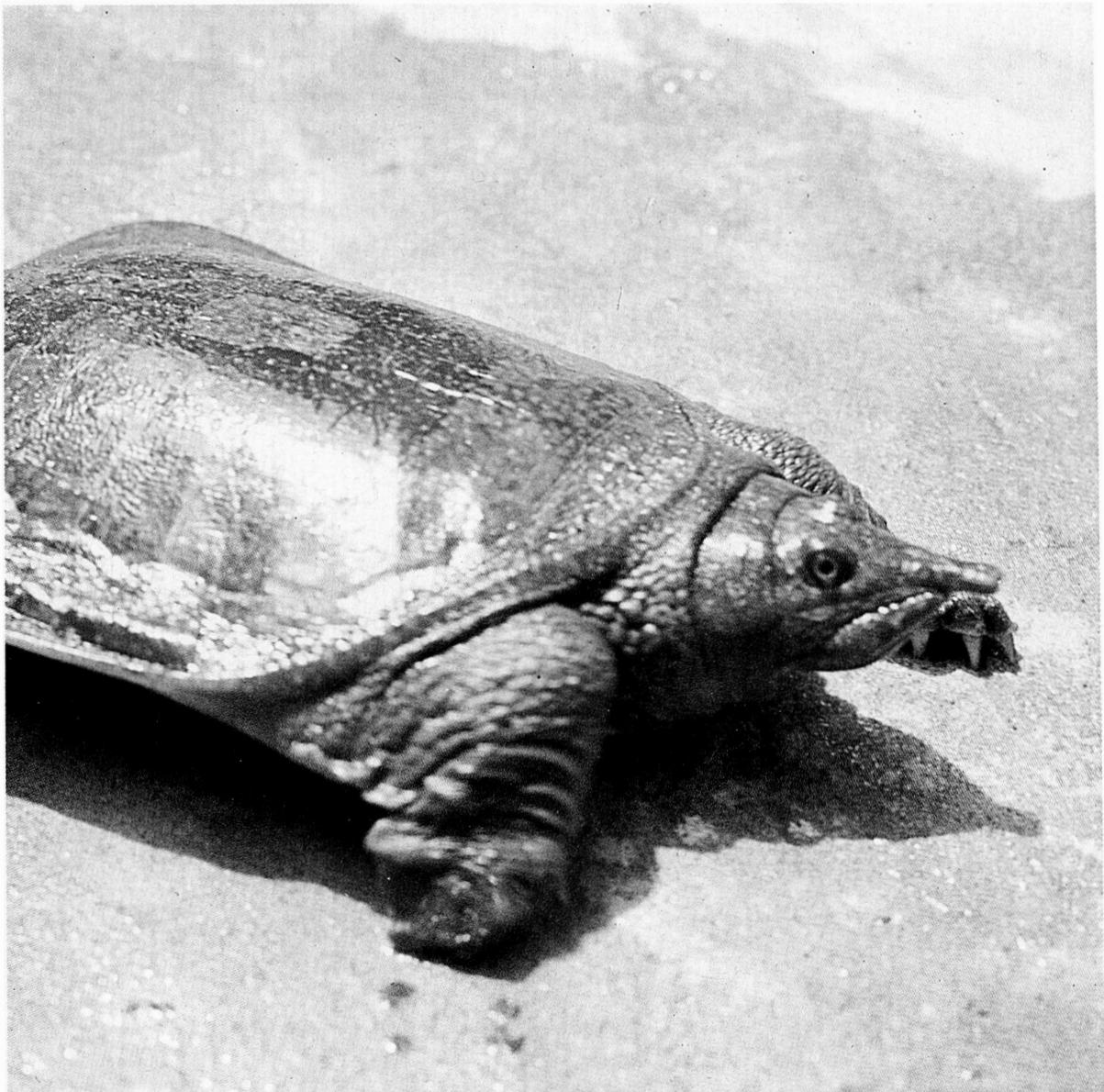


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AERIAL AND AQUATIC RESPIRATION IN THE BLACK-RAYED SOFTSHELL TURTLE *AMYDA CARTILAGINEA*

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ABSTRACT

Black-rayed softshell turtles (*Amyda cartilaginea*) from Malaysia were shown to be capable of extracting oxygen from water by a combination of cutaneous and buccopharyngeal respiration. Given access to air as well as water they consumed a mean 81 ml O₂ g⁻¹h⁻¹; when submerged and respiring aquatically the uptake fell to a mean value of 21 ml O₂ g⁻¹h⁻¹ (ratio 3.86:1). Behavioural data show that the turtles cannot survive indefinitely by aquatic respiration alone as they incur an oxygen debt, even when inactive. Scope for activity is substantially reduced, even when air becomes available, until the oxygen debt is repaid. Buccopharyngeal respiration is a normal feature of behaviour, and is not used solely during prolonged submergence. The turtles pump some 40–80 ml water min⁻¹ through the pharynx at 30°C. Turtles display dilated cutaneous blood vessels when they are submerged for long periods.

INTRODUCTION

Reptiles are in general air breathers, which rely wholly upon pulmonary gas exchange. However, over the past century a number of aquatic chelonians have been shown to gain some of their oxygen directly from the surrounding water. Gadow (1901) described the vascularized cloacal sacs of emydid turtles through which water is circulated, while Cahn (1937) demonstrated that water was moved in and out of the pharynx by emydid chelonians of the genus *Chrysemys*. Most interest in chelonians has centred upon the soft-shelled turtles (Family Trionychidae). Softshells are specialized, highly aquatic turtles, rarely seen out of water. They have flattened, skin-covered shells, long necks and distinctive snorkel-like snouts which enable them to breathe inconspicuously at the water surface. Gage & Gage (1886) described filamentous pharyngeal viliform processes in *Trionyx* (= *Amyda*) *spinifer* LeSueur and *Trionyx muticus* LeSueur, and also showed that these animals could take up oxygen whilst under water. They believed that the viliform processes functioned as gills, and were responsible for most aquatic oxygen uptake. Several authors have noted that the thin, well-vascularized skin covering the shells of softshells is likely to function as a respiratory surface; as Pritchard (1979) remarks 'the soft, delicate skin would seem to be somewhat of a liability otherwise'. Three decades ago two workers (Dunson, 1960; Girgis 1961) demonstrated that some softshell species (*Trionyx spinifer* and *Trionyx triungis* Forskal) could definitely extract oxygen from water (earlier workers other than Gage and Gage had assumed a respiratory function for skin and buccopharynx, but presented no direct evidence in support of the hypothesis). Girgis (1961) claimed that aquatic uptake of oxygen was sufficient to support the metabolism of an inactive, submerged Nile turtle.

The study reported here was carried out on a softshell species which has previously attracted little study, none of it physiological. The black-rayed softshell turtle *Amyda cartilaginea* is a large species (< 70 cm carapace length) widely distributed in S.E. Asia (Pritchard, 1979). It lives in muddy rivers as well as clear hill streams and is usually described as carnivorous, though a dead specimen dissected for the present study had a large intestine packed with palm kernels, suggesting a more

omnivorous lifestyle. Like many other Asian softshells, the species is exploited commercially for its flesh, being caught in traps or by rod and line. Black-rayed softshells were studied to determine whether they were able to take up oxygen from water, to assess the importance of such uptake, and to investigate the ventilatory behaviour, both pulmonary and buccopharyngeal, before, during and after periods of submergence.

MATERIALS AND METHODS

COLLECTION AND MAINTENANCE

Four animals were purchased from a food market in Penang, Malaysia; they had been collected from streams on the west coast of peninsular Malaysia. One had been badly bitten by the others and died shortly afterwards; it was used in anatomical investigations. The rest were held in fresh water at 30±2°C and fed three times per week on chicken liver and trash fish. They were returned to the wild at the conclusion of the study.

APPARATUS

Breathing patterns and oxygen uptake in air and water were studied with the aid of the perspex apparatus shown in Fig. 1 which was held in a temperature bath. All experiments were carried out at 30°C. The turtles could each be exposed to four experimental situations (modes A–D). In mode A the animal was supplied with flowing, aerated water (< 2 l min⁻¹) and had access to an air space (volume 3096 ml). The animal's breathing in air and pharyngeal pulses under water could be observed. In mode B an oxygen electrode connected via a pH meter (both manufactured by Strathkelven Instruments) to a chart recorder was fitted to lid 1 of the apparatus. This arrangement allowed the measurement of oxygen tension within the air space. To make an oxygen uptake measurement, a turtle was settled within the apparatus for 2 hr with water flowing through the apparatus (as in mode A). The flow was then cut off by closing taps 1 and 2. Magnetic stirring of the water continued so that the aquatic and aerial phases were in equilibrium and the animal was free to take up oxygen from both phases. In mode C, lid 2 was applied so that the animal could only take up oxygen from water, but water still flowed through the apparatus. Finally, in mode D, lid 2 was fitted with the oxygen electrode and taps 1 and 2 were

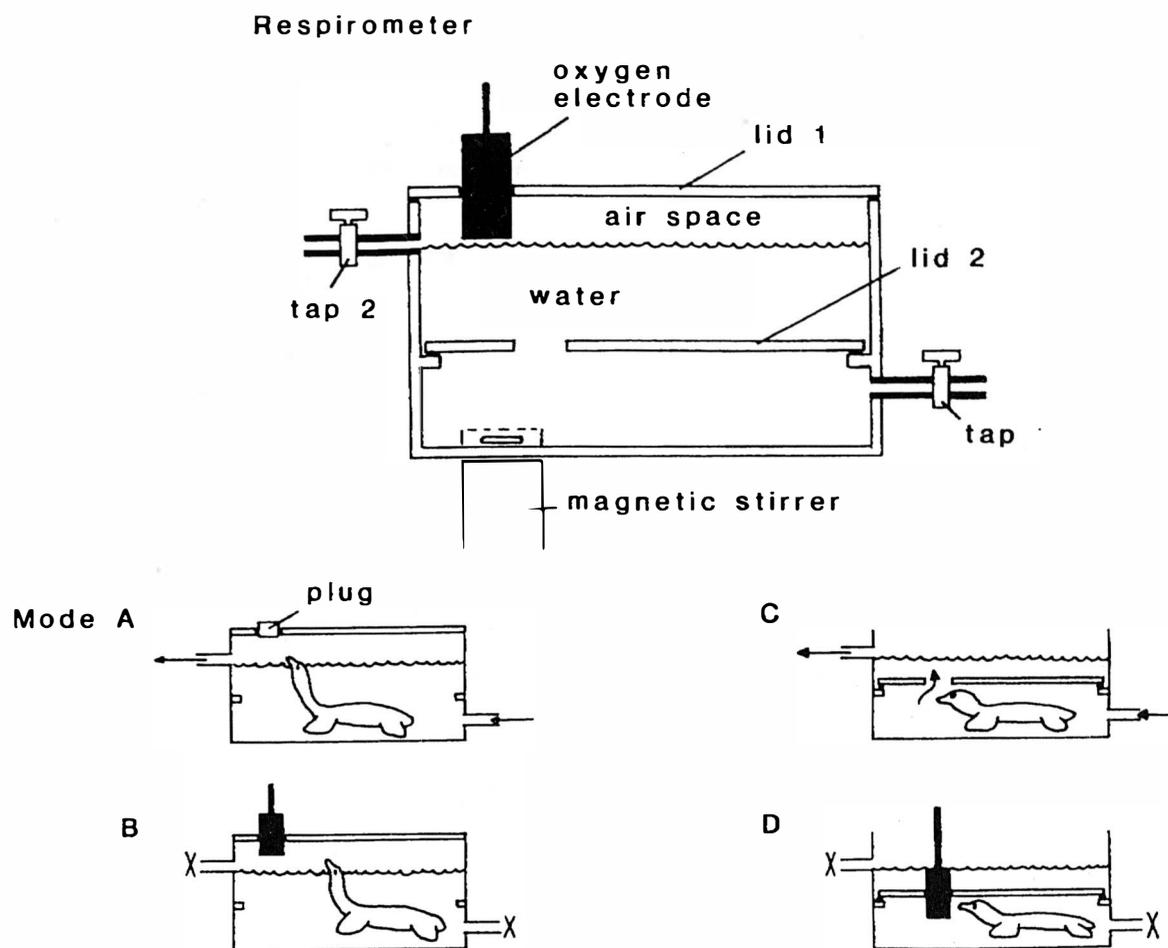


Fig. 1. Apparatus used in behavioural and respirometric studies. The four modes of use of the apparatus are also illustrated.

closed so that aquatic oxygen uptake could be measured. For modes C and D, lid 2 was gently put in position without disturbing the animal under investigation.

EXPERIMENTAL PROTOCOL

Behavioural responses. The three turtles, in turn, were each studied before (apparatus mode A), during (mode C) and after (mode A) a period of sustained immersion. The following features of behaviour were monitored throughout; physical activity (measured as the number of movement cycles exhibited by the left forelimb per min), the number of breaths taken at the surface, the number of times the animal surfaced (i.e. projected part of the head above the water surface), and the number of pharyngeal pulses per min. Turtle 1 was held immersed for 90 min, turtle 2 for 120 min and turtle 3 for 50 min. At intervals, in all experiments, the appearance of the unpigmented plastron was inspected with the aid of a mirror to determine the degree of vasodilation.

Oxygen uptake measurements. Each turtle, in turn, was subjected to the following procedure. First the animal was allowed to settle in the apparatus for 2 hr (mode A). Next the oxygen uptake in both air and water was measured over a period of 4 hr (mode B). The animal was then returned to the mode A configuration for 2 hr. Finally, aquatic oxygen uptake was measured

over a period of 20-30 min (mode D). Due allowance was made for the volume of the animal (measured by displacement) in all calculations. No attempt was made to measure aerial oxygen uptake in softshells held out of water as this risks skin damage and the animals usually exhibit prolonged activity.

ANATOMICAL OBSERVATIONS

Live animals. To determine the water flow characteristics associated with pharyngeal pulsations, a vital dye (methylene blue) was introduced into the water around the snout of a softshell resting on the bottom of its holding tank.

To estimate the tidal volume of water exchanged with each pharyngeal pulsation, a second softshell was filmed when respiring underwater (with a Panasonic F10 videocamera fitted with a high speed shutter) from in front and from the side against a 1 cm grid background. To make measurements, fine fibretip drawings were made from frozen videofields by placing acetate sheets over a video monitor screen.

Dissection. A dissection and latex injection of the anterior arterial system was carried out to determine whether *Amyda cartilaginea* possesses the rich pharyngeal vascularization described for *Trionyx spinifera* by Gage & Gage (1886) and for *Trionyx triunguis* by Girgis (1964).

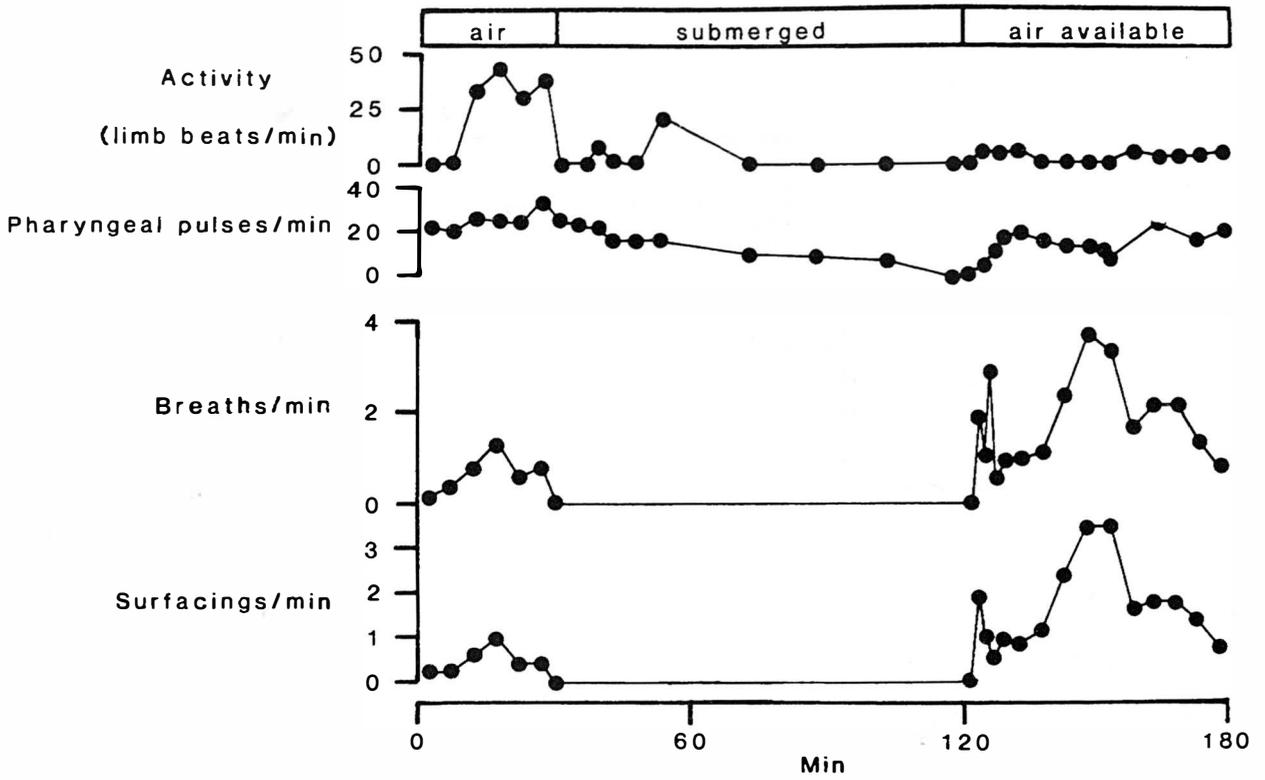


Fig. 2. Patterns of behaviour of turtle 1 before, during and after prolonged submergence.

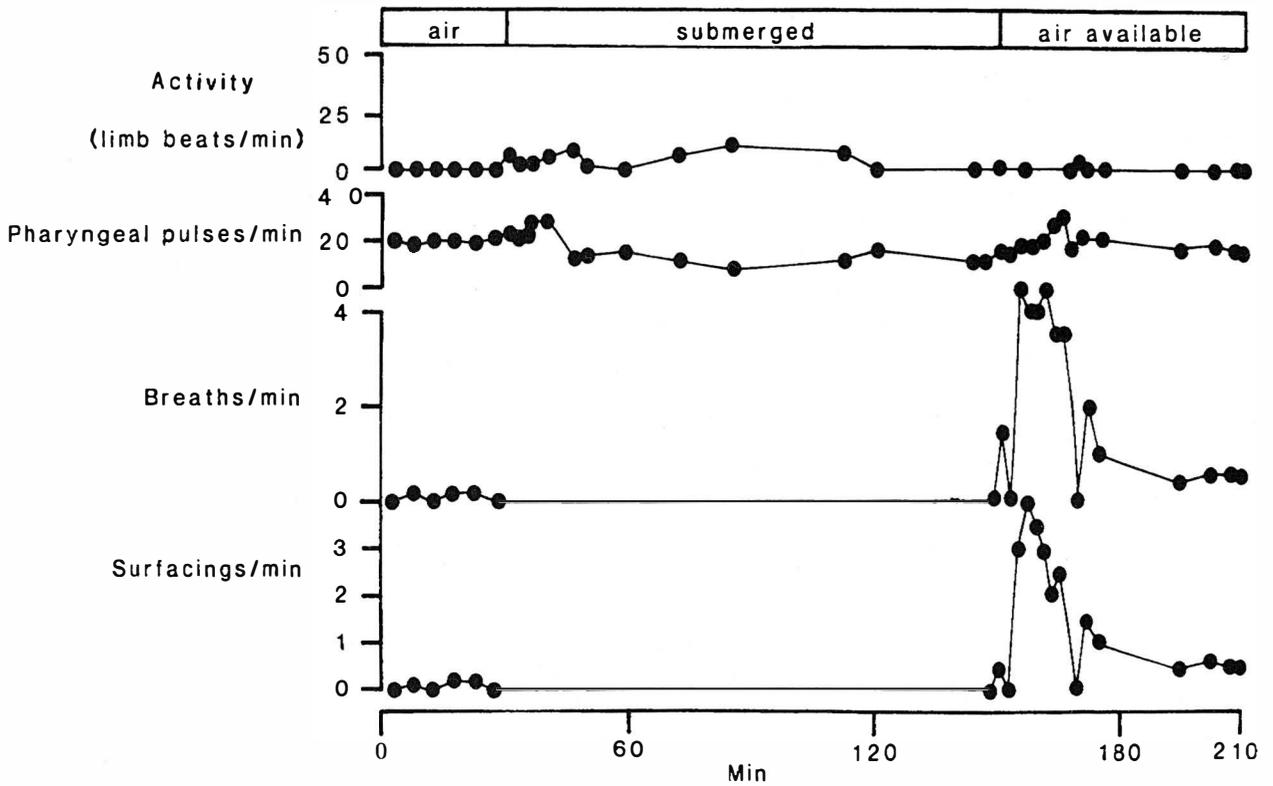


Fig. 3. Patterns of behaviour of turtle 2 before, during and after prolonged submergence.

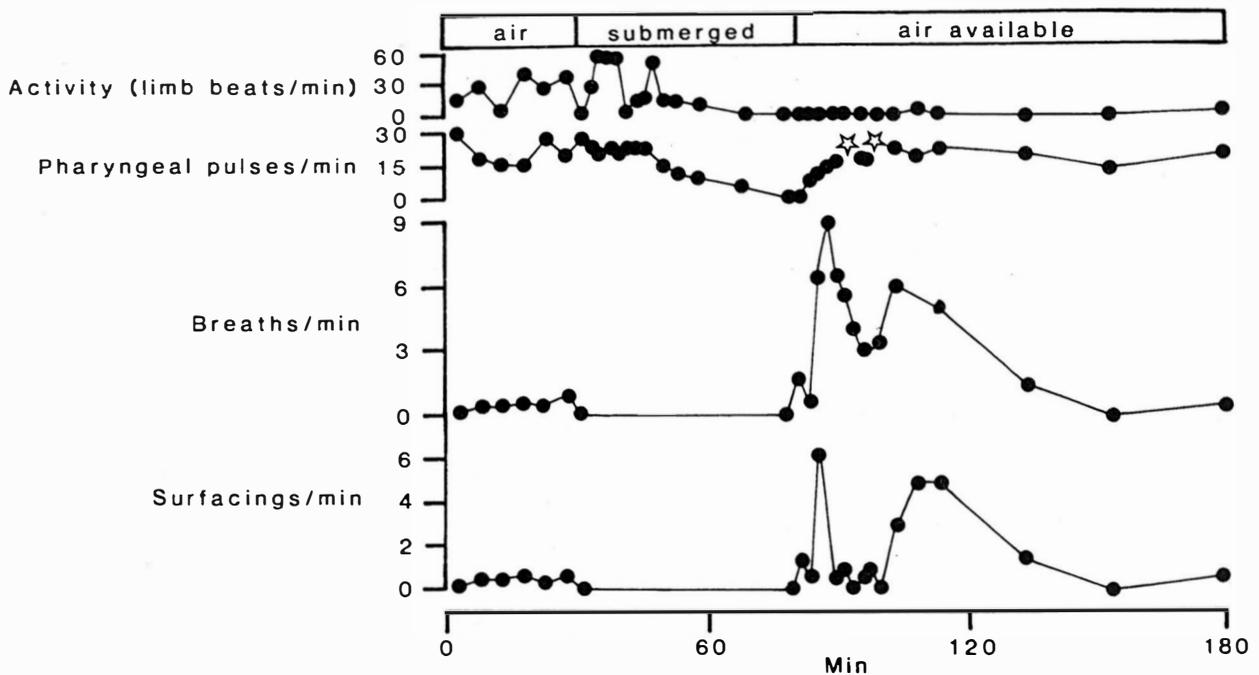


Fig. 4. Patterns of behaviour of turtle 3 before, during and after prolonged submergence. The stars indicate periods of time when the animal maintained its head out of water so that pharyngeal respiration was impossible.

RESULTS

BEHAVIOURAL RESPONSES TO IMMERSION

Figs 2-4 show the responses of the three turtles. Animal 1 (Fig. 2) showed a burst of swimming activity in the 20 min period prior to submergence. During this activity there was a rise in pharyngeal pulsation rate (from about 20 to 34 pulsations min^{-1}), while the breathing rate rose from 0.2 breaths min^{-1} to a peak of 1.4 breaths min^{-1} . During submergence physical activity fell to zero within 40 min. The pharyngeal pulsation rate fell steadily, reaching zero after 87 min. By this stage the normally greyish-white plastron was noticeably pink in colour and dilated surface blood vessels could be discerned. On gaining access to air, the turtle showed very little swimming activity, but the animal immediately surfaced several times by extending its neck and head to the surface (usually taking more than one breath per surfacing episode). After taking a number of deep breaths in the first 5 min after gaining access to air, the breathing rate of the animal dropped back to 0.5 breaths min^{-1} . Over the next 20 min the breathing and surfacing rates rose, breathing reaching a maximum of 3.8 breaths min^{-1} - far higher than during the burst of activity before submergence. Pharyngeal pulsations restarted as soon as breathing recommenced, and within 10 min had returned to 20 pulsations min^{-1} . The breathing rate fell over a further 40 min to less than 1 breath min^{-1} , and the animal again began to breathe only once per surfacing. This sequence showed that (a) pharyngeal pulsations were more frequent during activity, indicating that aquatic respiration was a normal feature of respiration, and not limited to periods of prolonged submergence, and (b) that the greatly enhanced rate of breathing after the 90 min submergence period represented repayment of an oxygen debt (since the turtle showed negligible physical activity when the rapid breathing was taking place). However, because the turtle had been active before submergence, it was not possible to be certain that the oxygen debt had been incurred during submergence.

Animal 2 (see Fig. 3) was inactive for a long time before submergence, showed little swimming during the 120 min of immersion (during which period the pharyngeal pulsation rate fell by half), but this animal still showed an enormous increase in breathing rate (to a maximum of 4.5 breaths min^{-1}) after gaining access to air. This response confirms that the increased ventilation after submergence reflects an oxygen debt acquired during that submergence. Turtle 3 (Fig. 4) was active before submergence and for the first 20 min of immersion. Activity and pharyngeal pulsation rate then fell gradually to zero. On gaining access to air the animal exhibited a rapid increase in breathing rate (to 9 breaths min^{-1}) in the first 10 min, followed by a fall, a rise and then a slow decline in breathing rate so that by 70 min after regaining access to air, the breathing rate had returned to resting levels and the animal began to swim again, albeit at a very low level of limb beat. As with the other turtles the pharyngeal pulsation rate rose to 20 pulsations min^{-1} within about 20 min of gaining access to air, but there was no sign of an overshoot in pharyngeal respiration, indicating that an oxygen debt is paid off by aerial respiration alone. Turtle 3 exhibited a pink plastron at the end of the period of submergence; the colour had disappeared by the time breathing rates had returned to normal.

OXYGEN UPTAKE

Oxygen uptake was recorded in two circumstances; when oxygen in both air and water was available, and when dissolved oxygen alone was accessible. From Table 1 it may be seen that oxygen uptake is much higher when air is available, by a factor of 3-6 (mean 3.9:1). The mean aquatic oxygen uptake rate ($21 \mu\text{l O}_2 \text{ g}^{-1} \text{ h}^{-1}$) recorded was about three times the level reported by Girgis (1961) for specimens of *Trionyx triunguis*. However, the latter were bigger animals (605-1520 g) and were investigated at a lower temperature (23°C), so this difference is probably not significant.

| Animal No. | Body wt (g) | Oxygen uptake in air + water (ml O ₂ g ⁻¹ h ⁻¹) | Oxygen uptake in water alone (ml O ₂ g ⁻¹ h ⁻¹) |
|------------|----------------|---|---|
| 1. | 371 | 68 | 23 |
| 2. | 310 | 102 | 16 |
| 3. | 326 | 72 | 25 |
| | mean | 81 | 21 |
| | ratio | 3.86 | 1 |

TABLE 1. Oxygen uptake in *Amyda cartilaginea*

ANATOMICAL OBSERVATION

Dye tests showed that there was two-way movement of water through the external nares, that some inhalation was through the mouth, but that exhalation (more forceful) was mainly through the nose. Videotape records showed that the pharyngeal region of the animal studied (turtle 3, 326 g body weight) changed in volume by about 2-4 ml between maximum inhalation and maximum exhalation. This suggests that, at rest, the animal would have been moving about 40-80 ml of water through the pharynx per minute. Roughly speaking this would result in 12-24 ml O₂ passing over the pharyngeal epithelium per hour.

Given an aquatic oxygen uptake rate of about 8 ml O₂ h⁻¹ for this turtle (see Table 1), it is conceivable that aquatic respiration could be sustained by buccopharyngeal uptake alone (though this would imply a rather high extraction efficiency and steep gradients of oxygen tension between blood and water).

Dissection showed that the vascular arrangements in the pharyngeal region of *Amyda cartilaginea* are very similar to those described for the Nile softshell by Girgis (1964). Paired pharyngeal arteries supply a rich arterial vascularization in the floor of the pharynx (a 'rete mirabile'); the pharyngeal arteries arise from the carotids.

DISCUSSION

Amyda cartilaginea like other softshelled turtles, is clearly capable of extracting oxygen from water. It may also be seen from the breathing pattern data that the animals would not be able to survive indefinitely by aquatic respiration alone, even when inactive, since a considerable oxygen debt (presumably reflecting accumulation of by-products of anaerobiosis) is incurred during prolonged submergence. Since resting animals with access to air consume oxygen at nearly four times the rate of animals able to take oxygen only from water, this finding is not unexpected, but conflicts with the conclusion of Girgis (1961) that Nile softshells could sustain an inactive metabolic rate by aquatic respiration alone. Certainly the evidence presented here strongly suggests that *Amyda cartilaginea* cannot remain submerged for periods of more than a few hours at most, and the resulting oxygen debt will restrict scope for activity for a considerable period thereafter.

Previous investigators have suggested that aquatic respiration in softshells is an inefficient process, only of survival value during prolonged submergence (e.g. Girgis, 1961). There has also been debate about the relative contribution of

cutaneous and buccopharyngeal oxygen uptake (Dunson, 1960; Girgis, 1961).

As far as overall efficiency is concerned, the aquatic respiration rate of *Amyda cartilaginea* corresponds to about a quarter to one third of the aerial respiration rate (the precise value is difficult to assess because in the experiments reported here 'aerial' oxygen uptake probably included some aquatic uptake of oxygen). This proportion is appreciably greater than that recorded by Root (1949) for hard shelled musk turtles, in which the aquatic oxygen uptake rate was only about 12% of the aerial value. This finding reinforces the idea that softshelled turtles are especially adapted for a life in which surfacing is reduced to a minimum.

The behavioural data collected in the study reported here clearly indicate that buccopharyngeal respiration is a normal feature of this species whenever the head is under water. Because the pharyngeal pulsation rate rises with increasing activity, it seems probable that uptake of oxygen by the pharynx is an auxiliary respiratory function at all times; its existence will delay a return to the surface for air, whether this be at intervals of a minute or so during violent activity, or at hourly intervals when the animal is quiescent. Girgis (1961) argued that pharyngeal uptake could not be important except during prolonged submergence, because the blood supply to the pharyngeal rete was from the carotid arteries, so would be fully oxygenated and therefore would not allow uptake of oxygen from water. However, Agassiz (1857) long ago showed that softshell turtles have unusually small lungs as far as chelonians are concerned. In consequence, the oxygen tension in alveolar air is likely to decline rapidly after the turtle takes a breath (particularly when the animal is active), so that arterial pO₂ will also fall, allowing oxygen uptake across the villi of the pharyngeal lining. As far as hard shelled chelonians are concerned, Vos (1936), McCutcheon (1943) and Root (1949) all considered that flow of water in and out of the pharynx was primarily carried out for olfactory purposes. The findings reported here for *Amyda cartilaginea* definitely indicate a respiratory rôle; not only is the rate of water flow through the buccopharyngeal region much greater than would be needed for olfaction, but the rate of pulsation is linked to activity.

While Dunson (1960) argued that buccopharyngeal oxygen uptake made the major contribution to aquatic respiration (in *Trionyx spinifer*), and that cutaneous uptake was less important, Girgis (1961) conducted experiments which appeared to show that 70% of the aquatic oxygen uptake in *Trionyx triunguis* took place across the skin of the shell, limbs and body, while only

30% of uptake was by buccopharyngeal respiration. Unfortunately the design of Girgis' experiments was flawed, so it is impossible to be confident about his results. Girgis held turtles in water-filled, sealed tanks that were divided into two compartments, one containing the head and neck, the other the rest of the turtle. Oxygen concentrations (not tensions) in both compartments were measured. The underlying rationale behind the experiment was that oxygen uptake in each compartment was independent. However, blood flow around the body provides a route of gaseous exchange between the two compartments. Given that the pharynx and body skin are both permeable to oxygen, it may be predicted that the oxygen tension in the two compartments (and the body fluids) will tend to equilibrate, so that the apparent oxygen uptake from each compartment will simply reflect the size of the compartments - as is the case if Girgis' data and experimental technique are inspected carefully! In the present study no attempt was made to differentiate between the uptake across pharynx and integument, not least because it is difficult to see how this could be done in an unstressed animal. The technique pioneered by Root (1949) on the hardshelled musk turtle (*Stemotherus odoratus* (Latreille)) involved sealing off the body surface so that only buccopharyngeal respiration could take place. Girgis also employed this method on the Nile softshell, but in both cases the assumption that the animal will extract oxygen across the buccopharyngeal wall at the same rate as it would if other possible respiratory surfaces were functioning is rather dubious. However, in the case of *Amyda cartilaginea*, some indirect assessments may be made. Firstly, during prolonged submergence, the pharyngeal pulsation rate tends to decline, sometimes to negligible levels, while the plastron surface shows evidence of increased cutaneous blood flow. This suggests that the balance between cutaneous and buccopharyngeal uptake is a dynamic one, with uptake across the skin becoming more important with duration of submergence. Secondly, the rate of water flow through the pharynx, combined with the measured oxygen uptake when submerged, suggests that the black-rayed softshell would have to extract about one-third to two-thirds of the available oxygen from the flow of water if it was to rely on buccopharyngeal exchange alone. Such efficiency of extraction seems most unlikely, despite the two-way flow of water, so it is probable that cutaneous uptake was at least as important as buccopharyngeal gas exchange in the experiments reported here. On the other hand, it needs to be remembered that softshells, *Amyda cartilaginea* included, are burrowing forms in their natural habitat. When

much of the body surface is covered with sand or mud, it will be impossible for significant amounts of oxygen to be taken up across the skin. Indeed, since the interstitial pO_2 of sediments is usually low due to a combination of microbial respiration and poor water circulation, it may be necessary for the turtle to constrict peripheral blood vessels to avoid loss of oxygen to the sediment. In these circumstances the buccopharyngeal route is likely to become dominant.

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