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RESPIRATION OF THE AMPHIBIOUS FISHES *PERIOPHTHALMUS CANTONENSIS* AND *BOLEOPHTHALMUS CHINENSIS* IN WATER AND ON LAND

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SUMMARY

1. The routine oxygen consumption by *Periophthalmus cantonensis* and *Boleophthalmus chinensis* in water increased geometrically, whereas that in air increased logarithmically with temperature. At temperatures of more than 20 °C the oxygen uptake of both species was greater in water than in air.
2. When the fishes were able freely to select either an aquatic or terrestrial habitat, the total oxygen consumption of *Periophthalmus* and *Boleophthalmus* was 236 and 110 ml/kg.h at 20 °C respectively; 66% (*Periophthalmus*) and 70% (*Boleophthalmus*) of the total uptake was from water, and 34 and 30% of the total uptake was from air at 20 ± 1 °C.
3. Oxygen uptake of fish limited to aquatic or terrestrial life was less than when they could freely select their habitat; for *Periophthalmus*, uptake was reduced to 83% when confined in water and to 50% in air, and for *Boleophthalmus*, to 65% in water and to 43% in air.
4. The proportion of oxygen uptake by the gill in water was 52% for *Periophthalmus* and 59% for *Boleophthalmus*; in air the corresponding figures were 27 and 52%.
5. The proportions of oxygen uptake via the skin in water was 48% for *Periophthalmus* and 36% for *Boleophthalmus*; in air the corresponding figures were 76 and 43%.
6. It is concluded that, on land, *Periophthalmus* relies mainly on its skin and *Boleophthalmus* relies mainly on its gills.

INTRODUCTION

Measurements of the oxygen uptake from water and air have been reported for the amphibious fishes *Periophthalmus sobrinus* and *Sicyaces sanguineus* by Gordon *et al.* (1968), *Anabas testudinius* by Hughes & Singh (1970*a*, *b*), *Clarias batrachus* by Singh & Hughes (1971), *Saccobranchus fossilis* by Hughes & Singh (1971) and *Amia calva* by Johansen, Hanson & Lenfant (1970). Recent reviews are given by Graham (1976) and Singh (1976). The partitioning of the oxygen uptake by specimens which were freely allowed to breathe from water and air was determined in the studies with *C. batrachus* and *S. fossilis*, but the relative amounts of oxygen taken up by the gill and skin of amphibious fishes in water or air have not been compared.

Table 1. *Body weight and body length of specimens*

Species	Age	Body weight (g)	Body length (mm)
Periophthalmus	Adult	4·0–8·0	48–87
Boleophthalmus	Adult	33·0–46·5	138–154

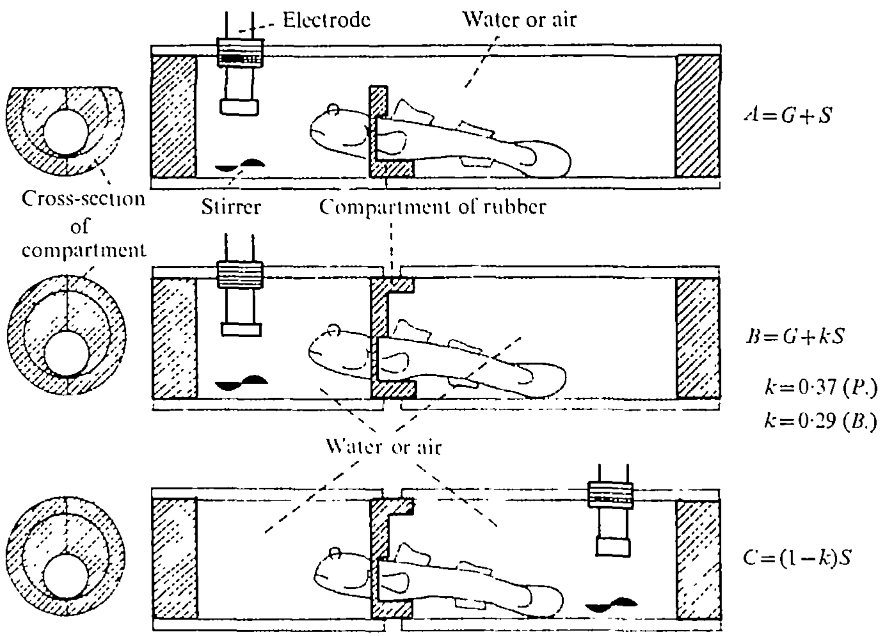

Fig. 1. Method for the estimation of oxygen-absorbing ability of different respiratory surfaces in air or water. *G*, Oxygen uptake at gill; *S*, Oxygen uptake at skin; *K*, Ratio of the area of head skin to the area of body surface excluding the fins; A, B, C, Oxygen uptake under the three different conditions, *P*, *Periophthalmus*; *B*, *Boleophthalmus*.

In this paper, the oxygen uptake and relative roles of the skin and gills in respiration of two Japanese amphibious fishes, *Periophthalmus cantonensis* (Osbeck) and *Boleophthalmus chinensis* (Osbeck), in aquatic and terrestrial habitats were studied. A comparison was made between specimens of the two species with respect to oxygen uptake by the gill or the skin alone. Adaptations of the two species to terrestrial life were also studied in relation to their respiration.

MATERIALS AND METHODS

Ranges in body weight and length of the specimens used are given in Table 1. They were sampled from the estuary of the Honmyo River along Ariake Bay. The amphibious fishes were kept in a circulation containing 50 % sea water (specific gravity 1·011–1·013). The fishes were acclimated for more than one day at the same temperature as that used in the experiment. Two to four individuals of the amphibious fishes were used for every experiment, but one individual each for estimations of oxygen uptake by the gill or skin alone. Oxygen consumption was estimated either

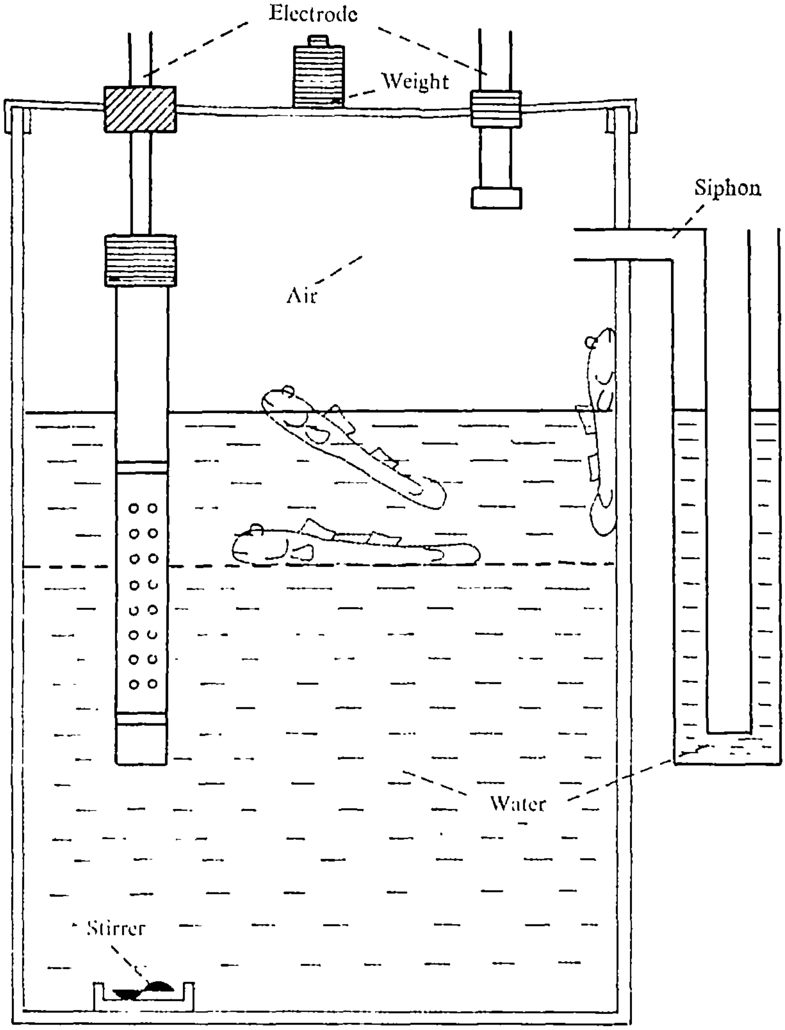

Fig. 2. Apparatus for measurement of respiration in freely selected habitat using revised Morooka's method.

(*a*) by a revised version of Morooka's method (Tamura & Morooka, 1973) in open aquaria of 2 l capacity beginning measurements when the water was almost fully saturated with dissolved oxygen, or (*b*) directly from the decrease in dissolved oxygen content in a closed vessel. The oxygen uptake from air or water alone was measured in a closed perspex cylinder in which the head and body regions of the fish were separated by a thin rubber septum. From measurements of the rate of oxygen uptake under three conditions (Fig. 1 A, B, C), it was possible to calculate values for the uptake by the gills and skin (Fig. 1). Cutaneous respiration was also measured by sealing the mouth and the gill openings using an adhesive paste, Aron Alpha. Oxygen uptake at each of the respiratory surfaces in fish that were free to breathe either water or air was measured in a closed two litre vessel containing 1·5 l of water and 0·5 l of air at atmospheric pressure (Fig. 2). In this experiment the estimation of oxygen consumption

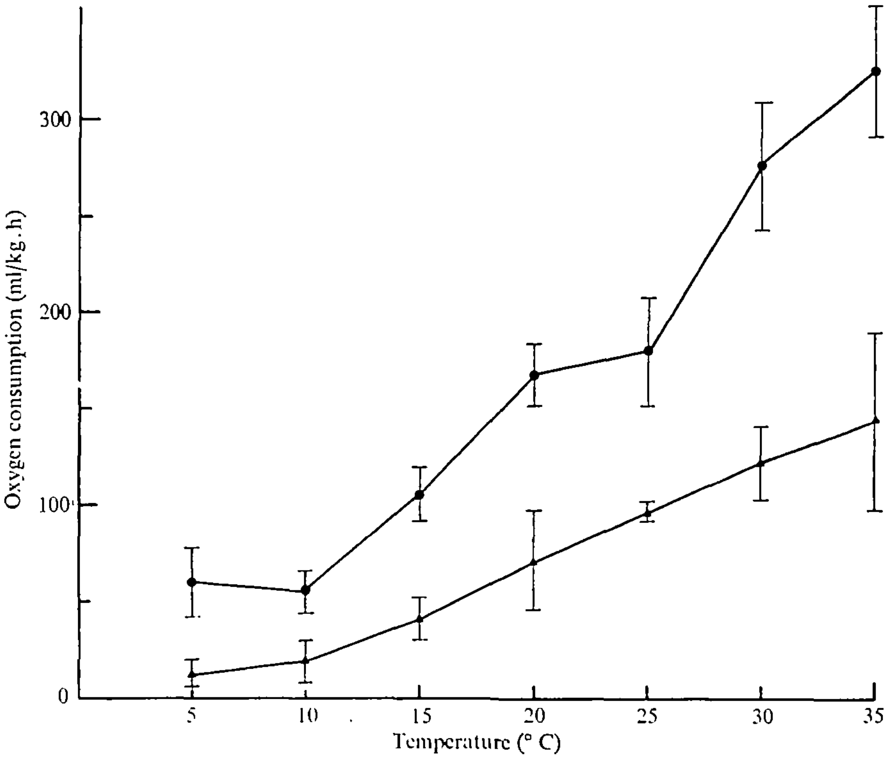

Fig. 3. Comparison of oxygen consumption in *Periophthalmus* (●) and *Boleophthalmus* (▲) at various temperatures in water (estimated by revised Morooka's method).

in air was corrected for the volume of oxygen dissolved during the experiment. Since the time for measurement was always 1 h, the carbon dioxide produced by the fish during this period was considered to have a negligible effect on the oxygen measurements. The experiments were carried out at 20 ± 1 °C. For the calculation of Q_{10}, the oxygen consumption was measured at 5 °C intervals for a specimen acclimated to each temperature for 1 day.

RESULTS

(*a*) *Effect of temperature on oxygen consumption*

Routine oxygen consumption of fish confined to water increased geometrically with ambient temperature, whereas when they were confined to air the increase was logarithmic. In both species the opercular movements stopped in water at 5 °C but they started again at 10 °C in *P. cantonensis* and at 15 °C in *B. chinensis* (Fig. 3) (Table 2)

(*b*) *Effect of light intensity on oxygen consumption*

The oxygen consumption, estimated by the revised Morooka's method, of both species suddenly doubled following an increase in light intensity during a period of 15–30 min, but it gradually returned to the initial value after a period of about 1 h (Fig. 4).

Table 2. *Opercular movements of* Periophthalmus *and* Boleophthalmus *in water at various temperatures (freq./min)*

Temp. (°C) ...	5	10	15	20	25	30	35
Species							
Periophthalmus	0	4	17	21	38	59	101
Boleophthalmus	0	0	15	22	51	58	79

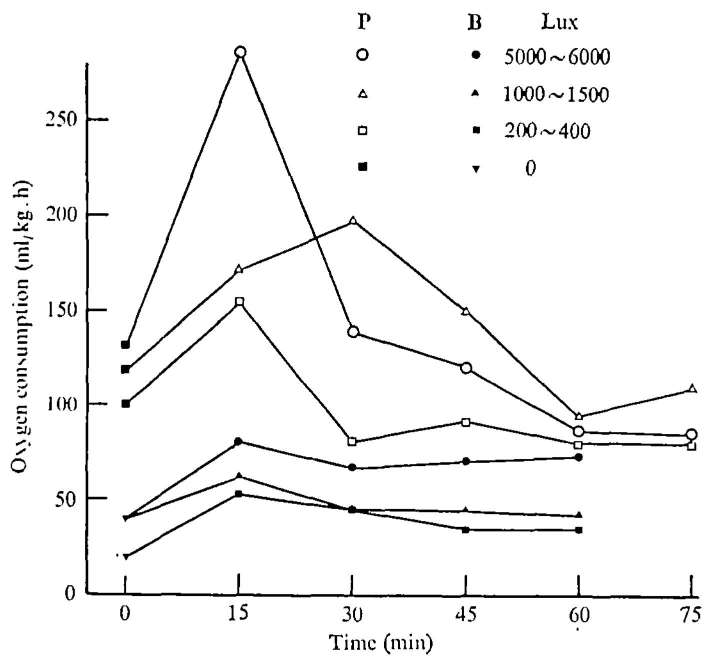

Fig. 4. Influence of stimulation by light on oxygen consumption of *Periopthalmus* (P) and *Boleophthalmus* (B) from air alone.

(c) Comparison of oxygen uptake from air and water

At temperatures of 20 °C and above, the oxygen uptake of both species was greater in water than in air, when fish were confined to each habitat: in *Periophthalmus* the oxygen uptake from air was about 60% of that from water and in *Boleophthalmus* about 70% at 20 °C (Fig. 5). When these two species were able to select freely between air-breathing and water-breathing it was found that *Periophthalmus* took 66% of its oxygen from water whereas *Boleophthalmus* obtained 70% of its total oxygen requirement from water (Table 3). Thus under these conditions both species depend more on water than on air. The total uptake was 236 ml/kg.hr in *Periophthalmus* and 110 ml/kg.hr in *Boleophthalmus* at 20 ± 1 °C (Fig. 2, Table 3). When the latter was restricted to water- or air-breathing, consumption was less than that in the freely-selected conditions, namely 65% when confined to water and 43% when confined to air (Fig. 1).

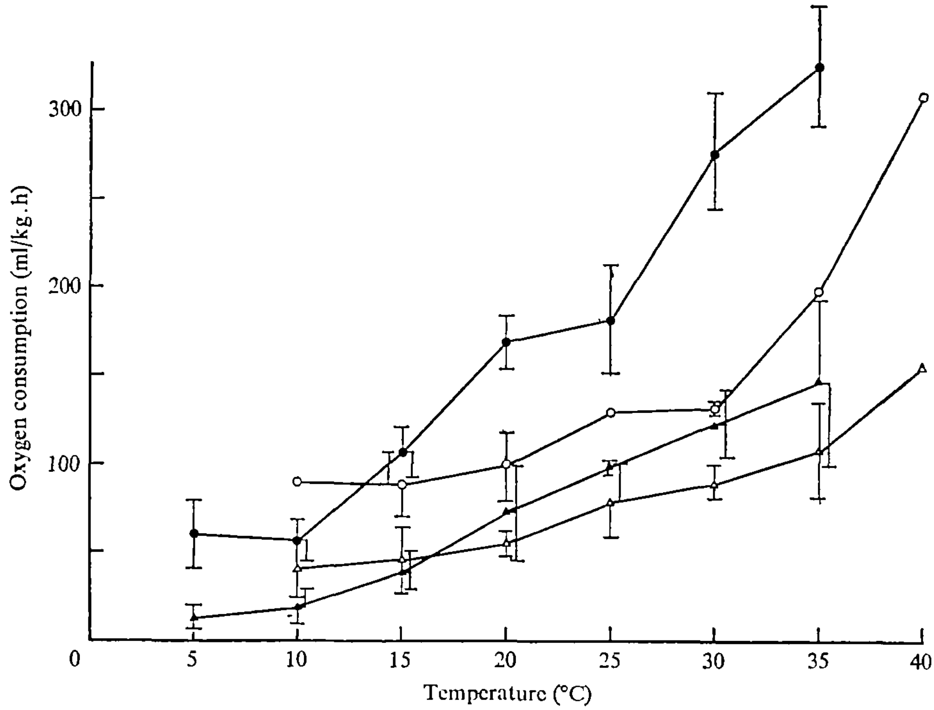

Fig. 5. Comparison of oxygen consumption of *Periophthalmus* and *Boleophthalmus* confined to aquatic or aerial habitats at various temperatures. *Periophthalmus*: ●, in water; ○, in air. *Boleophthalmus*: ▲, in water; △, in air.

Table 3. *Comparison of oxygen consumption of* Periophthalmus *and* Boleophthalmus *in freely selected habitat and in forcibly limited habitat* (20 °C)

Species	Freely selected habitat (ml/kg.hr) In water	In air	Total	Limited in water (ml/kg.h)	Limited in air	
Periophthalmus	155 ± 29 (66%)	81 ± 15 (34%)	236 (100%)	196 ± 14 (83%)	119 ± 13 (50%)	
Boleophthalmus	77 ± 11 (70%)	33 ± 19 (30%)	110 (100%)	72 ± 6 (65%)	47 ± 4 (43%)	
	(ml/ind.h)			(ml/ind.h)		body weight (mean)
Periophthalmus	1·04	0·54	1·58	1·31	0·80	6·7 g
Boleophthalmus	3·19	1·37	4·56	2·98	1·94	41·5 g
	(estimated by revised Morooka's method)			(estimated directly from dissolved oxygen decrease)		

(*d*) *Relative roles of gills and skin during oxygen uptake* (*Table* 3, *Fig.* 6)

In *Periophthalmus* confined to water, the gills and skin were used to an almost equal extent: 52 ± 12 % of oxygen uptake at the gills and 48 ± 12 % at the skin. In air the corresponding figures were 27 ± 7 % and 76 ± 10 % respectively. For *Boleophthalmus* the corresponding figures were slightly different namely 59 ± 17 % and 36 ± 8 % in water, and 52 ± 13 % and 43 ± 11 % in air. If the oxygen uptake of each organ in

Table 4. *Oxygen uptake by each organ per unit body surface area and body weight when fish is confined to each habitat*

(Estimated directly from dissolved oxygen decrease.)

Species ...		*Periophthalmus*	*Boleophthalmus*
Gill ml/dm².h	In water	1·80 (100%)	1·15 (100%)
	In air	0·56 (31%)	0·67 (58%)
ml/kg.h	In water	102	43
	In air	32	25
Skin ml/dm².h	In water	1·66 (100%)	0·69 (100%)
	In air	1·59 (96%)	0·54 (78%)
ml/kg.h	In water	94	26
	In air	90	20

animals restricted to water breathing is taken as a standard, the proportion entering through the gill of *Periophthalmus* in air is 31% of that in water. Thus the ability of the gills to take up oxygen from the air seems remarkably small. On the contrary, the oxygen uptake via the skin in air is 96% of that in water so that the skin is utilized to almost the same extent under both conditions. On the other hand, for *Boleophthalmus*, oxygen uptake via the gill in air is 58% of that in water, and the proportion entering via the skin in air is 78% of that in water. Thus in this species the ability of the skin for oxygen uptake in air is smaller, and that of the gill for oxygen uptake in air is greater, than in *Periophthalmus*.

(*e*) *Oxygen uptake per unit area of the skin*

For *Periophthalmus* in air, the uptake of oxygen per unit area of skin was almost the same (96%) as that in water, and for *Boleophthalmus*, the oxygen uptake per unit area of skin in air was 78% of that in water (Table 4).

DISCUSSION

Respiratory and ecological aspects

Individuals of *Periophthalmus* at Ariake Bay begin to move onto the dried mud of the estuaries from March onwards (Kobayashi *et al.* 1971), the dominant individuals leave the water for the land at temperatures greater than 18 °C. Corresponding specimens of *Boleophthalmus* start to move onto the dried mud from May onwards and the dominant individuals migrate to land at temperatures above 22 °C (Tamura, in press). They become active for feeding on land at these temperatures. Since the temperature variations are rapid and radiant heat is severe in air, these fishes often dive into puddles during the day time.

Respiration and light intensity

The effect of light is probably caused by changes in intensity and not the intensity itself. Effects due to the increase in body temperature caused by the radiant heat of sunlight are probably not involved in the results shown in Fig. 4.

Table 5. *Comparison of oxygen uptake of amphibious fishes in forcibly limited habitat*

Species	Oxygen uptake (ml/kg.h)		Temp. (°C)	Reference
	In water	In air		
Amia calva	19–20	72–99	19–27	Johansen *et al.* (1970)
Anabas testudineus	76	106	25	Hughes & Singh (1970*a*, *b*)
Clarias batrachus	65	71	25	Singh & Hughes (1971)
Periophthalmus sobrinus	84	92	25	Gordon *et al.* (1968)
Saccobranchus fossilis	65	55	25	Hughes & Singh (1971)
Periophthalmus cantonensis	196	119	20	Present authors
Boleophthalmus chinensis	72	47	20	Present authors

Table 6. *Proportion of oxygen uptake obtained from water and from air in some air-breathing fishes*

Species	From water (%)	From air (%)	Temp. (°C)	Reference
Periophthalmus cantonensis	66	34	20	Present authors*
Boleophthalmus chinensis	70	30	20	Present authors*
Amia calva	65	35	20	Johansen *et al.* (1970)
Saccobranchus fossilis	59·2	40·8	25	Hughes & Singh (1971)
Anabas testudineus	46·4	59·6	25	Hughes & Singh (1970)
Clarias batrachus	41·6	58·4	25	Singh & Hughes (1971)
Electrophorus electricus	22	78	25–27	Farber & Rahn (1970)
Protopterus aethiopicus	11	89	20	Lenfant & Johansen (1968)
P. aethiopicus	10	90	20	McMahon (1970)

* Data of present authors are the result of free selection of habitat by fish.

Comparison of oxygen uptake in water and in air

In measurements of oxygen uptake in closed or open vessels, the activity of both species differs in relation to temperature. *Periophthalmus* is more active than *Boleophthalmus* at 20 °C, the former move their pectoral or caudal fin several times or change the body position a little, and in the dark the activity is routine but not very marked. On the contrary *Boleophthalmus* hardly move at all and activity is near to standard metabolism. Therefore the difference between the amount of oxygen uptake of both species when showing the same activity may not be so great as indicated by Tables 3 and 5. The oxygen uptake of Japanese amphibious fishes confined to water was greater than when they were restricted to air breathing. In freely selected habitats, the oxygen uptake was also greater from water than from air in both species. Therefore with respect to their respiration these amphibious species appear to be less well adapted to a terrestrial habit than other species of tropical amphibious fishes that have been previously investigated (Tables 5, 6).

Share of oxygen uptake in each respiratory organ

The respiratory organs of amphibious fishes show a number of functional changes including reduction in gill area, a change in the number of gill filaments and total filament length, reduction in size of the gill opening and the degree of vascular development of blood capillaries of the suprabranchial wall or skin etc. (Schöttle, 1931; Carter, 1957; Stebbins & Kalk, 1961; Tamura, in press). A number of authors

Table 7. *Water-to-blood distance in* Periophthalmus cantonensis, Boleophthalmus chinensis *and* Oryzias latipes

Organ	*Periophthalmus* (mean)	*Boleophthalmus* (mean)	*Oryzias latipes* (mean)
Gill	7·7 μm	—	5·7 μm
Skin of head	7·5 μm	—	—
Skin of trunk	8·5 μm	17·3 μm	41·5 μm

have discussed the relative roles of the gill and skin in oxygen uptake, for example, Hughes & Singh (1971) reported that in *Clarias batrachus* and *Saccobranchus fossilis* confined to water breathing, 83·9 and 83·0 % of the oxygen was absorbed through the gill and 16·6 and 17 % through the skin. Berg & Steen (1965) found that for *Anguilla vulgaris* in water the shares of the gill and skin in the total oxygen uptake were 85–90 and 15–10 % respectively. Thus for these three species the gills are responsible for much greater proportions of the total oxygen uptake than in the Japanese species. Teal & Carey (1967) determined the proportions of the total oxygen uptake through the gill and skin in both habitats for *Periophthalmus sobrinus* and obtained values of 45 %:55 % in water and 37 %:63 % in air, relationships which are close to those found for *Periophthalmus cantonensis* in the present study.

Cutaneous respiration

As mentioned above, *Periophthalmus cantonensis* showed greater cutaneous respiration in air per unit of body weight and surface area than did *Boleophthalmus*, therefore it is suggested that the former is more adapted to air breathing. In a histological study, Matsuo (unpublished, Table 7) has shown that the water-to-blood distance for the skin of *Periophthalmus* is 9 μm on average, whereas for *Boleophthalmus* it averaged 17 μm. Thus the respiratory efficiency for these two species would differ for fish of the same surface area. The observation that oxygen uptake is reduced in air should be confirmed by further experiment, especially in high humidity and temperature, because the oxygen uptake by skin in air may be largely determined by the ambient humidity and temperature.

Terrestrial adaptation of the two species

Some indication of the terrestrial adaptation of these two species is given by changes in the proportion of the total oxygen uptake which enters through the gill when the fish is in water or on land. For *Periophthalmus*, gill oxygen uptake when confined to air decreased by 48 % (52–27 %) (Fig. 6) relative to that when it was confined to water; whereas in *Boleophthalmus*, gill oxygen uptake decreased by only 12 % (59–52 %) relative to that in water. Accordingly, the gill of *Boleophthalmus* can be regarded as being more adapted to a terrestrial life than that of *Periophthalmus*. The ratios of the gill area to body surface area were 0·38 in *Periophthalmus* and 0·56 in *Boleophthalmus* (Tamura, in press). These results showing a relationship between the size of the relative gill dimensions and the amount of oxygen uptake via gills in air can account for the terrestrial adaptation of *Boleophthalmus*. Further, adaptation of the gills in such fish usually involves an increase in stoutness of the gill as a whole which restricts their collapse in air. The gills are usually thicker and secondary lamellae are farther

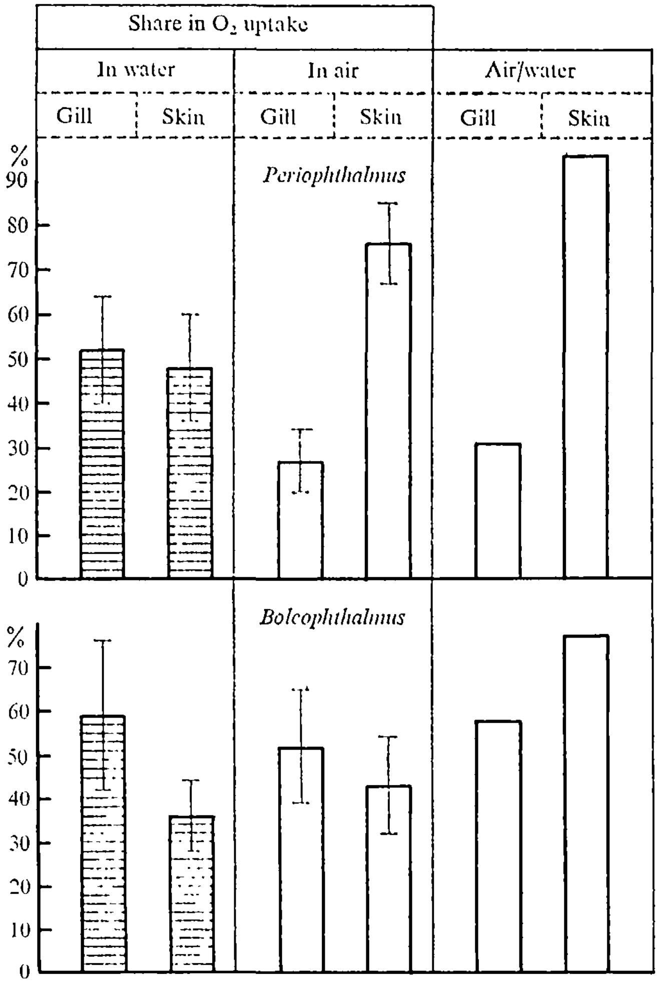

Fig. 6. Share of gill and skin of *Periophthalmus* and *Boleophthalmus* in oxygen uptake in both confined habitats and the uptake by each organ in air as a percentage of the uptake of each organ in water (estimated directly from a decrease in dissolved oxygen).

apart. In fact, in *Boleophthalmus* the secondary lamellae are more widely spaced than in *Periophthalmus* (Tamura, in press). It may be concluded that these differences in respiratory function are associated with an adaptation to air-breathing in the gill of *Boleophthalmus*, whereas the skin of *Periophthalmus* is more adapted to terrestrial life.

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