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A comparative allometric study of the morphometry of the gills of an alkalinity adapted cichlid fish, *Oreochromis alcalicus grahami*, of Lake Magadi, Kenya

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Abstract. A morphometric analysis of the gills of *Oreochromis alcalicus grahami* has been carried out on specimens from ecologically distinct lagoons and a water-holding tank of Lake Magadi, a highly alkaline salt lake situated in a volcanically active region of the southern part of the Great Rift Valley in Kenya. The data were compared with those from *Oreochromis niloticus*, a close relative that lives in fresh water and with data from other fresh water and marine fish. Our primary goal was to identify the possible adaptive features which enable the fish to survive in an environment characterized by severely fluctuating levels of oxygen, a condition exacerbated by factors such as high temperature, alkalinity and osmolarity.

The specimens of *O. a. grahami* from the south-western lagoons of the lake had gills better adapted for gas exchange with a body mass specific diffusing capacity for oxygen which was about 2 times greater than that of the gills of the specimens from the fish spring lagoons and 2.5 times that of those from the water-holding tanks. Some parameters of the gills of *O. a. grahami*, e.g. the gill filament length and number of gill filaments are significantly greater than those of *O. niloticus* but the number of secondary lamellae, area of secondary lamellae and the diffusing capacity of the gills are similar in the two species. Compared with most other fish, the gills of *O. a. grahami* appear to be particularly well adapted for gas exchange especially by having a thin water-blood barrier. Perhaps in no other extant fish have the gills had to be so exquisitely designed to meet environmental extremes and regulate complex and at times conflicting functions such as gas exchange, iono-regulation, acid-base balance and nitrogenous waste excretion as in *O. a. grahami*

Key words: Lake Magadi, lagoons, tilapia, alkalinity, gills, diffusion, respiration

Introduction

Oreochromis alcalicus grahami (Trewavas, 1983) is a tilapiine cichlid fish which subsists in the small peripheral lagoons of the Kenyan Lake Magadi

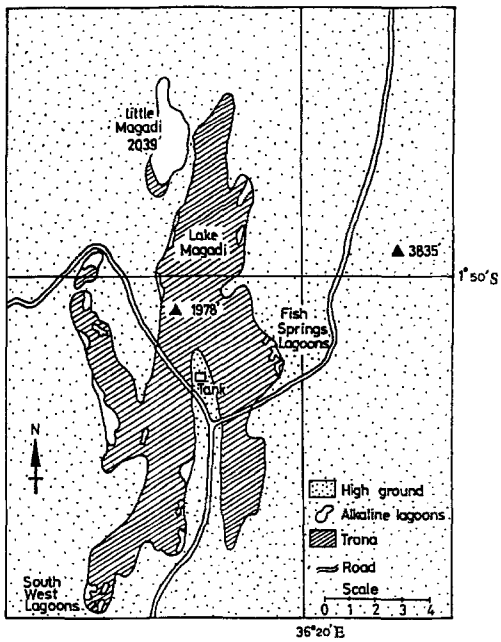

Figure 1. A map of the Magadi region showing the Greater Lake Magadi and the Little Magadi. Also shown are the three sites from which the fish were caught for the present study. These are, the Fish Spring Lagoons, the Water Holding Tank and the South-Western Lagoons. The Little Magadi is in elevation about 20 m above the Greater Lake Magadi from which it is separated by a volcanic cliff. The two lakes are only 25 m apart at their closest proximity. Scale 1 cm to 2 km.

(latitude 1°43′ to 2°00′ S; longitude 36°13′ to 36°18′ E), a tectonically highly unstable region of intense volcanic activity characterized by discharges of hot alkaline springs which merge to form hot water pools, swamps and lakes. Lakes Nakuru, Magadi, Natron and Eyasi (all in the Eastern arm of the Great Rift Valley) are considered by Talling and Talling (1965) to be the most alkaline lakes in Africa. Lake Magadi lies at an altitude of 600 m above sea level and covers an area of about 100 km^2. It essentially consists of two separate Lakes, the Greater Magadi and the Little Magadi (Figure 1). The main lake is largely covered by a thick crust of salt (trona) (Figures 2 and 3) which is recovered for commercial purposes by the Magadi Soda Company. The trona is composed of sodium salts, of which about 80% is sodium carbonate and sodium bicarbonate. The tilapia, *O. a. grahami* is the only vertebrate animal which lives in the shallow highly alkaline lagoons which are charged by scattered peripheral geothermal springs. The temperature of the water in some parts of the lagoons inhabited by the fish may be as high at 46°C (Coe, 1966), the osmolarity ranges from 525 to 600 $mOsm \cdot kg^{-1}$, the diel PO_2 is as

Figure 2. A view of Lake Magadi showing the trona (salt) which covers much of the surface of the lake. The trona is claimed by the Magadi Soda Company (PLC) for commercial purposes. The different colours of the trona are due to the algae and cyanobacteria which grow in the brine. Fish can only survive in the peripheral lagoons which are charged by volcanic springs.

high as 53 kPa (due to the photosynthetic activity of the algae) and during the night (as a result of activity of cyanobacteria) the level drops to below 3 kPa (Narahara et al., 1996), the conductivity of the water at 160,000 μmho·cm^{-1} is very high, the carbonate-bicarbonate concentration is about 200 mmol·L^{-1}, the Na^+ and Cl^- concentrations are respectively about 340 and 100 mmol·L^{-1} and the pH ranges from 9.6 to 10.5 (Fryer and Iles, 1972; Reite et al., 1974; Johansen et al., 1975; Eddy et al., 1981; Wood et al., 1989). Because of the high temperature and salinity of the water, the dissolved oxygen concentration ranged from 1.1 to 1.3 mg·L^{-1} at night (Reite et al., 1974) while due to the high buffering capacity of the water, the PCO_2 (the free CO_2) of 0.04 kPa is extremely low (Wood et al., 1989).

The adaptive stratagems of *O. a. grahami* are of great ecomorphophysiological interest and serve as a rare paradigm of the malleability of organisms in the face of environmental pressures. A number of studies have been carried out to establish the specific factors which have enabled the fish to survive in this extremely severe habitat. The ecology and the natural history of *O. a. grahami* have been reported by Coe (1966), Fryer and Iles (1972) and Trewavas (1983), the osmoregulatory and ionic metabolism by Reite et al.

Figure 3. A close up of the surface of Lake Magadi showing the thick trona which covers much of it. The water next to such sites is too hot and concentrated to support fish life.

(1974), Leatherland et al. (1974), Johansen et al. (1975), Maloiy et al. (1978), Maetz and De Renzis (1978), Eddy et al. (1981), Eddy and Maloiy (1984) and Wright et al. (1990), while the oxygen carrying characteristics of the blood were investigated by Lykkeboe et al. (1975). The gills have a relatively large number of mitochondria-rich cells with profuse intracytoplasmic microtubular network (Maina, 1990, 1991, 1996) and accessory chloride cells which frequently occur in marine fish have been reported by Laurent et al. (1995). Atypical of aquatic teleosts, *O. a. grahami* has a complete ornithine-urea cycle in the liver and excretes urea instead of ammonia (Randall et al., 1989; Wood et al., 1989, 1994; Walsh et al., 1993). The thermostabilities of the hemoglobin and muscle mitochondria, respectively, have been evaluated by Franklin et al. (1994) and Johnston et al. (1994) while the effect of temperature on muscle pH and enzymes were studied by Johnston et al. (1983). The scaling of oxygen consumption with body mass was recently investigated by Franklin et al. (1995) and the general respiratory physiology examined by Narahara et al. (1996).

The specific objectives of the present study were:

1. To quantify the structural parameters which determine the gas exchange capacity of the gills of *O. a. grahami* collected from physically isolated sites around Lake Magadi and relate them to body size and habitat.

2. Compare the gill parameters of *O. a. grahami* with those of *Oreochromis niloticus*, a close freshwater relative, to find out possible adaptations for survival in an alkaline, hyperosmotic and generally hypoxic medium and,
3. Compare the gills of *O. a. grahami* with other groups of fish (freshwater and marine) to assess possible differences or commonalities in gill functional design and size scaling.

Materials and methods

The specimens used in this study were obtained from three sites of the Greater Lake Magadi (here subsequently referred to as Lake Magadi) namely two peripheral lagoons, the Fish Springs Lagoon (FSL) and the South-Western Lagoons (SWL) and from a cement Water-Holding Tank (WHT) (Figure 1). The water contained in the holding tank is pumped from the FSL, over a distance of about 2.5 km and is used in the factory operations of the Magadi Soda Company (MSC). We believe that the fish are sucked into the pumping system at the FSL and survive the distance to the tank. The fish were caught with a seine early in the mornings (between 7.30 and 9 a.m.) in January and February, 1992: this is a particularly dry time of the year in the region. They were immediately transported to a temporary laboratory set up on the balcony of the chemical laboratory of the MSC and placed in buckets of continuously aerated lagoon water. Following completion of our experiments, the excess fish were released back into the respective collection sites.

The fish spring lagoons

The FSL are located on the north-eastern side of the Lake (Figure 1). They are the largest and the most conspicuous and accessible lagoons which contain fish. To maintain an adequate supply of water for processing the trona, a retaining wall has been constructed to hold back the water from the discharging peripheral geothermal springs, thus forming a large relatively deep (about 1.5 m) artificial lagoon. Fish can move via an overflow shute into the shallower lagoons which are closer to the main body of the lake. The daytime temperature of the water in the lagoon ranges from 35 to 38 °C and the diurnal oxygen tension in the water fluctuates remarkably: the water is supersaturated with oxygen in the afternoon while it is virtually anoxic at night (Narahara et al., 1996). The lagoons are visited by a large number of birds, mainly egrets, avocets, plovers, marabou storks, ibises, pelicans and flamingos which feed on the algae and fish.

The south-western lagoons

The SWL are scattered over a wide area of this rather shallow and rugged part of the lake. Some of the lagoons dry up during protracted spells of drought while some are too hot to contain fish. The average daytime temperature of the water in these lagoons is 40 °C. There are, however, remarkable thermal gradients within individual lagoons depending on the proximity to and size of the geothermal springs. In some areas of the lagoons, the temperature has been reported to be as high at 46 °C (Coe, 1966): we observed that most fish preferred to stay in the cooler sections with a temperature below 38 °C. The average PO_2 in these lagoons was 22 kPa. As in the FSL, the area is frequented by a large number of algae and fish eating birds. Perhaps due to the intense degree of predation, the fish seined in one of the SWL were generally very small, ranging in body mass from 0.54 to 1.29 g.

The water-holding tanks

Because the tanks are fairly deep (about 4 m), are surrounded by a wire mesh fence, and are closer to areas of human activities (as they are located at the factory site), the level of predation (at least from birds) is relatively less compared to the other two sites. Furthermore, the numerical density of fish and hence competition for resources is probably lower in the tanks than in the FSL and SWL. This may be one of the factors which allows these fish to grow to a much larger size. The largest fish seined from the FSL was about 22 g while the largest specimen from the WHT was 54 g. More than half of the fish caught from the WHT weighed more than 15 g, whereas fish of this size were extremely rare in the FSL. The daytime average temperature of the water in the WHT is 32 °C.

Fixation of the gills

Small, medium and large size fish were selected for the study. They were sacrificed, weighed and the gills carefully dissected out, mopped with tissue paper, weighed and fixed by immersion in 2.3 per cent glutaraldehyde buffered in phosphate (total osmolarity, 460 $mOsm \cdot L^{-1}$; pH 7.4).

Morphometric analysis of the gills

Gill respiratory surface area measurements and the determination of the morphometric diffusing capacity of oxygen were carried out on 7 specimens from the FSL, 6 from the WHT, and 8 from the SWL. Wet body weight ranged from 0.57 g (SWL) to 54.21 g (WHT). The gills were rinsed with physiological saline and measurements made by the method described by Muir and

Hughes (1969) and Hughes (1984, 1990). The total surface area (mm^2) of the secondary lamellae, A, was estimated as:

$$A = (2L \cdot d) \cdot ba \tag{1}$$

where L is the total length of the gill filaments (mm); d, the average number of secondary lamellae per mm on one side of a gill filament; and ba the average bilateral area of the secondary lamellae (mm^2). A binocular microscope with a micrometer eyepiece was used to determine L by measuring the length of every filament in the small fish and every third or fifth filament in the larger fish. The spacing of the secondary lamellae on one side of the filaments (the lamellar density) was determined from measurements of all the lamellae on representative filaments from all the arches on the left side of the gill system. The bilateral area of the secondary lamellae was determined by choosing lamellae at regular intervals (from some of the filaments on which length was determined) after projecting a video image of carefully dissected horizontally oriented lamellae onto a screen of a television monitor. The images were traced out and the area determined by square-counting using a square lattice acetate grid mounted on the screen. Double the value of an individual lamellar face area in real units (i.e. after adjusting for magnification) was taken to be the total area of a lamella, as both surfaces are used in gas exchange. Measurements were made on the gill arches on the left side of the body and the values doubled to estimate the total value for a specimen.

Estimation of the diffusing capacity of the gills

The morphometric diffusing capacity of oxygen was estimated using the formula:

$$D_{O_2} = A \cdot \tau ht^{-1} \cdot K_{O_2} \tag{2}$$

where, D_{O_2} is the oxygen diffusing capacity (units: $ml \cdot min^{-1} \cdot mmHg^{-1}$); A is the gill respiratory surface area (i.e., the total surface area of the secondary lamellae, equation 1); τht, is the water-blood diffusion distance (expressed as a harmonic mean thickness); and K_{O_2} is Krogh's permeation coefficient for oxygen. The traditional K for poikilotherm tissue, originally determined for frog connective tissue, of 1.1×10^{-5} $ml \cdot cm^{-2} \cdot cm^{-1} \cdot min^{-1} \cdot atmosphere^{-1}$ at 20 °C (Krogh, 1919) was used. When converted into a value readily used in calculations, this becomes 1.45×10^{-6} $ml^{-1} \cdot mm^{-2} \cdot \mu m^{-1} \cdot mmHg^{-1}$. The value of τht used in determining D_{O_2} was 0.83 μm (Maina, 1990). The procedure recognizes the fundamental fact that oxygen transfer (the conductance) across the water-blood barrier takes place entirely by diffusion. The rate of

the flux is dependent on the prevailing partial pressure gradient and on the physical characteristics of the gas exchanger, the process being directly proportional to the area ($D_{O_2} \propto A$) available for gas exchange and inversely proportional to the thickness of the barrier ($D_{O_2} \propto \tau h t^{-1}$). Krogh's constant ($K_{O_2}$) defines the material properties of the barrier tissue with respect to oxygen permeation. The constant is not affected by increase in temperature since solubility decreases as diffusivity increases, the two processes essentially cancelling each other. It is noteworthy that D_{O_2}, as estimated here in fish, takes into account only diffusion across the tissue barrier (the water-blood barrier) and does not include the diffusion through the plasma layer nor the oxygen reaction rates with the red blood cells as modeling in the mammalian lung (e.g., Weibel, 1970) and the avian lung (e.g., Maina, 1989) has entailed. Further, the model does not take into consideration the mucus lining nor the unstirred boundary water layer. The results were compared with those of specimens of *Oreochromis niloticus* of similar range of body weight investigated by Kisia (1989) and Kisia and Hughes (1992).

Allometric regressions of the form $Y = a\mathrm{W}^b$ were computed on logarithmically transformed data where Y is a respiratory variable, W the body mass and a and b are derived constants where a is the y-intercept (the value of a respiratory variable at a body mass of unity) and b the scaling factor (the magnitudinal change) between the respiratory variable and body mass. Our morphometric values were not adjusted for the shrinkage which accompanies tissue fixation with glutaraldehyde. Firstly, this was because of the comparative design of our study whereby the biological materials were affected to the same extent, and secondly because fixation with iso-osmotic glutaraldehyde results in inappreciable tissue shrinkage (Mathieu et al., 1978). Hughes (1984) reported a shrinkage of 3 per cent on fish gills fixed with Bouin, Oikawa and Itazawa (1985) observed shrinkage of 2.9 per cent for the gill filaments and 5.7 per cent for gill area, while Kisia (1989) encountered 3.7 per cent shrinkage after Bouin fixation and transfer to 50 per cent alcohol.

Results

Comparison of the gills of the specimens of O. a. grahami

Due to the extremely small and narrow body mass range of the fish from the SWL (0.57 to 1.29 g) (Table 1), their data were not plotted against those of the FSL and the WHT but among the three groups, their gill morphometric superiority is clearly evident (Table 3): while the area of the secondary lamellae (ASL) and the diffusing capacity of the gills (D_{O_2}) are similar in the FSL and WHT fish, all the values of the SWL ones are significantly greater than those

Table 1. Gill parameters of specimens of *Oreochromis alcalicus grahami* collected from different sites[a] of Lake Magadi. NSL, number of secondary lamellae; GFL, gill filament length (mm); NGF, number of gill filaments; ASL, area of secondary lamellae (mm_2); D_{O_2}, morphometric diffusing capacity ($mlO_2 \cdot mm^{-2} \cdot min^{-1} \cdot mm\ Hg$).

Site	Body Mass	NSL	GFL	NGF	ASL	D_{O_2}
FSL						
1	1.28	157,900	1,579	1,132	1,169	0.00204
2	2.15	156,152	2,142	1,234	1,273	0.00222
3	5.8	233,900	3,602	1,340	2,643	0.00462
4	8.42	236,792	4,298	1,474	3,552	0.00621
5	12.29	276,493	5,797	1,554	3,982	0.00696
6	20.59	306,751	6,727	1,724	6,166	0.0108
7	21.47	174,938	6,834	1,772	4,076	0.00712
Mean body mass normalized values		44,283 SD 40,778	640 SD 348	308 SD 306	457 SD 239	0.0008 SD 0.0004
SWL						
1	0.57	66,920	1,615	874	375	0.0012
2	0.58	58,359	1,408	760	512	0.00154
3	0.60	69,341	1,879	836	499	0.00145
4	0.63	61,011	1,696	818	641	0.0018
5	0.82	81,174	1,484	926	877	0.00187
6	0.84	88,191	1,707	962	766	0.0016
7	0.93	85,423	1,493	952	609	0.00114
8	1.29	90,361	1,227	938	904	0.00123
Mean body mass normalized values		99,755 SD 14,879	1564 SD 203	1,195 SD 249	841 SD 159	0.0015 SD 0.0003
WHT						
1	20.47	350,815	6,839	1,702	6,666	0.0116
2	28.46	413,905	9,178	1,854	12,707	0.0222
3	31.24	413,595	10,418	2,058	11,705	0.0204
4	34.56	435,367	9,465	2,150	10,101	0.0176
5	49.05	452,844	10,723	1,950	13,767	0.0240
6	54.21	525,383	10,878	2,026	14,080	0.0246
Mean body mass normalized values		12,740 SD 2982	281 SD 60	59 SD 17	330 SD 70	0.0006 SD 0.0001

[a]FSL, Fish springs lagoons; SWL, south-western lagoons; WHT, water-holding tank.

Table 2. Comparison of gill morphometric parameters of *Oreochromis alcalicus grahami* with those of *Oreochromis niloticus*.[a] NSL, number of secondary lamellae; GFL, gill filament length (mm); NGF, number of gill filaments; ASL, area of secondary lamellae (mm^2); τht, harmonic mean thickness of the water-blood barrier (μm); D_{O_2}, morphometric diffusing capacity of the gills (ml $O_2 \cdot mm^{-2} \cdot min^{-1} \cdot \mu m^{-1} \cdot$ mm Hg).

Species	Body mass	NSL	GFL	NGF	ASL	τht	D_{O_2}
O. a. grahami							
1	0.57	66,920	921	873	375	–	0.00066
2	0.58	59,360	817	760	512	–	0.00089
3	0.60	69,341	1,128	836	499	–	0.00087
4	0.63	61,011	1,069	818	641	–	0.00112
5	0.82	81,173	1,217	926	877	–	0.00153
6	0.84	88,191	1,434	962	766	–	0.00134
7	0.93	85,423	1,389	952	609	–	0.00106
8	1.28	157,900	1,579	1,132	1,169	–	0.00204
9	1.29	90,361	1,583	938	904	–	0.00158
10	2.15	156,152	2,142	1,234	1,273	–	0.00222
11	5.8	233,900	3,604	1,340	2,643	–	0.00462
12	8.42	236,792	4,298	1,474	3,552	–	0.00621
13	12.29	276,493	5,797	1,554	3,982	–	0.00696
14	20.47	350,815	6,727	1,702	6,666	–	0.01160
15	20.59	306,751	6,727	1,724	6,166	–	0.01080
16	21.47	174,938	6,834	1,772	4,076	–	0.00712
17	28.46	413,905	9,178	1,854	12,707	–	0.02220
18	31.24	413,594	10,418	2,058	11,705	–	0.02040
19	34.56	435,367	9,465	2,150	10,101	–	0.01760
20	49.05	452,844	10,723	1,950	13,767	–	0.02400
21	54.21	525,383	10,878	2,026	14,080	–	0.02460
Mean body mass normalized values		56,403 SD 44,200	889 SD 606	621 SD 580	567 SD 280	0.83[b] SD 0.21	0.00099 SD 0.00052
O. niloticus							
1	0.12	–	–	–	–	0.87	0.000194
2	0.14	–	–	–	–	0.99	0.000199
3	0.19	–	–	–	–	1.09	0.000213
4	0.31	–	–	–	–	0.87	0.000431
5	0.48	48,843	630	794	362	1.17	0.000449
6	1.13	72,086	764	782	704	0.94	0.00109
7	2.22	98,223	1,015	1,174	1,326	1.47	0.00135
8	3.89	130,769	1,952	1,324	1,622	1.18	0.00200
9	6.00	186,087	2,853	1,501	2,925	1.78	0.00238

Table 2. Continued.

Species	Body mass	NSL	GFL	NGF	ASL	τht	D_{O_2}
10	7.74	157,693	2,400	1,446	2,113	1.28	0.00239
11	10.8	210,536	3,116	1,702	4,042	1.20	0.00488
12	19.1	236,952	4,149	1,802	6,066	1.55	0.00567
13	37.2	326,717	5,389	1,986	7,515	1.83	0.00595
14	53.5	429,981	7,313	2,068	19,005	1.27	0.02170
Body mass		34,352	453	251	440	1.25[c]	0.00076
normalized		SD	SD	SD	SD	SD	SD
values		29,370	348	213	173	0.31	0.00048

[a]Data on *Oreochromis niloticus* are those of comparable range of body weight investigated by Kisia (1989) and Kisia and Hughes (1992).
[b]Mean harmonic mean thickness of water-blood barrier in *O. a. grahami* reported by Maina (1990) on six specimens.
[c]Mean of the individual specimen values.

of the other two groups. The allometric regression lines of the morphometric parameters of the FSL and WHT fish are given in Table 5.

Comparison of the gills of O. a. grahami *with* O. niloticus

The body mass normalized gill parameters of *O. a. grahami* and *O. niloticus* are given in Table 2, the allometric equations summarized in Table 6 and the plots presented in Figures 4, 5, 6, 7 and 8. The only parameters which are significantly greater in *O. a. grahami* are the GFL and NGL (Table 4). The water-blood barrier in *O. a. grahami* is significantly thinner than in *O. niloticus* (Figure 7 and Table 4) but the diffusing capacities of the gills are similar (Figure 8 and Table 4) in the two groups of fish.

Comparison of the gills of O. a. grahami *with other fish species*

The total gill filament length *O. a. grahami* is comparable to that of the highly aerobic tuna (Figure 6) but greater than that of other species reported by Hughes (1966) and Hughes and Morgan (1973) such as the horse mackerel (*Trachurus trachurus*), the tench (*Tinca tinca*), the brown trout (*Salmo trutta*) and the European eel (*Anguilla anguilla*) (Figure 6). The number of secondary lamellae (Figure 5) and the total area of the secondary lamellae (Figure 4) in *O. a. grahami* are lower than in the tuna and the mackerel but comparable to the rainbow trout, *Oncorhynchus mykiss*, the carp (*Cyprinus carpio*), the

Table 3. Statistical comparisons[a,c] of the gill morphometric parameters[b] of specimens of *O. a. grahami* collected from the fish spring lagoons (FSL), south western lagoons (SWL) and a water-holding tank (WHT).

Parameter	Comparisons and probability levels		
	A	B	C
NSL	$0.01 < P < 0.001$ (S)	$0.1 < P < 0.05$ (NS)	$P < 0.001$ (S)
GFL	$P < 0.001$ (S)	$0.02 < P < 0.01$ (S)	$P < 0.001$ (S)
NGF	$P < 0.001$ (S)	$0.05 > P > 0.02$ (S)	$P < 0.001$ (S)
ASL	$0.01 > P > 0.001$ (S)	$0.5 > P > 0.1$ (NS)	$P < 0.001$
D_{O_2}	$0.01 > P > 0.001$ (S)	$P > 0.5$ (NS)	$P < 0.001$ (S)

[a]Student's *t*-test (NS – nonsignificant difference; S – significant difference).
[b]Definition of the comparisons:
A = FSL Vs SWL
B = FSL Vs WHT
C = SWL Vs WHT
[c]The structural parameters are defined in Tables 1 and 2.

Table 4. Statistical comparisons[a] of the gill morphometric parameters[b] of *O. a. grahami* and *O. niloticus*

Parameter	Probability levels	Comment
NSL	$0.5 > P > 0.1$	NS
GFL	$0.05 > P > 0.02$	S
NGF	$0.05 > P > 0.02$	S
ASL	$0.5 > P > 0.1$	NS
τht	$0.01 > P > 0.001$	S
D_{O_2}	$0.5 > P > 0.1$	NS

[a]Student's *t*-test (NS – nonsignificant; S – significant difference).
[b]Parameters defined in Tables 1 and 2.

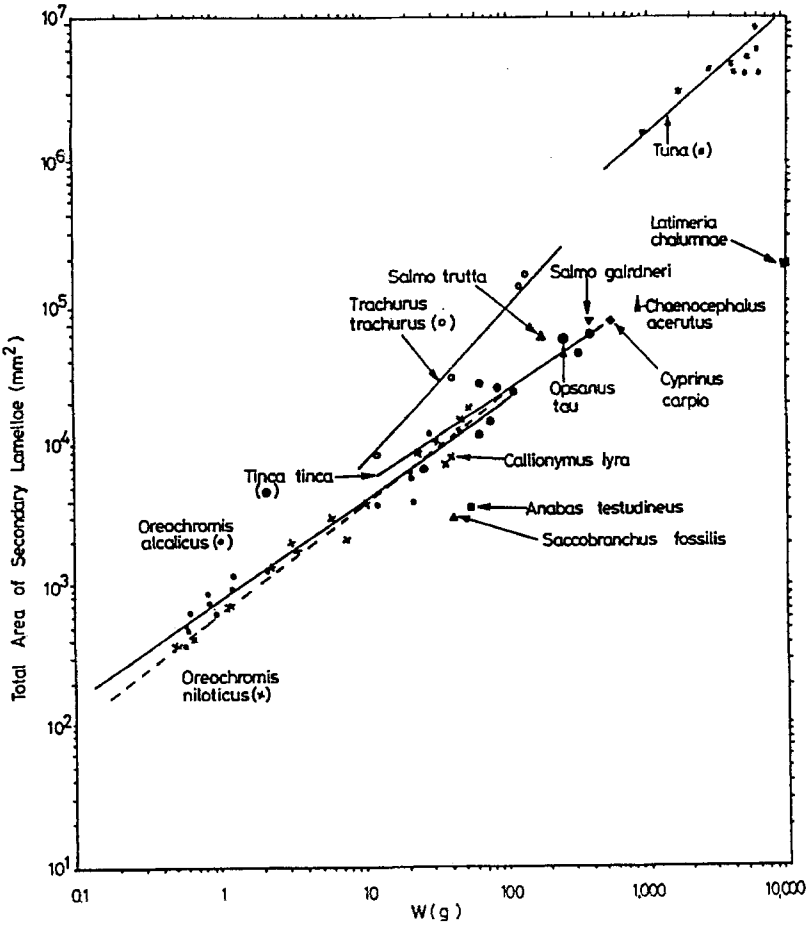

Figure 4. Plot of the total area of the secondary lamellae (ASL) against body mass in *O. a. grahami*, *O. niloticus* and other selected species of fish on which comparable data are available. The tuna, the mackerel (*Trachurus*) and the tench (*Tinca*) have a greater ASL than both *O. a. grahami* and *O. niloticus* while air-breathing fish like *Anabas* and *Saccobranchus* have appreciably lower values. Data on the non-tilapiine fish plotted in this figure and in the subsequent ones (i.e. Figures 4 to 8) are largely from Hughes and Morgan (1973) and the references given therein.

Antarctic hemoglobinless fish (*Chaenocephalus aceratus*) and the toadfish (*Opsanus tau*). Bimodally breathing fish like the climbing perch (*Anabas testudineus*) and the air-breathing siluroid fish (*Saccobranchus fossilis*) (Hughes and Munshi, 1973a,b) have a lower surface area of the secondary lamellae than *O. a. grahami* (Figure 4). Compared with fish on which data are available (Hughes and Morgan, 1973), only the tuna has a thinner water-blood barrier than *O. a. grahami* where it ranged from 0.83 to 1.45 μm (Maina,

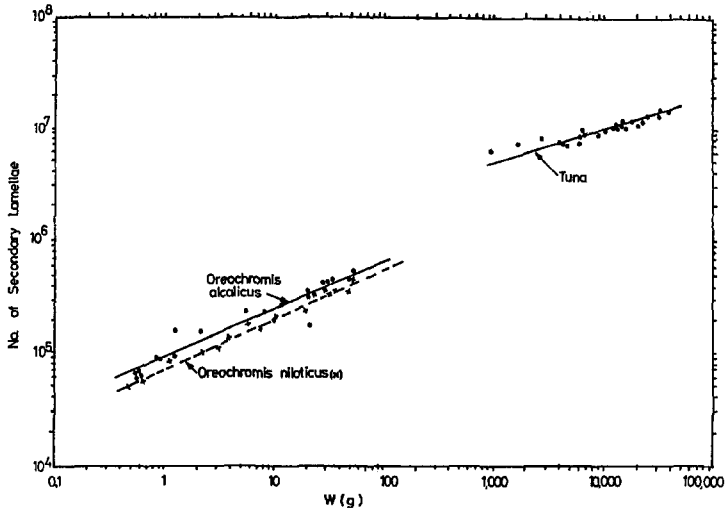

Figure 5. Plot of the number of secondary lamellae (NSL) against body mass in *O. a. grahami*, *O. niloticus* and the tuna. The tuna have a greater NSL than *O. a. grahami*.

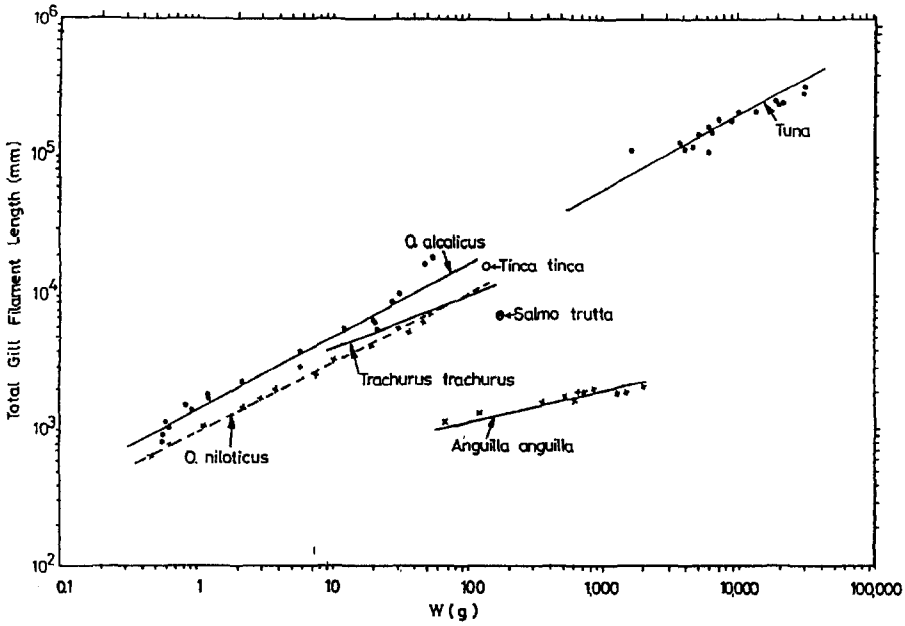

Figure 6. Plot of the total gill filament length (TGL) against body mass in *O. a. grahami*, *O. niloticus* and other fish on which data are available. The TGL in the tuna is comparable to that of *O. a. grahami*. Fish like the tench (*Tinca*) and the mackerel (*Trachurus*) have smaller TGL than *O. a. grahami*.

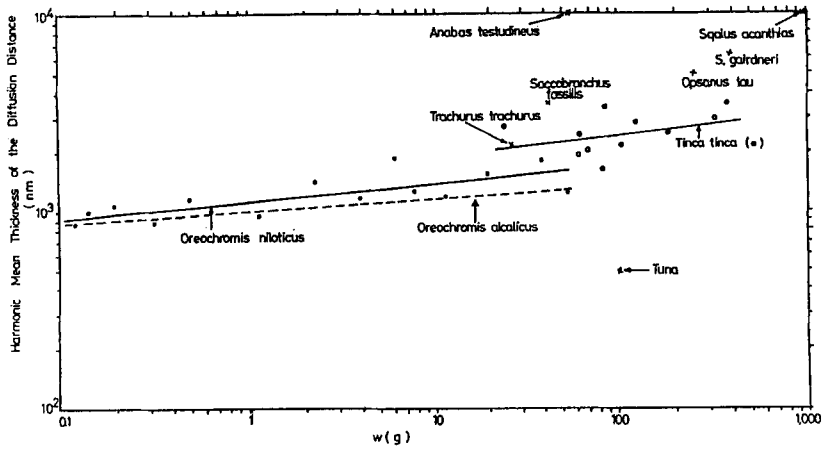

Figure 7. Plot of the harmonic mean thickness of the water-blood barrier (τht) for *O. niloticus*, *O. a. grahami* (from six specimens examined by Maina, 1990) and other species of fish on which data are available against body mass. Only the tuna have a thinner water-blood barrier than *O. a. grahami* while other fish like the tench (*Tinca*), the horse mackerel (*Trachurus*) and the toadfish (*Opsanus*) have thicker barriers.

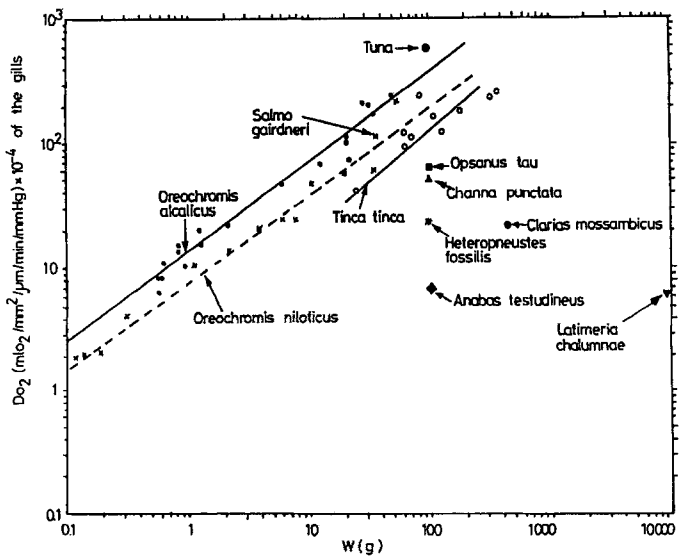

Figure 8. Plot of the morphometric oxygen diffusing capacity (D_{O_2}) of the gills of *O. a. grahami*, *O. niloticus* and other fish on which data are available against body mass. Only the tuna have a greater D_{O_2} than *O. a. grahami* and only the trout (*Salmo gairdneri*) has a D_{O_2} greater than *O. niloticus*, a feature which may illustrate the gill morphometric superiority of the tilapiine fish in general.

Table 5. Allometric regression lines [$Y = aW^b$] and correlation coefficients [r] of the body length, gill mass, and gill respiratory structural parameters of specimens of *Oreochromis alcalicus grahami* collected from different sides[a] of Lake Magadi. W, wet body mass.

Parameter	$Y = aW^b$	r	Units
Mass of the gills			
FSL	$0.06W^{1.1349}$	0.9852	g
WHT	$0.07W^{0.5873}$	0.8422	–
SWL	$0.08W^{1.010}$	0.9394	–
Gill filament length			
FSL	$1{,}421W^{0.5247}$	0.9870	mm
WHT	$2{,}131W^{0.4215}$	0.8735	–
Number of gill filaments			
FSL	$1{,}077W^{0.1523}$	0.9870	–
WHT	$1{,}175W^{0.1434}$	0.6171	–
Number of secondary lamellae			
FSL	$54{,}170W^{0.1690}$	0.6736	–
WHT	$124{,}509W^{0.3494}$		
Area of secondary lamellae			
FSL	$62.5W^{0.9695}$	0.9695	mm^2
WHT	$11{,}361^{0.6457}$	0.8290	–
Morphometric diffusing capacity			
FSL	$16.79W^{0.5572}$	0.9692	ml $O_2 \cdot mm^{-2} \cdot min^{-1} \cdot \mu m^{-1} \cdot mmHg^{-1} \cdot 10^{-4}$
WHT	$19.65W^{0.6481}$	0.8284	–

[a]FSL, fish springs lagoons; WHT, water-holding tank and SWL, south-western lagoons. The narrow weight range of the SWL fish did not allow meaningful computation of the regression lines on gill structural parameters.

1990; Laurent et al., 1995) (Figure 7). Fish such as *Tinca tinca, Trachurus trachurus, Opsanus tau* and *O. mykiss* and particularly air-breathing fish such as *A. testudineus* and *S. fossilis* (Hughes and Morgan, 1973) and *Clarias mossambicus* (Maina and Maloiy, 1986) have a thicker water-blood barrier. The gills of the tuna have a greater gill diffusing capacity than those of *O. a. grahami* which in turn has higher values compared with other fish on which data are available (Figure 8).

Table 6. Allometric regression lines [$Y = aW^b$] and correlation coefficients [r] of the respiratory structural gill parameters of *Oreochromis alcalicus grahami* compared with those of *Oreochromis niloticus*[a].

Parameter	$Y = aW^b$	r	Units
Number of gill filaments			
O. a. grahami	$953W^{0.2023}$	0.9849	–
O. niloticus	$981W^{0.1980}$	0.9890	–
Length of gill filaments			
O. a. grahami	$1{,}369W^{0.5442}$	0.9959	mm
O. niloticus	$951W^{0.5122}$	0.9955	–
Number of secondary lamellae			
O. a. grahami	$90{,}282W^{0.4255}$	0.9633	–
O. niloticus	$69{,}119W^{0.4400}$	0.9940	–
Area of secondary lamellae			
O. a. grahami	$76{,}736W^{0.7238}$	0.9866	mm^2
O. niloticus	$62{,}400W^{0.7977}$	0.9890	–
Harmonic mean thickness of the blood-gas barrier[+]			
O. a. grahami	$1{,}059W^{0.07537}$	0.9867	nm
O. niloticus	$1{,}130W^{0.0865}$	0.7552	–
Morphometric diffusing capacity of the gills			
O. a. grahami	$13.4W^{0.7236}$	0.9866	ml $O_2 \cdot mm^2 \cdot \mu m^{-1} \cdot min \cdot mmHg^{-1} \cdot 10^{-4}$
O. grahami	$7.91W^{0.6885}$	0.9850	–

[a]The data on *Oreochromis niloticus* are those of specimens of comparable range of body weight investigated by Kisia (1989) and Kisia and Hughes (1992). [+]Based on the data reported by Maina (1990).

Discussion

Gills of O. a. grahami *from different sites*

The Lake Magadi tilapia is exposed to diverse physiological challenges. The most important of these are ionic balance, acid-base balance, nitroge-

nous waste excretion and gas exchange. The two different groups of lagoons around Lake Magadi known to contain fish namely the Fish Spring Lagoons (FSL) and the South-Western lagoons (SWL) constitute remarkably different ecosystems. In general, temperature in the SWL is on average greater (at about 40 °C) but can be as high as 46 °C in some fish containing lagoons (Coe, 1966), while in the FSL, it ranges from 35 to 38 °C (average 37 °C). The oxygen concentration (measured at about the same time of the day and at the same ambient temperature) was generally lower in SWL than in FSL: we determined the PO_2 of the water in the SWL (at 12 noon) to be 22 kPa while that in FSL was 47 kPa. The significant differences between the morphometric parameters of the gills of the SWL fish compared with those of the FSL and WHT fish (Table 3) suggests an adaptive specialization for operation in a more stressful habitat. The comparability of the parameters between FSL and WHT fish (Table 3) conforms with the observation that the two groups are the same, (WHT fish being derived from the FSL stock through the water pipes). Furthermore, the habitats in which they subsist are similar though the water at the WHT is relatively much cooler. Body mass scaling effects may account for the significant differences in some gill morphometric parameters such as the gill filament length and number of gill filaments between the two groups of fish (Table 3).

Longstanding physical isolation of the two groups of Lake Magadi tilapia in different ecologically demanding habitats could have lead to genetic divergence and subsequent allopatric speciation. It has been suggested, however, that separate pools (within the same lagoon) may be connected during heavy rains and the fish may mingle during such rare instances (Coe, 1966; Trewavas, 1983). Molecular genetic studies will help shed some light on the phylogenetic affinities of the fish in the various lagoons of Lake Magadi including those in the Little Magadi (Figure 1) where fish have been sighted but not studied) and those in the other Rift Valley Lakes, particularly Lake Nakuru where the species is said to have been introduced three times in the last 40 years (Fryer and Iles, 1972; Trewavas, 1983) and in Lake Natron which from stratigraphic studies is thought to have been continuous with Lake Magadi until the recent geological past (Coe, 1966). Fish may have moved between the different Rift Valley lakes during the early geological formative years of the continental Africa. There is a possibility that in Lake Magadi, where there has been longstanding physical isolation and intense inbreeding in the different local habitats, different strains, subspecies, or even different species of fish may have developed. The current dearth of data on the evolution of the Lake Magadi tilapia and on the geomorphology of the Great Rift Valley and the East African lakes in particular (Cooke, 1958; Greenwood, 1960; Grove and Pullan, 1963; Temple, 1967) does not permit resolution of this problem.

From observations on the fossiliferous beds around Lake Megadi, Copley (1958), Coe (1966), Albrecht (1967) and Butzer et al. (1972), have suggested that in the past, the lake may have been much larger and probably more dilute than it is now. Since the cichlid fish in the African lakes are of riverine origin (Fryer and Iles, 1972), it is plausible that the fish adapted to increasing salinity in an initially fresh water background. This may explain why *O. a. grahami* has not entirely lost the capacity for adaptation, survival and reproduction in fresh water (Maina, 1990; Kisia, unpublished observations).

Gills of O. a. grahami *versus those of* O. niloticus

The cichlids of the genus *Oreochromis* formerly *Tilapia*, before the taxonomic review by Trewavas (1983), are known to thrive in remarkably diverse habitats. Though they are basically a fresh water tropical and Near-East fish, they are ecologically and physiologically a highly versatile group which is now widely distributed across the tropical and subtropical regions of the world (Philippart and Ruwet, 1982; Chervinski, 1982). A few tilapiine species can reproduce in sea water while most species can tolerate brackish water and survive in moderately cool and even fairly high temperatures (Fryer and Iles, 1972; Magid and Babiker, 1975; Bardash et al., 1972; Benech and Lek, 1981; Fernandes and Rantin, 1986a,b,c, 1987). Some species endure very low dissolved oxygen concentrations and can even withstand temporary anoxia (Welcomme, 1964; Coulter, 1967). Reite et al. (1974) found that *O. a. grahami* could tolerate a range in environmental pH from 5 to 11, lower (3 to 4) and higher values (12) being fatal within 6 hours.

O. a. grahami appears to be a particularly energetic fish. The resting oxygen uptake ($VO_{2(resting)}$ of *O. niloticus* (at 25 °C) has been estimated to be 75.5 $ml \cdot kg^{-1} \cdot h^{-1}$ by Kisia (1989) and 58.7 $ml \cdot kg^{-1} \cdot h^{-1}$ by Fernandes and Rantin (1989) and that of *Sarotheradon mossambicus* 74.2 $ml \cdot kg^{-1} \cdot h^{-1}$ (Coulton, 1978). Franklin et al. (1995) estimated the VO_2 of *O. a. grahami*, to be 415 and 542 ml $O_2 \cdot kg^{-1} \cdot h^{-1}$ at 37 °C and 42 °C respectively. In the same species, Narahara et al. (1996) estimated $VO_{2(resting)}$ to be 148 and 773 $ml \cdot kg^{-1} \cdot h^{-1}$ at 27 °C and 36 °C, respectively. The Q_{10} of *O. niloticus* within the temperature range of 25 to 35 °C (Fernandes and Rantin, 1989) was 1.49 while in *O. a. grahami*, between 27 and 36 °C, the Q_{10} is 6.2 (Narahara et al., 1996). The oxygen demands in *O. a. grahami* thus should drop significantly during the night when the temperature drops sometimes by as much as 14 °C. This atypically large drop in the water temperature may be due to a high radiation of heat from the shallow lagoons, the high conductivity of the water, that of the rocky lagoon floor and the surrounding terrain. It would be interesting to establish the maximum oxygen consumption, $VO_{2(max)}$, of *O.*

a. grahami at different temperatures. These values could likely be some of the highest for fish: $VO_{2(max)}$ in *Tilapia niloticus* (Farmer and Beamish, 1969) was estimated at 320 $ml \cdot kg^{-1} \cdot h^{-1}$ at 25 °C. The ability of *O. a. grahami* to maintain bursts of high energy in a hypoxic environment suggests presence of certain structural and/or functional respiratory adaptation(s). From our observations, the gills of *O. a. grahami* appear to be generally morphometrically better adapted for gas exchange than those of *O. niloticus*, a close relative adapted to neutrality. Physiologically, especially with respect to excretion of end products of nitrogen metabolism (Randall et al., 1989; Wright et al., 1990; Wood et al., 1994), the two species are remarkably different. It is envisaged that the morphometric adaptations of the gills have evolved especially in response to the severe environment the fish lives in.

Gills of O. a. grahami *versus those of other fish*

Fish gills are a multifunctional organ which is involved in gas exchange, osmoregulation, excretion of nitrogenous metabolic wastes, and acid-base balance. Increased surface area and thin water-blood barrier are two of the structural adaptive features which enhance gas exchange. A compromise must be established between the essential functions which in some instances require conflicting structural designs. This state is well recognized in fish gills and has been termed 'the osmoregulatory compromise' (Muir and Hughes, 1969; Randall et al., 1972; Nilsson, 1986; Heisler, 1989; Gonzales and McDonald, 1992). In Lake Magadi, over a 24-hour cycle, oxygen concentration in the water fluctuates dramatically ranging from virtual anoxia to a supersaturation state (Narahara et al., 1996). During the day, at high PO_2, *O. a. grahami* could minimize ionic flux over the respiratory surface area by utilizing only a fraction of the available surface area through preferential perfusion of the gills, a process well documented in some fish (Randall, 1970; Randall et al., 1972; Booth, 1978; Nilsson, 1986). During the night, however, it is plausible that most of the surface area is recruited to extract enough oxygen from the almost anoxic water. In *O. a. grahami*, the respiratory surface area appears to have been reduced to the basic minimum (the values are not very much different from those of *O. niloticus* – Figure 4) but gas transfer is compensated for by a relatively thin water-blood barrier (Figure 7). We observed that *O. a. grahami* resulted to breathing at the surface of the water during the day particularly after post-exercise (e.g. after being chased by a seine). Although the fish could simply be pumping the well aerated top layer of the water over the gills, there are indications that the buccal cavity and especially the swim bladder (Maina et al., 1995) may serve as an accessory respiratory organ during episodes of extreme hypoxia. Some tropical aquatic fish living in water at low PO_2 are known to result to air-breathing by utilizing their buccal

cavity and the swim bladder as gas exchange sites (Kramer, 1987; Saint-Paul and Soares, 1987, 1988). In a normoxic situation (PO_2 = 14.5 kPa), in *O. a. grahami*, all the oxygen needs are derived from the water (through the gills), while in hypoxic water (PO_2 = 5.5 kPa), 87.5 per cent of the oxygen needs come from the water and the rest (12.5 per cent) from the air (Narahara et al., 1996).

In fish, there exists a strong correlation between gill respiratory surface area and oxygen demand (Gray, 1954; Hughes, 1966; Steen and Berg, 1966; Hughes and Morgan, 1973; Palzenberger and Pohla, 1992). Gill area increases approximately to the same power [b = 0.850] as the value of the resting metabolism in fish (Winberg, 1956). From our observations at the lagoons, *O. a. grahami* appears to be a very active fish, at least over short distances. The tuna which is one of the most energetic fish studied attains a speed of 21 $m \cdot s^{-1}$ in 10- to 20-second bursts (Blaxter, 1969; Videler and Wardle, 1991). It has a respiratory surface area larger than normally found in other fish (Muir, 1969; Muir and Hughes, 1969; Palzenberger and Pohla, 1992). The cruising speed of *O. a. grahami* has been estimated to be about 0.5 m (3.2 BL)$\cdot s^{-1}$ (Wright et al., 1990), a rather low value compared with that of the tuna. However, though the tuna has a greater D_{O_2}, in an exceptional feat, *O. a. grahami* extracts oxygen at extremely low PO_2 during the night, in circumstances which with all likelihood would be fatal to the tuna.

Conclusion

The survival of *O. a. grahami* in Lake Magadi is dependent on a complex interaction between physiological, ecological, behavioural, structural and geophysical factors. The algae which flourishes in the lake and the fish appear to lead a somewhat symbiotic relationship: the algae derive nitrogenous excretory waste products from the fish (mainly in form of urea) and in turn provide oxygen to the fish. The large drop in temperature of the water during the night enables the fish to drastically reduce its oxygen demands at a time when the PO_2 is critically low. There is a strong possibility that the air-bladder and the buccal cavity epithelia function as accessory respiratory organs especially in times of extreme hypoxia. Physiological factors such as a high oxygen affinity of blood and absence of Bohr shift (Narahara et al., 1996), constitute important factors in this intricate survival strategy.

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