

Respiratory physiology of the sandfish (Squamata: Scincidae: *Scincus scincus*) with special reference to subharenal breathing

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Abstract. The sandfish (*Scincus scincus*) is a desert-dwelling lizard that has evolved the ability to swim in loose sand. It can also bury itself deep in sand, remaining there for prolonged periods while staying physiologically active. We investigated the basic parameters of its respiratory physiology – pulmonary volumes, oxygen consumption, and carbon dioxide production – and the physical properties of its natural habitat. The latter were also simulated using a mathematical model, which was then applied to a more naturalistic system. The lungs, and thus the tidal volumes, of the sandfish are very small, and its oxygen consumption and carbon dioxide production are low. Our simulation suggests that the diffusion of gases through sand alone is sufficient to fully sustain physiological conditions. The animal's low need for oxygen enables it to inhabit a large biotope within the sandy desert, where it can passively regulate its body temperature (and consequently its metabolism), thus reducing its need for food and water, and where it can rest in locations that are safe from predators.

Key words. *Scincus*, respiratory, physiology, subterranean, adaptations.

Introduction

General information

The Saharo-Sindian scincid lizards of the genus *Scincus* GARSALT, 1764, which currently comprises four species and several subspecies (ARNOLD & LEVITON 1977), are among the few squamates that are adapted to subharenal (under sand) life. Their ability to move in an almost fish-like manner in the sand has earned them the name الرمال سمكة (pronounced “smkh armal”) in Arabic countries, which literally translates into “sandfish”. The most prominent member of the genus is probably *Scincus scincus* (LINNAEUS, 1758).

The main habitat of *S. scincus* is loose sand with minimal vegetation such as grass and dry shrubs (HARTMANN 1989). Its diet consists of desert insects such as orthopterans, but it also feeds on grass, fruits and seeds, which classifies it as an omnivorous animal (HARTMANN 1989). Due to the extreme aridity of its habitat, *S. scincus* is adapted to a lifestyle in which it obtains all necessary water from its animal prey, even though it may drink if water is accessible (HARTMANN 1989). Although *S. scincus* must come to the surface to hunt, feed and reproduce, it is capable of spending the greater proportion of the day in the sand. This suggests particular morphological and physiological adapta-

tions, of which those related to the respiratory system are probably the most important ones.

Sand-related issues

Sandy deserts offer very particular habitats. The small, sharp-edged sand particles can damage the integument and are also dangerous if they enter the body. In addition, the dry climate and the loose and moving sand strongly limit plant growth and consequently hiding places and food resources for animals. Extreme temperature oscillations, and high daytime temperatures in particular, amplify these effects.

The sandfish has evolved morphological and metabolic adaptations in response to these issues: smooth, scratch-resistant skin and a way of life that allows it to stay hidden deep in sand. To survive in such an environment, organisms need to minimise their water loss and overall metabolism or find alternative ways of acquiring vital resources.

The slow conduction of heat to lower sand layers creates a vertical gradient down to a depth where the temperature remains constant (NOFZIGER 2005). At night, air temperatures drop rapidly, slowly turning the gradient around. Such extreme changes are hostile to most animals. Howev-

er, the gradients and the deeper layers with constant temperatures enable sand-dwelling poikilothermic animals to adjust their body temperature by moving between layers. The temperature gradient allows *S. scincus* not only to evade extreme changes, but also to find the layer with the optimal temperature and thus to reduce its temperature-dependent metabolism to a minimum.

The pentagonal cross-section of its body with a dorsal apex (BAUMGARTNER et al. 2008) and the ability to hold the thoracic and abdominal portions of the body in a slightly concave manner between the two ridges facilitate ventilatory movements. This ensures ventilation even when the animal is buried deeply in the sand.

Subharenal respiration

In his monographic account, PERRY (1998) focused amongst the scincomorphic families (according to more recent molecular studies, these are no longer considered a valid phylogenetic group, see PYRON et al. 2013) on lacertids, teiids (including gymnophthalmids), and cordylids, stating that “the lungs of scincid lizards are very similar to those of teiids”. The only relatively detailed recent study of the lungs of some scincids, including *S. scincus*, is that by KLEIN et al. (2005), who reported that the lungs of all skinks are relatively long and slender, which had already been pointed out by BRANDT & RATZEBURG (1829) in their comparison of *S. scincus* and *Lacerta agilis*. Furthermore, KLEIN et al. (2005) described a slightly heterogeneous parenchymal distribution with faeoli in the cranial part and ediculae in the caudal-most part, while niches are absent from scincid lungs. They also provided data on the visceral topology and several measurements of the respiratory system of *Scincus* compared to some other scincid genera, namely *Chalcides*, *Eumeces* (as *Novoeumeces*), *Mabuya*, *Tiliqua*, and *Tribolonotus*.

The possibility of fine sand particles entering and blocking or even damaging the respiratory system is problematic. The particles vary in shape and size, but typically have a diameter of 0.1 to 0.5 mm (HARTMANN 1989). Due to their irregular shapes, they create interconnected interstitial pockets that are filled with air. These are small and consequently have a high airflow resistance (HOLDICH 2002). Forced air convection should therefore be slow, which creates the risk of local O₂ depletion and CO₂ accumulation. To overcome these phenomena in a compact sand bed, an animal must either frequently change position to layers containing “fresh air” or lower its metabolic rate to values at which physiological conditions can be sustained mainly by a diffusion-driven exchange of respiratory gases within the surrounding medium.

Motivation

Morphological adaptations of the locomotory apparatus of the sandfish have frequently been described and illustrated (ARNOLD & LEVITON 1977, HARTMANN 1989), but speciali-

zations related to breathing in sand have received little attention.

We therefore sought to shed light on how the sandfish achieves oxygen homeostasis while remaining buried in fine sand for hours or even days and whether it has evolved any specializations related to this problem.

For comparison, we additionally investigated a genetically closely related species as a reference species. In contrast to *Scincus scincus*, Schneider's opaque-lidded skink (*Eumeces schneiderii*) does not bury itself in loose sand, but creates caves and therefore does not face the problem of high airflow resistance. It reaches total lengths of 30 to 35 cm, half of which is due to the tail. *Eumeces schneiderii* is found mostly in the same geographical regions as *S. scincus* (SCHMITZ et al. 2004), but – unlike it – usually stays in rocky areas and prefers to hide in cavities that it finds or excavates itself. In the rare event that it does bury itself in sand, it will stay close to the surface.

Materials and methods

Scincus scincus and *E. schneiderii* were kept in our laboratory under appropriate conditions, following the guidelines suggested by the European Union and the protocol developed by HARTMANN (1989) for the husbandry of *S. scincus*.

Oxygen consumption

To quantify the amount of oxygen consumed, an animal was placed in a sealed tank with a sandy bottom substrate for burying, a piece of wood for hiding under, a small fan secured in a cage for air circulation, and an oxygen sensor (GOX 100, Greisinger electronic, Regenstauf, Germany) to measure the loss of elemental oxygen in the chamber. Temperature-controlled water was circulated around the tank to keep the sand at 29°C. In all experiments, the animals were kept in the measuring chamber for four hours, and the oxygen concentration values were logged every two minutes. The container was monitored to ensure that the animals were indeed buried. To calculate standardized values from simple oxygen concentrations, the weight of the animals and the exact amount of air in the tank were recorded beforehand. An ANOVA test and a two-tailed t-test were performed to establish whether different metabolic rates were equivalent or corresponded to different metabolic states.

Lung volumes

To quantify the total lung volume, the lungs were removed from a fixated sandfish specimen and filled with casting plastic (Technovit 7143, Heraeus Kulzer GmbH, Wehrheim, Germany). The excised lungs were then macerated in 20% KOH at 45°C for three days. The total lung volume was regarded as equal to the displacement volume of the

cast. Tidal volumes were measured with a spirometer; to this end, a spirometric mask was constructed that could be placed on the head of a sandfish and sealed airtight. A 10-ml plastic pipette containing a small water droplet was used as a measuring unit: every time the volume in the mask changed due to inspiration or expiration, the droplet moved in the pipette by the same amount. After fitting the mask, the time-dependent position of the drop was recorded on video, and the volume changes due to breathing were analysed.

Investigating lung volume and anatomy by means of corrosion casting is not a standard method, for which reason additional methods were used for comparison. A section preparation of the lungs and a magnetic resonance imaging (MRI) analysis were conducted.

Lung preparation

The thorax was carefully opened and the rib cage cut away. The heart was removed to expose the lungs. The lungs were extracted from a frozen specimen. A lung was soaked in and filled with phosphate-buffered saline (PBS) solution. The PBS was exchanged three times. The lung volume was measured by weighing the liquid inside the lungs, where the density used for the calculation was approximately 1 g/ml. The lungs were also compared to the plastic replica in terms of size and form. One lung was slit open to compare its inner structure with that of the plastic replica.

Magnetic resonance imaging

All magnetic resonance imaging experiments were performed on a 7T Biospec (Bruker Biospin GmbH, Ettlingen, Germany) with the sandfish placed in ethanol, and images were acquired using a 3D spin echo sequence. The imaging parameters were set to a field-of-view of $100 \times 60 \times 60$ mm and a matrix size of $1024 \times 512 \times 512$ points, resulting in a nominal resolution of $98 \times 117 \times 117$ μm . The repetition time was 300 ms, and the echo time was set to 11 ms. The total imaging time was 22 h.

Diffusion coefficient

The values of diffusion coefficients of gases in air at different temperatures are standard constants and were taken from the literature (CUSSELER 2009). In a porous medium filled with air, such as sand, the diffusion coefficient is somewhat smaller, and therefore the effective diffusion coefficient for these parameters had to be identified by measuring the gas concentration change over time in one spatial dimension. The measurements were performed using a straight distilling adapter ($l = 17$ cm, $\varphi = 1$ cm), which was connected to the oxygen sensor on one side, filled with sand and flooded with nitrogen gas. This apparatus was then sealed until the oxygen concentration reached equi-

librium. Subsequently, it was opened on one side, and the oxygen concentration was measured on the other at regular intervals. A schematic overview of the measuring setup is shown in Figure 1. The experiment was repeated with different volumes 'V' of sand (Fig. 2). The system was also simulated by means of an algorithm, implemented in GNU Octave, and the results of the measurements and the simulation were compared to adjust the diffusion coefficient. The simulation was conducted with a finite volume model using Fick's law of diffusion, which states that the diffusion flux 'J' is directly proportional to the diffusion coefficient 'D' and the concentration gradient ' ∇x ':

$$1) J = -D\nabla x$$

For every discrete volume unit, the fluxes at its borders, the concentration change 'dx' and the end concentration 'x' were calculated for every time step. The volume units had the same width as the cylinder, which rendered diffusion one-dimensional. The calculation was performed for the same volumes of sand used in the measurement, and the simulation was repeated with an adapted diffusion coefficient until the curve fit optimally for all sand volumes. The differential equations used in the simulation were constructed as follows: fluxes are directly proportional to the concentration difference in the previous time step and the diffusion coefficient:

$$2) J_h(t) = \frac{D}{\Delta h} (x_{h-1} [t-1] - x_h [t-1])$$

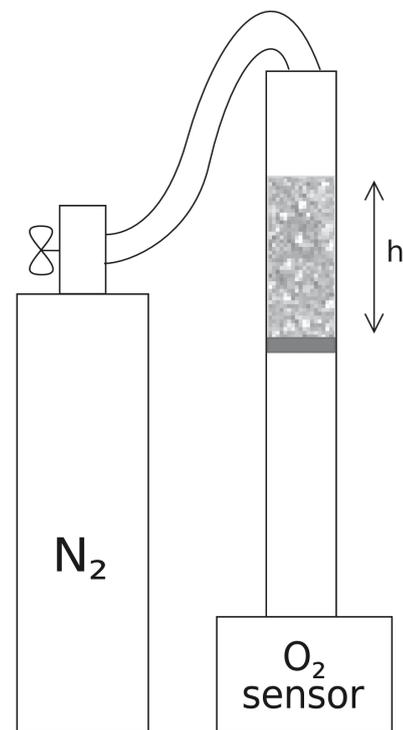


Figure 1. Schematic overview of the diffusion measurement setup. The depth of the sand in the cylinder is represented by 'h'.

The concentration change of a unit of volume equals the sum of all entering and exiting fluxes in a time step:

$$3) \Delta x_h = \frac{J_h(t) - J_{h+1}(t)}{\Delta h}$$

The final concentration is then calculated using a simple difference equation, where the previous concentration is updated with the concentration change of the current time step.

$$4) x_h(t) = x_h(t-1) + \Delta h_h \Delta t$$

The ratio between the diffusion coefficients of O_2 and CO_2 in air at $20^\circ C$ was calculated from data obtained from the literature (WELTY et al. 2008). Under these conditions, CO_2 diffusion is slower by a factor of 1.4. Supposing that this ratio remains constant in sand, the effective diffusion coefficient for CO_2 was approximated.

Simulation of the natural model

To model how oxygen behaves in sand with a breathing animal present, Fick's second law of diffusion was used in the analogue form of the heat equation with a point consumer (Poisson equation):

$$5) \frac{\partial c}{\partial t} = D \nabla^2 c + f$$

where 'f' is the consumer function. If the "natural" model of the sand were very thick and the animal buried very deeply, we could assume uniform diffusion in all directions and consequently calculate it in a spherical coordinate system with only one dimension. Since this was not the case, the horizontal surface of the sand had to be taken into account. Therefore, the calculations were performed in a cylindrical coordinate system. For this specific problem, a simple exact solution of Fick's second law exists, which saves a great amount of computation time. The Poisson equation was integrated, and thus the general form was obtained, where 'r' and 'z' represent the radial and vertical coordinates, and 'h' the depth of the buried consumer:

$$6) c(a) = \int \varphi(a-\xi) \cdot f(\xi) d\xi$$

$$7) c(a) = \frac{K}{4 \pi D} \left[\frac{1}{\sqrt{r^2+z^2}} - \frac{1}{\sqrt{r^2+(z-2h)^2}} \right] + c_0$$

To use the oxygen consumption data in the simulations, it had to be converted from the standard form of $mlg^{-1}h^{-1}$ to an absolute one, 'K' in $mols^{-1}$. Simulations were performed for both *S. scincus* and *E. schneiderii*. Considering the standard metabolic pathway, the rise in CO_2 is directly proportional to the decrease in O_2 . Since the normal oxygen concentration in air is approximately 21%, the safe oxygen

concentration (with 3% CO_2) can therefore be assumed to equal 18%. Hypothetical spheres (around the nostrils) were determined in which the maximum oxygen concentration remained above the 18% boundary. The size of the spheres was determined by the oxygen consumption of the animal and should be small enough for the air inside to be mixed by ventilation. The rest of the oxygen was supplied by diffusion, which follows a sigmoid progression (see results and discussion section).

Results and discussion

Lung reconstruction

The lung resembles a simple sac with little surface enlargement by invagination (Fig. 3). The micro-ornamentation resembles rounded, approximately hexagonal plates that are divided by thin imprints of the trabecular network. The total pulmonary volume measured by means of water displacement of the lung replicas was approximately $400 \mu l$.

Since *S. scincus* inhabits hot, arid regions it would be interesting to compare it to similarly-sized animals that live in other climates. *Lacerta viridis*, which is slightly larger in size but has approximately the same weight, inhabits Mediterranean, moderate as well as continental regions. It also has simply shaped lungs, but their total volume is almost 10

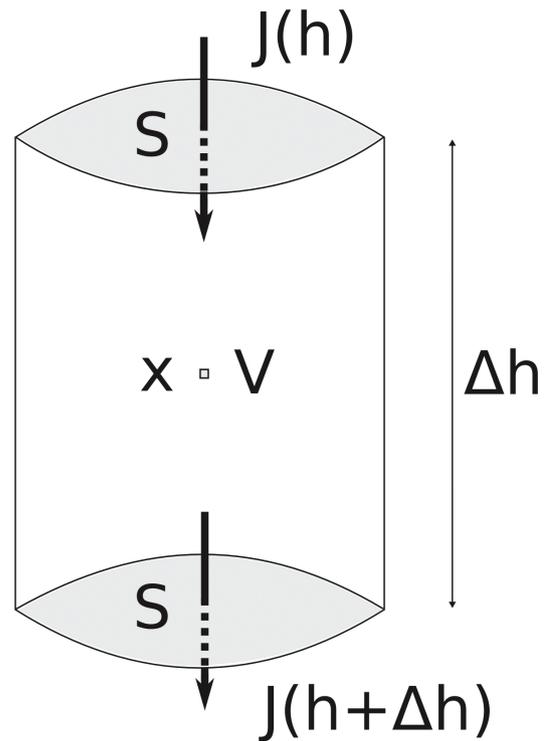


Figure 2. Schematic overview of the discrete volume unit used in the simulation. The gas concentration 'x' and 'V', the volume, are calculated as the product of the bottom or top surface area 'S' and the height 'h'.

times larger, i.e., approximately 5 ml (PERRY & DUNCKER 1978). A larger volume creates a larger surface area that facilitates greater gas exchange. The exchange surfaces, however, must be kept moist at all times, so that a larger surface results in increased water loss, which would be disadvantageous in regions where water is difficult to acquire. In an arid habitat, having smaller, simple lungs would therefore seem to be beneficial.

Lung preparation

The total lung volume of the sandfish, as measured by the weighing method, was approximately 370 μl . This agrees well with the results obtained from the replica ($\sim 400 \mu\text{l}$, or 1.6 ml per 100 g of body mass) (Fig. 3B). Furthermore, the form of the lung does not seem to vary much, but it is difficult to comment on the visceral micro-ornamentation based on observation.

Magnetic resonance imaging analysis

MRI analysis yielded a lung volume of 389 μl , which agrees with the volumes of the replica and the preparation. The low resolution of this method renders evaluating the mi-

cro-ornamentation difficult (see video in Supplementary material).

Since the replica, the lung preparation with liquid filling, and MRI analysis all provided similar result, we can assume that the average lung volume of the sandfish is in the range of 400 μl . It is not possible to state how accurate the replica is in terms of visceral ornamentation, since it is difficult to evaluate the reference material. The corrosion cast does, however, exhibit delicate structures that follow a template for the most part, and it has been shown that this method is suited to casting more complex mammalian lungs (the method was applied to rat lungs, and even single alveoli were cast; data not shown), which suggests that it should also be suitable for the simple lungs of lizards. The downside of this approach, however, is that only a negative of the actual lung is produced, which is not the case with traditional dry preparations (e.g., LAMBERTZ et al. 2015).

Oxygen consumption

A sample measurement regression of the normalized reduction in elemental oxygen in the chamber can be seen in Figure 4. The measurements showed that *E. schneiderii* has an average oxygen consumption of 0.44 $\text{mlg}^{-1}\text{h}^{-1}$, while

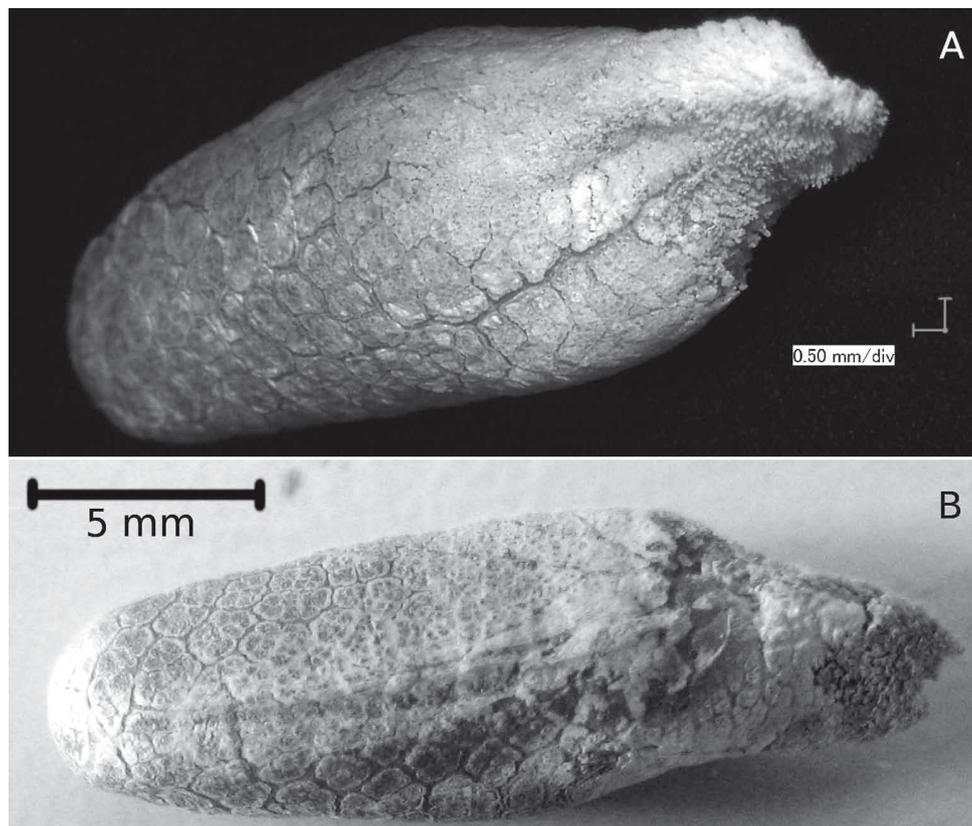


Figure 3. Anatomy of the sandfish lung. A) shows the *Scincus scincus* lungs replica, obtained by corrosive casting with Technovit 7143 and subsequent dissolving of the lungs with KOH, viewed from the front and the cranio-lateral side; B) shows the ventral side of the lung replica.

S. scincus generated two values (Fig. 5). According to an ANOVA test with a level of significance of 0.01% ($\alpha = 0.05$), the two values in fact correspond to two different levels of metabolic activity. The results indicate that *S. scincus* can switch between an active state with an average oxygen consumption of $0.45 \text{ ml g}^{-1} \text{ h}^{-1}$ and a passive state with much lower values of approximately $0.15 \text{ ml g}^{-1} \text{ h}^{-1}$ at a constant temperature. A two-tailed t-test ($\alpha = 0.05$) confirmed that the values for active *E. schneiderii* and *S. scincus* are equivalent ($p = 0.95$).

A comparative study of lizard metabolic rates in different climates showed that small lizards (3–20 g) living in hot arid or colder humid regions have lower metabolic rates at the same average temperature than, for example, Mediterranean lizards (AL-SADOON & SPELLERBERG 1985). The metabolism of desert-inhabiting lizards is even slower. At 30°C , the lowest O_2 consumption rate found was that of *Chalcides ocellatus* (approx. $0.10 \text{ ml g}^{-1} \text{ h}^{-1}$), a skink species inhabiting the same geographic regions as *S. scincus*, and the highest was that of the Mediterranean lizard *Podarcis muralis* (approx. $0.50 \text{ ml g}^{-1} \text{ h}^{-1}$) (AL-SADOON & SPELLERBERG 1985). An extensive study of *Scincus mitranus* showed a resting oxygen consumption rate of approximately $0.16 \text{ ml g}^{-1} \text{ h}^{-1}$ at 30°C for adult specimens (17–25 g) (AL-SADOON 2002, AL-SADOON et al. 1999).

The resting oxygen consumption identified for *S. scincus* fits well the values recorded for other lizards living in arid areas. Considering its size, its consumption is in the same ranges as those of *S. mitranus* and their relative *Chalcides ocellatus*, the latter of which is not a sand swimmer but is of similar size and inhabits a similar habitat. Their consumption rates are lower than those of lizards inhabiting colder and moister environments, and almost half that of lizards

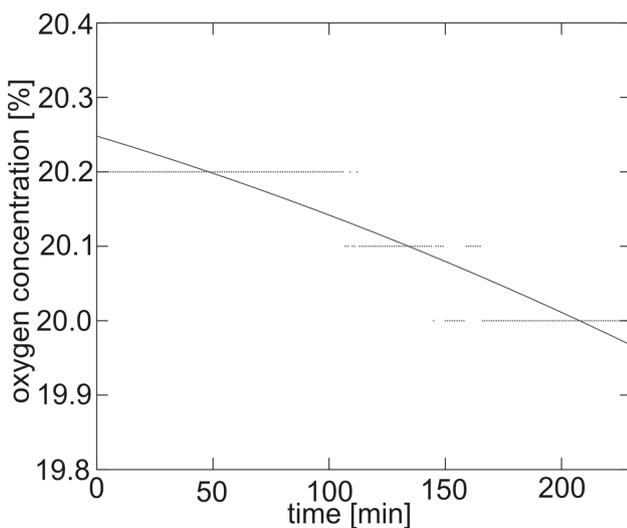


Figure 4. Sample regression of *S. scincus* oxygen consumption. The vertical axis represents the change in oxygen concentration in %, the horizontal axis shows time. The dots represent individual measurements and the curve the calculated quadratic polynomial regression, $P = -1.23 \times 10^{-6} x^2 - 9.38 \times 10^{-4} x + 20.25$. The accuracy of the O_2 sensor was 0.1%.

living in Mediterranean regions (AL-SADOON 2002; AL-SADOON & SPELLERBERG 1985).

Another interesting group for comparison are the amphisbaenians. These are fossorial reptiles that live in tropical and subtropical regions, but can also be found in arid areas and are known for their undulating locomotion and burying behaviour (ABE 1986, DIAL et al. 1987). Amphisbaenians can downscale their oxygen consumption to a fraction of the sandfish's: to approximately $0.03\text{--}0.05 \text{ ml g}^{-1} \text{ h}^{-1}$ (ABE 1986, DIAL et al. 1987). Genera for which such investigation was performed, like *Bipes*, do not bury themselves as deeply, however (DIAL et al. 1987) as the sandfish (50 cm deep and below as described by HARTMANN 1989). They therefore probably cannot escape the high temperatures and passively regulate their body temperature to the same extent. Furthermore, a portion of their gas and water exchange takes place via the integument (ABE 1986). It is very likely that they must slow their metabolism even further to survive in such harsh conditions.

Gas transport in sand

Since the air pockets between sand particles have a small diameter, airflow resistance is high and air-mixing strongly reduced, despite the air pockets being interconnected. However, diffusion is a constant process, so that the question arises of whether it suffices to prevent local O_2 depletion and CO_2 accumulation. Fick's law of diffusion states that diffusion in a medium depends only on the concentration gradient and the diffusion coefficient, which is material-specific. Our measurements of one-dimensional oxygen diffusion and the simulation progressions with the best fit are plotted in Figure 6.

Both measurement and simulation exhibited a sigmoid behaviour typical of diffusion. Figure 6 illustrates the sim-

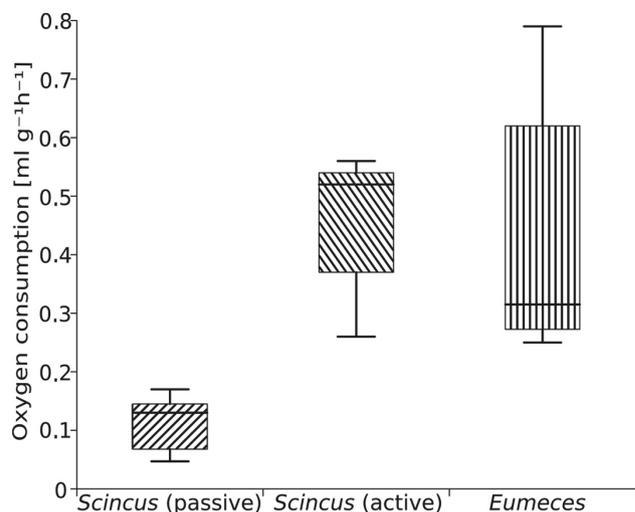


Figure 5. Boxplot of the oxygen consumption of *S. scincus* (in passive and active states) and of *E. schneiderii*.

ulations with the best possible curve fit after adapting the diffusion coefficient to the value of $4 \times 3 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$, as this showed to be the best possible fit for all sand depths in the simulation. At greater sand depths, minimal aberrations of the model can be observed. Nevertheless, the simulation describes the natural model well. Using the calculated diffusion coefficient for oxygen, the effective diffusion coefficient of carbon dioxide through sand was estimated at approximately $3 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$.

A simple one-dimensional diffusion system is relatively easy to measure and compare with calculations. A complete biological system containing a living organism, however, is much more complex. It is therefore rational to first test the mathematical model with a simple system and only then apply it to the complex one.

Simulations

To model nature as closely as possible, the geometrical properties of a large horizontal layer of sand were approximated. If a point consumer is buried in the sand, diffusion occurs in all directions, but not at the exact same rate, because the surface of the sand layer is flat and in contact with a large volume of air, where the oxygen concentration remains constant. Horizontally, the diffusion rate should be equal in all directions, depending only on the distance from the consumer. Vertically, the diffusion rate additionally depends on whether the consumer is close to the surface. The system can be described by a buried point consumer and a diffusion gradient that expands in two dimensions: a radial horizontal and a vertical one. If the consumption rate remains constant, the gradient should eventually reach equilibrium, where it also remains constant.

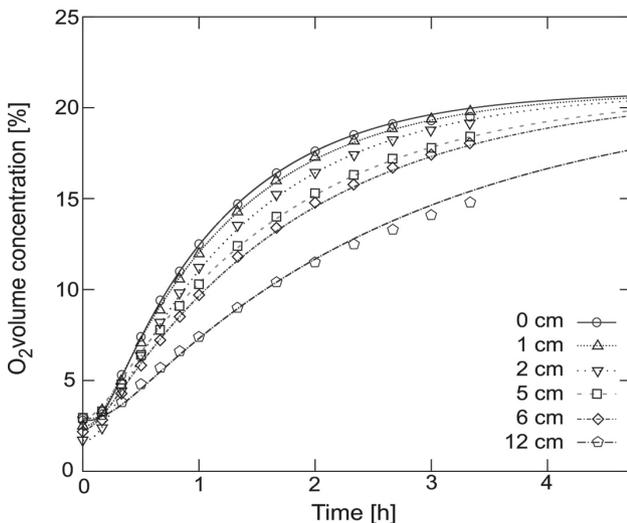


Figure 6. Oxygen diffusion measurements and simulation. Diffusion was measured and simulated for different discrete values of sand depth through which oxygen had to pass. The dots represent measured oxygen concentrations and the curves simulated data.

When the oxygen concentration is calculated in relation to space, it is low in the immediate vicinity of the consumer and high farther away. The concentration function should therefore have a funnel form that changes asymptotically over time until it reaches the constant state (Fig. 7).

If air in the direct environment can be mixed by respiration, the consumed oxygen and the produced carbon dioxide can be evenly distributed in a small volume, which would sustain physiological conditions. This volume can be calculated by using the diameter of the funnel at safe oxygen concentrations (Fig. 8). Identifying exactly how exhaled air behaves under sand is not trivial, but for reasons of simplicity, we supposed a spherical volume of air that needs to be mixed. If the consumption rate rises, the minimum volume of air that needs to be mixed also increases. The relation between these two variables is illustrated in Figure 9.

For *S. scincus*, the minimal volume of sand in which air needs to be mixed was below $100 \mu\text{l}$ in the active state. At rest, the consumption was even below $10 \mu\text{l}$. For *E. schneiderii*, the total consumption is much higher due to its size even though it has a similar normalized oxygen consumption per gram of body mass. Consequently, it requires much more air ($> 1.5 \text{ ml}$) to be mixed with its breath to sustain physiological conditions. The carbon dioxide production curve has the same form as the O_2 consumption curve, but is somewhat steeper due to the slower diffusion of CO_2 . *S. scincus* requires only $10 \mu\text{l}$ to be mixed in the passive state and $110 \mu\text{l}$ in the active state. For Schneider's skink, the difference is more significant, since it needs to mix approximately 2 ml of air (Fig. 9). Next, the question of whether these values are within the physiological range of the animals had to be addressed. For this purpose, the total and tidal breathing volumes of *S. scincus* were measured. On average, the tidal volume was $227 \mu\text{l} \pm 48 \mu\text{l}$ (mean \pm standard deviation; $n = 4$). The volume of the sample lung

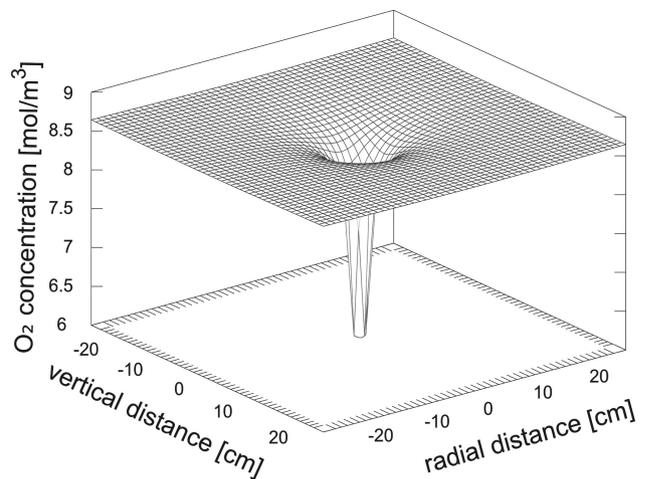


Figure 7. Oxygen consumption simulation at $2 \times 10^{-7} \text{ mol}^{-1}$. 3D-representation of a sample simulation with the consumer buried 50 cm deep in a sand bed that has a perfectly flat surface.

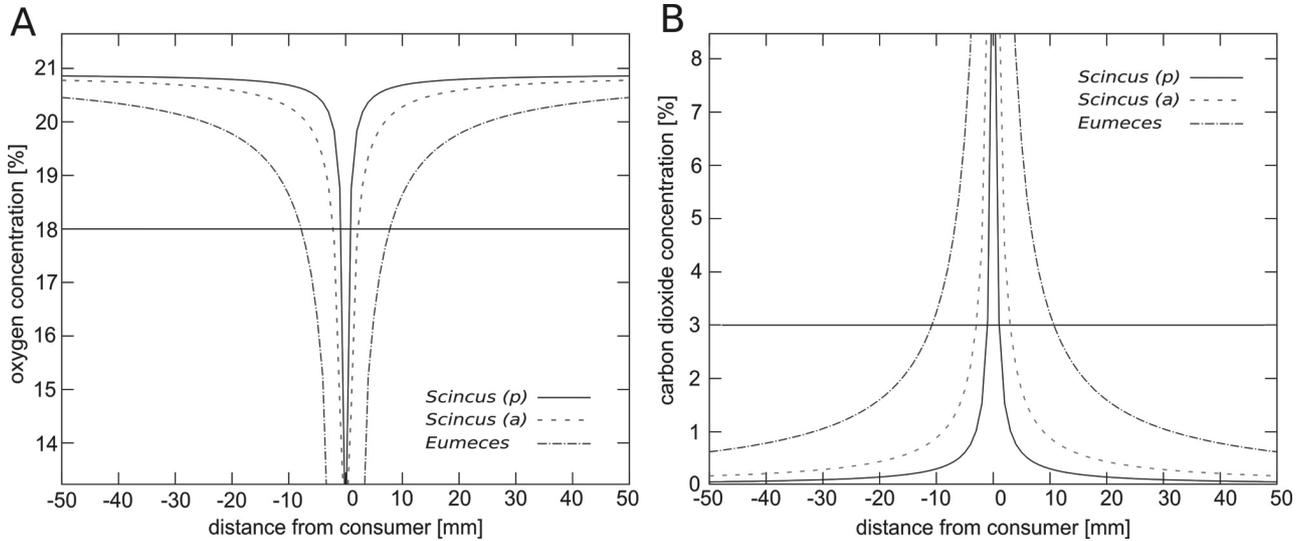


Figure 8. Simulation of the natural system. Cross-sections in the horizontal plane of the funnels, simulated for three different oxygen consumption (A) and carbon dioxide accumulation (B) rates. *Scincus scincus* in the passive state: *Scincus (p)*; in the active state: *Scincus (a)*; and *E. schneiderii*. The diameter of the funnel at the critical concentration is also indicated (horizontal line).

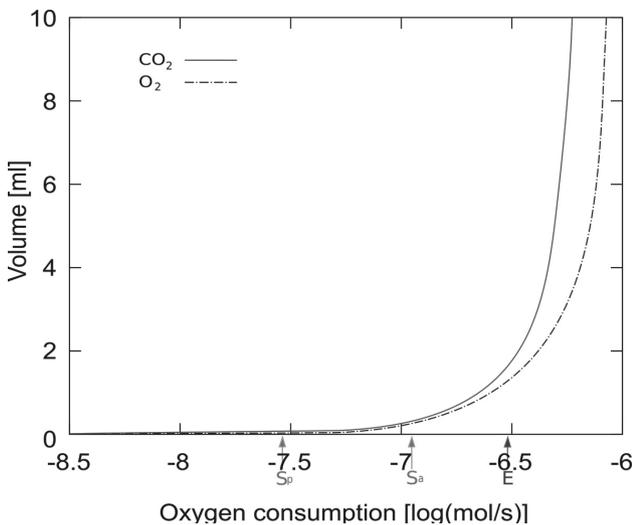


Figure 9. Minimum mixing volumes for diffusion to provide sufficient air transport. The curves represent the minimum volumes required to supply oxygen and remove residual carbon dioxide by diffusion alone. The arrows indicate the oxygen consumption/carbon dioxide production rates of *S. scincus* in passive (Sp) and active (Sa) states, and of *E. schneiderii*.

cast was approximately 400 μl , which is not quite double the average tidal volume. Assuming that tidal volumes equal the volume of air mixed in the immediate vicinity of *S. scincus*, the necessary air-mixing volume in sand is well within the limits of the tidal volume. Even the smallest measured tidal volumes are above the threshold of the minimum required mixing volumes, which also applies to the active state of *S. scincus*.

Conclusion

Animal metabolism depends heavily on body temperature (HILL et al. 2008). Mammals and birds are able to regulate their body temperatures actively and therefore sustain it at an optimal value. Poikilotherms such as *S. scincus*, however, are more dependent on ambient temperatures. A faster metabolism increases the conversion of food and oxygen to carbon dioxide and water. In environments where resources are abundant, organisms can afford higher metabolic rates than in environments where these are scarce.

The oxygen consumption rate depends on the activity level. In our oxygen consumption measurements in *S. scincus*, we found two distinct levels of oxygen metabolism. For *E. schneiderii*, only one mode was measured, but this is probably due to selective activity; comparative studies of other lizards suggest that it also has other consumption levels (AL-SADOON & SPELLERBERG 1985).

We have shown how *S. scincus* is capable of surviving prolonged periods buried in dry sand. Simulation suggests that gas diffusion through sand with minimal air-mixing promoted by ventilation provides sufficient air transport. Even if the animal is in an active state, the gas exchange is sufficiently low to sustain physiological conditions for extended periods. This may, however, not even be necessary, given that an active *S. scincus* moves about and thus constantly enters areas with “fresh air”. Lapsing into a passive state, where oxygen consumption drops to a third of the active state to sustain physiological gas conditions, does not seem necessary in dry sand. HARTMANN (1989) noted that the natural habitat of *S. scincus* contains some xerophilic plants such as grasses and dry shrubs, which would suggest that the soil in these areas is not completely dry. Since diffusion of oxygen in water is three orders of magnitude

lower than in air (WELTY et al. 2008), moisture would also have a great effect on diffusion in porous media. Water loss due to respiration may also contribute to dampening of the sand, especially in the direct vicinity of the nostrils.

Since *S. scincus* is known to dig deep into the ground (where oxygen is depleted more quickly), its lower oxygen consumption could therefore not only give it the advantage of a wider range of temperatures, but also the opportunity to shelter in areas where predators with higher oxygen consumption rates, such as snakes, are less likely to occur. According to our simulations, even a reduction of the diffusion coefficient that increases the minimum mixing volumes by a factor of 20 would not be problematic for a passive sandfish.

Further investigation is, however, needed to understand (i) gas transfer in sand with different moisture levels and (ii) possible exhalation-drying mechanisms in the respiratory system. It remains unclear which portion of the tidal volume equals the effective air-mixing volume in the immediate vicinity of the sandfish's nostrils. The minimum air-mixing volume of *Scincus scincus* in the passive state is one order of magnitude smaller than the tidal volume, and therefore does not seem to pose a problem. This should be investigated with the lower diffusion rates that exist in wet sand.

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Supplementary material

Additional information is available in the online version of this article at <http://www.salamandra-journal.com>

Supplementary video

Online Supplementary data

VIHAR, B., C. WOLF, W. BÖHME, F. FIEDLER & W. BAUMGARTNER (2015): Respiratory physiology of the sandfish (Squamata: Scincidae: *Scincus scincus*) with special reference to subharenal breathing. – *Salamandra*, 51(4): 326–334.

Supplementary video: Click on image in Adobe Acrobat or Reader Version 6 or higher to activate the video.

