

## ASPECTS OF THE PHYSIOLOGY OF TERRESTRIAL LIFE IN AMPHIBIOUS FISHES

### I. THE MUDSKIPPER, *PERIOPHTHALMUS SOBRINUS*

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Bony fishes which could spend periods of time out of water, but which lacked special anatomical modifications for survival out of water, were probably involved in the early stages of the evolution of terrestrial life in the vertebrates. The rhipidistian crossopterygians were apparently the group ancestral to all higher vertebrates (Romer, 1966; Schmalhausen, 1968), but they are unavailable for study of possible physiological adaptations to life on the land. One way we may obtain some idea of the possible range of physiological adaptations which might have been possessed by these ancestral forms is to make comparative studies of pertinent aspects of the physiology of living amphibious fishes. We define amphibious fishes as those which spend periods of time out of water, on or above the ground surface, as normal parts of their life-histories. The most relevant forms are those lacking specialized accessory respiratory organs.

Present-day amphibious fishes are all products of long periods of evolution. It is probably impossible to find any living form which completely lacks any structural modifications for terrestrial life. However, there are many forms, belonging to a wide range of systematic categories, which are only minimally modified when compared with their closest purely aquatic relations. Limited information relating to survival and physiological mechanisms of adaptation to life out of water are available for a few of these: the European eel, *Anguilla anguilla* (Berg & Steen, 1965, 1966); two species of synbranchid 'eels', *Monopterus albus* from south-east Asia and Indonesia (Liem, 1961, 1967); and *Symbranchus marmoratus* from Central and South America (Johansen, 1966); the Chilean clingfish, *Sicyases sanguineus* (Vargas & Concha, 1957; Gordon, Fischer & Tarifeño, in preparation); the Indo-Pacific blenniid *Andamia heteroptera* (Rao & Hora, 1938); and the western North American goby, *Gillichthys mirabilis* (Todd & Ebeling, 1966; Todd, 1968). The Old World gobiid mudskippers (families Periophthalmidae, Boleophthalmidae, and relations) are among the most widely known amphibious fishes, but even they have been little studied in physiological terms (Bertin, 1958 (review of early literature); Stebbins & Kalk, 1961; Garey, 1962; Teal & Carey, 1967; Bandurski, Bradstreet & Scholander, 1968; Gordon *et al.* 1969).

The present paper provides additional information on the physiology of terrestrial

life in one species of mudskipper, *Periophthalmus sobrinus* Eggert (also known in the literature as *P. cantonensis* and *P. koelreuteri*). Working with the population on the island of Nosy Bé, off the north-western coast of Madagascar, we have studied survival time out of water, rates of evaporative water loss, and upper lethal temperatures, three aspects of the 'diving syndrome' (metabolic rates, heart rates, and blood lactic acid levels), and changes in nitrogen excretion. Related observations on natural history and the physiology of salinity adaptation in the same fish are presented by Gordon *et al.* (1965*a*, 1968). A preliminary summary of the present results was presented by Gordon *et al.* (1965*b*).

#### MATERIALS AND METHODS

Mudskippers ranging in size from recently metamorphosed post-larvae to large adults weighing up to 15 g. were abundant during the period June–August 1964 in the intertidal mangrove areas and on rocky stretches of coast along the shores of the island of Nosy Bé. They were easily captured in small dipnets during low-tide periods at night. Fish ranging in weight from 1 to 15 g. were used for experimental purposes. No attempt was made to feed the fish, nor to separate the sexes. Fish were maintained in the laboratory in 10 l. plastic aquaria with wire covers, with up to twenty fish per aquarium. The aquaria were tipped slightly, so that the 2 l. of water present covered only about half the bottom surface. The fish thus had their choice of being in or out of water. Water salinity was constant at 34 ‰.

Laboratory air temperatures varied with ambient temperature, and ranged diurnally from 23° to 30° C.

The following measurements were carried out on intact fish: *Survival out of water, evaporative water loss, and lethal temperatures.* Groups of fish were taken from sea water and lightly blotted on paper towels to remove excess water. Twelve were placed in a 4 l. volume plastic box, closed except for some small air holes, on paper towels dampened with sea water. Survival time was noted for these animals. Five other fish were placed individually in tared, dry, screen-covered plastic dishes in the shade of a building directly adjacent to the mangrove area in which they had been captured. Serial weighings were made to determine rates of evaporative water loss. Air movements over the boxes were nil or slight throughout observation periods. Precision of weighings was  $\pm 0.1$  g. Similar weight-change measurements were also made on other fish, in similar chambers, placed in full sunlight. Body temperatures of these fish were measured immediately after their deaths, via cloacal insertion of Schultheis mercury thermometers. The precision of temperature readings was  $\pm 0.1^\circ$  C.

*Metabolic rates.* Small fish (1–5 g. weight) were placed in the chambers of Scholander volumetric microrespirometers (Scholander & Iversen, 1958). Runs were made with the chambers either dry or partly filled with 10 ml. water; 6–12 hr. were allowed for temperature equilibration and adjustment of the animals to the apparatus. Oxygen consumption was measured for periods of about 1 hr. All measurements were made at temperatures between 23.0 and 24.5° C. Precision was  $\pm 2$  mm.<sup>3</sup> O<sub>2</sub> (STP)/g. hr.

*Heart-beat frequencies.* Frequencies of heart beat in fish in and out of water were measured by the method of Garey (1962), using a portable Sanborn Visette electrocardiograph, model 300. Rates were calculated on the basis of 12 sec. observation periods.

**Blood lactic acid concentration.** Fish were killed by a blow on the head. Blood samples were taken directly from the exposed heart in disposable glass micropipettes of 25  $\mu$ l. capacity. Colorimetric lactic acid determinations were carried out using *p*-phenylphenol (Natelson, 1961, p. 272); precision:  $\pm 2$  mg. %. Field preparation of lactic acid samples and standards was carried through the penultimate concentrated sulphuric acid stage. Samples and standards were sealed in glass ampoules and stored at room temperatures until analysed several months later. Control checks involving before-and-after analyses of similarly prepared and treated samples and standards showed that this storage had negligible effect on the results.

**Ammonia and urea excretion.** Fish were placed in covered plastic boxes of 0.5 l. capacity containing 100 ml. of water. Water samples of 0.25 ml. were taken at intervals and analysed for excreted ammonia and urea. Analyses were carried out using urease and the microdiffusion method of Conway (Natelson, 1961, p. 440); precision:  $\pm 0.05$  mM ammonia or urea/kg. hr.

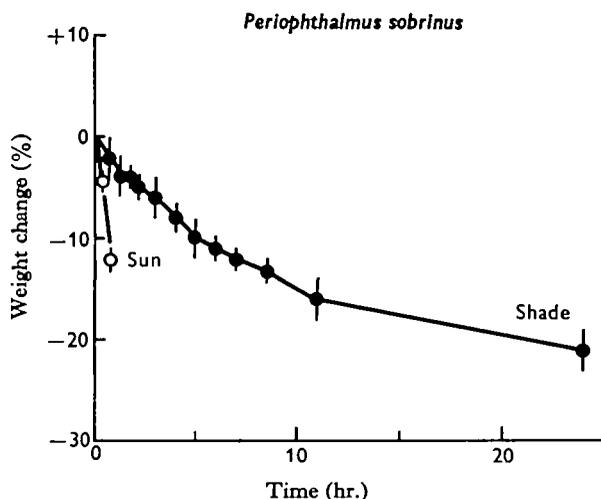


Fig. 1. Evaporative water loss (expressed as percentage change in body weight) in groups of mudskippers in air in shade (●) and full sun (○). Five fish in shade group, four in sun group. Lines join means of groups of observations; vertical lines,  $\pm 1$  s.e.

## RESULTS

Mudskippers can live completely out of contact with liquid water for extended periods, if not subjected to excessively severe thermal stresses. Fish maintained out of water, but in an atmosphere near saturation with water vapour, survived for up to 37 hr. (range 30–37 hr., twelve fish, 10–15 g. weight). Three of five fish exposed to nearly still air at 70–80% relative humidity, in shade, survived 24 hr. of this treatment. In contrast, four fish placed in bright sun, beginning at 9.20 a.m. on a clear day, all died within 50 min. Figure 1 summarizes data on rates of evaporative water loss from the fish in air at 70–80% relative humidity in shade, and the fish placed in full sun. Average weight loss from the fish during the first 6 hr. in the shade was linear with time at the rate of 1.8% of the original weight per hour. The slope decreased after

this to 0.4% of original weight per hour in the last 12 hr. of the experiment. The fish in the sun lost an average 12% of body weight in their short period of survival.

The fact that three of the five fish used for the shade experiment survived weight losses double those shown by the fish in the sun at the time of death of the latter makes it probable that weight loss (i.e. dehydration) *per se* was not the cause of death in the sun experiment. Elevated body temperatures appear to be a more probable cause. Body temperatures at death in these fish ranged from 33° to 35°C. Air temperatures in the chambers at the time of death (with thermometer bulb shaded) were 43° to 44°C.

Table 1. *Oxygen consumption of mudskippers in and out of water*

Environment	Oxygen consumption (mm <sup>3</sup> (STP)/g. hr.) [ $\bar{X} \pm \text{s.e.}(N)$ ]
100% SW	
Immersed	84 ± 9 (8)
Dry 3 hr.	94 ± 6 (6)
Dry 8½ hr.	90 ± 6 (5)
60% SW	
Immersed	54 ± 4 (6)
Dry 20 min.	76 ± 11 (6)
Dry 5 hr.	64 ± 8 (6)
FW	
Immersed	65 ± 9 (4)
Dry 3 ¾ hr.	79 ± 8 (4)

Metabolic rates of small mudskippers in sea water and out of it for periods up to 8½ hr. showed no statistically significant variations (Table 1). The same situation existed in other groups of fish adapted to environments of low salinity.

Electrocardiographic measurements of heart-beat frequencies showed considerable changes in rates from times immediately after handling and insertion of electrodes until experimental fish became moribund, usually 1½–2 hr. later. High initial rates were probable due to shock and agitation resulting from handling during insertion of electrodes. However, as Fig. 2 shows, there were no large changes in heart rate following subsequent gentle movement of fish, in nets, from aerated sea water to air or vice versa. Absolute heart rates varied considerably between individuals, so, for clarity, Fig. 2 presents data for only one individual. Six other fish were also studied in this fashion. All showed the same pattern.

Only one statistically significant change occurred in blood lactic acid concentrations in mudskippers maintained in and out of sea water for various periods (Table 2). As compared with control fish allowed to move into or out of water *ad libitum*, fish kept completely immersed for 40 min. increased blood lactic acid levels by an average of 9 mg. %, or 36% (significant at  $P = 0.05$  level by *t* test).

There was no statistically significant change in the relative amounts of ammonia and urea excreted over 24 hr. periods by intact mudskippers kept out of water for 12 hr. and then replaced in water, as compared with control fish kept continuously in water (Table 3). However, this result may not reflect physiological reality. Rates of excretion of both compounds were more than doubled, compared with controls, in the 24 hr. immediately following 12 hr. out of water (both increases significant at  $P = 0.02$  level by *t* test).

The magnitudes of these increases are considerably greater than would have occurred if there had been no change in the rate of nitrogen metabolism while the fish were out of water, as compared with the rate in water. A short calculation demonstrates this.

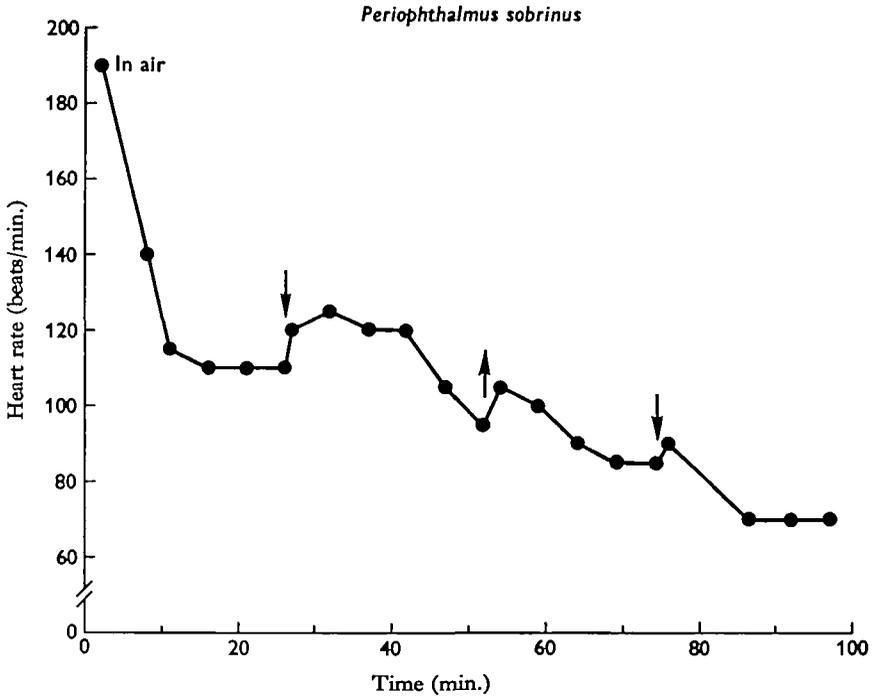


Fig. 2. Heart rates in a mudkipper in and out of water. Data are for one fish. Six other fish showed similar patterns. Downward pointing arrow indicates placement of fish in aerated sea water; upward pointing arrow indicates removal into air.

Table 2. *Blood lactic acid concentration in mudskippers in different environments*

Environment	Blood lactic acid (mg %) [ $\bar{X} \pm \text{s.e. (N)}$ ]
Control—in and out of 100% SW <i>ad lib.</i>	25 ± 2 (6)
Immersed, 20 min.	23 ± 3 (6)
Immersed, 40 min.	34 ± 2 (7)
In air, 20 min.	26 ± 3 (6)
In air, 40 min.	26 ± 2 (6)
In air, 40 min., immersed, 5 min.	27 ± 3 (6)

Table 3. *Ammonia and urea excretion from mudskippers in and out of water*

Environment	24 hr. rates of excretion [ $\bar{X} \pm \text{s.e. (N)}$ ]	
	Ammonia (mM/kg. hr)	Urea (mM/kg. hr)
100% SW	0.49 ± 0.05 (5)	0.36 ± 0.05 (5)
Air 12 hr., return to 100% SW	1.03 ± 0.12 (5)	1.02 ± 0.22 (3)

Assume the accumulation of all waste nitrogen production during the period out of water, and complete release of all production out of water within the first 24 hr. back in water. Subtracting the control rates from the experimental rates, and noting that the period in air was only 12 hr., indicates that the rate of ammonia production out of water was approximately double the rate of production in water; the rate of urea production increased by approximately 3.5 times. The ammonia:urea ratio appears to have shifted considerably in the direction of urea during the period out of water.

#### DISCUSSION

*Periophthalmus sobrinus* is a highly terrestrial fish which is physiologically well adapted to its mode of life. It does not show any of the symptoms of adjustment to asphyxia ('diving syndrome') which are characteristic of air-breathing higher vertebrates diving into water (Andersen, 1966) or of purely aquatic fishes 'diving' into air (Leivestad, Andersen & Scholander, 1957; Serfaty & Raynaud, 1957, 1958*a, b*; Garey, 1962; Scholander, Bradstreet & Garey, 1962; Eliassen, 1963; Serfaty & Waitzenegger, 1964; Caillouet, 1968). It appears not to suffer any deleterious changes during periods out of water which are far longer than those it normally chooses to spend in this situation in nature (Gordon, *et al.* 1968). Like other mudskippers it can also survive indefinitely completely submerged in well aerated water, deprived of access to air (Stebbins & Kalk, 1961; Hora, 1935, 1939).

These properties, however, appear not to be general for all types of amphibious fishes. Indeed, other species of mudskippers differ fundamentally. The Australian *Periophthalmodon australis* is so well adapted to terrestrial life that it reacts to immersion in water in much the same way as normally air-breathing higher vertebrates (Garey, 1962; Bandurski *et al.* 1968). Further, each of the other species of amphibious fishes in which one or more of the major aspects of the 'diving syndrome' has been studied appears to differ from all the other forms (Berg & Steen, 1965, 1966; Johansen, 1966; Gordon *et al.* in preparation). It seems improbable that the full range of diversity in these respects has been explored, since many amphibious species having very different phylogenetic histories remain unstudied.

An important part of the mechanism permitting the metabolic and cardiovascular independence of environment shown by *P. sobrinus* must be its capacity (shared with other mudskippers) to respire buccopharyngeally and cutaneously, as well as branchially (Harms, 1929; Schöttle, 1931; Willem & Boelaert, 1937; Teal & Carey, 1967). This species appears to have the capacity to balance changes in the rates of oxygen uptake by these various routes so exactly that no detectable changes occur in total metabolic rate. Contrary to reports for other mudskippers, *P. sobrinus* at Nosy Bé does not seem to need to carry water about in its buccal cavity in order to breathe while out on land (Gordon *et al.* 1968). We do not know the cause of the small, but statistically significant, increase in blood lactic acid levels found in fish kept completely immersed for 40 min. These fish may have been physically more active than the other groups sampled.

Evaporative water loss rates in *P. sobrinus* are low compared with those found in anuran amphibians, including the crab-eating frog (*Rana cancrivora*) of south-east Asia, which shares the same environment (Gordon, Schmidt-Nielsen & Kelly, 1961). This difference probably relates to the presence of scales in the fish skin and

perhaps also to different surface/volume ratios (excluding gills in the fish). Our results indicate that thermal stresses are a far more serious hazard for these fish than are dehydration stresses. The body temperatures associated with death in the fish we exposed to drying in full sunlight were only a few degrees above ambient air temperatures on the mud flats at Nosy Bé at midday (Gordon *et al.* 1968). Since our observations were made in mid-winter, it seems probable that the situation becomes still more marginal at other times of year. However, we have no information on the capacity of *P. sobrinus* for thermal adaptation.

*P. sobrinus* resembles anuran amphibians in its apparent shift towards ureotelism while out of water. Even primarily aquatic and ammoniotelic anurans like *Xenopus laevis* shift towards ureotelism under similar circumstances (Balinsky, Cragg & Baldwin, 1961; Balinsky *et al.* 1967). *Xenopus* also increases its rate of urea synthesis when subjected to restrictions in water supply (McBean & Goldstein, 1967).

We have no direct information on the possible cause of death in *P. sobrinus* kept out of water for about 1½ days and protected from severe dehydration or extreme temperature. It seems possible, however, that nitrogen metabolism may be an important factor in this. Our nitrogen excretion data make it probable that these fish halt urine release while out of water, as do anuran amphibia (Schmidt-Nielsen & Forster, 1954; Shoemaker, 1964). They certainly cannot eliminate metabolically produced ammonia by way of their gills, as do other teleosts (Goldstein & Forster, 1961; Goldstein, Forster & Fanelli, 1964). Under these circumstances ammonia concentrations in the body fluids may well build up to toxic levels. The indicated shift towards urea production while out of water acts to slow this process down, hence giving the fish more time, but the shift is far from complete.

#### SUMMARY

1. A study has been carried out of major aspects of the physiological adaptations for terrestrial life possessed by the amphibious mudskipper fish, *Periophthalmus sobrinus*, on the island of Nosy Bé, Madagascar.

2. These fish can survive for approximately 1½ days out of water, if not exposed to severe dehydration or thermal stresses. Evaporative water-loss rates while out of water are relatively low. Upper lethal temperatures are only a few degrees above normal midday environmental temperatures.

3. These fish lack the symptoms of the 'diving syndrome'. Metabolic rates (oxygen consumption), heart rates, and blood lactic acid concentrations are not affected by shifts of fish between water and air.

4. Rates of ammonia and urea production increase in fish out of water. The ratio of urea/ammonia also increases.

5. The generality of the results, also their physiological significance, are discussed.

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