



**The ecophysiology of terrestrial nesting
in Australian ground frogs (Anura: Myobatrachinae)**

Nicola J. Mitchell

Department of Environmental Biology
University of Adelaide

October 2000

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text

I consent to this copy of the thesis, when deposited in the University Library, being available for photocopying and loan

Nicola Mitchell

The ecophysiology of terrestrial nesting in Australian ground frogs (Anura: Myobatrachinae)

<i>Abstract</i>	7
Acknowledgements	9
1 Introduction	10
2 The energetics of endotrophic development in the frog <i>Geocrinia vitellina</i> (Anura: Myobatrachidae)	14
<i>Abstract</i>	14
INTRODUCTION	15
METHODS	17
<i>Study species and site</i>	17
<i>Egg collection and measurement of nests</i>	17
<i>Egg maintenance</i>	17
<i>Egg staging and morphology</i>	18
<i>Rates of oxygen consumption</i>	19
<i>Bomb calorimetry</i>	20
<i>Statistical analysis</i>	20
RESULTS	22
DISCUSSION	29
<i>Embryonic and larval development</i>	29
<i>Ecological implications</i>	29
<i>Energy cost of development</i>	31
<i>Phylogenetic comparisons of energy densities and production efficiencies</i>	33
Acknowledgements	36

3	Effects of temperature on energy cost and timing of embryonic and larval development of the terrestrially breeding moss frog <i>Bryobatrachus nimbus</i>	37
	<i>Abstract</i>	37
	INTRODUCTION	38
	METHODS	40
	<i>Field measurements</i>	40
	<i>Laboratory maintenance of eggs</i>	42
	<i>Rates of oxygen consumption</i>	42
	<i>Bomb calorimetry</i>	44
	<i>Statistical analysis</i>	44
	RESULTS	45
	<i>Characteristics of eggs</i>	45
	<i>Field incubation times and natural nest temperatures</i>	45
	<i>Effect of temperature on development time</i>	45
	<i>Effect of temperature on $\dot{V}O_2$</i>	48
	<i>Masses and energy contents of ova, hatchlings and froglets</i>	49
	DISCUSSION	52
	<i>Effect of temperature on incubation time and differentiation rate</i>	52
	<i>Effect of temperature on $\dot{V}O_2$</i>	53
	<i>Energy cost of development</i>	54
	<i>Incubation in natural nests</i>	55
	<i>Ecological implications of protracted development</i>	57
	Acknowledgements	59
4	Constraints on the size of terrestrial masses of large eggs: oxygenation of embryos and larvae of <i>Bryobatrachus nimbus</i>	60
	<i>Abstract</i>	60
	INTRODUCTION	61
	METHODS	64
	<i>Study site</i>	64
	<i>Measurement of clutch parameters</i>	64
	<i>Field measurement of clutch PO_2</i>	64
	<i>Field measurement of larval lashing rate</i>	65

<i>Measurement of critical PO₂</i>	65
<i>Laboratory measurement of perivitelline PO₂ near hatching</i>	66
<i>Effect of temperature on jelly PO₂ and larval behaviour</i>	66
<i>Statistical analysis</i>	68
RESULTS	69
<i>Characteristics of clutches in the field</i>	69
<i>Critical PO₂ of larvae</i>	73
<i>Perivitelline diameters, PO₂ through jelly capsules of isolated embryos, and measurement of PO₂_{in}</i>	74
<i>The effect of temperature on jelly PO₂ and larval behaviour</i>	76
DISCUSSION	81
<i>The oxygen environment of natural nests</i>	81
<i>PO₂ in the perivitelline space of single embryos</i>	82
<i>Oxygenation of a globular mass of embryos</i>	84
<i>Larval responses to low PO₂</i>	87
<i>Limits to clutch size</i>	88
Acknowledgements	90
5 Incubation water potential affects survival, growth, metabolic rate and morphology of terrestrial embryos of the Australian frog, <i>Bryobatrachus nimbosus</i>	91
<i>Abstract</i>	91
INTRODUCTION	92
METHODS	94
<i>Fieldwork and egg collection</i>	94
<i>Response of B. nimbosus embryos to dehydration</i>	94
RESULTS	97
<i>Field observations of breeding pairs</i>	97
<i>Effects of water potential on laboratory eggs</i>	99
DISCUSSION	106
<i>Susceptibility of B. nimbosus embryos to dehydration</i>	106
<i>Factors ensuring adequate hydration</i>	108
Acknowledgements	110

6	Nest characters, site selection, and egg mortality in a frog with protracted development	111
	<i>Abstract</i>	111
	INTRODUCTION	112
	METHODS	114
	<i>Study sites and data collection</i>	114
	<i>Frequency of nest use</i>	114
	<i>Measurement of nest characters</i>	114
	<i>Statistical analysis</i>	116
	RESULTS	117
	<i>Characters of B. nimbus nests in sub-alpine and lowland sites</i>	117
	<i>Frequency of nest use</i>	117
	<i>Do nests containing clutches differ from vacant nests?</i>	119
	<i>Frequency of clutch mortality</i>	122
	DISCUSSION	124
	<i>The Incubation climate of B. nimbus nests</i>	125
	<i>Causes of egg mortality</i>	126
	<i>Potential for nest-based male selection in B. nimbus, and male nesting strategies</i>	127
	Acknowledgements	129
7	Males call more from wetter nests: effects of substrate water potential on reproductive behaviours of the terrestrial toadlet, <i>Pseudophryne blbronli</i>	130
	<i>Abstract</i>	130
	INTRODUCTION	131
	METHODS	133
	<i>Study species and site</i>	133
	<i>Experimental design and watering procedure</i>	133
	<i>Experimental measurements</i>	134
	<i>Measurement of the plot environment</i>	137
	RESULTS	138
	<i>Experimental conditions</i>	138
	<i>Distribution of nesting males</i>	138

<i>Calling effort of males in wet and dry nests</i>	140
<i>Male mating success</i>	142
<i>Egg hatching success in wet and dry nests</i>	142
DISCUSSION	143
Acknowledgements	147
References cited	148
Appendices	162
1 Measurement of oxygen consumption of amphibian eggs	162
2 Nest and egg mass characteristics of <i>Geocrinia lutea</i>	164
3 Estimating substrate water potential with filter papers	166
4 Characters of <i>Bryobatrachus nimbus</i> nests at a lowland site in Implicate rainforest.	168

Abstract

Variability in the microclimate of oviposition sites is a component of biological fitness that has only recently received serious attention. The fittest phenotypes will be produced if nest characters are consistently optimal; therefore selection should favour the ability of parents to select quality nests. In this study of three species of Australian Myobatrachine frogs I examine how the microclimate of terrestrial oviposition sites influences a), phenotypic traits of embryos, and b), the reproductive strategies and success of adults.

1. *Geocrinia vitellina* and *Bryobatrachus nimbus* develop without larval feeding and fuel their metamorphosis from yolk (endotrophism). Their production efficiencies are respectively, 59.2% and 61.5%, the first such measures for endotrophic amphibians, and their egg energy densities (26.4 and 26.0 J mg⁻¹) are greater than those of exotrophic amphibians (with feeding larvae).
2. Eggs of *B. nimbus* require 13 months to complete metamorphosis in sub-alpine moss nests, at an effective temperature of 8.5° C, and overwintering exhausts most of 249 J of energy contained in the ovum. Eggs in warmer nests (about 14.9° C) can reach metamorphosis in 5 months, and save 123 J, however, this saving is inconsequential if winter temperatures are too cold for froglets to feed.
3. The globular egg masses of *B. nimbus* are vulnerable to hypoxia because of substantial diffusion distances created by extremely large jelly capsules. Models of a spherical, hatching-stage egg mass show that the central embryo in larger clutches (13-21 eggs) experiences profound hypoxia at temperatures above 5° C. However, because broader nests are used as oviposition sites, real masses are typically hemispherical, and embryos are confined to 1-2 layers, which enhances their oxygen supply. Moreover, the photosynthetic nest material enriches jelly oxygen in daylight, when metabolic demands of embryos and larvae are greatest. Laboratory experiments demonstrate that larvae in warm nests (15°-20° C) seek out oxygen-rich jelly at the surface and walls of nests.

4. Incubation of *B. nimbus* embryos at water potentials between 0 and -25 kPa produces normal hatchlings at 0 and -5 kPa, but markedly stunted and asymmetric hatchlings at -10 and -25 kPa, with reduced rates of oxygen consumption. However, similar effects are not observed in natural nests, where desiccation contributes only a small portion (<7%) of embryonic mortalities. This suggests that embryos are able to uptake sufficient water from the surrounding substrate of bryophyte and lichens, and the capacious jelly capsule may be a valuable moisture reservoir.

5. Previous studies have shown broader tolerance to low water potentials in *Pseudophryne bibronii* embryos (0 to -200 kPa), but wetter incubation produced larger hatchlings. Patterns of male *P. bibronii* nest site selection and call advertisement are examined in a field experiment. Males prefer to nest on substrates of high water potential (≥ -15 kPa) and call from these nests at greater rates, and on three times as many nights as males occupying drier areas, presumably because they are not constrained by a risk of dehydration. Male mating success is coupled with calling effort; consequently, females choose between hydrated males, and in the majority of cases, oviposit in a wet nest that produces viable embryos.