J. Exp. Biol. (1970), **53**, 559–572
With 7 text-figures
Printed in Great Britain

ASPECTS OF THE PHYSIOLOGY OF TERRESTRIAL LIFE IN AMPHIBIOUS FISHES

II. THE CHILEAN CLINGFISH, *SICYASES SANGUINEUS*

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(*Received* 14 *May* 1970)

INTRODUCTION

The first paper of this series (Gordon *et al.* 1969) defined our usage of the phrase 'amphibious fishes', surveyed the literature on the physiology of terrestrial adaptations in those amphibious fishes lacking specialized accessory respiratory organs, and presented data on aspects of related adaptations in the amphibious mudskipper fish, *Periophthalmus sobrinus*. The point was made in that paper that each species of amphibious fish appears to have adapted to terrestrial life in a different way.

The present paper describes the results of a study of physiological adaptive responses shown by another amphibious fish, one phylogenetically quite distinct from all others previously studied. The Chilean clingfish *Sicyases sanguineus* belongs to the teleostean superorder Paracanthopterygii (Greenwood *et al.* 1966), which diverged from the other superorders of teleost fishes in the Cretaceous or Paleocene periods some 60–120 million years ago (Rosen & Patterson, 1969).

S. sanguineus is one of the largest and most terrestrial of the clingfishes (Briggs, 1955). It occurs in large numbers intertidally and subtidally along rocky coastlines of western South America from southern Peru to southern Chile (de Buen, 1960). Only limited physiological studies have been carried out on it previously, specifically aspects of respiratory metabolism and heart rate while out of water (Vargas & Concha, 1957; Concha & Vargas, 1957). We have studied the population living on the shore of Montemar, just north of Viña del Mar, Chile. Measurements made included survival-time out of water, rates of evaporative water loss, upper lethal temperatures, three aspects of the 'diving syndrome' (metabolic rates, heart rates and blood lactic acid levels), and changes in nitrogen excretion. Ebeling, Bernal & Zuleta (1970) made related observations on behaviour in the field, aspects of the species' ecology, and aspects of respiratory physiology. Gordon (1970) discusses some of the implications of our studies of nitrogen excretion.

MATERIALS AND METHODS

Clingfish ranging in size from juveniles weighing about 1 g to moderately large adults weighing up to 120 g were common during the period September–November 1967 on exposed surfaces of wave-washed rocks along the shore at Montemar. They

were captured in long-handled dip nets during periods of low tide. Fish ranging in weight from 1 to 110 g were used for experimental purposes. No attempt was made to feed the fish, or to separate the sexes. Fish were maintained in the laboratory in uncovered 20 l plastic aquaria, containing up to 25 fish each. The aquaria were filled about two-thirds full with sea water, and were continuously aerated. Sea water was changed twice each day. Water salinity was constant at 34 ‰. Water temperature varied with, but not as much as, laboratory air temperatures. Air temperatures were the same as outdoor temperatures and ranged diurnally from 12–16 °C in mid-September to 15–19 °C in late October. Along-shore sea-water temperature varied from 12–13 °C during the same period. Reyes (1965) summarizes climatological data for the locality.

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 the aquaria and lightly blotted on paper towels to remove excess water. Care was taken to make sure that no water was retained in the branchial chambers or the buccopharynx of any fish. Single fish were placed in individual tared thin plastic boxes, each covered with a piece of plastic window screen. The fish in the boxes were then exposed to the experimental environmental conditions, with determinations of survival and body weight (precision ± 0·1 g, on 500 g capacity torsion balance) made at intervals. Three experimental conditions were studied, all at ambient humidities (60–75 % relative humidity): (1) still air in shade, (2) moving air in shade, and (3) moving air in full mid-afternoon sun. Air was circulated in the shade by a small electric fan; in the sun by the regular afternoon sea breeze. Body temperatures of fish in the sun were measured immediately after their deaths, via cloacal insertion of Schultheis mercury thermometers (precision ± 0·1 °C).

S. sanguineus is inert under most circumstances, especially when it is firmly attached to a solid surface out of water. In the laboratory it is difficult to determine whether such a fish is alive or dead. Criteria for life used in survival studies were either visible small spontaneous movements or movements elicited by breaking the suction between the ventral attachment disk and the substrate by means of a fine dissecting needle inserted under one edge of the disk.

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 air-equilibrated sea water; 0·5–1 h was allowed for temperature equilibration. Oxygen consumption was measured for periods of 2–3 h. All measurements were made at temperatures of 14–15 °C. Precision was ± 2 mm^3 O_2 (s.t.p.)/g h.

Large fish (30–80 g weight) were placed in closed glass jars. Aerial respiration was measured in jars of 270 ml volume, capped with tight-fitting Bakelite screw caps, each cap perforated by a 6 mm diameter hole sealed with a rubber serum-bottle stopper. Single fish in sea water were placed in the jars, with plastic window screen over the openings, in their own aquaria 18–24 h before experiments began. At the start of measurements the jars were gently removed from the aquaria, emptied of water, and capped, all without touching or visibly disturbing the fish. Jars were placed upright in a 14 °C water bath, the enclosed fish attached to the sides in head-upward position. Gas samples of 0·2–0·3 ml were taken at intervals through the serum stoppers using 1 ml tuberculin syringes lubricated with concentrated alkaline citrate solution and fitted

with 12 mm long no. 27 gauge hypodermic needles. The tips of the needles were immediately placed beneath the surface of additional concentrated alkaline citrate solution in a nearby dish, and a large part of the sample was ejected to remove possible contamination by room air in the needle. An aliquot of the remainder was then bubbled into the alkaline citrate filled cup of a Scholander dissolved-gas analyser (Scholander *et al.* 1955), which was also held beneath the liquid surface in the dish. The fraction of oxygen in the aliquot of sample was then determined using alkaline pyrogallol (Scholander *et al.* 1955). Precision was ±0·3 % oxygen. Oxygen consumption was measured for periods up to 11·5 h.

Aquatic respiration was measured in jars containing 3·9 l of sea water and capped with tight-fitting, gasketed metal screw caps, each cap penetrated by two no. 16 gauge hypodermic needles cut off short and cemented into place with epoxy cement. One needle was used for taking water samples and was fitted with a piece of plastic tubing extending to the bottom of the jar. Single fish were placed in the jars in sea water, with aeration, 12–24 h before experiments began. The jars stood vertically in a 14 °C. water bath. At the start of measurements the aeration stones were removed, and water levels were topped off, and the covers were put on, care being taken not to trap air bubbles. The fish were undisturbed throughout, remaining quietly attached to the jar walls. Water samples of 4–5 ml were taken at intervals with glass syringes. Two syringes were used, one initially filled with air-equilibrated sea water. Water was vigorously pumped back and forth between these syringes to mix the contents of the jars thoroughly, and the sample was taken via the sampling needle fitting. One ml aliquots of the sample were transferred without exposure to room air to the extractor of a Scholander dissolved-gas analyser (Scholander *et al.* 1955) and the oxygen content was determined. Precision was ±0·2 ml oxygen (s.t.p.)/l. Oxygen consumption was measured for periods up to 12 h.

Heart-beat frequencies. Frequencies of heart beat were measured in fish of 60–95 g weight, in and out of water. Electrocardiograph (ECG) leads were made from straightened small metal fish hooks coated, except for their points, with epoxy cement and soldered to insulated wires. Two leads were used per fish, one inserted into the muscles directly beneath the heart (located on the mid-line just above the anterior edge of the ventral sucking disk), the other inserted into the lateral muscles of the tail. Following attachment of leads, fish used for short-period emersion experiments were placed in individual thin plastic boxes covered with plastic window screening and returned for 30 min to aerated sea water in an aquarium. The box plus fish was then gently removed from the water, drained dry via holes in its bottom, and placed on an adjacent table. At the end of the emersion period the box was gently replaced in the aquarium and the fish was observed for another 30–60 min. In long-period emersion experiments leads were attached to fish already in air in dry aquaria. These fish were then handled as the other fish. Recordings were made on a Picker model VS-III portable ECG machine, usually for 15 sec at each observing time. Heart rates were calculated from the recordings by determining the number of beats and the elapsed time between the first and last beats detectable. Occasional irregularity of intervals between beats was ignored. In these experiments water temperatures were 14–16 °C, air temperatures 15–19 °C.

Concentration of lactic acid in the blood. Fish (60–160 g weight) were stunned by a

blow on the head. Blood samples were immediately taken directly from the exposed heart in disposable glass micropipettes of 50 μl capacity. Colorimetric determinations of lactic acid were carried out using *p*-phenylphenol (Natelson, 1961, p. 272); precision: $\pm$ 1 mg%.

Blood concentrations and excretion of ammonia and urea. Blood concentrations of ammonia were determined in fish of 43–69 g weight on 100 μl samples taken by hypodermic syringe from the caudal artery. Blood concentrations of urea were determined in fish of 27–50 g weight on 100 μl samples taken by hypodermic syringe directly from the heart.

Fish of 18–59 g weight maintained either in sea water or in air for varying periods were placed in glass jars containing 100 ml air-equilibrated sea water. Water samples of 1·0 ml were taken at intervals over periods of 1–16 h and analysed for excreted ammonia and urea. Jars were kept in a water bath at 14 °C. Analyses of both blood and water samples were carried out using urease and the microdiffusion method of Conway (Natelson, 1961, p. 440); precision: $\pm$0·05 mM/l blood ammonia, $\pm$0·2 mM/l blood urea, $\pm$0·05 mM excreted ammonia or urea/kg h.

RESULTS

Sicyases sanguineus can live indefinitely completely submerged in well-aerated water. It also can live completely out of contact with liquid water for extended periods, if not subjected to excessively severe dehydration or thermal stresses (Fig. 1).

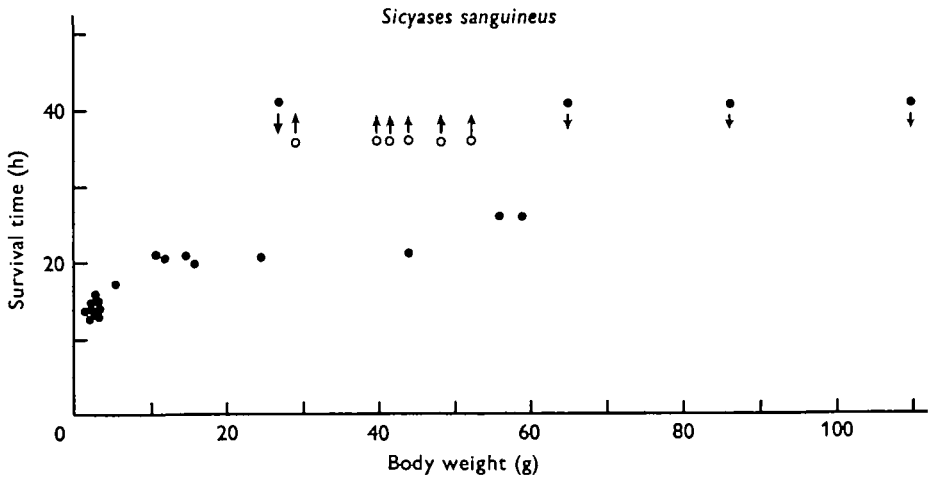

Fig. 1. Survival time out of water of *Sicyases sanguineus* of differing body weights. Fish in calm air, shade, 12–14 °C, 60–70% relative humidity. Each point represents one fish. ●, First experimental group; ○, second experimental group. Points with downward-pointing arrows are fish found freshly dead at times indicated. Points with upward pointing arrows are fish found moribund at times indicated, with death likely to occur soon afterward.

Fish maintained out of water in calm air and shade, at seasonally normal temperatures and relative humidities, survived from 13 h (2–5 g weight juveniles) to 35–40 h (half-grown and adult fishes, weighing more than 25 g). The eventual death of fish under these conditions cannot have been due to dehydration. Total loss of body weight

during 40 h averaged less than 10 %. Other fish, subjected to moving air in the shade, at the same temperatures and relative humidities, tolerated more rapid weight losses of 20–25 % before dying (Fig. 2). Fractional rates of weight loss under given conditions were independent of body weight over the range 10–130 g.

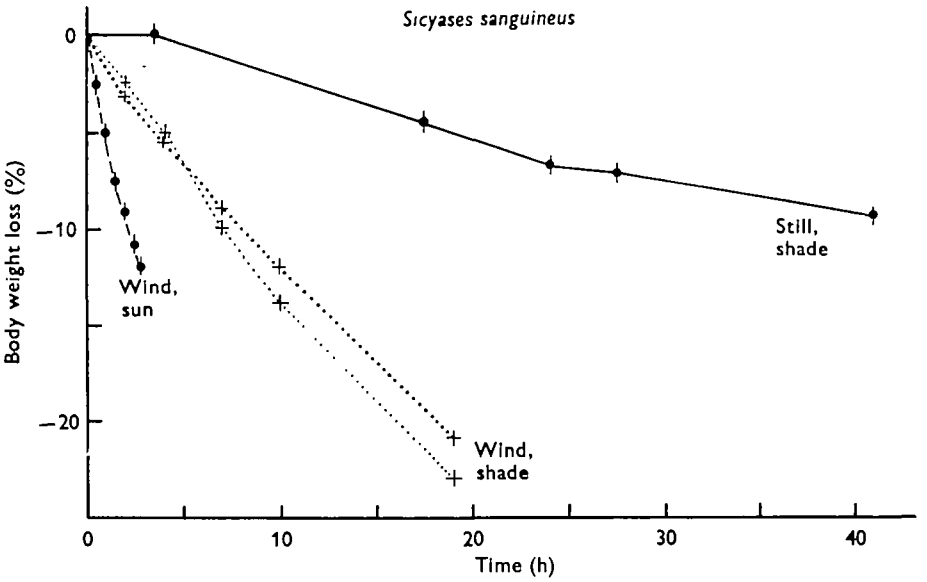

Fig. 2. Evaporative water loss (expressed as percentage change in body weight) in groups of *Sicyases sanguineus* in three combinations of still or moving air, shade, or full afternoon sunlight. Air temperatures 13–16 °C, 65–76 % relative humidity. Points on still, shade and wind, sun lines indicate $\bar{X} \pm$ S.E. for successive weighings of, respectively, groups of 12 fish (12–128 g body weight) and six fish (48–72 g body weight). Wind–shade lines join points for two individual fish of 12 (59–90 g body weight), the two shown having been exposed to the highest wind velocity used in the experiment (13–15 km/h). Wind–sun fish were exposed to gusty, variable natural breezes fluctuating from 0–8 km/h.

Exposure of fish to moving air and full early afternoon sun produced very high rates of evaporative water loss, and mortality after 2–3 h. Even under these circumstances, however, weight losses of 12–13 % were tolerated before death (Fig. 2). Death under conditions of combined wind and sun was probably due to overheating. Body temperatures at death in these fishes ranged from 21 to 24 °C. Air temperatures in the plastic chambers at times of death (with thermometer bulb shaded) were 15–16 °C.

Metabolic rates of small (1–5 g body weight) *S. sanguineus* in sea water and out of it for periods up to 13 h increased progressively with duration of stay out of water (Fig. 3). This phenomenon could not have been a result of desiccation, as the measurements were made in closed, moist respirometer chambers.

The metabolic effects of emersion on larger fish may be similar to those just described for small ones, but the data are more complex. Larger *S. sanguineus* (30–80 g body weight) in water respired continuously, as do other fishes. Weight-specific metabolic rates under these conditions show the usual relationship to size (Fig. 3). However, larger fish out of water appeared to consume oxygen only intermittently. This was particularly the case during at least the first 12 h out of water, but also occur-

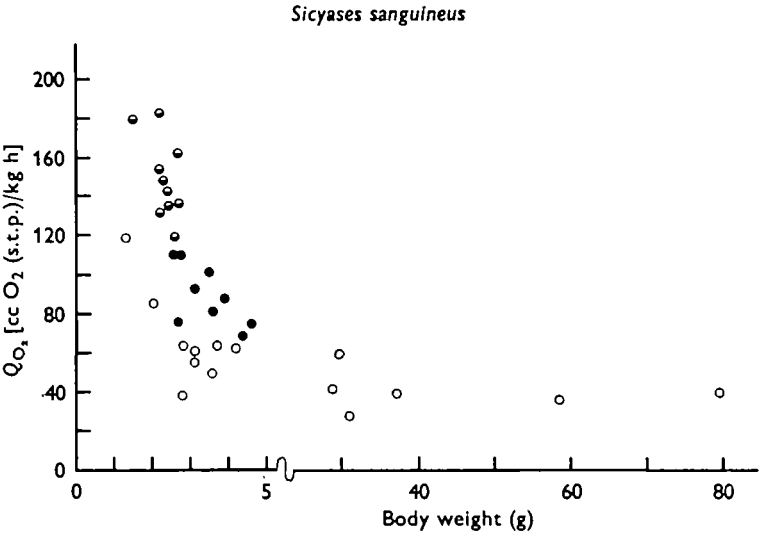

Fig. 3. Weight-specific oxygen consumption of *Sicyases sanguineus* of differing body weights in and out of water for differing periods. Each point represents one fish. ○, Fish in water; ●, fish out of water 1–3 h, ◒, fish out of water 11–13 h.

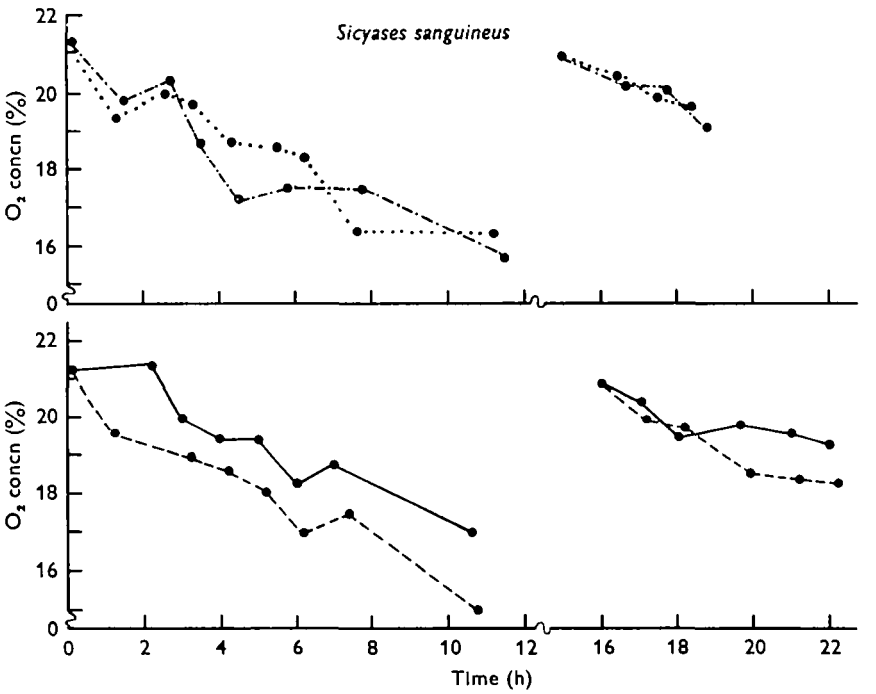

Fig. 4. Oxygen consumption (expressed as change in oxygen content of air enclosed in respirometer) of medium-size *Sicyases sanguineus* (25–43 g body weight) out of water for differing periods. Each line represents one fish. The eight fish shown are representative of a total of 15 fish studied.

red to some extent after emersions of as long as 22 h (Fig. 4). Periods without detectable oxygen uptake lasted as long as 4 h. The occasional indications in Fig. 4 of oxygen production by the fish may be attributed to analytical error. All such upswings are within the precision of the procedures used (see Methods).

The frequency of gas analyses in these experiments was limited by the nature of the oxygen-determination procedure to no more often than every 30–40 min. As a result, it was impossible to determine the true pattern of oxygen consumption by large fish in air. During periods when uptake occurred, it might have occurred at variable rates, or at some fixed rate, but continued for different fractions of different intervals, or in some combination of these possibilities. Considering this situation, it seemed best to estimate both average overall metabolic rates, and minimum and maximum metabolic

Table 1. *Oxygen consumption of larger* Sicyases sanguineus *in and out of water* (*cc* O_2 (*STP*)/*kg h;* 30–80 g *fish*)

($\bar{X} \pm$ S.E. (*N*).)

In water	40 ± 4 (6)
In air, based on least-squares regression analyses	
Air, 0–12 h	32 ± 2 (6)
Air, 15–23 h	18 ± 3 (7)
In air, based on individual observation intervals	
Air, 0–12 h	
Minimum	2 ± 2 (6)
Maximum	93 ± 18 (6)
Air, 15–23 h	
Minimum	4 ± 2 (7)
Maximum	44 ± 8 (7)

rates. The former calculations were made via least-squares regression analyses of the points relating oxygen percentage to time over the duration of the entire experiment. The latter estimates were derived from the slopes of the lines joining successive pairs of individual observations (i.e., between points 1 and 2, then 2 and 3, etc.). Table 1 summarizes these data, and compares them with the average metabolic rates for larger fish in water. The regression analysis shows that average rates in air were statistically significantly lower than rates in water. The minimum–maximum calculations demonstrate that there were intervals in which the fish consumed no oxygen, and that metabolic rates during uptake periods in shorter-term emersions may be at least double rates in water. Active periods during the later stages of longer-term emersions showed maximal rates equal to rates in water.

Electrocardiographic measurements of heart-beat frequencies showed some changes in rates between times immediately after handling and insertion of electrodes and later on, but rates became stable within 10–15 min (Figs. 5, 6). Gentle removal of fish from the water produced an immediate, sharp slowing of the heart, rates declining by 20–25 % within 30 sec. Additional slowing, by another 15–20 % of the basal rate in water, developed gradually over the ensuing 1·5–2 h. These low rates persisted, with minor fluctuations, as long as the fish were kept out of water. Gentle replacement of the fish into water, whether after 4 h or 24 h emersion, produced a brief flurry of strong

swimming movements, a resumption of respiratory movements (initially of large amplitude) and, in all but one fish (Fig. 6, right side), an immediate (within 30–60 sec) restoration of the heart beat frequencies measured initially in water. There is no detectable variation in heart rate with body size, at least over the size range used in these experiments.

The lactic acid concentration in the blood was low in fish in sea water (Fig. 7).

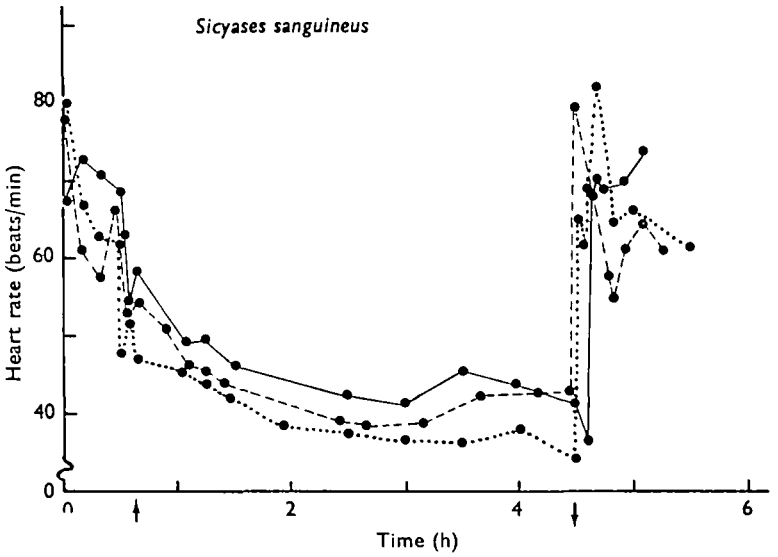

Fig. 5. Heart-beat frequencies in medium-size (60–80 g body weight) *Sicyases sanguineus* in water and out of water for short periods. Each line represents one fish. The three fish shown are representative of a total of seven fish studied. Arrow pointing upward indicates time of removal from water, downward arrow return to water.

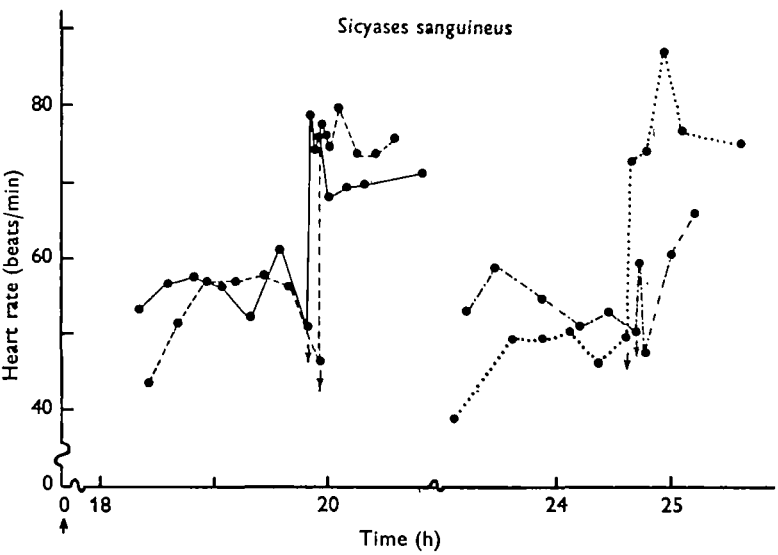

Fig. 6. Heart-beat frequencies in medium-size *Sicyases sanguineus* out of water for long periods, then replaced in water. Each line represents one fish. Arrows pointing down indicate times of return of fish to water.

Increasing duration of emersion produced a gradual increase in concentration over a period of about 5 h, following which the level remained constant, at approximately × 3 the concentration in water, for the maximum length of the experiments (25·5 h). Two groups of fish were put back into water after different times in air, then sampled after 20–40 min in water. The group which had been in air for 14·5 h made a substantial return toward control lactate levels, while the group which had been in air for 22 h showed no change from the higher level.

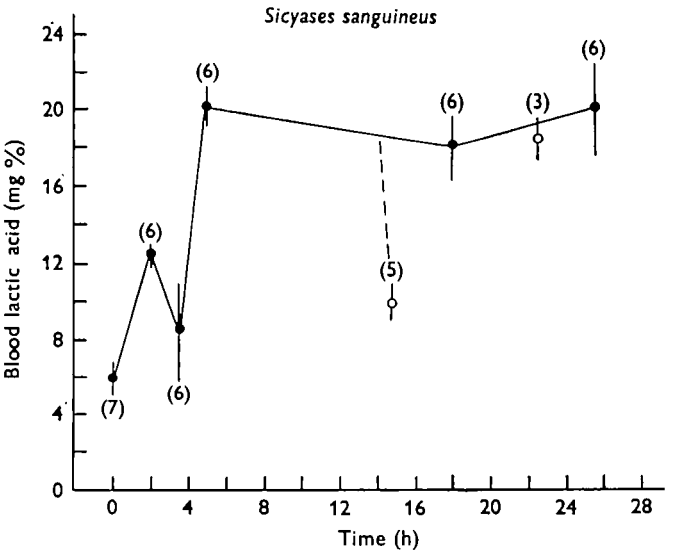

Fig. 7. Lactic acid concentrations in the blood in groups of medium to large size (60–160 g body weight) *Sicyases sanguineus* in water (0 time), out of water for differing periods, and returned to water for 20–40 min after differing periods in air. Points indicate $\bar{X} \pm 1$ S.E. for groups of numbers of individuals noted. ●, Fish in air for indicated times; ○, fish returned to water after indicated periods in air.

Blood concentrations of ammonia and urea in fish in sea water were within the normal range for many terrestrial vertebrates (Table 2). Rates of ammonia and urea excretion by fish in sea water were substantial, with urea containing almost three-quarters of the total nitrogen excreted in these two molecular forms (ratio of urea N:ammonia N $\approx$ 3:1). Time limitations prevented a full study of the effects of increasing emersion time on the pattern of nitrogen excretion, but the data warrant two conclusions. First, most, if not all, of the nitrogenous wastes produced by fish while out of water are retained by them until they are back in water. This is indicated by the considerable elevations in rates of total nitrogen excretion in groups of fish returned to water for 1–4 h after emersion periods of 20 or 36 h. Secondly, emersion produces a marked shift towards urea in the form of nitrogen excreted. Fish which had been in air for 20 h and then returned to water excreted × 13 more nitrogen as urea than as ammonia. This ratio for fish which were in air for 36 h was near × 45. The fact that rates of ammonia excretion immediately after long periods of emersion were comparable with (actually somewhat lower than) rates of ammonia excretion from fish in water may indicate that virtually all waste nitrogen produced while fish were in air

was in the form of urea. There was no odour of ammonia detectable around fish in air. There were no detectable variations in rates of either ammonia or urea excretion with body size in any of these experiments. Our data are not adequate to permit a calculation as to whether or not rates of waste nitrogen production during periods out of water were different from these rates in water.

Table 2. *Some parameters of nitrogen metabolism in* Sicyases sanguineus

($\overline{X} \pm$ S.E. (N)).

Blood concentrations, fish in sea water (mM/l)	
Urea	3·2 ± 0·2 (6)
Ammonia	0·20 ± 0·01 (8)
Rates of excretion (mM/kg h)	
Fish in sea water	
Urea	0·38 ± 0·05 (6)
Ammonia	0·27 ± 0·02 (6)
Fish in air 20 h, sea water 1–4 h.	
Urea	1·31 ± 0·14 (6)
Ammonia	0·21 ± 0·02 (6)
Fish in air 36 h, sea water 1–4 h	
Urea	4·29 ± 0·52 (6)
Ammonia	0·16 ± 0·02 (6)

DISCUSSION

Sicyases sanguineus in nature remains closer to water than the mudskipper fish, *Periophthalmus sobrinus* (Ebeling *et al.* 1970; Gordon *et al.* 1968, 1969). However, it can survive periods of total emersion equally well. Our data on survival agree well with those of Vargas & Concha (1957). The mudskipper makes the greater physiological adjustment, however, since it lives at environmental temperatures about 20 °C above those in the clingfish's environment. Differences in rates of evaporative water loss between the two species, both under calm air in the shade, appear to be largely accounted for by differences in water-vapour pressure deficits in the two experimental situations.

The pattern of physiological adjustments to emersion shown by *S. sanguineus* is unlike that of any previously studied amphibious fish, or other amphibious vertebrate. Metabolic rate changes for small fish are in the opposite direction from those expected in the diving syndrome. The Australian lungfish (*Neoceratodus*) is the only other fish we know of which shows a higher metabolic rate when given access to air than when restricted to breathing water—and it is highly specialized for breathing air (Grigg, 1965). The intermittent pattern of aerial oxygen consumption shown by the larger fish has no known counterpart elsewhere among amphibious lower vertebrates, though various terrestrial reptiles behave similarly (Schmidt-Nielsen, Crawford & Bentley, 1966; Pough, 1969; Crawford & Schultetus, 1970), as do at least some diving mammals while out of water (Bartholomew, 1954).

The occurrence of periods without oxygen uptake is circumstantial evidence that larger *S. sanguineus* do not respire continuously through the skin. Occasional cutaneous gas exchanges may occur across areas of thinner skin on the ventral surface, just anterior to the attachment disk. Ebeling *et al.* (1970) observed that this region occasionally became reddish in colour, due to suffusion with blood, at times when the fish actively

raised its head from the attachment surfaces. Analogous integumentary colour changes have been observed in an Indian torrent-dwelling catfish out of water (Mahajan, 1964) and in captive blue sharks (*Prionace glauca*) subjected to anoxia during transport (Newman, 1969). It should be noted that the ability to raise the head, which is rare among fishes generally, appears to be a widespread characteristic of clingfishes (Fishelson, 1968).

Larger clingfish in air also appear to make use of buccopharyngeal respiration, but in an unusual manner. Ebeling *et al.* (1970) found that the fish gulp air, which is then held in the gill cavities, from which they extract oxygen while keeping their oral and branchial openings tightly closed. It is not apparent why they should do this, since keeping the gill cavity openings agape presumably would permit continuing respiratory exchanges without requiring any effort by the fish. Avoidance of dehydration does not appear to a probable explanation, since the fish rarely emerge on to rocks not frequently washed over by waves.

We never observed head-lifting and cutaneous perfusion with blood in larger fish in air in our respirometer jars. Intermittent occurrence of the pattern of buccopharyngeal respiration just described could account for the observed intermittency of oxygen consumption by larger fish. The continuous uptake shown by small fish might have been due to cutaneous respiration across their thinner skins. We have no explanation for the progressively higher rates of metabolism shown by the small fish out of water.

We also have no explanation for a large difference existing between our oxygen-consumption data and those of Vargas & Concha (1957). Vargas and Concha made metabolic-rate measurements on 115 *S. sanguineus* in air for up to 90 min, the fish ranging in weight from 100 mg to nearly 1 kg. Their analytical procedure was imprecise and they had no facilities for controlling temperature. Their facilities for maintaining fish in captivity were also less than ideal, and produced major variations in the performance of fish of given weights. Despite these technical limitations and the relatively short durations of their runs, it is curious that they encountered none of the intermittency in uptake we have described for larger fish. Their data for fish in the 1–5 g body weight range agree well with ours for fish out of water for 1–3 h.

The pattern of heart-rate changes observed is classically that of the diving syndrome (Elsner, 1969) or of fish exposed to asphyxia in water (Serfaty, Labat & Bernat, 1965; Randall & Smith, 1967; Hughes & Umezawa, 1968; Spitzer, Marvin & Heath, 1969). Our data agree fairly well with those of Concha & Vargas (1957), who measured heart rates on fish in air for unspecified periods, at 18 °C, with the animals placed on their backs. Our data agree well with observations of Jones (1966), that recovery of heart rate to normal aerial rates in diving frogs is completely dependent upon resumption of respiratory movements. Clingfish out of water almost never make respiratory movements, but they behave like ordinary fish when in water. We have no way of determining the extent to which our results are artifacts of our having studied forced dives, rather than natural ones. Lund & Dingle (1968) indicate that unrestrained frogs making spontaneous dives may not show diving bradycardia at all.

The pattern of changes in blood lactate concentration found in *Sicyases* also does not fit the usual diving syndrome. Perhaps the fish are able to tolerate a moderate degree of tissue anaerobiosis while out of water, and they may have some internal reserves of oxygen which slow the development of this condition for at least the first

few hours of emersion. Their capacities for cutaneous and buccopharyngeal respiration appear to be inadequate to prevent partial anoxia, but are sufficient to maintain a steady state quite distant from complete anaerobiosis. The results from the groups replaced in water after differing, fairly long periods out of water indicate that *Sicyases* does not use a general shutdown of peripheral circulation as an adjustment to restricted oxygen supply. The lack of movement back toward control levels in the group out of water for 22 h may indicate a decline in recovery capacity after long exposure to air. The number of fish in that group was small however. We are aware of no similar patterns of change in blood lactic acid in other amphibious or diving vertebrates.

S. sanguineus appears to shift its nitrogen metabolism strongly in the direction of ureotelism when it is taken out of water. They resemble mudskippers (*Periophthalmus sobrinus*) in this regard, although there may be important quantitative differences between the two forms. Specifically, *Sicyases* does not appear to accumulate ammonia in its body fluids while out of water, while mudskippers may (Gordon *et al.* 1969). If this inference is correct, the clingfish are also significantly different from non-amphibious fishes taken from the water (Pequin & Serfaty, 1962). Our data on ammonia concentrations in the blood of clingfish in well-aerated water are comparable with those reported for systemic blood of a marine sculpin (Goldstein, Forster & Fanelli, 1964) and for the aquatic frog *Xenopus laevis* in water (Unsworth & Crook, 1967), but are significantly lower than concentrations reported for freshwater rainbow trout in waters of low ammonia content (Fromm & Gillette, 1968).

The stimulus for the shift towards ureotelism is not specifically identifiable from our data. The traditional interpretation is that it is a response to restriction of water supply. It may indeed be at least partly that, but it may also be partly a response to asphyxiation (Furukawa & Ogasawara, 1955). Sharma (1969) indicates that changes in inorganic ion concentrations in the blood of freshwater crayfish (*Orconectes rusticus*) may induce similar shifts, but he also states that it is unlikely that the ornithine-urea cycle exists in the crayfish. It is therefore uncertain whether teleost fish react similarly, since they all appear to use the ornithine-urea cycle as at least a major source of urea (Huggins, Skutsch & Baldwin, 1969). It should be noted that fish may also use uric acid as a substrate for urea production (Goldstein & Forster, 1965).

In view of the considerations just mentioned, it is probable that the capacity to make metabolic shifts towards or away from ureotelism is a widespread property of many kinds of fishes. Thus the presence of this capacity in amphibious fishes does not necessarily represent a feature of special phylogenetic significance (Gordon, 1970).

SUMMARY

1. A study has been made of major aspects of the physiological adaptations for terrestrial life possessed by the amphibious clingfish, *Sicyases sanguineus*, on the coast of central Chile.

2. These fish can survive for more than 1½ days out of water, if not exposed to severe dehydration or thermal stresses. Rates of evaporative water loss while out of water are low. Upper lethal temperatures are also low, reflecting the uniformly cool water temperatures of their environment. In nature the fish rarely leave areas in which they are frequently washed over by waves.

3. These fish demonstrate an unusual combination of metabolic and cardiovascular adjustments to emersion. Metabolic rates (oxygen consumption) of small (1–5 g weight) fish are higher out of water than in water. Larger fish show a pattern of intermittent oxygen uptake when out of water. Heart rates respond to emersion (and associated cessation of breathing movements) in the pattern of the diving syndrome. Lactic acid concentrations in the blood gradually increase above control levels during the first few hours of emersion, then remain constant at about $\times 3$ control level. There is no indication of peripheral vascular shutdown during emersion.

4. Emersion produces a marked shift towards ureotelism in waste nitrogen production. There appears to be no systemic accumulation of ammonia during emersion.

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

These studies were carried out at the Laboratorio de Fisiología Celular, Facultad de Ciencias, Universidad de Chile, Montemar, Chile. The work was supported by the Ford Foundation through the University of California—Universidad de Chile Convenio program; National Science Foundation grants to the senior author (GB 5661, GB 15180); and ARO grant (U.S.) DAHC-19-68-G-0018 to S. Fischer. We wish to thank the following people for their kind assistance and co-operation: Mr Eugene Menzies, Resident Co-ordinator of the Convenio in Santiago, Chile and his staff; Sr Anelio Aguayo, Acting Director of the Estacion de Biología Marina, Montemar, and his staff; Drs Fernando Vargas and Mario Luxoro of the Laboratorio de Fisiología Celular; and Dr Alfred Ebeling of the Department of Biological Sciences, University of California, Santa Barbara. José Soto, Robert Sahara and Shwu-jen Chiu provided technical assistance.

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