

Cocoon formation and metabolic depression by the aestivating hylid frogs *Cyclorana australis* and *Cyclorana cultripes* (Amphibia: Hylidae)

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Abstract. Two northern Australian burrowing hylid frogs, *Cyclorana australis* and *C. cultripes*, form a cocoon when induced to aestivate. The cocoon completely encases the frog except for the external nares and it consists of shed multiple layers of outer epidermal cells interspersed with ground substance. The cocoon of *C. australis* was about 24 μ thick and had about 34 layers (formed at about 0.7 layers day⁻¹), and the cocoon of *C. cultripes* was about 16 μ thick with about 51 layers (about 0.6 layers day⁻¹). Evaporative water loss decreased from 15.8 to 1.1 mg g⁻¹ h⁻¹ for cocooned *C. australis*, and 15.1 to 1.3 mg g⁻¹ h⁻¹ for cocooned *C. cultripes*; these correspond to cutaneous resistances of 3 to 112 sec cm⁻¹ for *C. australis*, and 0 to 223 sec cm⁻¹ for *C. cultripes*. Metabolic rate declined significantly with aestivation by 70% from 0.160 to 0.049 mg O₂ g⁻¹ h⁻¹ for *C. cultripes* after about 6 weeks of cocoon formation, and respiratory exchange ratio decreased significantly from 0.98 to 0.68. Metabolic rate declined insignificantly by 40% from 0.158 to 0.095 ml O₂ g⁻¹ h⁻¹ for aestivating *C. australis*, which remained more responsive during aestivation than *C. cultripes*. All species of *Cyclorana* so far investigated aestivate and form a cocoon of similar structure and efficiency, but the most tropical species *C. australis* does not appear to as effectively depress metabolism as other species.

Key words: hylid frog, *Cyclorana*, aestivation, cocoon, evaporative water loss, metabolic depression

Introduction

Frogs that inhabit the arid areas of Australia often have anatomical, physiological and behavioural strategies to enhance their survival over extended dry periods. For example, the burrowing frogs so far investigated (the hylids *Cyclorana platycephala*, *C. maini* and *Litoria alboguttata*; and the myobatrachids *Neobatrachus* spp) aestivate during dry conditions. They form a cocoon of multiple layers of shed epidermal cells, which is an effective barrier to evaporative water loss, and depress their metabolic rate to about 25% of resting to conserving energy reserves (Lee & Mercer, 1967; van Beurden, 1984; Withers, 1993, 1995, 1998; Withers & Richards, 1996; Christian & Parry, 1997).

This study continues these previous investigations of cocoon formation and metabolic depression during aestivation, and examines cocoon formation, evaporative water loss and metabolism for two hylid species, the tropical-zone *C. australis* and the central-northern *C. cultripes*.

Materials and Methods

Specimens of *C. australis* and *C. cultripes* were collected near Kununurra, Western Australia, after heavy rain in January 2000. The frogs were air-freighted to Perth, washed in tap water, and placed individually in plastic chambers with a

small hole in the lid for ventilation. The chambers were placed in a dark cupboard and the frogs left undisturbed except for inspection every 2 to 4 days. Ambient temperature was 24 \pm 1 °C.

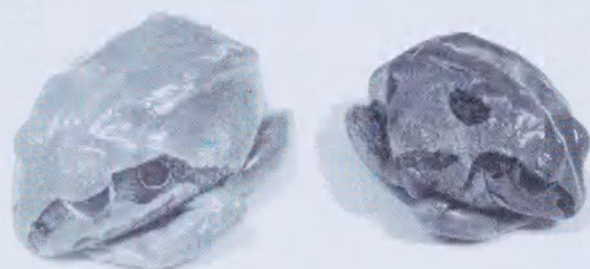


Figure 1. Aestivating *C. australis* (left) and *C. cultripes* (right), showing semi-transparent cocoon enveloping the entire body, except for the nares.

After 2 days to acclimate the frogs to the laboratory conditions, resting metabolic rate and evaporative water loss were determined by flow-through respirometry. The frog was placed in a cylindrical glass chamber, on a plastic mesh grid to expose the ventral as well as dorsal skin to a flowing airstream (100-400 ml min⁻¹). Excurrent oxygen, carbon dioxide and water contents were measured with a Servomex paramagnetic O₂ analyser, a Qubit infra-red CO₂ analyser, and a Vaisala thin-film capacitance humidity meter (see Thompson & Withers, 1998). After a further 46 to 50 days, aestivating frogs were re-examined, then their cocoon removed for examination using a Phillips 505 scanning electron microscope. Both air-dried and formol saline-preserved pieces of cocoon were examined, to measure thickness and to count the number of cocoon layers (see Withers 1995).

Results

Both species became quiescent, assumed the water-conserving posture, and formed a cocoon (Fig 1). *C. australis* remained more responsive to handling than *C. cultripes*, and would more often move their eyes and limbs when disturbed than *C. cultripes*.

The cocoon of both species formed a semi-transparent, shiny film, closely adherent to the skin over the entire body surface



Figure 2. Scanning electron micrographs of cocoon showing the normally compacted structure (left; air-dried specimen, *C. cultripes*) and the layered structure that is more obvious after the cocoon is soaked in formol saline (right; formol saline fixed, *C. australis*). Scale bars are 10 μ .

Table 1. Number of days for cocoon formation, number of cocoon layers, cocoon thickness (μ), layers formed per day, thickness per layer, resistance per layer, and resistance per thickness (see Table 2 for resistance) for two individuals of both *C. australis* and *C. cultripes*.

	<i>C. australis</i>	<i>C. cultripes</i>
days	46	51
layers	34	32, 33
μ	23.1, 24.5	16.7, 14
layers d ⁻¹	0.74, 0.74	0.65, 0.63
μ layer ⁻¹	0.68, 0.72	0.51, 0.44
R layer ⁻¹	3.3, 3.3	6.8, 7.0
sec cm ⁻²	4.6-4.9 10 ⁴	13.4-15.9 10 ⁴

except for the external nares through which the frogs continued to breathe. It was sealed over the eyes, mouth and cloaca. The cocoon consisted of multiple thin layers of shed epidermis (Fig 2). *C. australis* shed 34 layers of skin in 46 days, with a mean thickness of 0.7, compared with *C. cultripes* that formed 32.5 layers in 51 days with a mean thickness of 0.48 μ per layer. (Table 1).

Evaporative water loss declined significantly for both species during aestivation, with an increase in resistance from about 1 to 223 for *C. cultripes* and 112 for *C. australis* (Table 2). The resistance per layer, and resistance per unit thickness, was lower for the cocoon of *C. australis* than *C. cultripes* (Table 1).

The resting metabolic rate (RMR) of *C. cultripes* declined significantly by 70% during aestivation to about 30% of RMR. The metabolic rate of *C. australis* declined, but not significantly, to about 60% of RMR (Table 2). The carbon dioxide production declined similarly. For *C. cultripes*, the respiratory exchange ratio declined significantly from 0.98 for awake frogs to 0.68 for aestivating frogs, but RER was extremely variable and did not alter significantly for *C. australis*.

Discussion

This study indicates that two further species of *Cyclorana* aestivate and form a cocoon, similar to the more arid *C. maini* and *C. platycephala*, and sub-humid to tropical *C. novaehollandiae* (Withers 1995, 1998). Cocoon structure is similar for the five species of *Cyclorana* investigated, with cocoon layers (about 0.2-0.7 μ thick) added to the cocoon at a rate of about 0.6-1.1 layers per day, conferring a resistance of about 3-10 sec cm⁻¹ per layer. It is of interest to determine whether the further seven species of *Cyclorana* that have yet to be studied (*brevipes*, *cryptotis*, *longipes*, *maculosa*, *manya*, *vagita*, *verrucosa*; Cogger 1992), will also aestivate and form a similar cocoon.

The extent of metabolic depression by *C. cultripes* is similar to that of other aestivating *Cyclorana* spp, and also various *Neobatrachus* spp. Withers (1993) reports depressed metabolic rate (DMR) as 8–33% of RMR for six *Neobatrachus* spp and two *Cyclorana* spp. In contrast, metabolic depression was to only 60% of RMR for *C. australis*, which remained more responsive during aestivation than *C. cultripes* and other *Cyclorana* spp previously examined. Perhaps energy conservation from metabolic depression is not as important for this larger frog that inhabits wet tropics, as its higher body mass confers an energy consumption advantage (lower mass-specific metabolic rate) and rainfall in the monsoonal tropics is more predictable than in the arid areas where irregular rainfall might require burrowing frogs to aestivate for one or more years and necessitate metabolic adaptive strategies to survive these long periods without feeding. Whether metabolic depression during aestivation will be similarly less pronounced for other tropical *Cyclorana* species (*brevipes*, *cryptotis*, *longipes*, *maculosa*, *manya*, *vagita*; Cogger

Table 2. Body mass, oxygen consumption rate ($\dot{V}O_2$; ml g⁻¹ h⁻¹), carbon dioxide production rate ($\dot{V}CO_2$; ml g⁻¹ h⁻¹), respiratory exchange ratio (RER = $\dot{V}CO_2/\dot{V}O_2$), evaporative water loss (EWL; mg g⁻¹ h⁻¹) and resistance to water loss (R; sec cm⁻¹). Values are mean \pm standard error, with sample size (n). P is probability of difference between awake and aestivating values (t-test).

<i>C. cultripes</i>	Awake (n=10)	Aestivating (n=6)	P
Mass	11.7 \pm 1.0	9.8 \pm 1.2	
$\dot{V}O_2$	0.160 \pm 0.021	0.049 \pm 0.007	0.001
$\dot{V}CO_2$	0.145 \pm 0.011	0.033 \pm 0.006	<0.001
RER	0.98 \pm 0.086	0.68 \pm 0.09	0.024
EWL	15.1 \pm 1.7	1.30 \pm 0.17	<0.001
R	-0.9 \pm 0.7	223 \pm 30	0.001

<i>C. australis</i>	Awake (n=8)	Aestivating (n=12)	P
Mass	44.2 \pm 9.4	32.1 \pm 5.4	
$\dot{V}O_2$	0.158 \pm 0.037	0.095 \pm 0.011	>0.05
$\dot{V}CO_2$	0.100 \pm 0.030	0.082 \pm 0.014	>0.05
RER	0.67 \pm 0.11	0.96 \pm 0.20	>0.05
EWL	15.8 \pm 2.1	1.1 \pm 0.1	<0.001
R	2.7 \pm 0.1	112 \pm 12	<0.001

1992) remains to be determined.

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