami* and *T. mossambica*. Pp. 213–228 in K. SCHMIDT-NIELSEN, L. BOLIS, and S.H.P. MADDRELL, eds. Comparative Physiology: Water, Ions and Fluid Mechanics. Cambridge University Press, Cambridge.

MAGID A.M.A. and M.M. BABIKER. 1975. Oxygen consumption and respiratory behavior of three Nile fishes. Hydrobiologia 46:359–367.

MAINA J.N. 1990. A study of the morphology of the gills of an extreme alkalinity and hyperosmotic adapted teleost *Oreochromis alcalicus grahami* (Boulenger) with particular emphasis on the ultrastructure of the chloride cells and their modifi-cations with water dilution: a SEM and TEM study. Anat. Embryol. 181:83–98.

MAINA J.N., C.M. WOOD, A.B. NARAHARA, H.L. BERGMAN, P. LAURENT, and P.J. WALSH. 1996. Morphology of the swim bladder of a cichlid teleost: *Oreochromis alcalicus*

grahami (Trewavas, 1983), a fish adapted to a hyperosmotic, alkaline and hypoxic environment: a brief outline of the structure and function of the swim bladder. In J.S. MUNSHI and H.M. DUTTA, eds. Horizons of New Research in Fish Morphology in the 20th Century. Oxford and IBH, New Delhi (in press).

MALOIY G.M.O., G. LYKKEBOE, K. JOHANSEN, and O.S. BAMFORD. 1978. Osmoregulation in Tilapia grahami: a fish in extreme alkalinity. Pp. 229–238 in K. SCHMIDT-NIELSEN, L. BOLIS, and S.H.P. MADDRELL, eds. Comparative Physiology: Water, Ions and Fluid Mechanics. Cambridge University Press, Cambridge.

MORGAN P.R. 1972. Causes of mortality in the endemic tilapia of Lake Chilwa (Malawi). Hydrobiologia 40:101–119.

PERRY S.F. and D.G. McDONALD. 1993. Gas exchange. Pp. 251–278 in D.H. EVANS, ed. The Physiology of Fishes. CRC, Boca Raton, Fla.

RANDALL D.J., C.M. WOOD, S.F. PERRY, H.L. BERGMAN, G.M.O. MALOIY, T.P. MOMMSEN, and P.A. WRIGHT. 1989. Urea excretion as a strategy for survival in a fish living in a very alkaline environment. Nature 337:165–166.

REITE, O.B., G.M.O. MALOIY, and B. AASEHAUG. 1974. pH, salinity and temperature tolerance of Lake Magadi tilapia. Nature 247:315.

SCHEID P. and M. MEYER. 1978. Mixing technique for study of oxygen-hemoglobin equilibrium: a critical evaluation. J. Appl. Physiol. 45:818–822.

SKADHAUGE E., C.P. LECHENE, and G.M.O. MALOIY. 1980. Tilapia grahami: role of intestine in osmoregulation under conditions of extreme alkalinity. Pp. 133–142 in B. LAHLOU, ed. Epithelial Transport in Lower Vertebrates. Cambridge University Press, Cambridge.

STUENKEL E.L. and S.D. HILLYARD. 1981. The effects of temperature and salinity acclimation on metabolic rate and osmoregulation in the pupfish, Cyprinodon salinus. Copeia 2:411–417.

TREWEVAS E. 1983. Tilapiine Fishes of the Genera Sarotherodon, Oreochromis and Danakilia. British Museum of Natural History, Dorchester.

VERHEYEN E., R. BLUST, and C. DOUMEN. 1985. The oxygen uptake of Saratherodon niloticus L. and the oxygen binding properties of its blood and hemolysate (Pisces: Cichlidae). Comp. Biochem. Physiol. 81A:423–426.

WALSH P.J., H.L. BERGMAN, A.B. NARAHARA, C.M. WOOD, P.A. WRIGHT, D.J. RANDALL, J.N. MAINA, and P. LAURENT. 1993. Effects of ammonia on survival, swimming and activities of enzymes of nitrogen metabolism in the Lake Magadi tilapia (Oreochromis alcalicus grahami). J. Exp. Biol. 180:323–387.

WOOD C.M., H.L. BERGMAN, P. LAURENT, J.N. MAINA, A. NARAHARA, and P.J. WALSH. 1994. Urea production, acid-base regulation, and their interactions in the Lake Magadi tilapia, a unique teleost adapted to a highly alkaline environment. J. Exp. Biol. 189:13–36.

WOOD, C.M., S.F. PERRY, P.A. WRIGHT, H.L. BERGMAN, and D.J. RANDALL. 1989. Ammonia and urea dynamics in the Lake Magadi tilapia, a teleost fish adapted to an extremely alkaline environment. Respir. Physiol. 77:1–20.

WRIGHT P.A., S.F. PERRY, D.J. RANDALL, C.M. WOOD, and H.L. BERGMAN. 1990. The effects of reducing water pH and total CO_2 on a teleost fish adapted to an extremely alkaline environment. J. Exp. Biol. 151:361–369.

ZEIDLER R. and D.H. KIM. 1977. Preferential hemolysis of postnatal calf red cells induced by internal alkalinization. J. Gen. Physiol. 70:385–401.