

Respiration by mangrove ants *Camponotus anderseni* during nest submersion associated with tidal inundation in Northern Australia

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Abstract. The ant *Camponotus anderseni* lives exclusively in twigs of the mangrove tree *Sonneratia alba*, which forms the fringe at the wettest part of the mangrove zone. During inundation, which can last up to 3 h, the entrance hole to the nest cavity is blocked with a soldier's head that effectively prevents flooding, but simultaneously blocks gas exchange with the surroundings. The ants and brood, together with their mutualistic Coccid, *Myzolecanium* sp. 1, occupy an average of 23% of the volume of the nest cavities (maximum of 50%). Measurements of CO₂ production in the laboratory indicate respiratory rates of 1.90 and 0.41 $\mu\text{L CO}_2 \text{ h}^{-1} \text{ mg}^{-1}$ live mass at 25 °C for workers and larvae, respectively. Measurements of sealed natural nests show that mean respiratory rates decrease to 18.9% and 1.8% of the normoxic rate at CO₂ concentrations of 10% and 25%, respectively. In artificial nests where the initial CO₂ is elevated, the respiratory rates after 1 h are reduced to 48% and 2.3% of the normoxic rate when exposed to CO₂ concentrations of 10% and 25%, respectively. Air samples from natural nests in the field taken more than 12 h after inundation have mean CO₂ concentrations of up to 4–5%, which means that the CO₂ concentration in the parts farthest from the entrance must be much higher. In sealed nests in the laboratory, the O₂ concentration after 1 h decreases by 6.8% and, in the same period, the CO₂ concentration increases by 12.1%, which suggests that the ants have partly switched to anaerobic respiration. The rapid and extreme depression of the respiratory rates of *C. anderseni* represents an outstanding physiological adaptation that allows their survival under the extreme conditions of tidal inundation.

Key words. *Camponotus anderseni*, carbon dioxide, inundation, mangrove, nests, oxygen uptake, respiration.

Introduction

The intertidal environment presents a range of physiological challenges for air-breathing animals that must contend

with the twice-daily inundation of their habitat. A few species of ants have successfully invaded this habitat, avoiding drowning and suffocation by various behavioural and physiological adaptations, including relocating their nests in trees above the water (Adis, 1982), exploiting air pockets in their mud nests chambers (Nielsen, 1997a; Nielsen *et al.*, 2003) and tolerating immersion in seawater (Nielsen, 1981).

The mangrove communities in Northern Australia are extensive and inhabited by fish and crustaceans, and they also contain a rich, but relatively uninvestigated, insect

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fauna. The ant fauna in the Darwin Harbour mangrove include at least 24 species and have been studied by Clay & Andersen (1996), Nielsen (1997a,b, 2000) and Nielsen *et al.* (2003).

The ant *Camponotus anderseni* McArthur & Shattuck lives exclusively in twigs of the mangrove tree *Sonneratia alba* J. Smith in a mutualistic relationship with the Coccid, *Myzolecanium* sp. 1. A colony consists of many nest chambers, each the length of one internode. When colonies of *C. anderseni* are exposed to fresh air, gas exchange between the nest and the atmosphere is restricted to the single entrance hole. Direct observations reveal that the entrance hole is blocked with a soldier's head during inundation that effectively prevents flooding, but simultaneously blocks gas exchange with the atmosphere (Nielsen, 2000). If the ants and coccids, which may form up to 50% of the volume in a crowded nest, maintain normal respiratory rates, they would presumably consume the small amount of available oxygen soon after inundation begins.

The present study aims to examine the metabolic responses of *C. anderseni* to the microenvironment of the sealed twigs to which they retreat twice daily during periods of high tides, by measuring the concentrations of CO₂ and O₂ in the nests during inundation. To further explore the relationship between ants and CO₂ concentrations, CO₂ production by the ants is also measured at different CO₂ concentrations in the air.

Materials and methods

Study site

The ants were studied in the mangrove around Darwin Northern Territory, Australia (12°30'S, 131°E), which is one of the most diverse mangroves in Australia, containing at least 36 tree species (Wightman, 1989). The Darwin Harbour supports more than 20 000 ha of mangrove, and the width of the mangrove zone is in the range 25–700 m (Brocklehurst & Edmesdes, 1996). The tree species *S. alba* covers only 5% of the total area, but is very visible because it always forms the fringe at the wettest part of the mangrove zone. Therefore, the trees are most accessible from a boat at medium to high tides. Tidal movements are up to 8 m, resulting in frequent inundation of the nests of *C. anderseni* for up to 3 h (Nielsen, 2000).

Nests volume and nest contents

The air volumes of the nests were determined by three methods at the end of the experiments. (i) The cavities were filled with water injected into the nest through one of the syringe needles used for gas sampling (see below). The volume of liquid used to fill the nest was assumed to be the volume of the air space. On occasions when the nest had to be stored for later examination, alcohol was used as a measuring liquid to preserve the nest contents. (ii) The

twig with the nest was weighed before and after filling with liquid. The air space of the nest was estimated as the mass of liquid divided by its density. (iii) The nest was split and the length and the diameters of the nest cavity were measured at every 1 cm with a Vernier caliper to the nearest 0.1 mm. The total volume was calculated, and the air volume was estimated as the total volume minus the volume of the nest contents. The air volume used in the calculations was the mean of the three methods.

The nest contents were removed and the number of each life-stage counted and, subsequently, the wet and dry masses were measured. Some samples were stored in alcohol to ensure that the wet mass was calculated from the dry mass. The nest contents were assumed to have a density very close to one; therefore, fresh mass (mg) is equivalent to the volume (µL). The percentages of the total nest volume occupied by nest contents (all ant-stages and coccids) were calculated for every nest.

Carbon dioxide and O₂ in natural nests

The nests of *C. anderseni* were cut from the *S. alba* trees, and the entrances were covered with tape or gauze to prevent the ants from leaving the nests. In the field investigations of natural CO₂ and O₂ concentrations in the nests, holes (approximately 0.8 mm in diameter) were drilled from the ends of the twig into the nest chamber and the nest entrance was sealed with rubber tape. A glass syringe with a three-way valve and a needle was used to take 2–5-mL air samples through one of the holes in the nest. The syringes were taken to the laboratory for analysis.

The air samples from the nests and the respiratory measurements were analysed for CO₂ using a flow-through analyser model LI-6251 connected to a data acquisition and analyser system (Sable Systems International; Las Vegas, Nevada; using Datcan V software) (Nielsen *et al.*, 1999). The airflow was kept constant at 150 mL min⁻¹.

Oxygen concentrations in air samples and in the nests were measured with a micro-oxygen electrode with a tip diameter of 1.1 mm (Revsbech, 1989; Christensen *et al.*, 1994). Air samples from the field experiments were injected into a small cell (0.1 mL) with the oxygen electrode, and this cell was connected to the flow system in the CO₂-analyser. The flow-through cell with the oxygen electrode was then flushed with CO₂-free air to allow the total amount of CO₂ in the sample to be measured. The concentrations of O₂ and CO₂ in the nest cavity could then be calculated using the volume of the nests, the volume taken up by ants and coccids, and the 'dead volumes' in the system. The sensitivity of the O₂ probe is ± 0.1% O₂ and, when 5 mL air is taken from nests smaller than 0.3 mL, the 'nest air' is diluted by more than 16-fold with atmospheric air, and the concentration in the nest air is measured with a precision of less than 1.6%. Therefore, to minimize errors, it was decided that only nests above the mean volume of 0.3 mL would be used.

Respiration of workers and larvae

The respiratory rate, as determined by CO₂ production, was measured for workers, brood and alates of *C. anderseni* and for the coccids *Myzolecanium* sp. 1 at 25 and 35 °C. The number of individuals in each set of measurements was in the range 11–26 for workers and brood and 4–11 for alates and coccids. Furthermore, the respiratory rates of anaesthetized (enflurane) workers were measured at 35 °C using a previously described method (Holm-Jensen *et al.*, 1980; Duncan & Newton, 2000). The respiration chambers (cylindrical glass tubes; 60 mm in length, 13 mm in diameter) were connected to the air flow system in the CO₂ analyser. The chambers were placed in a temperature regulated water bath during measurements (Nielsen *et al.*, 1999). Coccids were measured when sitting with the stylet inserted into the wood, and the respiratory rate was calculated as the difference between the respiration of the coccids with wood and the respiration of wood alone. Each set of measurements consisted of 512 determinations of the CO₂ concentration in the air stream during a 5-min period. Three sets of measurements were taken from each group of individuals at both temperatures.

Carbon dioxide in sealed nests

The nests used in the laboratory experiments were placed in plastic bags in the field and kept cool during transport. Holes of the same size were drilled into the nest chamber from both ends of the twig, and a 0.7-mm gauge syringe needle with a three-way valve was inserted into the hole and sealed with household thermo glue. The natural entrance to the nest was also sealed.

The respiratory rates, expressed as CO₂ production, were measured in natural sealed nests in the laboratory at 25 ± 1 °C. Each nest was typically measured six times with respiratory periods in the range 0.5–240 min. The sealed nests with three-way valves were flushed with CO₂-free air immediately before the nest was sealed and, after each of the respiratory periods, the nest was flushed again with CO₂-free air, and the amount of CO₂ produced was measured in the air expelled from the nest.

Artificial nests

The respiratory rates of workers and larvae at 25 ± 1 °C were measured at different concentrations of CO₂ in the

initial air. Between 25 and 78 individuals (mean 42.0) were placed in a 5-mL glass syringe in which the piston was replaced with a rubber cork with a glass tube, allowing the syringe to be placed in the flow system of the CO₂ analyser. Five-minute periods were used to determine the normal respiratory rate in CO₂-free air. Next, the syringe was connected to another syringe (through a three-way valve) containing a mixture of atmospheric air and pure CO₂. The gases in the two syringes were mixed, and the 5.0-mL syringe with the ants was moved to the CO₂ analyser and 1.0 mL was pumped into the flow system to determine the exact CO₂ concentration of the initial air. After 1 h, three 1.0-mL samples were analysed for CO₂.

Oxygen and CO₂ in sealed nests

The oxygen concentrations in sealed nests were continuously measured by placing the tip of the glass electrode in a hole drilled into the nest cavity in the central part of the nest. The nests were flushed with CO₂-free air before the experiment, and the small space around the electrode was sealed with vaseline. At the end of the experiment, the CO₂ content in the nest cavity was determined by pushing CO₂-free air through the nest into the flow system of the CO₂ analyser.

The experiments were carried out at 25 ± 1 °C.

Results

Nests volume and nest contents

A total of 107 nests were investigated in this study, and the mean ± SD air volume of the nests was 0.31 ± 0.22 mL (range 0.04–1.13 mL). The nest content of worker ants, brood, alate and coccids is summarized in Table 1. The mean ± SD live mass of the nest contents was 72 ± 47 mg, of which 13 ± 6% consisted of coccids. The mean ± SD volume of nest contents was 22.7 ± 9.4% of the total nest volume (range 1.5–49.6%).

Carbon dioxide and O₂ in natural nests

The concentration of CO₂ and O₂ in nests under natural conditions in the mangrove is shown in Figure 1. The slope of the regression equation represents the respiratory

Table 1. Mean numbers, standard deviations, and range of the different types of inhabitants in *Camponotus anderseni* nests ($n = 107$).

	Workers	Pupae	Larvae	Males	Alate queens	Coccids
Mean number per nest	21.5	3.0	9.2	1.1	0.5	8.8
Standard deviation	14.9	2.9	7.2	1.8	1.1	5.7
Maximum number per nest	86	14	27	14	7	28
Minimum number per nest	1	0	0	0	0	0
Percentage presence in all nests	100	77.6	88.8	43.9	27.1	98.1

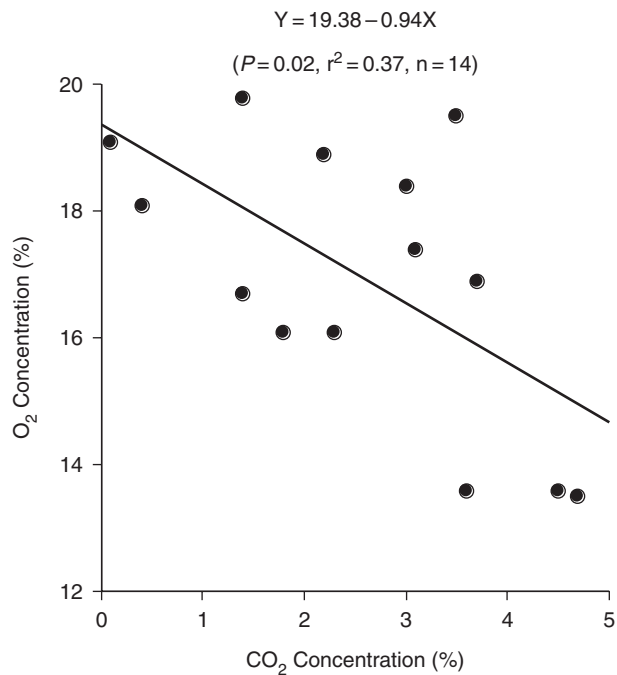


Fig. 1. The concentration of CO₂ and O₂ in nests of *Camponotus anderseni* under natural conditions in the mangroves of Darwin Harbour. The air samples were taken from nests larger than 0.3 mL and more than 12 h after inundation.

quotient for the nest content, and the high value confirms that mainly carbohydrate is metabolized.

Respiration of workers and larvae

The respiratory rates at 25 and 35 °C for different stages of *C. anderseni* and the coccid *Myzolecanium* sp. 1 are shown in Table 2, as well as the respiratory rate of anaesthetized workers at 35 °C. The mean fresh mass of all stages is given and the SD is shown for the experiments where all individuals were weighed. The mass ratio (dry/fresh) can be used to convert the fresh mass respiratory

rates to dry mass respiratory rates. The Q₁₀ values for workers, males, and queens were 2.17, 2.16 and 2.29, respectively; and, for larvae and pupae, the Q₁₀ values were 1.70 and 1.78, respectively.

Carbon dioxide in sealed nests

For all individual sealed nests the increase in the CO₂ production (*Y*) is described by:

$$Y = a + b \times \ln(\text{time}) \quad (P < 0.007, 0.94 < r^2 \leq 0.99)$$

The respiratory rate (dy/dt_x) was calculated at the time of each measurement. Assuming that the respiration rate during the first minutes was unaffected by the lack of access to fresh air, the mean respiratory rate for this period ($1.7 \pm 0.6 \mu\text{L CO}_2 \text{ mg}^{-1} \text{ fresh mass h}^{-1}$) was taken as the control (normoxic and normacapnic) rate for the nest at 25 °C.

Figure 2 shows the relationship between ant respiratory rate (*Y*) and CO₂ concentration (*X*) in the sealed nests. The respiratory rate is expressed as percentage of the (normoxic) control rates under atmospheric conditions.

The respiratory rates are expressed as percentages of the initial respiratory rates, and show a decrease to 18.9% and 1.8% of the normoxic rate at CO₂ concentrations of 10% and 25%, respectively. All ants survived these high levels of CO₂. A logarithmic correlation ($P < 0.001, r^2 = 0.70, n = 96$) between the time the nest was sealed and the CO₂ concentration in the nests indicated that the concentrations of 10 and 20% CO₂ in the nests were reached after 22 and 140 min, respectively.

Artificial nests

The CO₂ production (*Y*) of worker ants after being enclosed in artificial nests with different concentrations of CO₂ (*X*) for 60 min is shown in Figure 3. Values are calculated from the initial respiratory rates measured in CO₂-free air and the CO₂ production during 1 h. Because the respiratory rates decrease logarithmically in sealed nests with increasing concentration of CO₂, logarithmic

Table 2. The respiratory rates at 25 and 35 °C of different stages of *Camponotus anderseni* and the Coccid *Myzolecanium* sp. 1; the respiratory rate at 35 °C of anaesthetized workers is also given.

	Mean ± SD fresh mass (mg)	Number of experiments	Respiratory rate ± SD at 25 °C ($\mu\text{L CO}_2 \text{ mg}^{-1} \text{ fresh mass h}^{-1}$)	Respiratory rate ± SD at 35 °C ($\mu\text{L CO}_2 \text{ mg}^{-1} \text{ fresh mass h}^{-1}$)	Mass ratio, dry/fresh
Workers	1.39 ± 0.31	25	1.90 ± 0.18	4.13 ± 0.63	0.34 ± 0.01
Workers anaesthetized	1.47	5	–	2.68 ± 0.59	0.29
Pupae	2.73	6	0.37 ± 0.09	0.63 ± 0.04	0.25
Larvae	2.4 ± 0.25	12	0.41 ± 0.10	0.73 ± 0.10	0.33 ± 0.01
Males	1.6	6	1.42 ± 0.26	3.07 ± 0.26	0.30
Females	4.85	8	1.08 ± 0.17	2.47 ± 0.43	0.37
Coccids	1.37 ± 0.21	12	2.39 ± 1.48	–	0.39 ± 0.05

The SD for the fresh mass is shown for the experiments where all individuals were weighed.

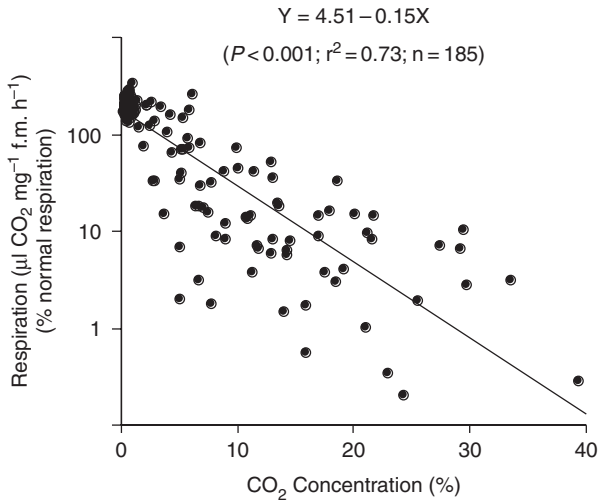


Fig. 2. Respiratory rate ($\mu\text{L CO}_2 \text{ mg}^{-1}$ fresh mass h^{-1}) of *Camponotus anderseni* as a function of CO_2 concentration in sealed nests. Respiratory rate is expressed as percentage of the (normoxic) control metabolism under atmospheric conditions.

transformations are used in the calculations. The relationship is best described by:

$$\ln(Y + 1) = 0.8205 - 0.0341X \quad (P < 0.001, \quad r^2 = 0.62, \quad n = 41)$$

Thus, the respiratory rates are reduced to 48 and 2.3% of the normoxic rate when exposed to CO_2 concentrations of 10 and 25%, respectively.

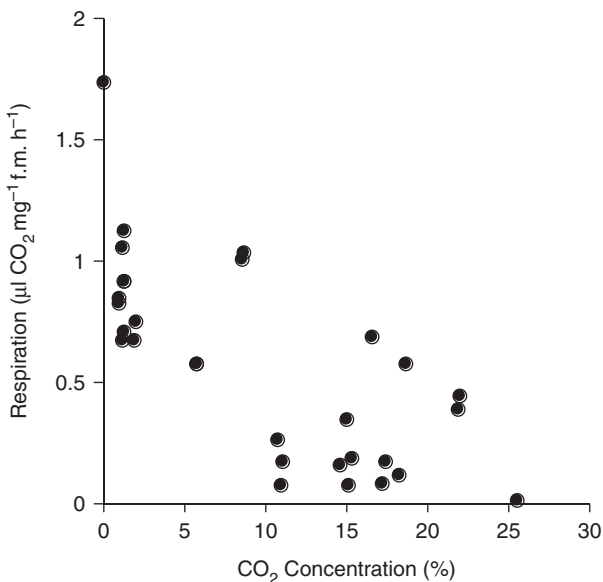


Fig. 3. Respiratory rates ($\mu\text{L CO}_2 \text{ mg}^{-1}$ fresh mass h^{-1}) of *Camponotus anderseni* workers after 60 min in sealed artificial nests with different concentrations of CO_2 .

Oxygen and CO_2 in sealed nests

The oxygen concentrations in 13 sealed nests were measured over 60 min. For each of the nests, the decrease in O_2 concentrations was described by a linear logarithmic function: ($0.86 < r^2 \leq 0.99$). The mean \pm SD concentration of O_2 in the nests 60 min after sealing was $14.2 \pm 1.8\%$. Extrapolating the decrease in O_2 concentrations for each nest gives an estimated mean \pm SD O_2 concentration of 13.1 ± 2.4 and $12.4 \pm 2.6\%$ after 120 and 180 min, respectively. The mean \pm SD CO_2 concentration in the nests after 60 min was $12.2 \pm 3.6\%$. The O_2 concentrations in the nests after 60 min were negatively correlated with the total nest volume: $Y = 16.609 - 0.004X$ ($P < 0.003$, $r^2 = 0.55$, $n = 13$) and positively correlated with the percentages of the total nest volume occupied by nest contents: $Y = 10.94 + 0.12X$ ($P < 0.027$, $r^2 = 0.37$, $n = 13$)

Discussion

In *S. alba* trees, approximately 20% of all twigs are hollowed by *C. anderseni*. Of these, 80% of the nest cavities are still occupied by *C. anderseni*, but the others are occupied by other ant species (Nielsen, 2000). The nests used in the present study were selectively chosen to maximize the size range. Unfortunately, the experiments have to be carried out before the volume, ant species, and nest contents can be determined. The sizes of the nests are not always as expected, and several experiments had to be excluded because the nests were occupied by other ant species. The mean nest size in this investigation is only 0.31 mL, and the mean number of workers is only 22, but the numbers of other developing stages and of coccids are relatively high (Table 1). This suggests that eggs or small larvae are efficiently transported from the queen nest to the other nests in the colony, which is surprising because very little worker activity outside the nest is observed during either the day or night.

The gas exchange between the nest and the surroundings can only take place through the entrance hole and, during inundation, the head of a major worker ant or queen blocks the entrance completely, preventing gas exchange and water intrusion. After inundation, the opened entrance hole allows CO_2 to diffuse out of the nest and O_2 to diffuse into the nest. After some time, depending of the nest volume, equilibrium between the inside and outside concentrations occurs. The concentrations inside the nest depend on the length and volume of the nest and the respiratory activity, which in turn depends on the mass of nest contents and temperature.

The mean CO_2 concentrations are 4–5% in nests measured more than 12 h after inundation, at which time equilibrium with the surroundings is expected. Given that the CO_2 concentration at the entrance would be approximately 0%, and the mean concentration is 4–5%, suggests that the CO_2 concentration in the parts farthest from the entrance must be much higher. Therefore, even under ‘normal’

conditions, some of the ants and coccids in the nest must contend with high CO₂ concentrations. Similar concentrations in ant nests are only found during inundation in the mud-nesting ant *Polyrhachis sokolova* (Nielsen *et al.*, 2003).

Workers and alates of *C. anderseni* show a high respiratory rate compared with most other ant species (Nielsen, 1986), but this is consistent with the results found in the sealed natural nests. This high respiratory rate may be partly due to high levels of activity, and the much lower respiratory rates of the anaesthetized workers is consistent with this assumption. Another factor that may have contributed to the high respiratory rate in CO₂-free air is that the ants normally live in nests with increased levels of CO₂. Larvae and pupae show 'normal' respiratory rates, and the respiratory rates of coccids are variable, possibly due to a large variation in size and unnatural experimental conditions. The Q₁₀ for all stages is clearly within the normal range of other ant species (Nielsen, 1986).

The CO₂ concentration in each of the sealed natural nests could be described by a logarithmic function. The initial respiration, the speed of the increase and the maximum level of CO₂ differ between the nests depending on the nest volume and the density and distribution of the ants and coccids. Therefore, to compare the decreases of the respiratory rates of the different nests, the percentage of the initial respiratory rates is used. As shown in Figure 2, the CO₂ concentrations reach surprisingly high concentrations despite the fact that the respiratory rates are depressed to extremely low levels. The metabolic depression in *C. anderseni* is initiated within a few minutes of the nest being sealed and, in crowded nests, respiratory rates drop to less than 1% of normal within 2.5 h.

The advantage of the artificial nests is primarily that the air volume and density of nest contents can be measured accurately and controlled. Thus, the other independent factor, the initial concentration of CO₂ in the nests, can also be controlled. The results confirm data from the sealed natural nests. The respiratory rates in artificial nests are depressed to 2.3% of the normoxic rate after 1 h at 25% CO₂ compared with 1.8% in natural nests under similar conditions.

The mean O₂ concentration after 1 h in sealed natural nests decreases by 6.8% and, in the same period, the CO₂ concentration increases by 12.1% (RQ = 1.8). This high respiratory quotient can only be achieved if the ants transform carbohydrates to fat or partly use anaerobic respiration (Wigglesworth, 1965).

Insects use a variety of anaerobic metabolic pathways to survive anoxic conditions, resulting in the accumulation of end products that include lactate succinate, alanine and ethanol (Chown & Nicolson, 2004). During the anaerobic period, CO₂ will be produced by the organism either as an end product of the pathways or as a consequence of the pH decrease due to the accumulation of lactate followed by liberation of CO₂ from bicarbonate in haemolymph and tissue (Keister & Buck, 1964; Wigglesworth, 1965). Although anaerobic respiration has not been demonstrated in ants, it is plausible in *C. anderseni* given the extreme

environmental conditions that this species encounters during nest inundation.

The review by Hoback & Stanley (2001) demonstrates that insects show a remarkable diversity of adaptations to allow them to handle hypoxia. Insect larvae living in soil, in dung or other hypoxic and hypercapnic environments have an ability to extract O₂ at very low concentrations or switch to anaerobic metabolism (Hoback *et al.*, 2002; Holter & Spangenberg, 1997; Zerm & Adis, 2003). The extreme conditions of these insects are the result of environmental factors such as the respiration of microbes. By contrast, the extreme conditions experienced by *C. anderseni* are created entirely by the respiration of the ants and the coccids sealed inside the nest. Both the extent of metabolic depression and the speed at which it is achieved are unusual for insects. The rapid and extreme depression of the respiratory rates of *C. anderseni* represents an outstanding physiological adaptation, which allows their survival under the extreme conditions of tidal inundation.

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