The seasonal energetics of three species of Australian tropical frogs (Anura: Hylidae).

Lorrae Jean McArthur
B. Sc. (Hons) (Monash)

Faculty of Education, Health and Science
Charles Darwin University

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Declaration

"I hereby declare that the work herein, now submitted as a thesis for the degree of Doctor of Philosophy by Professional Doctorate of the Charles Darwin University, is the result of my own investigations, and all references to ideas and work of other researchers have been specifically acknowledged. I hereby certify that the work embodied in this thesis has not already been accepted in substance for any degree, and is not being currently submitted in candidature for any other degree."

Signed:

Date:
This dissertation is dedicated in memory of Daniel Frederick McArthur (1927-2004)
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Abstract

Some aspects of eco-physiology were examined for one cocoon forming (*Cyclorana australis*) and two non-cocoon forming frogs (*Litoria caerulea* and *Litoria dahlia*) that inhabit the wet-dry tropics. Seasonal ecology was examined to measure species’ activity and field body temperatures. Precipitation was a good predictor of frog activity. Species were most active in the wet season and least active during seasonal dry conditions. *Cyclorana australis* were dormant for seven months, and their body temperature varied little among seasons. However, *L. caerulea* and *L. dahlia* were generally active year-round, and their body temperatures varied among seasons. The digestive function and gastrointestinal flexibility of frogs was measured as a consequence of seasonal activity. *Cyclorana australis* ingested three and four fold more energy in the wet season than *L. caerulea* and *L. dahlia*, respectively, but ingested none when dormant, whereas *L. caerulea* and *L. dahlia* generally ingested energy year-round. Respiratory physiology was measured to examine for any seasonal changes in energy metabolism. Wet and dry season daily energy flow models were constructed based on those respiratory measurements and on estimated energy ingested in the field. *Cyclorana australis* incurred an energy deficit when dormant. However, the large amount of energy ingested in the wet season compensated for the dry season deficit. Furthermore, *C. australis* reduced the deficit by depressing energy metabolism to 35% of normal resting levels. Some of this reduction may have resulted from the significant down-regulation of the gastrointestinal tract. Cellular changes in the villi, specifically enterocyte volume, contributed to significant differences between wet season and dormant frogs. *Litoria caerulea* and *L. dahlia* ingested less energy in the dry than in the wet season, but the lower energy intake was offset by reduced energy expenditure. For these two species, expenditure was reduced as a consequence of low dry season body temperatures on respiratory metabolism, rather than metabolic depression.
Chapter One:

General Introduction and Background

East Point, Dry Coastal Vine Forest

Knuckey Lagoons, Lowland Wetland

Mickett Creek, Lowland Woodland

Howard River, Lowland Wetland
1.1 Physiological constraints of Australian frogs

Balancing energy and water requirements is generally challenging for frogs compared with other tetrapods. Frogs in general have simple lungs and therefore partly rely on cutaneous respiration for gas exchange (Boutilier et al. 1992). However, skin needs to be moist for gas exchange. This results in evaporative water loss that can lead to dehydration (Shoemaker et al. 1992). Furthermore, frogs are ectotherms, and body temperature generally conforms to the surrounding microclimatic conditions (Tracy 1976). Energy metabolism and most other physiological processes are temperature dependent (Withers 1992). Thus, temperature can be an important environmental variable (Rome et al. 1992). The physiological capacities of frogs in their environment, with respect to water and temperature, can be primary determinants of frog distribution and ecological limitations (Sinsch 1984, Huey 1991, Oseen and Wassersug 2002, Schwarzkopf and Alford 2002). Nonetheless, many species of frog are adapted to exchanging energy, ions, water and respiratory gases within a diverse array of environments, and even successfully endure extreme conditions on both a daily and seasonal basis.

1.1.1 The Australian climate: the need for adaptation?

The climate, with respect to precipitation and temperature, varies dramatically across the continent of Australia. Subsequently, a gradient exists (Figure 1.1). Several broad ecological zones are described: temperate, grassland, desert, subtropical, tropical and equatorial (Figure 1.1). Although these zones are based on the Köppen classification for world climate, zones are modified for the Australian climate (temperature, humidity and precipitation) and for the broadly defined assemblages of native vegetation (The Australian Government Bureau of Meteorology: http://www.bom.gov.au/climate/environ/other/kpn_group.shtml). The number of described frog species that inhabit these zones is positively correlated with precipitation (Main 1968).

The southern temperate zone is marked by wet, cold winters and mild to warm, dry summers. The major vegetation assemblages in this zone include open-forests,
woodlands and shrub-lands that are dominated by *Eucalyptus* spp., and less dominated by areas of alpine, rainforest and heath (Barlow 1994). The central subtropical, desert and grassland zones are marked by hot, dry summers, and cold-mild, low rainfall winters. The major vegetative assemblages in these zones include open shrub-lands dominated by *Acacia* spp., open-woodlands dominated by either *Eucalyptus* spp. or *Acacia* spp., and herb-lands that are either mixed or dominated by *Astrebla* (Barlow 1994). The northern equatorial and tropical zones are marked by wet, hot, humid summers and warm, dry winters. The major vegetation assemblages in these zones include savanna, and woodlands and open-forests that are both dominated by *Eucalyptus* spp., and minor areas of rainforest (Barlow 1994).

Figure 1.1. Climate classification of Australia based on a modified Köppen classification system. Classification is derived from 0.025 x 0.025 degree resolution mean rainfall, mean maximum temperature and mean minimum temperature grided data. All means are on a standard 30-year climatology (1961 to 1990) (Australian Government Bureau of Meteorology: http://www.bom.gov.au/climate/environ/other/kpnrp.gif). Numbers in parentheses are the estimated number of native species of frog in broad regions within classified climate zones (Tyler 1999).
1.1.1.1 Frogs adapted to temperate climate

The temperate zone generally provides ideal conditions for frogs when compared to other zones. For example, the giant frog, *Heleioporus australiacus*, from South-eastern Australia is normally active throughout the year because rainfall (at least 5mm), humidity (above 60%) and temperature (above 8°C) are appropriate for most of the year (Penman et al. 2006). However, in some regions temperature can get very low and rainfall distinctly seasonal. Some frogs alter behaviour in response to these variable seasonal conditions. Selecting appropriate retreat sites allows frogs to sit and wait out adverse climatic conditions. For example, some north American species of *Rana* bury themselves underground for short periods in response to extreme cold conditions (Watson et al. 2003). Alternatively, when water sources dry out, some species of frog migrate away from breeding areas to sites that provide permanent water or seeps (Lamoureux and Madison 1999, Mazerolle 2001, Muths 2003).

1.1.1.2 Frogs adapted to desert climate

The harsh central desert and semi-arid areas of Australia, which account for about 70% of the continent, experience low precipitation and a large temperature range (Main 1968). Nonetheless, about 30 desert-dwelling species of frog inhabit these zones (van Beurden, 1982). Traits shared among these species include fossorial habits, the ability to absorb water quickly and efficiently through a highly vascularised ventral patch, reduced metabolism (aestivation), ability to withstand considerable dehydration compared to other anurans, opportunistic reproduction and rapid development (Mayhew 1968, Ruibal et al. 1969, van Beurden, 1982).

Many desert-dwelling species burrow underground to avoid adverse dry and hot conditions (Mayhew 1968, Ruibal et al. 1969). While underground, some species form a cocoon that envelops the entire body surface, except for the nares. The cocoon can reduce water lose in some frogs to as little as 3% of that when not covered by a cocoon (van Beurden, 1982, Withers 1995). In addition to cocoon formation, some species store water. For example, the Australian central desert frog, *Cyclorana platycephala*, holds 57% of total body weight as water in its semi-
permeable bladder (van Beurden, 1982). Furthermore, when seasonally dormant, some species conserve both water and energy by depressing metabolism (aestivation) to 30 - 60% of that when normally resting (Withers 1993, 1998, Withers and Thompson 2000). Emergence from burrows is primarily dependent on precipitation, and in general is followed by a short burst of breeding behaviour (Mayhew 1968). As water resources dry out, frogs burrow underground and wait until the next event of rain.

1.1.1.3 Frogs adapted to tropical climate

The wet-dry tropics of northern Australia experience both annual drought and flooding. Frogs in general are more active during the wet summer period than in the dry winter period (Kam and Chen 2000). Similar to temperate zone frogs, some species either sit and wait out dry season conditions and even migrate distances to find suitable retreat sites in which to wait. The South and Central American toad, *Bufo marinus*, reduces its movements in the dry season (Schwarzkopf and Alford 2002), and selects burrows rather than retreat sites normally used in the wet season (Schwarzkopf and Alford 1996, Seebacher and Alford 1999). In contrast, the small rock hole frog, *Litoria meiriana*, migrates from wet season breeding sites to areas with permanent water sources (McAlpin 1995). Similar to desert-dwelling frogs, some frogs burrow underground during the dry season and remain dormant for prolonged periods (Withers 1993).


Few studies have investigated how cocoon and non-cocoon forming frogs adjust their behaviour and physiology in response to the drought-like conditions of the wet-dry
tropics. The tropical dwelling, cocoon forming frog, *Cyclorana australis*, has the same fossorial habits as desert-dwelling frogs, yet this species shows no significant depression in metabolism when dormant in laboratory conditions (Withers and Thompson 2000). Withers and Thompson (2000) suggested that the physiological capacity of *C. australis* has limited its distribution. However, the quandary remains as to how *C. australis* survive several months of dormancy without ingesting food. Furthermore, some temperate-dwelling, non-cocoon forming frogs show the ability to depress metabolism in laboratory conditions, but not in the field (Glass et al. 1997). It is possible that in more extreme climatic zones, such as the drought-like conditions of the wet-dry tropics, non-cocoon forming frogs may use metabolic depression in the field.

The primary focus of this research was to examine and compare the physiological adaptations of ecologically different species of frogs that live in the wet-dry tropics. The findings from this research may throw some light onto whether the physiology of frogs is constrained by either the ecological habits of species or by the climatic conditions in which they live.

### 1.2 Wet-dry tropics

#### 1.2.1 Climate of the wet-dry tropics

Research was conducted in the wet-dry tropics of Northern Australia around Darwin, a region that experiences strongly seasonal differences in humidity and rainfall and continuously high temperatures (Ridpath 1985, Bowman and Prior 2005). Four seasons are referred to in this dissertation that are defined by differences in climatic variables (Table 1.1): wet (December-February); late-wet (March-May); dry (June-August); and late-dry (September-November). In brief, the wet season is characteristically hot, humid and wet. In contrast, the dry season, although not much cooler than the wet season, is less humid and rainfall is negligible. The climatic conditions of the late-wet and the late-dry seasons reflect the transition between the wet and dry seasons.
Table 1.1 Climatic variables of the four seasons characteristic of the wet-dry tropics: wet (December-February), late-wet (March-May), dry (June-August) and late-dry (September-November). Means are based on data collected in no less than 60 years (Australian Government Bureau of Meteorology web site: http://www.bom.gov.au/climate/averages/).

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1.2.2 Frogs of the wet-dry tropics - Family Hylidae

Research was conducted using species of frogs belonging to the family Hylidae. The family Hylidae comprises 50% of the total number of species of frogs in the wet-dry tropics of the Northern Territory of Australia, and, of those, 90% belong to the genus *Litoria* and 10% belong to the genus *Cyclorana*. The *Litoria* are broadly described as ‘tree frogs’, but strictly, some species are terrestrial and others aquatic. However, one trait that is shared among this genus is the lack of cocoon formation, although the physiological resistance to water loss among this group is strongly related to species ecology (Young et al. 2005). In general, aquatic frogs are least resistant to water loss, terrestrial frogs are most resistant and arboreal frogs are intermediate. In contrast to the *Litoria* group, the genus *Cyclorana* has the ability to form a cocoon that provides an effective barrier against water loss (Withers 1998).

The ecological diversity among the Hylidae makes for interesting comparisons among frog species with different habits, but living in the same drought-like conditions of the wet-dry tropics. The species of frogs selected to compare the physiological response to contrasting seasonal conditions include the giant burrowing frog, *C. australis*, the green tree frog, *Litoria caerulea*, and the Dahl’s aquatic frog, *Litoria dahlii*. I selected these three species in particular because they are generally found easily in the field during optimum conditions, and are similar in size, which makes them suitable to compare with respect to their physiology.
1.2.2.1  *Cyclorana australis*

*Cyclorana australis* is a large, muscular frog, urostyle-vent length (UVL) 70-105 mm (Tyler and Davies 1986). This species is fossorial, cocoon forming and spends six to seven months underground during dry season conditions (Withers 1993, Christian and Parry 1997, Withers 1998, Withers and Thompson 2000). Their distribution extends across the tropical climatic zone of north Australia, and they inhabit seasonally dry open woodland (Tyler and Davies 1986, Cogger 2000).

1.2.2.2  *Litoria caerulea*

*Litoria caerulea* is a large frog, UVL 60-110 mm (Tyler and Davies 1986). This species is arboreal, non-cocoon forming and seeks refuge in tree hollows (Christian and Green 1994a, Reynolds 2005). Its distribution extends widely across the equatorial and tropical climatic zones in northern Australia, east across the subtropical climatic zone and inland into the desert zone (Shine et al. 1989, Cogger 2000). *Litoria caerulea* inhabit a wide range of environments including coastal dry monsoon vine thicket, inland wet monsoon rainforest, open woodland, riparian zones and suburban habitats.

1.2.2.3  *Litoria dahlii*

*Litoria dahlii* is a moderate to large, slender frog, UVL 50-70 mm (Tyler and Davies 1986). This species is aquatic, non-cocoon forming, and, when its ephemeral aquatic habitats disappear, it seeks refuge in either more permanent water sources or moist cracks in the soil (Barker et al. 1995). The distribution of this species extends narrowly across the tropical climatic zone of northern Australia, and they inhabit floodplain areas, including ephemeral ponds and swamps (Barker et al. 1995, Cogger 2000).
1.2.3 Some habitat assemblages of frogs in the wet-dry tropics

1.2.3.1 Lowland wetland (Howard River)

An ephemeral pond was used for studies of *C. australis* and *L. dahlii*. The pond (approximately 0.8 ha) is situated on crown land (12°32’15” S, 131°07’18” E) within the Litchfield shire, and is located approximately 40 km southeast of Darwin. The pond is part of a series of naturally revegetated depressions that were formed from sand mining several years earlier. Water generally remains in this pond from December to August each year. The maximum water depth of the pond that was recorded during this study was 1.2 m. The pond overflows during the peak of the wet season and water travels northeast into the Howard River. This river is situated about 500 m east of the pond, and, in most years, parts of this river retain water year-round. Rural properties (2 ha lots), located south and west of the pond (500 m away in either direction), potentially provide permanent water sources also, such as dams, manmade ponds and irrigation.

The Howard River pond study site lies within lowland wetland. Soil mainly consists of sand that supports both eucalyptus and wetland plant communities. Open forest fringes the west side of the area and is dominated by *Eucalyptus spp.* (*tetradonta* and *miniata*), and the annual grass *Sarga timoriense*. Soil becomes waterlogged during the wet season, and consequently, supports plant communities dominated by *Grevillea pteridifolia*, *Pandanus spiralus* and *Leptospermum longifolium*. Species of *Melaleuca* grow within the pond, such as *magnifica*, *nervosa* and *viridiflora*. However, the canopy of these trees covers only a small percentage (< 5%) of the pond’s surface area, providing minimal shade. Emergent species of sedge (*Cyperus* and *Eleocharis*) and aquatic plants (*Ceratophyllaceae demersum* and *Nymphaea violacea*) are found in the pond. The water remains clear until late in the dry season when the water level becomes very low. Public access and the lack of land management provide a potential for fire in the area, and one fire event was recorded at the study site the year prior to research.
1.2.3.2 Lowland wetlands (Knuckey Lagoons)

A lagoon was used for further study of *L. dahlii*. The lagoon (approximately 12 ha) is part of the Knuckey Lagoons conservation reserve (~130 ha) located (130°56’45” E, 12°26’00” S) approximately 15 km east of Darwin. The Parks and Wildlife Services of the Northern Territory manage the reserve. The lagoon is one of four in the reserve, and lies adjacent to the Stuart Highway. The maximum water depth recorded at this lagoon was 4 m, but the lagoon has the capacity to hold more water. The lagoon generally retains some water throughout the year, but during the course of this study it dried out completely in the late-dry season. The lagoon is situated in a semi-urban setting that potentially provides permanent water sources such as dams, manmade ponds and irrigation.

The Knuckey Lagoons study area lies within lowland wetland. Soil mainly consists of sand and clay that support both eucalyptus and grass communities. Mixed woodland fringes the west of the lagoon, dominated by *P. spirilas* and *G. pteridifolia*, and, further to the west, species of *E. tetradonta* and *E. miniata* dominate. Grassland encompasses the lagoon that is dominated by *Bothriochloa bladhii* and *Pseudoraphis spinescens*. *Eleocharis sundaica* and *S. timoriense* surround the lagoon and become seasonally inundated (Parks 2000). Aquatic plants, such as *N. violacea*, are found within the lagoon, but the lagoon is devoid of trees. The water remains clear until the water level drops to about 1 m in the late-dry season. At this depth, water clarity becomes poor because of the large numbers of water birds that aggregate and feed in the lagoon at this time. Fire is frequently used as a management tool to decrease fuel loading in the area, and one fire event was recorded during this research.

1.2.3.3 Lowland woodland (Mickett Creek)

Open woodland was used for further studies of *C. australis*. The study area (approximately 24 ha) is part of a recreation reserve (~120 ha) located (130°45’98” E, 12°12’27” S) approximately 15 km north east of Darwin, and managed by the
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Darwin City Council. The reserve supports many small ephemeral ponds and wet areas, but none are large enough to retain water throughout the year. Mickett Creek is situated in a semi-urban setting, which potentially provides permanent water sources such as dams, manmade ponds and irrigation.

The Mickett Creek study area lies within lowland woodland. Soil mainly consists of sand that supports both eucalyptus and wetland plant communities. Dominant tree species include *E. tetradonta* and *E. miniata*. Seasonally inundated areas that are interspersed throughout the woodland are dominated by *P. spiralis*, *G. pteridifolia* and spp. of *Melaleuca*. *Sarga timoriense* is the most dominant grass species within the woodland. Many dirt access tracks fragment the woodland. The reserve receives low priority land management due to its location (away from residential areas), and therefore fuel loading is not controlled. The high level of human activity and fuel loading in this area creates a potentially high fire risk and one area of the woodland was burnt during this research.

1.2.3.4  *Dry coastal vine forest (East Point)*

Coastal vine forest at East Point was used for studies of *L. caerulea*. East Point is a recreational coastal reserve (approximately 30 ha) located (130°49'98" E, 12°24'27" S) about 6 km north of Darwin and managed by the Darwin City Council. The reserve includes remnant plant communities, revegetated areas, cleared grasslands and developed parklands (Clouston and Associates 2000). Remnant plant communities include mangrove, eucalyptus, pandanus, and semi-deciduous monsoon vine forest that originally covered most of the reserve, but has since been reduced to about 70% of its original area. Management access roads mosaic the vine forest and define a number of areas. Two of those areas (9ha, 10ha), adjacent to the public access road, were used as the study site. Both temporary and permanent fresh water sources can be found at East Point. Small shallow depressions (max depth of 30 cm) lie within the vine forest and hold temporary water during the wet season. Amenities, horse troughs and irrigation exist in areas outside the study site and offer permanent sources of water. Outside the reserve (about 1.5 km), suburban backyards provide water potentially year-round.
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The East Point study site lies within dry coastal vine forest. Soil mainly consists of bauxite that dries out seasonally (Brock 2001). The vine forest canopy is typically short for coastal habitats (generally less than 15 m) and trees are densely distributed (between 60-90% canopy cover). Some of the dominant tree species include deciduous and evergreens: *Acacia auriculiformis*, *Bompax ceiba*, *Grewia breviflora*, *Ganophyllum falcatum*, *Litsea glutinosa*, *Sterculia quadridia*, *Strychnos lucida* and *Zanthoxylum parviflorum* (Brock 2001, Reynolds 2005). Vines are also prominent, such as *Opilia amentacea* and *Smilax australis*. Although the thicket is relatively dry compared to inland wet monsoon rain forests, the council regularly maintains the surrounding grassed areas. Consequently, the threat of fire is considered minimal and no fire was recorded during this research.

1.3 Scope and aims of the dissertation

The principal purpose of this research was to contribute to the knowledge of how frogs interact with their physical environment for the successful management of species in their natural habitat. The physiological adaptations of frogs in the wet-dry tropics may be compared to those found in frogs from other climatic zones to examine the eco-physiological constraints of frogs in general. The research is derived from ideas developed in other studies that have explored different aspects of frog physiology that are based on an energy perspective. The scope of this thesis encompasses the seasonal differences in the ecology, digestive physiology, gastrointestinal flexibility and respiratory physiology of three ecologically distinct species of frog that inhabit the wet-dry tropics. The last part of the dissertation uses wet and dry season energy flow models that combine some aspects of this present research, to summarise the interrelationship between the ecology and physiology of each species.
1.3.1 *Frog ecology*

The climatic conditions experienced in the field have implications for frog energy and water balance. Microclimatic can influence the activity of frogs on both a daily and seasonal basis (Ruibal et al. 1969, Lamoureux and Madison 1999, Hodgkison and Hero 2001). *Cyclorana australis* remain dormant for several months during the dry season (Cogger 2000, Withers and Thompson 2000), and while dormant they form a cocoon to minimise water loss (Withers 1998). However, it is not clear how non-cocoon forming species respond to similar dry season conditions (Cogger 2000). The first part of this dissertation examines the field activity of frogs in each season and the associated climatic conditions.

**Hypotheses:** I hypothesised that all three species would be more active in the wet season than in the dry season. Activity would be dependent on one or more of the climatic conditions, precipitation, temperature and humidity. I also predicted that the activity of the non-cocoon forming species would be greater than the cocoon forming species during the dry season. To determine these differences in activity I recorded the numbers of each species encountered in the field each season. I also attempted to determine the daily and seasonal refuge sites of frogs so that microclimatic conditions and animal body temperatures could be measured. I predicted that the range in body temperatures would be narrower for *C. australis* than for *L. caerulea* and *L. dahlii* as a consequence of their difference in activity and refuge sites. The significance for presenting these variables at the onset of the dissertation is related to the effect of body temperature on frog physiology, which follows in the subsequent chapters, but is also relevant here in relation to the effect of temperature on seasonal activity.

1.3.2 *Digestive physiology*

Both temperature and season influence how ectotherms assimilate energy by affecting digestive passage time and digestive efficiency (Harwood 1979, McConnachie and Alexander 2004). Temperature directly influences the activity of digestive enzymes, gastrointestinal motility and nutrient absorption, and thus the rate
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and efficiency of energy transformation (Carlos and Diefenbach 1975, Larsen 1992, Rome et al. 1992). The significance of temperature is related to its effect on digestive physiology in the field. Increased body temperature is positively related to digestive function, and subsequently, temperature is positively associated with growth (Freed 1980, Waldschmidt et al. 1985). The effect of season on digestive function is related to the frequency of feeding. For example, infrequent feeding significantly decreases digestive rate and increases passage time (Jaeger and Barnard 1981, Larsen 1984, Larsen 1992). Thus, the rate at which energy is assimilated is reduced. Morphological and histological down-regulation of the gastrointestinal tract (GIT) contributes to seasonal changes in digestive function (Geuze 1971, Secor et al. 1994, Starck and Beese 2002). The significance of regulating the GIT is that down-regulation reduces energy expenditure when the gut is not used (Secor and Diamond 1996, Overgaard et al. 2002, Starck 2003).

Hypotheses: I hypothesised that the digestive function of *C. australis* would show greater physiological variation between seasons, with respect to passage time and digestive efficiency, than that of *L. caerulea* and *L. dahlii*. *Cyclorana australis* are dormant during the dry season and do not consume food or water for several months (Cogger 2000, Withers and Thompson 2000). In contrast, it is expected that *L. caerulea* and *L. dahlii* feed more frequently and experience only short periods of fasting during adverse dry conditions. It is not known if temperature and season affect the digestive physiology of these frogs in response to changing seasonal conditions.

1.3.3 Gastrointestinal flexibility

The up and down-regulation of the gastrointestinal tract (GIT) are related to the frequency of feeding. Morphological and histological changes in the GIT, that are associated with changes in digestive function, are primarily related to changes within the villi of the innermost mucosal layer of the small intestine (Secor et al. 1994, Secor and Diamond 1995, 1998, Jackson and Perry 2000, Starck and Beese 2001). Up-regulation increases the mass and surface area of the villi. Consequently, the increased surface area promotes increased energy assimilation when digesting a meal.
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(Dykstra and Karasov 1992). In contrast, down-regulation of the GIT effectively decreases the mass and surface area of the villi. This results in decreased metabolic maintenance expenditure that subsequently conserves energy when the GIT is not being used (Starck and Wimmer 2005).

**Hypotheses:** Little is known about the GIT flexibility of species that may undergo only brief periods of fasting, but some studies show that GIT flexibility in seasonally dormant species of frogs is greater than species that are active year-round (Naya and Bozinovic 2004, Naya et al. 2005). Consequently, I conducted research on the flexibility of the GIT to complement the research conducted on the digestive physiology of species in response to season and periodic fasting. In this chapter I hypothesised that the seasonally dormant frog *C. australis* would show greater GIT flexibility between seasons and under fasting conditions, with respect to morphology and histology, than for either *L. caerulea* or *L. dahlii*.

### 1.3.4 Respiratory physiology

Some ectotherms have the innate ability to adjust respiratory physiology in response to seasonal conditions. Metabolic depression is the physiological adjustment of oxygen consumption to below standard metabolic rate (SMR) when dormant for prolonged periods (see Hochachka and Guppy 1987, Blaxter 1989, Guppy and Withers 1999). This functions to conserve both energy and water when resources are limited (Dimmitt and Ruibal 1980). Some desert-dwelling frogs are renowned for their aestivating ability (van Beurden 1984, Flanigan et al. 1991, Withers 1993, Flanigan and Guppy 1997, Withers and Thompson 2000). Species living in more arid regions possibly depress metabolic rate more than species living in areas with more predictable rainfall (Withers and Thompson 2000). However, non-cocoon forming species experience the same drought-like conditions as cocoon forming species, yet the metabolic response in this group of frogs to adverse seasonal conditions is unclear.

Metabolic depression in the wet-dry tropics may vary among frogs, as is the case among reptiles (Gregory 1982, Grigg et al. 1986, Kennett and Christian 1994, Abe
1995, Christian et al. 1995, Christian et al. 1999). For example, the arboreal frillneck lizard Chlamydosaurus kingii, significantly reduces energy expenditure during dry season conditions by depressing its metabolism to 43% of normal resting levels. Reduced activity and metabolic depression account for a significant energy saving in the field (Christian and Green 1994b). In contrast, the freshwater crocodile, Crocodylus johnstoni and the bluetongue lizard, Tiliqua scincoides, show no physiological response to dry season conditions (Christian et al. 1996, Christian et al. 2003), yet, the activity of these reptiles is markedly reduced in the dry season and subsequently results in reduced energy expenditure. Given these studies on reptiles, the physiological adaptations of frogs in the wet-dry tropics may also vary, depending on the ecology of species rather than on their environmental (climatic) constraints.

**Hypotheses:** In general, I examined the effects of season on oxygen consumption, with respect to the digestive and hydrated states of frogs. I hypothesised that the seasonally dormant frog, C. australis, would show a greater reduction in energy expenditure during the dry season than the seasonally active species L. caerulea and L. dahlia. Although it is predictable that fed frogs consume more oxygen than unfed frogs, it is not know how quickly frogs recover normal metabolism following dormancy. I hypothesised that all three species would recover normal metabolism following a period of fasting, as an adaptation for maximising short bursts of feeding activity during periodic favourable conditions.

**1.3.5 Energy flow models**

Temperature influences the respiratory physiology of ectotherms, and subsequently their partitioning of energy. The mobilization of oxygen during respiration, and the optimal working conditions of enzymes to provide energy during metabolism, are dependent on temperature (Jungermann and Barth 1996). Although temperature varies little in the wet-dry tropics, some ectotherms utilise the variation to modify body temperature and consequently alter energy metabolism. Basking behaviour increases body temperature and subsequently, energy turnover (Lillywhite et al. 1998). Alternatively, for example, the north Australian blue tongue lizard, Tiliqua
*scincoides*, selects microclimates that reduce body temperature to conserve water and reduce energy turnover during seasonally adverse dry conditions (Christian et al. 2003).

Few studies have modelled energy flow in amphibians (Fitzpatrick 1973), and to date no studies have modelled the energy flow of ecologically different species of frog that live in the wet-dry tropics. Overall, energy flow models account for energy ingested as food that is transformed into chemical energy and expended on various physiological processes in the body tissues (Brody 1964, Brafield and Llewellyn 1982, Blaxter 1989). I use a simple equation that is established on a temperature-specific basis, and states that energy expenditure is equal to that used for production (i.e. growth and fat stores), respiration (loss of heat to the environment), and energy lost in feces (Brafield and Llewellyn 1982).

Wet and dry season energy flow models were constructed to compare the physiological adaptations of ecologically distinct species in response to the same climatic constraints. Models were constructed for hypothetical free-ranging frogs based on laboratory measurements of energy metabolism (standard, resting and when digesting) over a range of temperatures (15, 20, 25 and 30°C) using known quantities of energy ingested and egested. Laboratory measurements were combined with field measurements of body temperature and egested energy. This combination was used to estimate the energy metabolism of frogs in the field.

**Hypotheses:** I hypothesised that *C. australis* would incur a greater debt in the dry season than *L. caerulea* and *L. dahliai* because of differences in seasonal activity among species. In addition to this, I hypothesised that, although energy flow varies between seasons and among species, dependent on their level of seasonal activity, wet season energy flow would offset that in the dry season.
1.4 Bibliography


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Chapter Two: Seasonal ecology

Giant burrowing frog, *Cyclorana australis*

Green tree frog, *Litoria caerulea*

Dahl’s aquatic frog, *Litoria dahlii*
2.1 Abstract

The tropical climatic zone of Australia experiences long drought-like conditions, yet many species of frogs persist in this region of extremes. This ability may be explained by the frogs’ level of seasonal activity and the microclimatic conditions of its refuge sites. In this chapter, I investigate some aspects of the seasonal ecology of Cyclorana australis, Litoria caerulea and Litoria dahlii. I examine the level of activity of each species by measuring the number of frogs observed and some associated climatic variables in four defined seasons. Frog body temperatures and associated microclimatic conditions of some refuge sites were also measured. As predicted, all three species were most active in the wet season. Cyclorana australis and L. caerulea were least active during the dry season, and, L. dahlii was least active during the late-dry season. Furthermore, the dry season activity of L. caerulea and L. dahlii was greater than C. australis. Air temperature, humidity and precipitation did not explain the variation in seasonal activity. Cyclorana australis sheltered above ground during the wet season, but burrowed underground (10 cm ± 2.0) and remained dormant for about seven months during seasonally dry conditions. Seasonal average body temperatures of this species varied little due to the burrow soil barrier that insulated frogs against fluctuating air temperatures. Litoria caerulea generally sit and wait out adverse conditions inside tree hollows. The humidity inside tree hollows is noticeably greater than outside hollows and is positively related to body temperature. Litoria dahlii generally sheltered in water, but in the late-dry season, their aquatic habitats dried out. Late-dry season refuge sites were not determined in this study. The aquatic medium that this species inhabits insulates frogs against fluctuating air temperature. The body temperature of L. dahlii was negatively related to air temperature, similar to C. australis. The differences among seasonal activity and microclimatic conditions of refuge sites possibly contribute to reducing the potential effects of dry season conditions on frog physiology, with respect to water and energy balance.
2.2 Introduction

Climatic conditions can influence the activity of frogs on both a daily (Hodgkison and Hero 2001) and seasonal basis, such as with the timing of breeding (Bertoluci and Rodrigues 2002), post-breeding activity (Richter et al. 2001), over-wintering (Lamoureux and Madison 1999) and dormancy (Ruibal et al. 1969). Frogs alter activity in response to changes in air temperature, absolute humidity, wind, solar and thermal radiation, soil water potential and precipitation (Tracy 1976, Spotila et al. 1992, Williams and Hero 2001). Precipitation is strongly correlated with the short term breeding activity of frogs (Duellman 1995, Marsh 2000, Bertoluci and Rodrigues 2002, Jensen et al. 2003), as well as with frog distribution and habitat selection (Williams and Hero 2001, Oseen and Wassersug 2002). Some frogs respond to dry periods by either moving long distances (McAlpin 1995, Watson et al. 2003), siting and waiting (McClanahan et al. 1978, Mazerolle 2001) or undergoing dormancy (Seymour and Lee 1974, van Beurden 1984). Generally, warmer summer periods are consistent with increased frog activity (Kam and Chen 2000) and cold winter periods with decreased activity (Lamoureux and Madison 1999).

Frogs select refuge sites during abating environmental conditions with microclimatic conditions that may have implications on both daily and seasonal water balance. Cocoon forming frogs minimise water loss during dormancy by burrowing underground and forming a cocoon (Seymour and Lee 1974, van Beurden 1984, Withers 1995) that significantly reduces water loss (Christian and Parry 1997, Withers 1998). Some non-cocoon forming frogs sit and wait out dry periods within suitable refuge sites (Pough 1980, Christian and Green 1994); some migrate distances to refuge sites that either provide year-round water or retain some moisture (McAlpin 1995, Mazerolle 2001). In contrast, however, some non-cocoon forming frogs expose themselves completely to all climatic conditions, and instead of using behavioural means, minimise water loss by physiological means, such as using skin secretions (McClanahan et al. 1978, Withers and Hillman 1984, Geise and Linsenmair 1986, Young et al. 2005).

The microclimatic conditions of refuge sites have implications on both daily and seasonal energy balance of frogs also, since most physiological processes are

Little is known about the seasonal ecology of many species of frog that live in the wet-dry tropics (Duellman 1995, McAlpin 1995, Seebacher and Alford 1999, Kam and Chen 2000), and experience a long drought-like period that is broken by a period of high precipitation (Ridpath 1985, Cook and Heerdegen 2001). Consequently, I conducted research that was based on the seasonal activity of Cyclorana australis, Litoria caerulea and Litoria dahlii. I hypothesised that all three species would be more active in the wet monsoon season than in the drought-like dry season. Similar to other taxa of frog that are most active during the rainy breeding period, the activity of all three species would be primarily positively related to precipitation. I also predicted that the activity of L. caerulea and L. dahlii would be greater than C. australis during the dry season. It is assumed that because a cocoon does not restrict these two species they would consequently be able to opportunistically exploit favourable conditions during the dry season. As an effect of differences in activity and refuge sites among species, I hypothesised that the range in body temperatures would be narrower for C. australis than for L. caerulea and L. dahlii.

To test these hypotheses, I used visual encounter field surveys to measure the seasonal activity of each species. These data were analysed for relationships between the numbers of frogs encountered and measured climatic variables (air temperature, humidity and precipitation). Using field sampling and radio telemetry, I measured the daily and seasonal body temperatures of frogs. These data were analysed for any relationships between body temperature and measured microclimatic variables (air and substrate temperatures, and humidity).
2.3 Materials and methods

2.3.1 Experimental protocol

The seasonal ecology of *C. australis*, *L. caerulea* and *L. dahlii* (see Chapter One for species descriptions) was examined in the four seasons: wet (December-February); late-wet (March-May); dry (June-August); and late-dry (September-November). The seasonal activity of the species and the associated climatic conditions were measured using visual encounter surveys (VES) at Howard River, East Point and Knuckey Lagoons (see Chapter One for details of field sites). The seasonal, night and day body temperatures ($T_b$) of the species and associated microclimatic variables were measured using field sampling and radio telemetry at Mickett Creek, East Point and Howard River.

2.3.2 Visual encounter surveys of seasonal frog activity

VES were used to provide an index of seasonal frog activity. The VES methods had minimal disturbance to frog behaviour and habitat (Crump and Scott 1994). Methods involved counting the number of frogs that were encountered on the surface (frogs/distance) between 21:00 and 24:00 hours every 10 days at Howard River (*C. australis*; August 2001-September 2002), Knuckey Lagoons (*L. dahlii*; September 2001-August 2002), and East Point (*L. caerulea*; August 2002-September 2003).

VES were conducted at a number of fixed transects at each field site (Howard River, 10 x 40 m; East Point, 6 x 80 m; Knuckey Lagoons, 10 x 120 m). Transects at Howard River and Knuckey Lagoons started from the midline of the pond and traversed outwards in a straight line, and, transects at East Point were set up along a defined walking track that meandered through the coastal vine forest. Permanent distance markers were used to mark transects so that transect distance could be recorded at the time of each survey because Howard River and Knuckey Lagoons become seasonally inundated. Transects were at least 20 m apart to minimize the
Chapter Two: Seasonal ecology

likelihood of recording an individual more than once during a survey (sensu Crump and Scott 1994). The focal area surveyed on each transect was within a 1 m radius of the observer. Each transect was surveyed at a consistent pace and only frogs encountered within transects were recorded. When frogs were encountered on transects, its position (above ground, or on ground, and distance from water) and a general description of its microhabitat (log, water, tree, or ground) were recorded.

Air temperature ($T_a$, °C), absolute humidity (AH, g cm$^{-3}$) and precipitation (mm) were measured at the time of each VES. Air temperature and relative humidity (RH, %) were measured from a fixed position in each field site using a hand-held meter (Vaisala: HM34C calibrated ± 5% RH; ± 1°C). Relative humidity was converted to AH using the Smithsonian water density table 108 (List 1971) against measured $T_a$. Precipitation was recorded at each site using RGI Onset data logging rain gauges that were downloaded in each season using the program BoxCar Pro 3.0.

2.3.3 Measurements of frog body temperature when active using field sampling

Field sampling was conducted between 21:00 and 24:00 hours once a week from September 2001 to August 2002. Field sampling involved traversing habitat in a random manner. Frog body temperature ($T_b$, °C) was measured each time a frog was encountered. These measurements were averaged in each season ($C. australis$, $n = 12 \pm 6$ SD; $L. caerulea$, $n = 18 \pm 6$ SD; $L. dahlii$, $n = 14 \pm 5$ SD), as an index of night time $T_b$ when active in the field.

To measure night time $T_b$, skin temperature was taken 1 cm away from the dorsal surface of frogs using a hand-held infrared thermometer (Raytek: Raynger MX2; emissivity ($\varepsilon$) = 0.98; ± 0.1°C resolution). I assumed that skin temperature was the same as body temperature (Wygoda 1984). Only frogs that remained stationary were included in the data set.
2.3.3.1 Measurements of microclimate during field sampling

Substrate temperature ($T_s$, °C), $T_a$ and RH were measured within 10 cm of the frog at the time of night time $T_b$ measurements. Substrate temperature was taken 1 cm away from the surface of the substrate using the hand-held infrared thermometer. Air temperature and RH were measured using the hand-held humidity and temperature meter.

2.3.4 Measurements of frog body temperature when not active using radio telemetry

Radio telemetry was used to determine the day and night seasonal $T_b$ of frogs when not active in the field and to determine the location and microclimatic conditions of refuge sites (C. australis, April-September 2003, Micket Creek; L. caerulea, April 2003-March 2004, East Point; L. dahlii, March-May 2004, Howard River). The same individual was measured once every 3-10 days, at different times of the day to maintain independence of observations (Swihart and Slade 1985). Day and night data were each averaged in each season, and, night time data for seasonal $T_b$ were combined with that taken during field sampling ($P > 0.05$).

Body temperature was measured using surgically implanted temperature sensitive transmitters (see 2.3.4.2 for details). Non-triangulation methods were used to locate transmitter signals (~40 pulses min$^{-1}$, ~18 ms pulse width) using a hand-held antenna and receiver (Telonics, USA). Frogs ($n = 10$ for each species) were tracked daily for the first few days following release and then every 3-10 days there after. When frogs were located, their position (above ground, or on ground, and distance from water), a general description of their microhabitat (log, water, tree, or ground) and their microclimatic conditions was recorded. Air temperature, RH and $T_s$ were measured once during the track session, as for field sampling.
2.3.4.1 Measurements of microclimate during radio telemetry

After locating the burrows of *C. australis*, some frogs were partially excavated (n = 3) to determine burrow depth (10 cm ± 0.5 SD). Subsequently, thermocouple lead wires (Omega) that could be attached directly to a hand-held digital thermometer (± 0.1°C resolution) were permanently fixed into the soil profile at 10 cm soil depth and within 10 cm of the frog burrow (n = 4). These methods were used because of a slight inaccuracy in determining the exact location of burrows and so as not to disturb the frog in its burrow. Measurements were taken every 3-10 days at the time of tracking frogs. Data were sorted into night (19:00-6:00 hours) and day (6:00-19:00 hours) and averaged for each season.

After locating some of the tree hollows used by *L. caerulea*, microclimate was measured inside (n = 3) and outside (n = 1) hollows. Vaisala (Finland) temperature and humidity sensors (linked to StowAway Volt data loggers that were powered by 12 V batteries) were used over a two weeks data logging period in each season. Air temperature and humidity were logged every hour in each logging period. Data were sorted into night and day and averaged for each season.

For *L. dahlii*, water resistant StowAway Tidbit data loggers were used to take a coarse measurement of $T_s$ (water) for two weeks in the wet, late-wet and dry seasons. Freestanding water was not present during the late dry season during this study. Data loggers were placed 2 m from the waters edge at 10 cm water depth (n = 3). Another logger was placed in the shade outside of water and above the ground to record $T_a$ (n = 1). Water and air temperature were logged every hour in each period. All data loggers were downloaded using the program BoxCar Pro 3.0. Data were sorted into night and day and averaged for each season.

2.3.4.2 Surgical procedures

Temperature-sensitive radio transmitters were surgically implanted at Charles Darwin University, Darwin. Two types of single stage transmitters were used, models E392 (short life: 17 weeks, 1.83 g ± 0.25) and E350 (long life: 9 months,
3.01 g ± 0.25), both of which had no external antennae (Sirtrack; Hawkes Bay, New Zealand). *Cyclorana australis* (47.6 g ± 25.5 SD, n = 10) and *L. caerulea* (52.5 g ± 18.3 SD, n = 10) carried long life transmitters that were less than 7.6% of their body mass, and *L. dahlii* (25.1 g ± 15.2 SD, n = 10) carried short life transmitters that were less than 9.3% of its body mass. Prior to implantation, transmitters were calibrated at 5°C increments between 5°C and 40°C in a water bath (Grant, USA) using a mercury thermometer that was traceable to a standard.

Surgical procedures closely followed established methods (K. Hatch pers. comm., Werner 1991, Goldberg et al. 2002). Following capture, frogs were anaesthetised by immersing their ventral surface in 0.1% MS222 (tricaine methansulfonate) that was dissolved in distilled water and buffered to pH 7 using sodium bicarbonate (sensu guidelines provided by the USGS National Wildlife Health Centre for the anesthesia of amphibians in the field: http://www.nwhc.usgs.gov/research/amph). The rate of sedation depended on species (*C. australis*, 10-30 min; *L. caerulea*, 10-40 min; *L. dahlii*, 5-20 min). Surgical instruments were cleaned then boiled for 15 minutes, and together with transmitters, cold sterilized in 90% isopropyl solution one hour prior to surgery. Alcohol was evaporated off instruments and transmitters before being used.

Anaesthetised frogs were placed in a supine position on a sterilized surface. A small lateral incision was made on the right side of the abdomen, where the abdominal skin meets the dorsal skin (recognized by change in skin colouration or pattern), inferior to the rib cage and superior to the pelvic girdle, to avoid opening the bladder. An incision (< 15 mm) was first made through the integument and then through the underlying muscular abdominal wall. After implanting the transmitter into the body cavity, the abdominal muscle layer and integument was each sutured separately, using Braun Safil green absorbable surgical suture (70 cm; USP 4/0). Frog welfare was monitored throughout the procedure by checking reflex points (knee jerk and eye twitch responses). The effects of the anesthesia lasted 10-60 min dependent on species; for example *L. dahlii* was the quickest to become sedated and longest to recover. Frogs were cared for in the laboratory for a 4-5 day recovery period prior to being released at their point of capture. At the end of the transmitter’s life, frogs were recaptured, transmitters were surgically removed or replaced, and the frogs were
released back into the field. Transmitters did not appear to interfere with frog movement or activity (sensu White and Garrott 1990).

2.3.5 Statistical analyses

Frog count data (frogs/distance) were normalised using square root transformation, and frog $T_b$, climatic ($T_a$, precipitation and AH) and microclimatic variables ($T_a$, $T_s$ and AH) were log-transformed, so that parametric tests could be conducted (Zar 1996). Repeated measures analysis of variance (ANOVA) in the program SuperAnova (Abacus Concepts Inc.) was used for frog count data in which transects were repeatedly measured in each season. Frog $T_b$, climatic and microclimatic variables were statistically compared among seasons using ANOVA. Relationships between frog activity and climatic variables, and between frog $T_b$ and microclimatic variables, were determined using multiple regressions in the program StatView (v 5.0; SAS Institute Inc.). Means (non-transformed) were used in tables and bar graphs (Packard and Boardman 1999). Differences among means were considered statistically significant when $P < 0.05$. Pair-wise Bonferroni Dunn $t$-tests were used as a post–hoc determination of differences among means following ANOVA, and $t$-values are expressed as determinants of the relationships between variables following multiple regressions.
2.4 Results

2.4.1 Seasonal frog activity and climatic variables

The activity of frogs, as indicated by numbers of frogs counted per distance, differed among seasons (Table 2.1). Frogs were most active in the wet season. Activity was clearly different between the wet and dry seasons for both *C. australis* and *L. caerulea*, but the dry season activity of these two species did not differ from that in the late-wet and late-dry seasons. For *L. dahlii*, activity was clearly different between the wet and late-dry seasons, but late-dry season activity did not differ from that in the late-wet and dry season.

Table 2.1. The number (as an index of activity) of *Cyclorana australis*, *Litoria caerulea* and *Litoria dahlii* observed on transects (frogs km\(^{-1}\)) and measured climatic variables at Howard River, East Point, and Knuckey Lagoons in the wet (December-February), late-wet (March-May), dry (June-August) and late-dry (September-November) seasons, at night between 2100 and 2400 hours. Values are means (untransformed data) ± standard deviation and n = the number of sampling periods. *P*-values are the probability of differences among seasons (ANOVA). Italic *P*-values are significant at the 5% significance level and different superscript letters indicate differences among seasons using Bonferroni Dunn post-hoc *t*-tests.

<table>
<thead>
<tr>
<th>Species</th>
<th>Wet</th>
<th>Late-wet</th>
<th>Dry</th>
<th>Late-dry</th>
<th><em>P</em>-value</th>
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<tr>
<td><em>Cyclorana australis</em></td>
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<tr>
<td>Activity (frogs km(^{-1}))</td>
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<td>0 ± 0(^a)</td>
<td>0.7 ± 2.5(^a)</td>
<td>&lt; 0.001</td>
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<tr>
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<td>22.7 ± 5.7(^ab)</td>
<td>19.3 ± 4.3(^a)</td>
<td>27.5 ± 1.1(^b)</td>
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</tr>
<tr>
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<td>16.4 ± 2.5(^b)</td>
<td>12.2 ± 3.0(^a)</td>
<td>21.2 ± 1.4(^a)</td>
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</tr>
<tr>
<td>Precipitation (mm)</td>
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<td>9 ± 16(^b)</td>
<td>11 ± 32(^a)</td>
<td>151 ± 116(^ab)</td>
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<td>1 ± 2(^a)</td>
<td>134 ± 114(^ab)</td>
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<td><em>Litoria dahlii</em></td>
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<td>Activity (frogs km(^{-1}))</td>
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<td>26.1 ± 1.7(^b)</td>
<td>17.7 ± 2.5(^a)</td>
<td>26.8 ± 2.0(^b)</td>
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</tr>
<tr>
<td>AH (g cm(^{-3}))</td>
<td>21.5 ± 1.1(^b)</td>
<td>20.4 ± 2.2(^b)</td>
<td>11.0 ± 2.3(^a)</td>
<td>19.5 ± 2.7(^b)</td>
<td>&lt; 0.001</td>
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<tr>
<td>Precipitation (mm)</td>
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<td>39 ± 66(^be)</td>
<td>0 ± 0(^a)</td>
<td>22 ± 28(^b)</td>
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Climatic variables differed among seasons (Table 2.1). Air temperature (\(T_a\)), absolute humidity and total precipitation were generally higher in the wet season and lower in
the dry season. Climatic variables in the late-wet and late-dry seasons were intermediate to the wet and dry seasons.

2.4.2 Relationship between frog activity and climatic variables

The numbers of observed frogs in the field were not related to any of the measured climatic variables.

2.4.3 General spatial use of microhabitat when active

Generally, *C. australis* sat on the ground close to water when active in the field (Fig. 2.1). About 55% of the total frogs observed (n = 14) were positioned within 1 m from water, 25% were positioned 1-10 m from water and the other 20% were positioned 20-40 m from water. Transects did not extend further than 40 m. Frogs were calling in 70% of observations, and of those, 80% were calling within 1 m from water and the other 20% were 1-10 m from water. No frogs called from distances greater than 10 m from water. Of the other 30% of frogs that were not calling, most (60%) were positioned greater than 20 m away from water.

![Figure 2.1. The proportion of total observations that *Cyclorana australis* was found sitting at measured distances from water, and the proportion found calling.](image-url)
**Litoria caerulea** generally perched above the ground when active in the field (Fig. 2.2). About 17% of the total number of frogs observed (n = 18) were perched on logs (> 5 cm diameter), 22% on vines (> 5 < 15 cm diameter), and 61% on branches (> 5 cm diameter) in trees that ranged between 2-50 cm (DBH: diameter at breast height) and 2-10 m in height. Of the total observations, 50% were perched less than 0.5 m above the ground, 10% were perched 0.5-1 m and 40% were perched greater than 1 m above the ground. Frogs were calling in 20% of observations, and of those, all were calling greater than 1 m above the ground. Of the other 80% of observed frogs that were not calling, most (60%) were positioned less than 0.5 m above the ground.

![Figure 2.2](image-url) **Figure 2.2**. The proportion of total observations that *Litoria caerulea* was found sitting above the ground, and the proportion found calling.

**Litoria dahlii** generally either sat in water or on the ground close to water when active in the field (Fig. 2.3). Frogs were sitting in water within a few meters of the water’s edge in 90% of the total observations (n = 180). Frogs were sitting within 10 m of the water in 7% of the observations and 3% were greater than 30 m from the water (Fig. 2.3). No *L. dahlii* were heard calling when traversing transects.
2.4.4 **Refuge sites of frogs when not active.**

*Cyclorana australis* sheltered on the soil surface or burrowed underground when not active. Shelter sites were never associated with exposed areas but rather with vegetation, such as leaf litter and/or grass and located near the base of a tree or shrub. All frogs (n = 10) that were repeatedly measured in the wet season during radio telemetry observations were sheltering on the soil surface during the day. Of the remaining four individuals that were regularly tracked in the late wet season, three burrowed underground in May, and one burrowed mid-June (dry season). Those four frogs remained buried in the late-dry season and emerged in November after substantial precipitation (150 mm recorded in a similar event of frog emergence; pers. obs.). Burrows were circular chambers about twice the size of the inhabitant, and the bottom of the chamber was 10 cm (± 0.5 cm SD; n = 3) below the soil surface.

*Litoria caerulea* sheltered in tree hollows during the day and night when not active. At night, frogs did not emerge from tree hollows in the wet season in 13% of total observations (n = 37), in the late-wet season in 43% of total observations (n = 28) and in the late-dry season in 7% of total observations (n = 10). The remaining individual that was tracked in the dry season was not observed (n = 8) outside of its tree hollow.
During the day, *L. dahlii* was found sitting in water among vegetation such as lilies, waterweeds and algae. During the night, frogs sat in the water close to the water edge. Unfortunately, the refuge sites used by this species when its aquatic habitats dry out completely in the late-dry season (August-November 2001; Howard River) were not determined. The battery life of the transmitters implanted in frogs expired when water at Howard River dried out after which no more frogs could found at this site to track.

2.4.5 The average seasonal night and day $T_b$ of frogs and associated microclimate

Frog $T_b$ differed among seasons and between day and night (Table 2.2). Generally, frog $T_b$ was highest in the wet season and lowest in the dry season, and higher during the day than in the night. However, the night $T_b$ of *C. australis* was higher in the dry season than in the wet season, and, in the late-wet and dry seasons when in burrows, night and day $T_b$ did not differ.

Clearly, the dry season night and day $T_b$ of *C. australis* were greater than that of *L. caerulea* and *L. dahlii* ($P < 0.001$). Generally, the $T_b$ of *L. caerulea* and *L. dahlii* did not differ. The range in *C. australis* $T_b$ was narrow than that for *L. caerulea* and *L. dahlii*.

Microclimatic variables differed among seasons and between day and night (Table 2.2). Generally, $T_a$, $T_s$, and, $AH$ were highest in the wet season and lowest in the dry season. Air temperature and $T_s$ were greatest in the day than in the night, and, $AH$ was greatest in the night than in the day. Soil $T_s$ of the burrows used by *C. australis* generally did not vary among seasons and between night and day. *Cyclorana australis* experienced higher $T_s$ when outside of burrows than when inside burrows. For *L. caerulea*, the $AH$ and $T_s$ inside tree hollows (shown as $^*$ in the day in Table 2.2) were generally greater than that outside tree hollows, particularly in the dry season ($P < 0.001$). The humidity inside tree hollows was noticeably greater than the outside humidity during both the day and the night.
Cyclorana australis experienced greater daytime $T_a$ than L. caerulea and L. dahlii ($P < 0.001$ in all seasons), but at night, L. caerulea generally experienced higher $T_a$ ($P < 0.001$ in most seasons). Day and night $T_a$ were generally greater for C. australis than for L. caerulea and L. dahlii ($P < 0.001$ in most seasons), but $T_a$ generally did not differ between L. caerulea and L. dahlii ($P > 0.001$). Absolute humidity was generally greater for L. caerulea (both inside and outside tree hollows) than for both C. australis and L. dahlii ($P < 0.01$ in most seasons, day and night).

2.4.6 Relationship between frog $T_b$ and microclimate

The $T_b$ of C. australis increased with increasing $T_s$ ($P < 0.001; t = 5.842$) and decreased with increasing $T_a$ ($P = 0.023; t = -2.35$), and, was not related to AH ($P = 0.499; t = -0.682$). The $T_b$ of L. caerulea increased with both increasing $T_s$ ($P < 0.001; t = 13.441$) and AH ($P < 0.001; t = 4.662$), but was not related to $T_a$ ($P = 0.231, t = -1.202$). The $T_b$ of L. dahlii increased with increasing AH ($P < 0.001; t = 7.571$) and decreased with increasing $T_a$ ($P < 0.001, t = -4.581$), and, was not related to $T_s$. 
Table 2.2. Night and day time body temperatures (T<sub>b</sub>, °C) and associated microclimatic variables for *Cyclorana australis*, *Litoria caerulea* and *Litoria dahlii* in the wet (December-February), late-wet (March-May), dry (June-August) and late-dry (September-November) seasons. Values are the means of untransformed data, ± standard deviation. n values = the number of frogs measured, and the statistical sample size. T<sub>a</sub> = air temperature (°C), T<sub>s</sub> = substrate temperature (°C), and AH = absolute humidity (g cm<sup>-3</sup>). For *L. caerulea*, AH outside (n = 1) and AH inside (n = 3) relates to tree hollows, and # refers to the temperature inside tree hollows; for *C. australis*, δ represents measurements taken at 10 cm soil depth (n = 4). P-values are the probability of differences among seasons, between day and night (2-Factor ANOVA). Italic P-values are significant at the 5% significance level. For *L. dahlii*, all late-dry season variables and late-wet season T<sub>b</sub> values are missing (-).

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2.5 Discussion

Clearly *C. australis*, *L. caerulea* and *L. dahlii* are more active in the wet season than in other seasons. The measured temperature, humidity and precipitation associated with frog activity in the field were highest in this season, but the numbers of observed frogs were not statistically related to any of these measured variables. Precipitation was not a good indicator for frog activity, as predicted in this study based on similar studies (Duellman 1995, Seebacher and Alford 1999, Mazerolle 2001, Penman et al. 2006). Other factors may have contributed to this result, such as, dry season reductions in prey availability (Bush and Menhinick 1962, Jaeger 1980, Schwarzkopf and Alford 2002, Hodgkison and Hero 2003).

Frogs were heard calling during the wet season only. Breeding activity is typical during events of precipitation (Oseen and Wassersug 2002, Jensen et al. 2003). *Cyclorana australis* consistently called within one meter of water, whereas *L. caerulea* called greater than one meter above the ground and away from breeding areas. However, the breeding ecology of *L. dahlii* remains obscure. The calling activity of *L. dahlii* was not observed during this study. This species presumably attracts, secures mates and breeds without leaving the water (Barker et al. 1995).

*Cyclorana australis* and *L. caerulea* were least active in the dry season when precipitation was negligible and other climatic variables, such as air temperature and humidity, were lower than in other seasons. In contrast to *L. caerulea* and *C. australis*, *L. dahlii* were least active in the late-dry season when air temperature and humidity were no different to that in the wet and late-wet seasons. For *L. dahlii*, freestanding water remains in ponds throughout the dry season, and generally, it is not until the late-dry season that water dries up completely. The aquatic habitats of *L. dahlii* may allow them to withstand the significant decrease in precipitation, air temperature and humidity in the dry season, as long as freestanding water is available. The presence of freestanding water may be a good predictor of the activity of this species rather than the climatic variables that were measured. Nonetheless, as expected, the activity of *C. australis* was considerably less than that of *L. caerulea* and *L. dahlii* during their least active periods.
Chapter Two: Seasonal ecology

*Cyclorana australis* generally sheltered on the ground among leaf litter during the day and during the night when not active, but not dormant. *Cyclorana australis* burrowed underground late in the late-wet season and remained dormant until a significant rainfall late in the late-dry season. Soil moisture (water potential) may be a good predictor of burrowing and emergence behaviour and terrestrial movements (Seebacher and Alford 1999, Smith et al. 2003). The significance of soil moisture may be strongly related to water economy and body fluid osmolarity, which subsequently affects frog physiology (Dole 1967, McClanahan 1972).

The burrows of *C. australis* were considerably shallower (10 cm ± 0.5) than those of other species of burrowing frog, but non-cocoon forming (Mayhew 1968, Ruibal et al. 1969). Species of *Scaphiopus*, *Breviceps* and *Pyxicephalus*, burrow to depths of 122-152 cm (Ruibal et al. 1969). The average burrow depth for *C. australis* was most similar to those of the Australian cocoon forming frog *Cyclorana platycephala*, which burrows down 10-25 cm (van Beurden 1982). Burrow depth may correlate with the soil moisture profile (Main et al. 1959), i.e. soil moisture increases with depth. Cocoon forming species may not need to burrow as deep as non-cocoon forming species because the cocoon may insulate the frog against the soil water potential.

*Litoria caerulea* sheltered in tree hollows during the day, and during the night when not active. Frogs selected trees ranging between 5-16 m in height and 6-60 cm DBH (Reynolds 2005). The opening to hollows ranged between 1.5-5 cm and depth ranged between 4-30 cm (Reynolds 2005). However, the strategy used to endure dry season conditions varied among individuals. Of the ten frogs tracked in the late-wet season, only one persisted in the vine forest during the dry season. The other frogs migrated several kilometres to unnatural habitats that provided water year-round (effluent processing plants and amenity blocks, pers. obs.). Individual *L. caerulea* use either sit and wait or migratory behaviour in response to adverse seasonal conditions.

*Litoria dahlii* generally sheltered in water during the day and night when active and not active. Unfortunately, I was unable to track the movements of *L. dahlii* during the late-dry season and determine refuge sites when freestanding water dries out completely. Some species of *Rana* rely on soil moisture as their primary source of
Litoria dahlii possibly hide in moist cracks in the clay soil (Barker et al. 1995) when and if water dries out completely at Knuckey Lagoons. However, at Howard River the ponds completely dry out late in the dry season. Furthermore, and in contrast to Knuckey Lagoons, the ponds at Howard River have a sandy base that does not crack and provide potential refuge sites. Given this, it is presumed that L. dahlii move away from this breeding sites, similar to other frogs that breed in temporary water sources (Lamoureux and Madison 1999, Mazerolle 2001, Richter et al. 2001, Watson et al. 2003). For example, the Australian tropical dwelling frog, Litoria meiriana, lives and breeds in temporary creeks (Cogger 2000), but moves to permanent creeks and waterholes when breeding sites dry out in the dry season (McAlpin 1995). Litoria dahlii may either sit and wait in habitats that retain soil moisture during dry periods or migrate to habitats with permanent freestanding water.

The type of site that a frog selects for refuge may be related to its physiological constraints. The most significant constraint that a frog has is its low resistance to water loss, when compared to that of other vertebrates (Tracy 1976). Cyclorana australis form a cocoon when underground in burrows to prevent water loss, similar to some desert dwelling species (van Beurden 1982). A three month old cocoon reduces C. australis’ area specific evaporative water loss (mg cm$^{-2}$ h$^{-1}$) to 36% of that when without a cocoon (Christian and Parry 1997). Litoria caerulea used tree hollows that maintained higher and more constant humidity than outside tree hollows, which was most noticeable in the dry season when climatic conditions were more variable and least favourable for frog activity. Litoria dahlii has the least resistance to water loss compared to arboreal and terrestrial frogs (Tracy and Christian 2005, Young et al. 2005). Moist shelter sites may be essential for L. dahlii to balance water requirements during dry periods (Dole 1967, Huey 1991, Beck and Jennings 2003).

The minimum and maximum body temperatures of C. australis (27.1-33.2°C) were higher than those of L. caerulea (21.1-29.2°C) and L. dahlii (22.2-31.4°C). However, the difference between the minimum and maximum body temperatures for C. australis (6°C) was smaller than for L. caerulea (8°C) and L. dahlii (9°C). These
results may be explained by differences in the microclimatic conditions of refuge sites among frog species.

The body temperatures of *C. australis* and *L. dahlia* were both negatively related to air temperature. *Cyclorana australis* are protected from low fluctuating dry season air temperatures by an insulating soil layer when dormant inside burrows (Tracy 1976). Similarly, *L. dahlia* are insulated from fluctuating air temperatures, but by a water barrier. Thus, the seasonal body temperatures of these two species remain relatively constant when seasonal ambient temperatures fluctuate.

Substrate surface temperature is positively related to the body temperatures of *C. australis* and *L. caerulea* suggesting that the ventral surface of frogs contributes to its thermoregulation. That is, dorsal surface evaporative water loss is not the entire means by which frogs potentially thermoregulate (Tracy and Christian 2005). However, unlike these two species, the body temperature of *L. dahlia* was not related to substrate temperature. This result was not unexpected, given that this species may use water thermo gradients to regulate body temperature similar to other species of aquatic frog (Lillywhite 1970). Even though the insulation effect of water was evident (i.e. body temperature was negatively related to air temperature), the result that substrate temperature was not related to body temperature may be due to the methods used to measure water temperature in relation to frog body temperature. The temperature of the water was not measured directly in relation to the frog.

Although challenged by long drought-like periods and continuously high temperatures, *C. australis*, *L. caerulea* and *L. dahlia* clearly change their activity among seasons and select refuge sites that presumably reduce the effects of adverse conditions on energy and water balance. Seasonal inactivity and changes in body temperature may both have significant implications for energy balance. Determining the environmental constraints and integrating the physiological adjustments in response to such limitations is important for broadening our understanding of the seasonal ecology of amphibians in the wet-dry tropics (Ridpath 1985). Understanding these complex relationships within an ecosystem provides us with better tools to effectively manage species and their natural environment (Hamer et al. 2002, Hazell 2003). To emphasis this, the seasonal shift in habitat preferences, as
observed for *L. dahlii*, has implications for amphibians and habitat conservation (Lamoureux and Madison 1999).
2.6 Bibliography


Chapter Two: Seasonal ecology


Chapter Three: Digestive physiology

This chapter has been submitted as:
3.1 Abstract

The digestive physiology of the three species was measured, with respect to passage time (PT) and digestive efficiency (DE), at different temperatures (15, 20, 25 and 30°C) and within seasons (wet and dry). In addition to this, the DE of free-ranging frogs in the field was estimated. PT was 60-90% longer at 15°C than at 20, 25 and 30°C for all three species. Temperature did not influence the DE of *C. australis* and showed no discernible relationship with DE for *L. caerulea* (DE at 20°C was 11% greater than at 15 and 25°C). However, the DE of *L. dahlii* was lower at 15°C than at other temperatures, which may be explained by PT. Although PT was significantly longer at 15°C than at other temperatures for all three species, PT for *L. dahlii* was significantly shorter than for *C. australis* and *L. caerulea* at this temperature. Season did not influence the PT of *L. caerulea* and *L. dahlii*, but for *C. australis*, PT took 57% longer following dry season dormancy than during the wet season. Season influenced the DE of *C. australis* and *L. dahlii*, but not *L. caerulea*. DE was 18% higher during the dry season for *C. australis*, and 37% higher during the wet season for *L. dahlii*. Seasonal differences in DE may be associated with the up and down-regulation of the gastrointestinal tract in relation to the digestive state of individuals. Estimated DE of free-ranging frogs averaged 90% for *C. australis* in the wet season, and ranged between 72-74% for *L. caerulea* and between 71-89% for *L. dahlii* in the wet and dry seasons.
3.2 Introduction

In ectothermic vertebrates, a wide range of factors influences each of the five phases of energy assimilation: food intake, passage time (PT), digestion, absorption and defecation (Skoczylas 1978, Jaeger and Barnard 1981, Bedford and Christian 2000). Factors include, species, size, sex, age, body condition, diet, the amount of food ingested, temperature and season (Waldschmidt et al. 1985, Larsen 1992, Hume 2005). Although activity patterns of some species of amphibians in the wet-dry tropics are strongly seasonal (Schwarzkopf and Alford 1996, Schwarzkopf and Alford 2002), it is not known if, and how, the environment of the wet-dry tropics affects frogs’ digestive physiology or energy partitioning.

Temperature and season mainly affect the energy assimilation of ectotherms by altering PT and digestive efficiency (DE) (Harwood 1979, McConnachie and Alexander 2004). Studies of the effects of temperature on the digestive physiology of terrestrial vertebrate ectotherms have focused largely on reptiles (Harlow et al. 1976, Christian 1986, Slade et al. 1994), with fewer studies on frogs (Smith 1976, Gossling et al. 1980, Larsen 1984). Temperature directly influences the activity of digestive enzymes, gastrointestinal motility and nutrient absorption, and thus the rate and efficiency of energy transformation (Carlos and Diefenbach 1975, Larsen 1992, Rome et al. 1992). For example, the gastric wall in some North American species of *Rana* becomes non-secretory at temperatures below 10°C, and the enzyme pepsin dramatically reduces activity, which means gastric secretions and digestive enzymes are not able to assist in the digestive process (Reeder 1964, Forte 1996, Secor 2003). As an extreme example, the complete passage of chyme in the leopard frog, *R. pipiens* takes 12-24 hours at 24°C, but takes about 340 hours when aestivating at 4°C (Gossling et al. 1980). Greater digestive rates at higher body temperatures are associated with greater growth rates, but reach an upper limit when digestive rate plateaus with increasing temperature (Freed 1980, Waldschmidt et al. 1985).

Digestive function is similarly related to the frequency of feeding, and so changes seasonally (McConnachie and Alexander 2004). Infrequent feeding significantly decreases digestive rate and increases PT (Jaeger and Barnard 1981, Larsen 1984,
Larsen 1992). Some species of Australian frogs are seasonally dormant during adverse environmental conditions and do not consume food or water for several months (Christian and Parry 1997, Cogger 2000, Withers and Thompson 2000). The PT of the Green Striped Burrowing Frog, *Cyclorana alboguttata*, significantly increases following three months of dormancy (Cramp and Franklin 2003). Morphological and histological down-regulation of the gastrointestinal tract of ectotherms (Geuze 1971, Secor et al. 1994, Starck and Beese 2002) contributes to changes in digestive function, reducing energy expenditure when the gut is not being used during dormant periods (Secor and Diamond 1996, Overgaard et al. 2002, Starck 2003). Although little is known about the physiological responses of frogs that undergo periods of dormancy compared to sympatric frogs that feed more frequently under the same seasonal conditions, I hypothesised that the digestive function of seasonally dormant frogs would show greater physiological variation with season.

In this chapter, the digestive function of *C. australis*, *L. caerulea* and *L. dahlii* was measured. The broad aim was to compare the physiological adjustments in energy assimilation of each species in response to temperature and season. Specifically, I determined the effect of temperature and season on PT and DE under laboratory conditions. Furthermore, I measured the mass of feces egested by frogs collected from the field, to provide a coarse index of seasonal ingested energy and to estimate seasonal DE in the field.
3.3 Materials and Methods

3.3.1 Experimental protocol

PT and DE were measured to determine the effect of season and temperature on the digestive function of *C. australis*, *L. caerulea* and *L. dahlii*. PT is defined as the time (hours) between ingestion and egestion (Harwood 1979, Bedford and Christian 2000). The percentage of energy retained by the animal, which is absorbed across the gut wall, is referred to as DE and reflects the completeness with which food is digested and absorbed (Harlow et al. 1976, McConnachie and Alexander 2004, Hume 2005). DE is measurable as the difference between the heat of combustion of food and feces using calorimetry (Paine 1971, Blaxter 1989). DE was expressed in percentage terms using the equation:

\[
\text{DE} = \frac{(I - F)}{I} \times 100
\]

where I is the energy content of food ingested, and F is the energetic content of feces (Kitchell and Windell 1972, Johnson and Lillywhite 1979, Waldschmidt et al. 1985). This measure is underestimated, however, because feces may contain substances in addition to undigested plant or animal matter, such as cells being sloughed from the gut lining, gastrointestinal secretions, parasites, bacteria, nitrogenous products and other metabolic wastes (Harwood 1979, Dimmitt and Ruibal 1980, McConnachie and Alexander 2004).

The PT and DE of species were measured at different temperatures and during different seasons. Four experimental temperatures (15, 20, 25 and 30°C) were selected within the general temperature range frogs experience in the field (Chapter Two, Table 2.2). The effect of temperature on digestive function was measured during the wet season, when it is assumed the physiology of frogs is least affected by climatic variables. For a seasonal comparison, I compared the wet season measurements taken at 25°C (± 1.0) with dry season measurements taken at the same temperature. *Litoria caerulea* and *L. dahlii* are relatively active during the dry season and were easily collected from the field (n = 6 per species). However, *C. australis* is a burrowing species and is therefore extremely difficult to find during the dry season. Therefore, I simulated dry season conditions in the laboratory for this species by
maintaining frogs (n = 6) individually in 2 L perforated containers inside a controlled temperature cabinet at 25°C (± 1.0), in the dark and without food and water for 90 days. While dormant, *C. australis* forms a cocoon, and therefore, immediately before measurements of PT and DE, I removed the cocoon and then followed the same procedures as for all other treatment groups and species.

Prior to temperature trials, frogs were fasted in a temperature-controlled cabinet at 25°C (± 1.0) until no feces were egested for three consecutive days. Frogs were then equilibrated to an experimental temperature (± 1.0°C) for 48 hours. The order of temperature trials was arbitrary among individuals (McConnachie and Alexander 2004). Following equilibration, frogs were force fed three live crickets in three events; each event was separated by 24 hours and occurred between 1700 and 1900 hours (Harwood 1979, Slade et al. 1994). The water content of crickets was about 63% and the mean energy content, as determined by bomb calorimetry (see below), was 19.3 ± 2.9 (SD) kJ g⁻¹ (Slade et al. 1994, Iglesias et al. 2003). *Cyclorana australis* (46.2 g ± 8.1 SD) and *L. caerulea* (42.0 g ± 13.6 SD), ingested crickets that were 6-10 weeks old and had a total wet mass equal to 3.8% (± 1.4 SD) of frog initial body mass. *Litoria dahlii*, (17.4 g ± 7.5 SD) ingested crickets that were 4-6 weeks old and had a total wet mass equal to 4.5% (± 2.7 SD) of frog initial body mass. The dry mass of crickets ingested during temperature trials was estimated (n = 12) using the regression of wet mass against dry mass:

\[
\text{dry mass} = 0.371 \times \text{wet mass} \quad (R^2 = 0.986)
\]

PT was measured using an inert, non-toxic, granular fluorescent marker (50-350 μm), which is visible under a compound microscope with the assistance of a fluorescent light source (Harlow et al. 1976, McConnachie and Alexander 2004). No marker was used in the first feeding event. Green and orange markers were used in the second and third event, respectively. The PT (hours) of each colour marker was averaged for individual frogs. PT did not differ between coloured markers for all species (*C. australis, P = 0.837; L. caerulea, P = 0.307; L. dahlii, P = 0.881*), and therefore were pooled for further analyses. The total dry mass of feces egested by individuals at each temperature was recorded.
In addition to the above procedures, frogs were captured from the field each season (n = 18 per species) and any feces egested were collected and the dry mass recorded to estimate the amount of energy ingested and DE. Cyclorana australis are dormant in the field during the dry season and it is assumed that the energy ingested and egested would equal zero. Energetic estimates were based on the assumption that only crickets were ingested in the field, and therefore do not consider variability in diet (Bush and Menhinick 1962). From the analysis of a known number and mass of field faecal samples, I found the percentage of indigestible matter (sorted as soil, plant matter and small stones) as follows: 35% for C. australis (n = 23), 23% for L. caerulea (n = 21) and 5% for L. dahlii (n = 10). The mass of feces collected from the field was subsequently corrected for this estimated percentage of indigestible matter.

I used linear regression to calculate two unknowns, mass of food ingested and the amount of energy ingested so that I could estimate DE in the field. I estimated the dry mass of food ingested by regressing the dry mass of feces against the dry mass of crickets ingested at 25°C during the wet season temperature trials, as follows:

\[
\begin{align*}
\text{Cyclorana australis:} & \quad \text{mass ingested} = 0.223 \times \text{feces} \quad (R^2 = 0.97) \\
\text{Litoria caerulea:} & \quad \text{mass ingested} = 4.569 \times \text{feces} \quad (R^2 = 0.80) \\
\text{Litoria dahlii:} & \quad \text{mass ingested} = 7.872 \times \text{feces} \quad (R^2 = 0.47)
\end{align*}
\]

(intercept set at 0 assuming zero feces equals zero ingested). I then solved these equations for each species using the known field faecal mass. Similarly, I estimated the amount of energy (joules) ingested in the field by first regressing the number of joules ingested with the dry mass of crickets ingested at 25°C during the wet season temperature trials, as follows:

\[
\text{joules} = 4590 \times \text{ingested} \quad (R^2 = 1),
\]

(intercept set at 0 assuming zero ingested equals zero joules). This equation was then solved using the estimated dry mass ingested the field (calculated above).

3.3.2 Calorimetry

The energy content of crickets ingested and feces egested was determined using bomb calorimetry, following methods used by Bedford and Christian (2000), Dimmitt and Ruibal (1980) and Paine (1971). Prior to calorimetry, crickets and feces
were oven dried at 60°C to a constant dry mass (Dimmitt and Ruibal 1980, Slade et al. 1994). Feces and crickets were ground to a fine consistency using a mortar and pestle. Small amounts (< 0.05 g) were pressed into pellets in a 15-ton press (Perkin-Elmer, England), using ten tons of pressure for two minutes. Faecal samples less than 0.02 g were made up to 0.05 g with a known mass and caloric value of calorific grade benzoic acid (Paine 1971). Pellets were placed inside a semi-micro bomb calorimeter (model 1421, Parr Instruments Inc., Moline, IL), and ignited using standard bomb calorimeter practices (Parr Instruction Manual). The heat from combustion of calorific grade benzoic acid was used as a standard (26.4 kJ g\(^{-1}\); Parr instruments, Inc.), with the contribution of fuse wire, and expressed on a joule ash-free g\(^{-1}\) basis (Paine 1971). The energy content of three samples of crickets containing four arbitrarily selected individuals were measured in this manner (Slade et al. 1994).

3.3.3 Statistical analyses

DE (%) data were normalised using arcsine-transformation and all other data were normalised using log-transformation so that parametric tests could be conducted (Zar 1996) in the program SuperAnova (Abacus Concepts Inc.). Repeated measures analysis of variance (ANOVA) was used to examine variables at different temperatures using the same individuals. Since oxygen consumption is dependent on body mass (Hochachka and Guppy 1987, Bedford and Christian 1998), body mass was used as a covariate in most analyses (ANCOVA), except for analyses of feces egested, energy egested and DE. In these cases mass ingested was used as the covariate. Least square mean oxygen consumption (non-transformed) values were used in tables and graphs (Packard and Boardman 1999). Differences among means were considered statistically different when \(P < 0.05\). Post-hoc pair-wise Bonferroni Dunn \(t\)-tests (at the 0.05 significance level) were used to determine differences among means following ANCOVA.
3.4 Results

3.4.1 Temperature effect on PT

Temperature influenced the PT of all three species (Fig. 3.1). PT ranged between 12-420 hours for *C. australis*; 14-442 hours for *L. caerulea*; and 6-216 hours for *L. dahlii*. PT at 15°C was significantly longer than at 20, 25 and 30°C (7-fold longer for *C. australis*, *P* < 0.001, 9-fold longer for *L. caerulea*, *P* = 0.002 and 2.8-fold longer for *L. dahlii*, *P* = 0.030), but the PT of *C. australis* at 15°C did not differ from that at 20°C. Excluding the PT of frogs during the dry season, PT did not differ between 25 and 30°C for all three species, and, at 20°C PT did not differ from PT at 25 and 30°C for *L. caerulea* and *L. dahlii*.

The PT of *C. australis* and *L. caerulea* was 1.5-fold longer than that of *L. dahlii* (*P* = 0.023) at 15°C, and did not differ between *C. australis* and *L. caerulea*. At 20°C, the PT of *C. australis* was 3.7-fold longer than that of *L. caerulea* (*P* = 0.034), and the PT of *L. dahlii* at this temperature did not differ from either *C. australis* or *L. caerulea*. PT did not differ among species at 25 and 30°C.

3.4.2 Seasonal effect on PT

Season did not influence the PT of *L. caerulea* and *L. dahlii* (Fig. 3.1). For *C. australis*, PT was 4.7-fold greater during the dry season following dormancy than during the wet season (*P* = 0.029; Fig. 3.1). PT did not differ among species during either the wet or dry seasons.
**3.4.3 Temperature effect on DE**

The DE of individuals ranged from 67-73% for *C. australis*, 73-83% for *L. caerulea*, and 71-94% for *L. dahlii*. Temperature had no effect on the DE of *C. australis* and *L. caerulea* (Table 1). However, for *L. dahlii*, DE at 15°C was 24% less than at 20, 25 and 30°C, but did not differ among 20, 25 and 30°C (Table 3.1).
DE differed among species for each experimental temperature except for 15°C. The DE of *C. australis* was less than that of *L. dahlii* at 20 (*P* = 0.015), 25 (*P* = 0.014) and 30°C (*P* = 0.003), 37, 24 and 28% less respectively. Furthermore, the DE of *C. australis* was 37% less than that of *L. caerulea*, but only at 20°C (*P* = 0.015). The DE of *L. caerulea* was 22% less than that of *L. dahlii* at 25°C (*P* = 0.014), but did not differ between these two species at 20 and 30°C.

### 3.4.4 Seasonal effect on DE

Season influenced the DE of *C. australis* and *L. dahlii*, but did not influence the DE of *L. caerulea* (Table 3.1). For *C. australis*, DE was 18% higher during the dry season following dormancy than during the wet season. In contrast, the DE of *L. dahlii* was 37% less during the dry season than during the wet season. Dry season DE did not differ among species (*P* = 0.118).

### 3.4.5 Field faecal analyses

The mass of feces egested in the laboratory at 25°C by individuals collected from the field during the wet season ranged between 0-1.3 g for *C. australis*, 0.017-0.580 g for *L. caerulea*, and 0.004-0.340 g for *L. dahlii*. During the dry season it is assumed that *C. australis* egest 0 g feces because they aestivate, but for *L. caerulea* and *L. dahlii*, the feces of individuals ranged between 0-0.50 g and 0-0.176 g, respectively. The mass of feces egested by *L. caerulea* and *L. dahlii* did not differ between seasons (Table 3.2), and it is assumed that feces egested by *C. australis* would be greater during the wet than during the dry season. Among species, the estimated mass of food ingested by *C. australis* during the wet season was 4-fold more than ingested by *L. caerulea* and *L. dahlii* (*P* < 0.001, Table 2), and the estimated mass of food ingested did not differ between *L. caerulea* and *L. dahlii*. 

60
The estimated DE of individuals that were collected from the field ranged from 80-96% for *C. australis*, 70-74% for *L. caerulea* and 87-92% for *L. dahlii*. Digestive efficiency did not differ between seasons for all three species (Table 3.2), although the DE of *C. australis* during the wet season was not compared to dry season measurements because they were aestivating (Table 3.2). The estimated DE of *C. australis* and *L. dahlii* was approximately 18% higher than that of *L. caerulea* during the wet season (P < 0.001), but DE did not differ between *C. australis* and *L. dahlii*. 
Table 3.1. Digestibility of the estimated dry mass of crickets (19.3 kJ g−1) ingested by *Cyclorana australis*, *Litoria caerulea* and *Litoria dahlii* at four experimental temperatures, and ingested in the wet and dry seasons at 25°C. Values represent least squares means (ANCOVA) ± standard deviation; n = sample size. Digestive efficiency (%) was calculated using the equation: DE = ((I – F) / I) X 100. The P-value is the probability of a difference among temperature and season means. Italic P-values are significant at the 0.05 level, and superscript letters indicate temperature groups with similar means (Bonferroni Dunn post-hoc t-test). * denotes values taken following 90 days of dormancy for *C. australis*.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>15</th>
<th>20</th>
<th>25 Wet</th>
<th>25 Dry</th>
<th>30</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cyclorana australis</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Frog mass (g)</td>
<td>47.7 ± 10.8</td>
<td>47.8 ± 7.6</td>
<td>45.0 ± 7.6</td>
<td>*40.6 ± 13.3</td>
<td>44.2 ± 7.3</td>
<td>0.571</td>
</tr>
<tr>
<td>Mass ingested (dry; g)</td>
<td>1.70 ± 0.11</td>
<td>1.35 ± 0.21</td>
<td>1.54 ± 0.21</td>
<td>*1.01 ± 0.15</td>
<td>1.90 ± 0.21</td>
<td>0.005</td>
</tr>
<tr>
<td>Energy ingested (J g−1)</td>
<td>32724 ± 6632b</td>
<td>25920 ± 7533b</td>
<td>29642 ± 7602b</td>
<td>*19389 ± 7600a</td>
<td>36592 ± 8633b</td>
<td>0.005</td>
</tr>
<tr>
<td>Feces egested (dry; g)</td>
<td>0.35 ± 0.03a</td>
<td>0.33 ± 0.02a</td>
<td>0.35 ± 0.06a</td>
<td>*0.09 ± 0.05a</td>
<td>0.36 ± 1.0b</td>
<td>0.003</td>
</tr>
<tr>
<td>Energy egested (J g−1)</td>
<td>8421 ± 3500b</td>
<td>8181 ± 3433b</td>
<td>7825 ± 3387b</td>
<td>*1143 ± 3784a</td>
<td>7984 ± 4019a</td>
<td>0.003</td>
</tr>
<tr>
<td>Digestive efficiency (%)</td>
<td>71.2 ± 14.8a</td>
<td>67.0 ± 14.5a</td>
<td>73.1 ± 14.3a</td>
<td>*89.3 ± 10.9a</td>
<td>66.8 ± 16.0a</td>
<td>0.006</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td><strong>Litoria caerulea</strong></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Frog mass (g)</td>
<td>41.8 ± 13.1</td>
<td>39.4 ± 14.6</td>
<td>38.8 ± 14.9</td>
<td>52.8 ± 16.5</td>
<td>39.1 ± 8.4</td>
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<tr>
<td>Mass ingested (dry; g)</td>
<td>1.61 ± 0.6b</td>
<td>1.57 ± 0.5ab</td>
<td>1.63 ± 0.6b</td>
<td>0.66 ± 0.7a</td>
<td>1.97 ± 0.3b</td>
<td>0.008</td>
</tr>
<tr>
<td>Energy ingested (J g−1)</td>
<td>31036 ± 11848b</td>
<td>30327 ± 11903ab</td>
<td>31442 ± 11932b</td>
<td>13291 ± 5430b</td>
<td>37878 ± 11919b</td>
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<td>Feces egested (dry; g)</td>
<td>0.30 ± 0.10ab</td>
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<td>0.24 ± 0.18ab</td>
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</tr>
<tr>
<td>Energy egested (J g−1)</td>
<td>8926 ± 2989b</td>
<td>5300 ± 2981b</td>
<td>9630 ± 2236b</td>
<td>8654 ± 3483a</td>
<td>5275 ± 2583ab</td>
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</tr>
<tr>
<td>Digestive efficiency (%)</td>
<td>72.6 ± 8.1ab</td>
<td>82.2 ± 6.8ab</td>
<td>73.3 ± 6.7ab</td>
<td>64.8 ± 9.5a</td>
<td>83.0 ± 7.7b</td>
<td>0.016</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td><strong>Litoria dahlii</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frog mass (g)</td>
<td>14.7 ± 6.1</td>
<td>14.6 ± 6.3</td>
<td>14.8 ± 6.0</td>
<td>24.7 ± 7.6</td>
<td>18.5 ± 7.1</td>
<td>0.093</td>
</tr>
<tr>
<td>Mass ingested (dry; g)</td>
<td>0.69 ± 0.14b</td>
<td>0.86 ± 0.3ab</td>
<td>1.15 ± 0.3b</td>
<td>0.48 ± 0.4a</td>
<td>0.98 ± 0.2b</td>
<td>0.008</td>
</tr>
<tr>
<td>Energy ingested (J g−1)</td>
<td>11338 ± 4659ab</td>
<td>14512 ± 4659b</td>
<td>20210 ± 4651b</td>
<td>9324 ± 6971ab</td>
<td>16425 ± 4781b</td>
<td>0.008</td>
</tr>
<tr>
<td>Feces egested (dry; g)</td>
<td>0.15 ± 0.04</td>
<td>0.03 ± 0.04ab</td>
<td>0.04 ± 0.04a</td>
<td>0.24 ± 0.06</td>
<td>0.04 ± 0.06ab</td>
<td>0.026</td>
</tr>
<tr>
<td>Energy egested (J g−1)</td>
<td>4346 ± 1377b</td>
<td>946 ± 862ab</td>
<td>888 ± 971ab</td>
<td>5514 ± 1248b</td>
<td>1009 ± 1218ab</td>
<td>0.028</td>
</tr>
<tr>
<td>Digestive efficiency (%)</td>
<td>70.9 ± 8.2</td>
<td>93.8 ± 7.9a</td>
<td>93.4 ± 9.5a</td>
<td>58.5 ± 9.3</td>
<td>93.7 ± 8.2a</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>4</td>
<td></td>
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</tr>
</tbody>
</table>
Table 3.2. Digestibility of the estimated dry mass of food ingested in the field, taken from faecal measurements, in the wet and dry seasons for *Cyclorana australis*, *Litoria caerulea*, and *Litoria dahlii*. Values represent least squares means (ANCOVA) ± standard deviation; n = sample size; est. = estimated; † = assumed (because of the seasonal dormancy of *C. australis*). DE = digestive efficiency (%), calculated using the equation: DE = ((I - F) / I) X 100. The *P*-value is the probability of a difference between season means.

<table>
<thead>
<tr>
<th></th>
<th>Cyclorana australis</th>
<th>Litoria caerulea</th>
<th>Litoria dahlii</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wet</td>
<td>Dry</td>
<td>Wet</td>
</tr>
<tr>
<td>Frog mass (g)</td>
<td>40.4 ± 10.0</td>
<td>-</td>
<td>47.1 ± 23.2</td>
</tr>
<tr>
<td>Mass ingested (est.; g)</td>
<td>2.41 ± 1.1</td>
<td>0†</td>
<td>0.8 ± 0.7</td>
</tr>
<tr>
<td>Energy ingested (est.; Jg⁻¹)</td>
<td>30146 ± 13712</td>
<td>0†</td>
<td>10333 ± 7933</td>
</tr>
<tr>
<td>Feces egested (dry; g)</td>
<td>0.33 ± 0.4</td>
<td>0†</td>
<td>0.22 ± 0.1</td>
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<tr>
<td>Energy egested (Jg⁻¹)</td>
<td>3659 ± 4174</td>
<td>0†</td>
<td>2901 ± 592</td>
</tr>
<tr>
<td>Digestive efficiency (est.; %)</td>
<td>89.8 ± 6.9</td>
<td>-</td>
<td>72.4 ± 3.3</td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>-</td>
<td>16</td>
</tr>
</tbody>
</table>
3.5 Discussion

Although temperature did not affect the DE of *C. australis*, PT was significantly longer at 15 and 20°C than at 25 and 30°C. PT may have adjusted to match enzyme activity and nutrient absorption, dependent on temperature (Forte 1996). The temperature independence of DE functions to maximize the assimilation of energy from ingested meals regardless of whether or not animals maintain preferred body temperatures (McConnachie and Alexander 2004).

Of the two active species, temperature affected the DE of *L. dahlii*, but not *L. caerulea*. *Litoria dahlii* egested significantly more energy at 15°C, even though PT significantly decreased at this temperature. However, the PT of *L. dahlii* at 15°C was significantly less than that of *C. australis* and *L. caerulea*. It is possible that the enzyme activity of *L. dahlii* during digestion was limited at 15°C, and although gastrointestinal motility decreased, PT did not adjust enough to match the reduction in enzyme activity; thus, DE was reduced at this temperature. It may also be possible that the nature of enzymes varies dependent on the ecological differences between species of frogs (Harwood 1979).

The PT of *C. australis* following three months of dormancy was five times longer than during the wet season, which is similar to some other seasonally dormant species (Gossling et al. 1980, Cramp and Franklin 2003), and DE was significantly greater. This is in contrast to the DE of similar species. The DE of the Australian Green Striped Burrowing Frog, *C. alboguttata*, did not differ between active and dormant frogs (Cramp and Franklin 2003). Nonetheless, the higher DE of *C. australis* suggests that enzyme activity is more efficient following dormancy than during the wet season and promotes greater nutrient absorption, which is most likely the consequence of a long PT.

Season had an effect on the digestive physiology of *L. dahlii*, but no influence on that of *L. caerulea*. DE was significantly greater during the wet season than during the dry season for *L. dahlii*, which is contrary to *C. australis*, and PT was unaffected by season.
in both *L. caerulea* and *L. dahlii*. The changes in the digestive physiology for *L. dahlii* suggest that, similar to other studies, the gastrointestinal motility and the activity of enzymes are more efficient and promote greater nutrient absorption during the wet season than during the dry season (Harwood 1979, McConnachie and Alexander 2004).

The effect of season on digestive physiology may be associated with the condition of the gastrointestinal tract, resulting from fasting rather than the effect of temperature on enzymes, gastrointestinal motility and absorption, although these factors may be secondarily involved (Jackson and Perry 2000, Starck and Wimmer 2005). The long PT of *C. australis* following dormancy indicates that considerable down-regulation of the gastrointestinal tract resulted from fasting. *Litoria caerulea* and *L. dahlii* feed less frequently during seasonally adverse conditions, which is expected to promote moderate down-regulation of the gastrointestinal tract (Secor 2001, 2005). For *L. dahlii*, PT may not have offset the moderate down-regulation of the gastrointestinal tract, and subsequently DE during the dry season was less than that during the wet season. Future research on the gastrointestinal histology and morphology of frogs in response to seasonal change would further illuminate seasonal gastrointestinal regulation in both active and seasonally dormant species.

Field measurements of egested energy provided a crude estimate of the seasonal food intake and digestive state of individuals (Reeder 1964). I expected *L. caerulea* and *L. dahlii* to consume significantly more food during the more active breeding period than during dry season conditions (Johnson and Christiansen 1976), but I found that the estimated food intake of *L. caerulea* and *L. dahlii* did not differ significantly between seasons. Interestingly, however, *C. australis* ingested about three and five times more than *L. caerulea* and *L. dahlii*, respectively, and the estimated food intake during the wet season did not differ between *L. caerulea* and *L. dahlii*. The ability of *C. australis* to ingest large amounts of food during brief active period is similar to other seasonally dormant species (Larsen 1992).
Chapter Three: Digestive physiology

Depending on the group of animals, DE generally varies between 55-95% and is influenced by the type and amount of food ingested and, in some cases, temperature (Harlow et al. 1976, Jaeger and Barnard 1981, Larsen 1992). DE ranges between 80-90% for many lizards and snakes, and between 70-90% for species of frogs (Smith 1976). In my study, the DE of frogs at temperatures between 15 and 30°C ranged between 67-73% for *C. australis*, 73-83% for *L. caerulea* and 71-94% for *L. dahlii*. The DE of *C. australis* was lower than the two active species at 20, 25 and 30°C, but at 15°C, there was no difference among species. Season affected the DE of *C. australis* and *L. dahlii*, but not that of *L. caerulea*. Seasonal DE of frogs ranged between 73-89% for *C. australis*, 65-73% for *L. caerulea* and 59-93% for *L. dahlii*. Furthermore, the estimated DE of frogs collected directly from the field (*C. australis* averaged 90%, *L. caerulea* ranged between 72-74% and *L. dahlii* ranged between 71-89%), were more similar to seasonal measurements at 25°C than to other measured temperatures in the laboratory.

In general, the digestive function of *C. australis* was more variable than that of *L. caerulea* and *L. dahlii*. Although seasonal responses are more discernible for seasonally dormant species, subtle adjustments by species that remain active throughout the year may be very important to their annual energy budgets because they experience the more extreme conditions of above-ground habitats (Christian et al. 1999). Although the amount of food consumed by the two active species during the wet season is considerably less than that of the seasonally dormant frog, their annual energy expenditure may be similar.
3.6 Bibliography


Chapter Four: Gastrointestinal Flexibility

This chapter has been submitted as:

Litoria caerulea

Field Volunteers

Litoria dahlii
4.1 Abstract

The gastrointestinal flexibility of the three species of frog was examined in response to season (wet and dry) in free-ranging frogs, and in response to fasting (60-90 days) under laboratory conditions. I hypothesised that the phenotypic flexibility of the digestive system would be greater for seasonally dormant frogs than for frogs that are active year-round. The effects of season and fasting were shown using measurements of gastrointestinal morphology and histology. Gastrointestinal mass was significantly greater in the wet season free-ranging frogs than in frogs that fasted in the laboratory. *Cyclorana australis* showed the greatest gastrointestinal flexibility in response to fasting (2.3-fold difference), termed ‘extreme’; *L. caerulea* showed the least flexibility (1.6-fold difference), termed ‘moderate’; and *L. dahlii* showed neither extreme nor moderate flexibility (2.2-fold difference), termed ‘intermediate’. The gastrointestinal mass of free-ranging *L. caerulea* in the wet season was 1.5-fold greater than in the dry season, but did not differ between seasons for free-ranging *L. dahlii*. Changes in gastrointestinal mass were attributed to changes in the villi of the innermost mucosal layer. In addition to this, enterocyte volume significantly differed between groups. *Cyclorana australis* showed the greatest change in enterocyte volume (6.4-fold difference), *L. caerulea* showed a moderate change (2.1-fold difference) and *L. dahlii* was intermediate (5.8-fold difference). The mass of only the kidneys and spleen was less after fasting in the dry season than those in the wet season for *C. australis*. For *L. caerulea* and *L. dahlii*, the masses of all other measured abdominal organs and fat bodies did not differ among groups. Although most abdominal organs generally showed no response to either season or fasting, a reduction in gastrointestinal mass when fasting may contribute significantly to the energy conservation of frogs that spend long periods dormant, but does not appear to be a trait used by frogs that feed more frequently.
4.2 Introduction

Many species of terrestrial frogs living in xeric and semi-mesic environments aestivate between six months to three years and reduce daily energy expenditure by 60–80% (van Beurden, 1984, Flanigan et al. 1991, Flanigan and Guppy 1997, Withers and Thompson 2000). Reducing daily energy expenditure increases the duration that frogs can survive adverse environmental conditions (Secor and Diamond 1996). Some of this saved energy may be attributable to a reduction in maintenance costs resulting from the down-regulation of the gastrointestinal tract (GIT) in response to long periods of fasting (Secor et al. 1994, Starck 2003, Secor 2005, Starck 2005). For example, the South American horned frog, *Ceratophrys ornata*, and the African bullfrog, *Pyxicephalus adspersus*, are dormant for up to eight months and show an 84% reduction in intestinal performance (Secor and Diamond 1996, Secor 2005). This reduction in intestinal performance is the result of morphological changes within the small intestine, which is made up of highly metabolically active tissue (Starck and Wimmer 2005).

Not all species of frogs living under the same environmental and climatic conditions spend long periods dormant. Some species are active year-round, but may periodically fast for short durations when seasonal conditions are unfavourable and feed opportunistically only when climatic conditions permit (McClanahan et al. 1978, Christian and Parry 1997, Warburg et al. 2000). Little is known about the GIT flexibility of species that do not undergo long periods of fasting, but some studies show that GIT flexibility in seasonally dormant species of frogs is greater than in species that are active year-round (Naya and Bozinovic 2004, Naya et al. 2005). For example, The American bullfrog, *Rana catesbeiana*, and the smoky jungle frog, *Leptodactylus pentadactylus*, show only a moderate 44% and 23% reduction, respectively, in intestinal performance during periods of fasting compared to the more noticeable reduction in seasonally dormant species (Secor and Diamond 1996).

The up and down-regulation of the GIT are primarily related to changes within the villi of the innermost mucosal layer of the small intestine (Secor et al. 1994, Secor and

Patterns of energy storage in other abdominal organs and fat bodies are also phenotypically flexible and reflect the frequency of feeding (Holzapfel 1937, Smith 1950, Mizell 1965, Dykstra and Karasov 1992). For example, in some infrequently feeding snakes, the mass of their lungs, heart, liver, gallbladder and kidneys is considerably less when fasted than when fed (Secor and Diamond 1995, 1998). In amphibians, the toad, *Bufo bufo* and the bullfrog, *Rana arvalis*, lose fat body mass over four months of winter dormancy and spring breeding, 60 and 45% respectively (Mazur 1967). Interestingly, however, changes to abdominal organ and fat body mass are evident only in seasonally dormant species or infrequent feeders, but have not been found in frequent feeders during fasts (Selcer 1987, Secor et al. 2000).

The broad aim of this study was to examine the GIT flexibility of *C. australis*, *L. caerulea* and *L. dahlia* in response to season and fasting. *Cyclorana australis*, fasts for six to seven months during seasonal estivation (Chapter Two). In contrast, *L. caerulea* and *L. dahlia* remain active year-round, although they may fast for short periods during adverse dry conditions (Chapter Two). I compared the GIT flexibility of species through morphological measurements of the GIT, other abdominal organs and fat bodies. I also compared the histological measurements of the small intestine for (1) all three species during the wet season (when it is expected that frogs feed frequently and the GIT is up-regulated); (2) *L. caerulea* and *L. dahlia* during the dry season (when it is expected that frogs feed less frequently and the GIT has down-regulated); (3) all three species following a period of fasting under laboratory conditions, to test the hypothesis that
seasonally dormant species down-regulate organs, fat bodies and GIT during fasting to a greater extent than species that are active year-round.
Chapter Four: Gastrointestinal Flexibility

4.3 Materials and Methods

4.3.1 Experimental protocol

*Cyclorana australis* (38.63 g ± 12.0 SD), *L. caerulea* (35.29 g ± 12.5 SD) and *L. dahlii* (16.72 g ± 6.4 SD), were caught by hand around Darwin in the wet (December-February), late-wet (March-May) and dry (June-August) seasons, and taken back to a laboratory at Charles Darwin University. In the laboratory, frogs were housed individually inside perforated 5.7 L rectangular plastic containers (dimensions: 31 x 10 x 21 cm), which were placed within a temperature control cabinet (Sanyo Incubator, Japan) regulated at 25ºC (± 1.0 SD). Water was provided *ad libitum* to frogs collected during the wet and dry seasons prior to morphological examination. However, for frogs collected in the late-wet season, food and water were withheld to mimic dry season conditions and induce dormancy. *Cyclorana australis* formed a cocoon after about 20 days of fasting and remained dormant for 90 days, and *L. caerulea* and *L. dahlii* remained dormant in the laboratory for 60 days.

Measurements of abdominal organs and gastrointestinal histology were compared among the groups: wet, dry and fasting. Wet and dry season measurements were taken from free-ranging frogs in the field, and fasting measurements where taken from frogs undergoing dormancy in laboratory conditions. *Cyclorana australis* are difficult to find in the dry season because they aestivate underground. Therefore, frogs were collected in the late-wet season and induced into dormancy. Laboratory measurements of these dormant frogs are representative of dry season conditions.

4.3.2 Morphology

Morphological examination of frogs was conducted early on the day following its capture. Frogs were cooled at 5ºC for 15 minutes before being double-pithed through the
spinal column, using a fine gauge steel needle. Frogs were weighed using a Shimadzu BX420H (Philippines) electronic balance (0.001 g). Snout-urostyle length (SUL) was measured using Mitutoya (0.01 mm) digital calipers (Japan). Abdominal organs (heart, kidneys, gall bladder, liver and spleen), fat bodies and GIT were immediately dissected and respective masses recorded. During the dissection, amphibian ringers solution was used to flood the abdominal cavity and prevent sections from drying out. Sections were blotted dry before mass was recorded. The GIT was dissected at the junction between the esophagus and the stomach, and distally between the intestine and rectum. Mesenteries were carefully removed. The GIT was immersed in ringer solution and set aside for about one minute to allow the GIT to relax following procedures and before total length was measured. Any digesta inside the GIT were flushed out using a syringe filled with ringers solution and the GIT was reweighed. The GIT was excised at the pyloric sphincter and the length and mass of the stomach and the intestine were subsequently measured separately.

Following gross morphological determinants, 0.5 cm of the proximal end of the small intestine was discarded and the next one-centimeter sleeve was excised and retained in FAA fixative (formalin:alcohol:acetic acid, buffered with ringers solution), in preparation for histological analyses.

4.3.3 Histology

The one-centimeter sleeves of small intestine were processed using a Reichert Lynx el Microscopy Tissue Processor. Then the sleeves were soaked, vacuumed and embedded in wax. Up to six tissue samples were sliced (4 μm) from each sleeve, set on slides and stained, using hematoxylin and eosin, ready for histology measurements.

Images were taken from prepared slides using an Olympus BH-2 light microscope (Olympus Optical Co. LTD, Japan) with a mounted Olympus DP11-P camera that had an Olympus U-PMTVC lens. Images were captured digitally and subsequently
downloaded into the program Optimas 6.51 (Media Cybernetics image analyses program) that was calibrated to match camera optics and configured to take measurements using line morphometry. Measurements were taken from images following the methods of Secor and Diamond (1999), Secor et al. (2000) and Starck and Beese (2001, 2002). I measured 1) the width of the muscularis externa, which includes the circular and longitudinal muscle layers (40x mag.; Fig. 4.1A); 2) the circumference ratio of the mucosal and serosal layers (40x mag.; Fig. 4.1A); 3) the height of five villi, taken at the innermost edge of the circular smooth muscle layer to the outermost edge of the mucosal layer (40x mag.; Fig. 4.1A); and 4) the height (h) and width (d) of five enterocytes (1000x mag.; Fig. 4.1B) to calculate enterocyte volume using the equation for a cylinder ($\pi(d/2)^2 h$).

**Figure 4.1.** Histological sections of the GIT tract of *Litoria dahlii*. (A) Cross section of the small intestine showing (1) the serosa; (2) the muscularis externa (including circular and longitudinal muscle layers); and (3) a villi (including mucosal layer) (40x magnification). (B) A section of villi within the small intestine showing (1) a single enterocyte (1000x magnification).
4.3.4 Statistical analyses

All data were log-transformed, except for circumference ratio data (mucosal:serosal layers), which was transformed by taking the reciprocal (Zar 1996). The data were based on independent samples within each treatment (i.e. different individuals tested in each treatment). Analysis of variance (ANOVA) was used to investigate differences among body mass and SUL length groups (wet, dry and fasting) for each species. Analysis of covariance (ANCOVA) was used to investigate differences among groups of GIT morphological mass and length variables using body mass and SUL as the covariate, respectively, and groups of intestinal histological variables using body mass as the covariate. Body mass as a covariate when each measured variable was compared among species in ANCOVA. Differences between means were considered statistically different when $P < 0.05$. Pair-wise Bonferroni Dunn $t$-tests (at the 0.05 significance level) were used for post-hoc determination of differences among means following ANOVA and ANCOVA analyses. The least square mean (untransformed, but corrected for body mass or SUL) values are used in tables. Linear regressions were conducted for each species, using pooled seasonal and fasting data, to examine the relationship (represented by $t$-values and $P$-values significant at $\alpha = 0.05$) between changes in intestinal mass and any measured histological variables. All analyses were conducted using the program SuperAnova (Abacus Concepts Inc.), except for linear regressions, for which the program StatView (SAS Institute Inc) was used.
4.4 Results

4.4.1 Morphology

Wet season GIT mass was significantly greater than that following fasting for all three species (2.3-fold greater for *C. australis*, 1.6-fold for *L. caerulea* and 2.2-fold for *L. dahlii*; Table 4.1). Changes in stomach and intestinal masses were attributed to the differences between the wet season and fasting GIT mass of *C. australis* and *L. dahlii*, but not for *L. caerulea*, although the intestinal mass of *L. caerulea* in the wet differed from that in the dry season. Dry season GIT mass was greater than after fasting for *L. dahlii* (1.9-fold), but not for *L. caerulea* (Table 4.1). For *L. dahlii*, changes to stomach mass contributed to the difference between dry season and fasting GIT masses, but intestinal mass did not differ between those groups for this species. The length of the GIT, stomach and intestine of frogs did not differ among groups (Table 4.1).

Of the other abdominal organs and fat bodies, none differed among groups for both *L. caerulea* and *L. dahlii*, but the kidney and spleen masses of *C. australis* were greater in the wet season than after fasting, 1.9 and 2.7-fold respectively (Table 4.1).

Heart, liver and fat body masses generally differed among species, but the masses of kidneys, gall bladder and spleen did not. In the wet season, the heart mass of *C. australis* was larger than that of *L. caerulea* and *L. dahlii*, 43% (*P < 0.001*) and 68% (*P < 0.001*) respectively, and the heart mass of *L. caerulea* was 15% larger than that of *L. dahlii* (*P < 0.001*). However, in the dry season and after fasting, heart mass did not differ among species. The liver mass of *L. dahlii* was larger than that of *C. australis* and *L. caerulea* in the wet season, 52% (*P < 0.001*) and 55% (*P < 0.001*) respectively, and 8% larger than that of *L. caerulea* during the dry (*P = 0.002*), but after fasting, liver mass did not differ among species. The fat body mass of *L. caerulea* was 44% larger than that of *L. dahlii* during the dry season (*P < 0.001*), but fat body mass did not differ among species in the
wet or after fasting. GIT, stomach and intestinal masses and lengths did not differ among species in the wet and dry seasons or after fasting.

### 4.4.2 Histology

For *C. australis*, some measured histological variables were significantly greater in the wet season than after fasting (2.3-fold for the mucosal depth, 3.1-fold for mucosal:serosal (m:s) ratio, 3.2-fold for villi height and 6.5-fold for enterocyte volume), but the muscle width of the muscularis externa did not differ between wet season and fasting frogs (Table 4.2).

For *L. caerulea*, the muscle width, mucosal depth, m:s ratio and villi height of the GIT did not differ among groups. However, wet season enterocyte volume was 3.4-fold that in the dry season, but enterocyte volume did not differ between dry season and fasting groups for this species (Table 4.2).

For *L. dahlii*, the follow histological variables were greater in the wet season than after fasting: 1.5-fold for muscle width, 1.7-fold for the mucosal depth, 1.9-fold for villi height and 5.8-fold for enterocyte volume. In addition to this, the following histological variables were greater in the dry season than after fasting: 1.3-fold for the mucosal depth, 2.2-fold of villi height and 5.5-fold of enterocyte volume, but dry season muscle width did not differ from that after fasting, and the m:s ratio did not differ among groups (Table 4.2). The wet season mucosal depth of *L. dahlii* was 1.3-fold that in the dry season, but muscle width, villi height and enterocyte volume did not differ between the wet and dry seasons.

In the wet season, the mucosal depth of *C. australis* did not differ from *L. caerulea* and *L. dahlii*, but the mucosal depth of *L. caerulea* was 30% smaller than that of *L. dahlii* (*P* = 0.047). The enterocyte volume of *C. australis* during the wet season was larger than that of *L. caerulea* and *L. dahlii*, 43 (*P* = 0.008) and 96% (*P* < 0.001) respectively, and
the enterocyte volume of *L. caerulea* was 93% larger than that of *L. dahlii* (*P* < 0.001). Muscle width, m:s ratio and villi height did not differ among species during the wet season. In the dry season, the mucosal depth of *L. caerulea* was 27% smaller than that of *L. dahlii* (*P* = 0.013), but muscle width, m:s ratio, villi height and enterocyte volume did not differ between these two species. After fasting, the mucosal depth for *C. australis* was 58% smaller than for *L. caerulea* and *L. dahlii* (*P* = 0.002), but mucosal depth did not differ between *L. caerulea* and *L. dahlii*. The villi height of *C. australis* was 54% less than that of *L. caerulea* (*P* = 0.001), but did not differ from *L. dahlii*, and the villi height for *L. dahlii* was 36% less than for *L. caerulea* (*P* = 0.001). Furthermore, after fasting, the enterocyte volume for *L. dahlii* was less than for *C. australis* and *L. caerulea*, 66% (*P* = 0.001) and 85% less (*P* < 0.001) respectively, but enterocyte volume did not differ between *C. australis* and *L. caerulea*. When fasting, muscle width and m:s ratio did not differ among species.

### 4.4.3 Relationship between intestinal mass and histology

For *C. australis*, changes in intestinal mass were significantly related to villi height (*t* = 5.108; *P* < 0.001), mucosal depth (*t* = 4.085; *P* = 0.004), and the m:s ratio (*t* = 5.901; *P* = 0.004). Enterocyte volume and muscle width were not related to changes in intestinal mass. For *L. caerulea* and *L. dahlii*, changes in intestinal mass were significantly related to villi height (*t* = 2.219; *P* = 0.039 and *t* = 3.897; *P* = 0.001, respectively). Muscle width, mucosal depth, m:s ratio and enterocyte volume did not relate to any changes in intestinal mass for *L. caerulea*. However, for *L. dahlii*, changes were also related to mucosal depth (*t* = 2.165; *P* = 0.044) and enterocyte volume (*t* = 3.238; *P* = 0.005).
Table 4.1 Morphological changes in mass (g) and length (mm) of measured abdominal organs and fat bodies in response to season and fasting in *Cyclorana australis*, *Litoria caerulea* and *Litoria dahlii*. Values represent least squares means (untransformed) of respective body mass and SUL length corrected data; ± standard deviation; n = sample size. *P*-values are the probability of differences among groups (wet, dry and fasted) using ANOVA (body mass and SUL) and ANCOVA (all other measured variables). Italic *P*-values are significant at the 5% significance level; superscript letters indicate similarities among groups using Bonferroni Dunn post hoc *t*-tests. For *C. australis*, fasting data is also representative of dry season data. SUL = snout urostyle length; GIT = gastrointestinal tract.
Table 4.2 Histological changes in the size (μm) of the small intestine in response to season and fasting for *Cyclorana australis*, *Litoria caerulea* and *Litoria dahlii*. Values represent the least squares means (untransformed) of data that are corrected for body mass; ± standard deviation; n = sample size. *P*-values are the probability of differences among groups (wet, dry and fasted) using ANOVA (body mass) and ANCOVA (all other measured variables). Italic *P*-values are significant at the 5% significance level; superscript letters indicate similarities among groups using Bonferroni Dunn post hoc *t*-tests. For *C. australis*, fasting data is also representative of dry season data. vol. = volume.

<table>
<thead>
<tr>
<th></th>
<th><em>Cyclorana australis</em></th>
<th><em>Litoria caerulea</em></th>
<th><em>Litoria dahlii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wet n = 4</td>
<td>Fasting n = 4</td>
<td>Wet n = 6</td>
</tr>
<tr>
<td><strong>Body mass (g)</strong></td>
<td>39.2 ± 8.7</td>
<td>41.3 ± 9.1</td>
<td>31.0 ± 7.8</td>
</tr>
<tr>
<td><strong>Muscle width (μm)</strong></td>
<td>99.9 ± 62.1</td>
<td>55.6 ± 21.1</td>
<td>89.1 ± 27.9</td>
</tr>
<tr>
<td><strong>Mucosal depth (μm)</strong></td>
<td>38.3 ± 5.6b</td>
<td>16.3 ± 2.4a</td>
<td>35.9 ± 4.7</td>
</tr>
<tr>
<td><strong>Ratio (m:s)</strong></td>
<td>7.5 ± 1.0b</td>
<td>2.4 ± 0.2a</td>
<td>4.7 ± 3.3</td>
</tr>
<tr>
<td><strong>Villi height (μm)</strong></td>
<td>458 ± 120b</td>
<td>145 ± 17a</td>
<td>487 ± 188</td>
</tr>
<tr>
<td><strong>Enterocyte vol. (μm³)</strong></td>
<td>43.8 ± 2.0b</td>
<td>6.76 ± 0.9a</td>
<td>26.9 ± 2.6b</td>
</tr>
</tbody>
</table>
4.5 Discussion

Changes in the abdominal organs of frogs examined in this study show that GIT flexibility is similar to that found in other studies on snakes and frogs. Seasonally dormant or infrequent feeders show greater GIT flexibility than species that are active year-round and feed frequently (Mizell 1965, Starck 1999, Piersma and Drent 2003, Naya et al. 2005). GIT mass showed the greatest flexibility in response to periods of fasting, specifically changes in intestinal mass. The change in intestinal mass could be explained by its histology.

For *C. australis*, wet season GIT mass was 2.3-fold greater than after fasting. The GIT flexibility of *C. australis* was not as marked as other seasonally dormant or infrequent feeders, such as the 3-4.5 fold increase in GIT mass of *Cyclorana ornata*, and *Pyxicephalus adspersus* following dormancy (Secor and Diamond 1996, Secor 2005). However, the GIT mass of those species were measured as the difference between the postprandial response and long periods of fasting. In my study, frogs were randomly collected from the field during the wet season and the digestive states of individuals were not known, and therefore, the postprandial response of frogs following prolonged fasting was not measured.

For *L. caerulea*, GIT mass in the wet season was 1.6-fold that in the dry season, but dry season and fasting GIT mass did not differ. These observations are similar to studies on other species of frog that are active year-round (Secor and Diamond 1996, Secor 2005). Frequently feeding snakes are described as showing only ‘moderate’ GIT flexibility to fasting compared to the ‘extreme’ flexibility of infrequently feeding snakes (Starck 1999, Wang et al. 2001). Similarly, I describe the GIT flexibility of *L. caerulea* to fasting as moderate when compared to that of *C. australis*. Since dry season measurements of frogs from the field did not differ from those measured during fasting in the laboratory, I assumed that *L. caerulea* moderately down-regulate GIT in response to adverse seasonal field conditions when this species significantly reduces activity (Chapter Two). The significance of only moderately down-regulating the GIT is that it is
less expensive to up-regulate following feeding than frogs that show considerable down-regulation, as shown in studies on snakes (Skoczylas 1978, Secor 2001, Starck and Beese 2001).

Interestingly, the GIT flexibility of \textit{L. dahliai} was intermediate to that of \textit{C. australis} and \textit{L. caerulea} (wet season GIT was 2.2-fold that after fasting and 1.9-fold that in the dry season). Since the response of \textit{C. australis} was not as ‘extreme’ as other seasonally dormant species, I suggest that \textit{L. dahliai} shows a physiological response in GIT flexibility that is ‘intermediate’ to \textit{L. caerulea} and \textit{C. australis}, a description applied to snakes that show neither extreme nor moderate GIT flexibility (Secor and Diamond 1996, Secor 2005). Unlike \textit{L. caerulea} under field conditions, the GIT mass of \textit{L. dahliai} did not differ between the wet and dry seasons. This lack of response may be related to the timing of field sampling. Samples were collected in the dry when the aquatic habits of this species persist (Chapter Two). Collecting \textit{L. dahliai} in the late-dry season, when its water habitats dry out completely and frogs are possibly physiologically challenged (in respect to water), may have produced different results.

The changes in the GIT mass of frogs were attributed to some measured histology variables, primarily in the villi of the innermost mucosal layer, similar to the findings of studies on some species of snakes and other frogs (Secor and Diamond 1998, Jackson and Perry 2000, Starck and Beese 2001). Other studies suggest that the increase in GIT mass is mainly attributed to increased enterocyte volume, as well as an increased blood and lymphatic loading in the vessels of the connective tissue (Secor et al. 1994, Starck and Wimmer 2005). In my study, enterocyte volume was related to changes in intestinal mass only for \textit{L. dahliai}. However, all species showed significant differences in enterocyte volume when compared among groups. Similar to gross morphological measurements, \textit{C. australis} showed greater GIT flexibility, but in the microscopic structure of the small intestine, than the moderate flexibility of \textit{L. caerulea} and the intermediate flexibility of \textit{L. dahliai} (6.4-fold for \textit{C. australis}, 2.1-fold for \textit{L. caerulea}, and 5.8-fold for \textit{L. dahliai}).
Only the heart and kidneys of *C. australis* decreased mass during fasting. These results follow those of other studies; however, I expected that other abdominal organs would show greater flexibility than found in my study, similar to finding for other species of seasonally dormant frogs or infrequently feeding snakes (Selcer 1987, Secor et al. 2000). Interestingly, *C. australis* fat bodies were not affected during fasting. This result was unexpected considering that about half the maintenance energy for dormancy comes from fat bodies in some xeric dwelling species of frogs (Seymour 1973b). For example, the American desert dwelling spadefoot toads, *Scaphiopus couchii* and *S. hammondii* could survive two or more years of dormancy with the presence of large fat bodies (Seymour 1973a, Seymour and Lee 1974, Heatwole 1984). In my study, fasting duration (90 days) may not have been long enough to produce a measurable effect on either fat bodies or the other measured organs, other than the heart and kidneys. Nonetheless, fasting duration was sufficient to show significant down-regulation of the GIT.

The results of this study reveal that the three species show varying responses to season and fasting. As predicted, the seasonally dormant frog showed greatest morphological and histological flexibility of the GIT compared to the other two species that are active year-round. The consequence of down-regulating the GIT and other abdominal organs is the saving of energy. The accrued energy savings over time may be significant and contribute to the overall energy efficiency of dormancy, but may not be as important for species that are active year-round and feed opportunistically.
Chapter Four: Gastrointestinal Flexibility

4.6 Bibliography


Chapter Five:

Seasonal Respiratory Physiology

This chapter has been submitted as:

![Cyclorana australis partly cocooned](image1.jpg)

![Set-up of Respiratory System](image2.jpg)
5.1 Abstract

The extreme seasonality of the wet-dry tropics of northern Australia provides a challenging environment for tropical dwelling frogs in which to balance their energy and water requirements. Oxygen consumption was measured to determine the respiratory physiology of the three species of frog in response to simulated dormancy (60-90 days) in the laboratory and to season (wet and dry) in the field. In addition, respiratory gas analysis was used to examine the response of species to hydration and feeding following dormancy as a measure of how quickly frogs recover. Under simulated aestivating conditions, *C. australis* reduced oxygen consumption by 67%, which is similar to desert-dwelling species, but *L. caerulea* and *L. dahlii* reduced oxygen consumption to a lesser extent, 54 and 55% respectively. Exposure to water following dormancy was sufficient to restore standard metabolic rate. However, the respiratory response to feeding following dormancy was lower than that when fed under optimal conditions, which may be related to down-regulation of the gastrointestinal tract in response to fasting. *Litoria caerulea* and *L. dahlii* showed no significant respiratory response to season in the field, but the oxygen consumption of *L. dahlii* when fed in the dry season was 66% of that in the wet season. Metabolic depression functions to reduce energy and water expenditure when conditions become unfavourable, and may be significant for seasonally dormant species that spend several months aestivating underground. Without the limitation of a cocoon, *L. caerulea* and *L. dahlii* can opportunistically exploit resources when conditions are favourable. By moderately adjusting physiology, these two species can also benefit from the reduced energy and water expenditure when conditions are unfavourable.
5.2 Introduction


Aestivation is the physiological adjustment of oxygen consumption to below standard metabolic rate (SMR) during dormant periods (see Hochachka and Guppy 1987, Blaxter 1989), and functions to conserve energy and water when climatic conditions become unfavourable. Some Australian desert-dwelling cocoon forming species, of the genera *Neobatrachus* and *Cyclorana*, depress oxygen consumption by 60-80% when aestivating (van Beurden 1984, Flanigan et al. 1991, Withers 1993, Flanigan and Guppy 1997, Withers and Thompson 2000). Such a reduction in metabolic rate would allow the Australian water holding frog *Cyclorana platycephala*, for example, to survive between 2-5 years in dormancy, given persistent drought conditions (van Beurden 1980, van Beurden 1984, Flanigan et al. 1991).

Recovery from aestivation has significant implications for balancing energy requirements, particularly for desert-dwelling frogs that have only short periods to breed, grow and lay down fat stores in anticipation of the next dormant period (Seymour and Lee 1974, Hudson and Franklin 2002). Some Australian desert-dwelling species of *Limnodynastes*, *Neobatrachus* and *Cyclorana* are known to recover quickly from aestivation and breed immediately following rain (Mayhew 1968). This ability to condense their life cycle into brief active periods must be conducive to quick recovery from aestivation.
Cocoon formation provides an effective barrier against water loss during aestivation and minimises possible effects of dehydration (Gatten 1987, Warburg 1997). As a consequence of dehydration, some species increase oxygen consumption two-fold after losing 25% of their standard body mass (Pough et al. 1983). Therefore, frogs would benefit from entering aestivation before climatic constraints have any effect on physiological function, so that the energetic benefits of depressing metabolic rate are not compromised (Guppy and Withers 1999).

The physiological and behavioural adaptations observed in desert-dwelling species may be important for species living in other strongly seasonal environments, such as the wet-dry tropics (Hochachka and Guppy 1987, Christian et al. 1999). However, Withers and Thompson (2000) found that *C. australis* do not reduce oxygen consumption by a significant amount when dormant in laboratory conditions, and suggested that cocoon forming frogs living in arid regions depress metabolic rate more than frogs living in areas with more predictable rainfall. In that study, *C. australis* lost almost 30% body mass during simulated aestivation conditions. Thus, the failure to notice significant metabolic depression in Withers and Thompson’s (2000) study may be attributed to dehydration rather than the suggested ecological differences between tropical and desert-dwelling species.

Non-cocoon forming species living in the wet-dry tropics are challenged with the same long drought-like conditions of the dry season as cocoon forming frogs, yet little is known about the physiological adaptations of this group of frogs. Studies on non-cocoon forming species show that this group only moderately depress metabolic rate when compared to the more extreme response of desert-dwelling species (Taigen and Pough 1981, Wells et al. 1995, Glass et al. 1997). For example, the non-cocoon forming and mesic dwelling South American toad, *Bufo paracnemis*, depresses metabolic rate by about 45% during aestivation (Glass et al. 1997).

The broad scope of this study was to examine the metabolic response of the three species to induced dormancy in the laboratory and to season in the field. I hypothesised that *C. australis* would show similar respiratory flexibility to desert-dwelling species, and that *L. caerulea* and *L. dahlii* would only moderately depress
metabolism when under the same conditions as *C. australis*. In addition to this, the oxygen consumption of the three species was measured during recovery from dormancy by supplying food and water, and these responses were compared with those of frogs fed and hydrated under optimal wet season conditions. I hypothesised that all three species would recover quickly from dormancy. Furthermore, I compared the oxygen consumption of 30% dehydrated and fully hydrated frogs under optimal wet season conditions with that of frogs under simulated dormancy in the laboratory to determine the effect of hydration on metabolic rate. In these cases, I hypothesised that 70% hydrated frogs would show a greater metabolic response than fully hydrated frogs.
5.3 Materials and Methods

5.3.1 Experimental protocol

Frogs were caught by hand from selected field sites around Darwin in the wet (December-February) and dry (June-August) seasons and taken back to a laboratory at Charles Darwin University. Frogs were housed individually inside perforated 5.7 L rectangular plastic containers (dimensions: 31 x 10 x 21 cm), which were placed within a temperature control cabinet (Sanyo Incubator, Japan) regulated at 25ºC (± 1.0). For three days following capture and prior to experiments, frogs were provided with water ad libitum, using wet paper towel, but food was withheld to exclude the effect of digestion on metabolic rate (Seale 1987).

Oxygen consumption was measured using an open flow respirometry system (Vleck 1987, Walsberg and Hoffman 2005, 2006). At the beginning of respiratory measurements, frogs were weighed to the nearest 0.01 g and placed individually into plastic cylindrical chambers with air tight lids as follows: 350 ml (dimensions: 6.5 x 10 cm) for *C. australis* and *L. caerulea*, and 125 ml (dimensions: 4.4 x 10.8 cm) for *L. dahlii*. An air inlet hose entered one end of the chamber and an exit hose entered the other, from which the air sample was drawn. The flow of air through each frog chamber was maintained using Gilian Low Flow Sampler (Gilian Instrument Corp., USA) pumps. The volume of air was measured using Top-Trak (Sierra Instruments, USA) mass flow meters. Flow meters were calibrated using a Gillibrator 2 (Gilian Instrument Corp., USA) bubble generator.

Three frogs were monitored concurrently inside the temperature control cabinet. Each frog chamber was sampled for one hour over the 12-hour daylight period by systematically switching between chambers, using a controller activated solenoid switch (EDD50; SMC Corporation, Japan) for all treatments except dormancy. During dormancy, chambers were sampled for only 30 minutes of each sampling day, by manually switching between each chamber. Airflow was turned off between sampling to minimise dehydration and turned on one hour prior to sampling.
Following the methods of Christian et al. (1996), the air sample was drawn from the frog chamber, through a column filled with desiccant (Drierite, USA), the air pump and flow meter. A sub-sample of air was taken for measurement after passing through a column filled with CO₂ absorbent (Dragersorb 800, Germany) and a second drying column before reaching the Applied Electrochemistry S-3A/II oxygen analyser (Ametek, Pittsburgh, PA). A MacLab (8e, ADInstruments: Australia) system recorded airflow and oxygen concentration at the rate of one data point every two seconds. The airflow averaged 37 ml min⁻¹ (± 6.2 SD) during experiments. The oxygen consumption of frogs was calculated using the equations of Withers (1977, 2001).

5.3.2 Experimental treatments

Frogs were collected late in the wet season and held in captivity until the early dry season before they were exposed to simulated dry season conditions in the laboratory to determine if an aestivation response could be induced (Withers 1995). A dark temperature controlled (25°C ± 1.0) cabinet, ventilated via two 15 cm diameter side vents, was used to simulate dry season conditions. *Cyclorana australis* individuals were randomly split into two groups to examine the effect of hydration during dormancy. One group (n = 9) was provided with water inside the respiratory chamber at the start of the experiment, using wet paper towel that absorbed about 13 ml (± 4.0 SD) of water. Water was not replenished during the experiment. The other group (n = 10) was not provided with water at either the start or during dormancy. At the end of dormancy, body mass increased by a mean of 6.5% for the group with water and decreased by a mean of 21% for the group not provided water. Both groups of frogs were held in simulated aestivation conditions for 90 days. During this period, oxygen consumption was measured on days one, three, five, seven, ten and then every ten days following until day 90.

*Litoria caerulea* and *L. dahlii* were provided with water inside the respiratory chamber, using wet paper towel at the beginning of the experiment; this was not replenished during dormancy. Both of the non-cocoon forming species were restless during measurements and consequently the experiment was terminated after 60 days.
rather than 90 days. Oxygen consumption was measured on days one, three, five, seven, ten and then every ten days following until day 60.

At the end of the experiment, body mass was recorded and then frogs were placed randomly into one of four aestivation recovery treatments: resting, hydrated, fed, and fed + hydrated. The cocoon formed by *C. australis* during dormancy was removed at the start of these treatments. Frogs placed in the resting group were neither fed nor hydrated but were placed in a dry box and exposed to artificial light for three hours before being returned to the respiratory chamber and measurements resumed. In the fed and fed + hydrated treatments, *C. australis* ingested crickets weighing 5.7% (± 2.0 SD) of their initial body mass, *L. caerulea* ingested 5.5% (± 2.1 SD), and *L. dahlii* ingested 4.7% (± 1.5 SD) of body mass before being placed back into respiratory chambers. Frogs in the hydrated and fed + hydrated treatments were placed in a shallow container with water at 1 cm depth. During hydration, frogs were weighed every five minutes until they voided their bladder (decreased mass). Frogs in these two treatments were then returned to respiratory chambers, which contained wet paper towel, and respiratory measurements resumed. Each day, after respiratory measurements were taken, paper towels were refreshed in all treatments and any faeces were removed. Respiratory measurements lasted until defecation occurred for frogs that were fed, which on average took 144 hours. Frogs in the other treatments were measured for the same duration as fed frogs.

In addition to the frogs that were used in the aestivation and recovery experiments, two groups of frogs were collected during each of the wet and dry seasons. One group was used to determine the seasonal oxygen consumption of frogs (fasted for 72 h) at 25°C (± 1.0). The oxygen consumption of this group of frogs is referred to in this study as the standard metabolic rate (SMR), which is the minimum amount of oxygen required for simple maintenance of tissue that is measured under a set of standard conditions (Hochachka and Guppy 1987, Blaxter 1989). *Cyclorana australis* are normally dormant underground during the dry season; therefore, for a seasonal comparison, aestivating frogs were compared with wet season frogs (fasted for 72 h). After the initial acclimation period (72 h), frogs were placed in chambers with wet paper towel and respiratory measurements were taken over 48 hours.
The second group of frogs was used to compare the seasonal oxygen consumption of fed frogs at 25°C (± 1.0). The fasted frogs used above were not used in this experiment in case laboratory conditions influenced respiratory physiology. For *C. australis*, frogs fed + hydrated following dormancy were compared to wet season fed frogs for a seasonal comparison. After the initial acclimation period, *C. australis* ingested crickets weighing 9.1% (± 2.3 SD) of their initial body mass, *L. caerulea* ingested 6.3% (± 1.8 SD), and *L. dahlia* ingested 7.0% (± 1.6 SD) of body mass. Frogs were placed inside respiratory chambers with wet paper towel and respiratory measurements were taken every day for three days, which was the expected passage time (Chapter Three). On each day of the measurements, wet paper towels were replenished inside respiratory chambers.

An additional group of frogs, collected during the wet season, was used to measure oxygen consumption when fully hydrated and 70% hydrated at 25°C (± 1.0). To fully hydrate frogs, frogs were partially immersed as for frogs treated in the hydrated recovery treatment following dormancy. To dehydrate frogs, frogs were housed individually inside dry perforated 5.7 L rectangular plastic containers and placed inside the temperature control cabinet without water until initial body mass dropped by about 30%. For *C. australis* and *L. caerulea*, dehydration took between 3-5 days, and it took 1-3 days for *L. dahlia*. *Cyclorana australis* lost 28.3% (± 14 SD) of their initial body mass, *L. caerulea* lost 30% (± 12.3 SD), and *L. dahlia* lost 25.4% (± 5.1 SD) of body mass. Fully hydrated frogs were placed inside respiratory chambers with wet paper towel and respiratory measurements were taken over 24 hours. The 70% hydrated frogs were treated in the same manner, except that wet paper towel was not provided inside the respiratory chamber.

### 5.3.3 Statistical analyses

Oxygen consumption (whole animal) data were analysed in 30-minute blocks corresponding to the period of lowest oxygen consumption. Data were normalised using log-transformation so that parametric tests could be conducted (Zar 1996) in
the programs SuperAnova (Abacus Concepts Inc.) and StatView (SAS Institute Inc.). Repeated measures analysis of variance (ANOVA) was used to compare the means of oxygen consumption taken over time, and logarithmic regression was used to examine the relationship between oxygen consumption and time. Analysis of covariance (ANCOVA) was used to investigate differences between pre-aestivating and recovery treatments, wet and dry season fed and unfed groups and 70% hydrated and fully hydrated frogs. Since oxygen consumption is dependent on body mass (Hochachka and Guppy 1987, Bedford and Christian 1998), body mass was used as a covariate in all analyses (ANCOVA). Least square mean oxygen consumption (non-transformed, but adjusted for body mass) values were used in tables and regression plots (Packard and Boardman 1999). Logarithmic regression curves, including R squared values, were used to express the relationship between oxygen consumption and time during the aestivation experiment. Differences among means were considered statistically significant when $P < 0.05$. Pair-wise Bonferroni Dunn $t$-tests were used as a post-hoc determination of differences among means following ANCOVA. Direct comparisons within treatments were analysed using paired $t$-tests.
5.4 Results

5.4.1 Aestivation

*Cyclorana australis* were quick to settle inside chambers within the first few days of simulated aestivation under laboratory conditions, and they remained dormant for 90 days without any obvious signs of distress. Frogs provided with water at the beginning of the experiment generally took longer to form a cocoon than frogs not provided with water, but both groups formed a cocoon within the first 20 days of dormancy. In contrast, *L. caerulea* and *L. dahlii* remained restless and were more easily disturbed during measurements. Turning on the airflow prior to respiratory measurements was often enough to provoke movement inside the chamber.

![Figure 5.1. Oxygen consumption of frogs over the laboratory simulated aestivation period. The points are least square mean oxygen consumption (ml O₂ h⁻¹) over time (days dormant) for: Cyclorana australis (n = 19), Litoria caerulea (n = 10) and Litoria dahlii (n = 13). Logarithmic regression curves are fitted for C. australis and L. caerulea, but a linear curve is fitted for L. dahlii. Standard error bars show the variation around the mean.](image)

The two groups of *C. australis* (provided with water and not provided water) consumed similar amounts of oxygen during the experiment (*t* = -0.195, *P* = 0.561), so the metabolic rates of these two groups were pooled for further analyses. The oxygen consumption of *C. australis* decreased significantly over the aestivation period (*P* < 0.001; $r^2 = 0.234$; Fig. 5.1). The rate of oxygen consumption decreased by 67% after 50 days of dormancy (Table 5.1), and did not significantly decrease further after 50 days in dormancy.
Similar to *C. australis*, but to a lesser extent, the oxygen consumption of *L. caerulea* decreased significantly over the aestivation period ($P < 0.001$; $r^2 = 0.124$; Fig. 5.1). The rate of oxygen consumption decreased by 54% after 60 days of induced dormancy (Table 5.1). Because of the unsettled behaviour displayed by *L. dahlii* during the experiment, oxygen consumption was variable over time and among individuals, and consequently the amount of oxygen consumed did not decrease consistently over the aestivation period ($P = 0.073$; $r^2 = 0.021$; Fig. 5.1). However, the minimum amount of oxygen that individuals consumed during dormancy, without regard to the time in dormancy, was 45% of pre-aestivating oxygen consumption (Table 5.1).

**Table 5.1.** Body mass (g) and oxygen consumption ($\bar{V}O_2$: ml O$_2$ h$^{-1}$) of pre-aestivating and aestivating *Cyclorana australis*, *Litoria caerulea* and *Litoria dahlii* at 25°C under laboratory conditions. Values are least square means ± standard deviation, with sample size (n). The $P$-value is the probability of a difference in oxygen consumption among frogs at the beginning of experiments (pre-aestivating) and during induced dormancy (aestivating) within species (ANCOVA). Pre-aestivating frogs are compared with aestivating frogs at day 50 for *C. australis*, day 60 for *L. caerulea* and the minimum oxygen consumption of individuals, without regard to dormancy time, for *L. dahlii*. Italic $P$-values are significant at $\alpha = 0.05$.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Body Mass (g)</th>
<th>$\bar{V}O_2$ (ml O$_2$ h$^{-1}$)</th>
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<tbody>
<tr>
<td><em>Cyclorana australis</em></td>
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<td></td>
<td></td>
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<tr>
<td>Pre-aestivating</td>
<td>19</td>
<td>38.2 ± 13.1</td>
<td>1.43 ± 0.7</td>
</tr>
<tr>
<td>Aestivating</td>
<td>19</td>
<td>42.1 ± 11.9</td>
<td>0.47 ± 0.2</td>
</tr>
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<td>$P$-value</td>
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<td><em>Litoria caerulea</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-aestivating</td>
<td>8</td>
<td>44.1 ± 10.3</td>
<td>2.02 ± 0.9</td>
</tr>
<tr>
<td>Aestivating</td>
<td>10</td>
<td>39.5 ± 10.2</td>
<td>0.92 ± 0.3</td>
</tr>
<tr>
<td>$P$-value</td>
<td></td>
<td>0.337</td>
<td>0.003</td>
</tr>
<tr>
<td><em>Litoria dahlii</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-aestivating</td>
<td>15</td>
<td>14.2 ± 2.5</td>
<td>0.69 ± 0.8</td>
</tr>
<tr>
<td>Aestivating</td>
<td>15</td>
<td>13.9 ± 1.4</td>
<td>0.38 ± 0.3</td>
</tr>
<tr>
<td>$P$-value</td>
<td></td>
<td>0.745</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Pre-aestivation oxygen consumption did not differ among the three species ($P = 0.091$). However, during aestivation, *C. australis* consumed less oxygen than *L. caerulea* ($P < 0.001$) and *L. dahlii* ($P < 0.001$), 62 and 58% less respectively, but *L. caerulea* and *L. dahlii* consumed similar amounts of oxygen ($P = 0.120$).
5.4.2 Recovery

Generally, resting (following dormancy) *C. australis* and *L. caerulea*, and pre-aestivating *L. dahlii* consumed less oxygen compared to the other treatments (Table 5.2). However, the oxygen consumption of resting *L. caerulea* did not differ from that of frogs in the hydrated treatment, and for *L. dahlii*, pre-aestivation did not differ from that of resting and hydrated treatments. Generally, fed + hydrated *C. australis*, and fed *L. caerulea* and *L. dahlii* consumed the most amount of oxygen compared to other treatments (Table 5.2). However, the oxygen consumption of *C. australis* when fed + hydrated did not differ from pre-aestivating, fed and hydrated values, and for *L. caerulea* and *L. dahlii*, fed did not differ from fed + hydrated frogs.

Table 5.2. Body mass (g) and oxygen consumption (\( \text{VO}_2 \): ml O\(_2\) h\(^{-1}\)) of *Cyclorana australis*, *Litoria caerulea* and *Litoria dahlii* during recovery following laboratory simulated aestivation. Values are least square mean ± standard deviation, with sample size (n). The \( P \) value is the probability of a difference among pre-aestivating and recovery treatments: resting, fed, hydrated, fed + hydrated within species (ANCOVA). Italic \( P \) values are significant at \( \alpha = 0.05 \), and superscript letters indicate similarities among treatment groups within each species using Bonferroni Dunn post hoc \( t \)-tests.

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Body Mass (g)</th>
<th>( \text{VO}_2 ) (ml O(_2) h(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cyclorana australis</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-aestivating</td>
<td>19</td>
<td>44.4 ± 13.1</td>
<td>1.48 ± 0.5(^{b})</td>
</tr>
<tr>
<td>Resting</td>
<td>6</td>
<td>40.0 ± 10.9</td>
<td>0.82 ± 0.7(^{a})</td>
</tr>
<tr>
<td>Fed</td>
<td>4</td>
<td>44.3 ± 10.1</td>
<td>2.0 ± 1.3(^{b})</td>
</tr>
<tr>
<td>Hydrated</td>
<td>4</td>
<td>38.3 ± 8.4</td>
<td>1.48 ± 0.8(^{b})</td>
</tr>
<tr>
<td>Fed + hydrated</td>
<td>4</td>
<td>43.2 ± 17.1</td>
<td>2.15 ± 1.5(^{b})</td>
</tr>
<tr>
<td>( P )-value</td>
<td></td>
<td>0.133</td>
<td></td>
</tr>
<tr>
<td>( &lt; 0.001 )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Litoria caerulea</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-aestivating</td>
<td>8</td>
<td>45.6 ± 10.2(^{a})</td>
<td>1.99 ± 0.9(^{bc})</td>
</tr>
<tr>
<td>Resting</td>
<td>2</td>
<td>34.7 ± 7.3(^{bc})</td>
<td>1.17 ± 1.0(^{a})</td>
</tr>
<tr>
<td>Fed</td>
<td>2</td>
<td>32.3 ± 3.1(^{bc})</td>
<td>3.7 ± 1.5(^{b})</td>
</tr>
<tr>
<td>Hydrated</td>
<td>4</td>
<td>36.7 ± 12.3(^{bc})</td>
<td>1.44 ± 1.2(^{ab})</td>
</tr>
<tr>
<td>Fed + hydrated</td>
<td>3</td>
<td>44.5 ± 3.4(^{a})</td>
<td>3.22 ± 2.2(^{c})</td>
</tr>
<tr>
<td>( P )-value</td>
<td></td>
<td>( &lt; 0.001 )</td>
<td>( &lt; 0.001 )</td>
</tr>
<tr>
<td><em>Litoria dahlii</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-aestivating</td>
<td>7</td>
<td>19.9 ± 7.2(^{a})</td>
<td>0.77 ± 0.2(^{a})</td>
</tr>
<tr>
<td>Resting</td>
<td>4</td>
<td>19.8 ± 9.9(^{ab})</td>
<td>0.91 ± 0.7(^{a})</td>
</tr>
<tr>
<td>Fed</td>
<td>4</td>
<td>16.3 ± 6.0(^{ab})</td>
<td>1.9 ± 1.1(^{b})</td>
</tr>
<tr>
<td>Hydrated</td>
<td>4</td>
<td>25.0 ± 7.5(^{bc})</td>
<td>0.8 ± 0.6(^{a})</td>
</tr>
<tr>
<td>Fed + hydrated</td>
<td>3</td>
<td>24.3 ± 4.3(^{a})</td>
<td>1.73 ± 1.1(^{b})</td>
</tr>
<tr>
<td>( P )-value</td>
<td></td>
<td>( &lt; 0.001 )</td>
<td>( &lt; 0.001 )</td>
</tr>
</tbody>
</table>

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Oxygen consumption differed among species during the resting ($P = 0.016$), the fed ($P = 0.003$) and the fed + hydrated ($P = 0.003$) treatments. Resting *C. australis* consumed less oxygen than *L. caerulea* and *L. dahlii*, 31 and 40% less respectively. *Cyclorana australis* consumed less oxygen than *L. caerulea* and *L. dahlii* when fed, 71 and 54% less respectively, and when fed + hydrated, 17 and 64% less respectively. Oxygen consumption did not differ between *L. caerulea* and *L. dahlii* when resting ($P = 0.09$), fed ($P = 0.322$) and fed + hydrated ($P = 0.622$). All three species consumed similar amounts of oxygen following the hydration treatment ($P = 0.452$).

### 5.4.3 Wet and dry season, fed versus unfed

For *C. australis*, frogs fed in the wet season consumed 33% more oxygen than frogs fed in the dry season following dormancy. For *L. caerulea* and *L. dahlii*, frogs fed in the wet season did not differ from frogs fed in the dry season (Table 5.3). Unfed frogs consumed less oxygen than fed frogs in both seasons. For *C. australis*, unfed dry season frogs consumed 61% less oxygen than unfed wet season frogs (Table 5.3), but the oxygen consumption of unfed *L. caerulea* and *L. dahlii* did not differ between the wet and dry seasons.

For *L. caerulea* and *L. dahlii*, I also compared frogs that were fed in the wet and dry season (Table 5.3) with frogs fed + hydrated during recovery following aestivation (Table 5.2). The oxygen consumption of fed + hydrated *L. caerulea* during recovery was less than in the wet and dry seasons, 77 and 63% respectively ($P < 0.001$). Similarly, the oxygen consumption of fed + hydrated *L. dahlii* during recovery was less than in the wet and dry seasons, 82 and 67% respectively ($P < 0.001$).

Oxygen consumption differed among species that were unfed during both the wet ($P = 0.004$) and dry ($P < 0.001$) seasons. Wet season unfed *C. australis* consumed less oxygen than *L. caerulea* and *L. dahlii*, 51 and 63% less respectively. Similarly, dry season unfed *C. australis* consumed less oxygen than *L. caerulea* and *L. dahlii*, 73 and 81% less respectively. Oxygen consumption did not differ between *L. caerulea* and *L. dahlii* when unfed in the wet ($P = 0.326$) and dry ($P = 0.254$) seasons.
Furthermore, oxygen consumption did not differ among species when frogs were fed during either the wet season \((P = 0.445)\) or the dry season \((P = 0.167)\).

### Table 5.3. Wet and dry season body mass (g) and oxygen consumption \((\text{VO}_2: \text{ml O}_2 \text{ h}^{-1})\) of unfed and fed *Cyclorana australis*, *Litoria caerulea* and *Litoria dahlii*. Values are least squares means ± standard deviation, with sample size \((n)\). The \(P\)-value is the probability of a difference among wet season and dry season unfed and fed values within species \((\text{ANCOVA})\). Italic \(P\)-values are significant at \(\alpha = 0.05\), and superscript letters indicate similarities among treatments within each species using Bonferroni Dunn post hoc \(t\)-tests. * denotes values derived from aestivation data, and ^ denotes values derived from the fed + hydrated recovery data for *C. australis*.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mass (g)</th>
<th>(\text{VO}_2) (ml O(_2) h(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cyclorana australis</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfed Wet</td>
<td>6</td>
<td>41.9 ± 9.7</td>
<td>0.84 ± 0.4(^b)</td>
</tr>
<tr>
<td><em>Dry</em></td>
<td>18</td>
<td>42.4 ± 12.2</td>
<td>0.37 ± 0.2(^a)</td>
</tr>
<tr>
<td>Fed Wet</td>
<td>6</td>
<td>37.0 ± 9.8</td>
<td>8.33 ± 2.7(^d)</td>
</tr>
<tr>
<td>^Dry</td>
<td>3</td>
<td>25.1 ± 5.4</td>
<td>3.0 ± 1.4(^c)</td>
</tr>
<tr>
<td>(P)-value</td>
<td>0.05</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td><em>Litoria caerulea</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfed Wet</td>
<td>11</td>
<td>40.6 ± 11.1</td>
<td>2.02 ± 1.0(^a)</td>
</tr>
<tr>
<td>Dry</td>
<td>4</td>
<td>52.6 ± 21.6</td>
<td>1.61 ± 1.4(^a)</td>
</tr>
<tr>
<td>Fed Wet</td>
<td>14</td>
<td>33.4 ± 10.2</td>
<td>6.77 ± 3.7(^b)</td>
</tr>
<tr>
<td>Dry</td>
<td>5</td>
<td>34.1 ± 11.1</td>
<td>4.76 ± 2.4(^b)</td>
</tr>
<tr>
<td>(P)-value</td>
<td>0.052</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td><em>Litoria dahlii</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfed Wet</td>
<td>5</td>
<td>17.5 ± 7.6</td>
<td>0.99 ± 0.5(^a)</td>
</tr>
<tr>
<td>Dry</td>
<td>6</td>
<td>14.5 ± 2.6</td>
<td>1.1 ± 0.2(^a)</td>
</tr>
<tr>
<td>Fed Wet</td>
<td>6</td>
<td>14.9 ± 5.1</td>
<td>5.06 ± 2.7(^b)</td>
</tr>
<tr>
<td>Dry</td>
<td>5</td>
<td>20.1 ± 2.9</td>
<td>3.02 ± 2.1(^b)</td>
</tr>
<tr>
<td>(P)-value</td>
<td>0.227</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>

**5.4.4 Fully hydrated versus 70% hydrated.**

Oxygen consumption when fully hydrated was less than when 70% hydrated for *C. australis* and *L. caerulea*, 43 and 64% respectively (Table 5.4). The oxygen consumption of *L. dahlii* did not differ between 70% and fully hydrated frogs (Table 5.4). The oxygen consumption of 70% hydrated frogs did not differ among species \((P = 0.11)\).

Fully hydrated and 70% hydrated frogs (Table 5.4) were compared with frogs that were hydrated during recovery following aestivation (Table 5.2). Oxygen consumption did not differ among these treatments for *C. australis* \((P = 0.232)\) and
L. dahlii \( (P = 0.120) \). However, for L. caerulea, frogs re-hydrated during recovery did not differ from fully hydrated frogs \( (P = 0.137) \), but consumed 48\% less oxygen than 70\% hydrated frogs \( (P = 0.002) \).

**Table 5.4.** Body mass (g) and oxygen consumption (\( \bar{V}O_2 \): ml O\(_2\) h\(^{-1}\)) of 70\% and fully hydrated Cyclorana australis, Litoria caerulea and Litoria dahlii. Values are least squares means ± standard deviation, with sample size (n). \( P \)-value is the probability of a difference between 70\% hydrated and fully hydrated values within species (ANCOVA). Italic \( P \)-values are significant at \( \alpha = 0.05 \).

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Body Mass (g)</th>
<th>( \bar{V}O_2 ) (ml O(_2) h(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cyclorana australis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70% Hydrated</td>
<td>6</td>
<td>35.5 ± 12.4</td>
<td>1.61 ± 0.4</td>
</tr>
<tr>
<td>Fully Hydrated</td>
<td>6</td>
<td>41.9 ± 9.7</td>
<td>0.92 ± 0.4</td>
</tr>
<tr>
<td>( P )-value</td>
<td></td>
<td>0.297</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Litoria caerulea</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70% Hydrated</td>
<td>6</td>
<td>15.2 ± 4.5</td>
<td>3.92 ± 1.2</td>
</tr>
<tr>
<td>Fully Hydrated</td>
<td>11</td>
<td>40.6 ± 11.1</td>
<td>1.4 ± 1.0</td>
</tr>
<tr>
<td>( P )-value</td>
<td></td>
<td>&lt; 0.001</td>
<td>0.007</td>
</tr>
<tr>
<td><strong>Litoria dahlii</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70% Hydrated</td>
<td>5</td>
<td>29.6 ± 5.4</td>
<td>1.29 ± 0.3</td>
</tr>
<tr>
<td>Fully Hydrated</td>
<td>5</td>
<td>17.5 ± 7.6</td>
<td>1.43 ± 0.5</td>
</tr>
<tr>
<td>( P )-value</td>
<td></td>
<td>0.014</td>
<td>0.686</td>
</tr>
</tbody>
</table>
5.5 Discussion

*Cyclorana australis* reduced oxygen consumption by 67% after 50 days of dormancy, which is similar to desert-dwelling frogs (Seymour 1973, Whitford and Meltzer 1976, McClanahan et al. 1983). This level of reduction in metabolism was comparable to that found in Australian desert-dwelling *Cyclorana* and *Neobatrachus sp.* (50-85%) (van Beurden 1980, Withers 1993, Flanigan and Guppy 1997), but contrasts with an earlier study of *C. australis* that found no significant reduction in oxygen consumption during dormancy (Withers and Thompson 2000). In that study experimental animals lost almost 30% of standard body mass during dormancy; therefore, I investigated if dehydration of the frogs may have affected metabolic depression (Pough et al. 1983, Gatten 1987, Preest et al. 1992, Warburg 1997). I found no difference in oxygen consumption between fully hydrated and 70% hydrated frogs during dormancy, even though non-aestivating dehydrated frogs consumed almost 50% more oxygen than when fully hydrated. Furthermore, Withers and Thompson noted that frogs remained responsive during their experiments, but in my study, *C. australis* were completely inactive. The disparity between my study and Withers and Thompson’s study may be due to differences in laboratory simulated aestivation conditions and/or the physical condition of the frogs. Nonetheless, *C. australis* were shown to depress metabolism to the same extent as desert-dwelling species in simulated laboratory dry season conditions.

In addition to the cocoon forming *C. australis*, I found that *L. caerulea* and *L. dahlii* were also capable of reducing oxygen consumption (54 and 55% respectively) under the same laboratory simulated aestivation conditions. This ability to moderately depress metabolism is similar to that reported for other non-cocoon forming species (Glass et al. 1997) and, as predicted, their level of depression was not as pronounced as for *C. australis*. However, the low rates of oxygen consumption were not sustained for *L. dahlii*. The inability of *L. dahlii* to sustain low metabolic rates and the observed restlessness of *L. caerulea* and *L. dahlii* during simulated aestivation may imply that non-cocoon forming species do not normally remain inactive during dry conditions for extended periods, which is in contrast to *C. australis*. *Cyclorana australis* remain completely inactive for six to seven months (Chapter Two).
The oxygen consumption of *L. caerulea* and *L. dahlii* measured immediately after being collected from the field did not differ between the wet and dry seasons. The only notable difference between field measurements was that fed *L. dahlii* consumed less oxygen during the dry season than during the wet season. This metabolic response to feeding, and the ability of both non-cocoon forming species to depress metabolism under simulated laboratory conditions suggests that under more stressful conditions, such as more extreme dry season conditions as occurs further inland from Darwin, non-cocoon forming species may depress metabolism in the field. The advantage of moderate metabolic depression and the lack of cocoon formation allow frogs to opportunistically exploit favourable conditions to balance energy and water demands.

With the exception of *L. caerulea*, hydration following dormancy was sufficient enough to restore SMR. Resting without food and water following dormancy was not sufficient to bring metabolism up to that when pre-aestivating. I expected that the level of metabolic response when fed + hydrated following dormancy would be greater for cocoon forming species than non-cocoon forming species. Instead, I found that the oxygen consumption of all three species was similar (20 to 35% of that when fed under optimal conditions during the wet season). All three species had undergone similar periods of fasting during dormancy. Similarly, when fed under natural conditions, *L. caerulea* and *L. dahlii* consumed less oxygen during the dry than during the wet season, 40 and 45% respectively, although oxygen consumption was not significantly different between seasons for *L. caerulea*. This reduced response to feeding may be attributed to down-regulation of the gastrointestinal tract (Secor et al. 1994, Secor and Diamond 1995, 1998, Jackson and Perry 2000, Starck and Beese 2001), which functions to minimise energy expended on gastrointestinal maintenance during periods of fasting (Cant 1996). The depressed metabolic response to feeding following simulated aestivation and under natural conditions during the dry season suggests that the recovery of normal metabolism may not immediate for both cocoon and non-cocoon forming species. However, this generalisation would need to be examined further in other species.

The ecological significance of reducing metabolism is that *C. australis* can spend between six and seven months underground on stored energy and water during the
dry season. This physiological response to dormancy under laboratory conditions is characteristic of other cocoon forming species (Abe 1995, Withers 1995), and this study has shown that this tropical species is physiologically similar to desert species. Furthermore, non-cocoon forming frogs show the ability to moderately depress metabolism under simulated aestivation conditions in the laboratory, but I did not observe this physiological response under dry season field conditions. However, this study indicates that *L. caerulea* and *L. dahlii* show some ability for physiological adjustment in response to season, which may be related to observations that non-cocoon forming species are not completely dormant and opportunistically take advantage of favourable conditions, as for the Cane Toad (Seebacher and Alford 1999). Studies of additional species in the wet-dry tropics would test the generality that the metabolic flexibility of tropical cocoon forming species is similar to that of desert species, and, that the physiological response of cocoon forming species is greater than non-cocoon forming species.
Chapter Five: Seasonal Respiratory Physiology

5.6 Bibliography


Chapter Five: Seasonal Respiratory Physiology


Chapter Six:

Energy Flow

Assimilated

Ingested

Metabolic costs

Tissue Production

Growth

Reproduction

Egested
6.1 Abstract

Energy flow equations were used to examine the acquisition and allocation of energy under laboratory conditions at four temperatures (15, 20, 25 and 30°C), and under field conditions (wet and dry seasons) for the three species of frog. Energy gained in ingestion, and expended in egestion and on respiration (standard (SMR), resting (RMR) and digestive (SDA) metabolism) was measured, and the available energy for other activities and production (growth and reproduction) was calculated. Under laboratory conditions, the oxygen consumption (SMR, RMR and SDA) of frogs had a logarithmic relationship with increasing temperature. This relationship between temperature and respiration, and the measurements of energy ingested and egested in the laboratory were combined with field measurements (body temperature and energy egested) to estimate the energy flow of free-ranging species. Under field conditions, the estimated available energy of *C. australis* was considerably greater in the wet season than in the dry season. *Cyclorana australis* ingested three and four fold more energy in the wet season than *L. caerulea* and *L. dahlii*, respectively. In the dry season, when *C. australis* are normally dormant, this species incurred a daily energy deficit (-0.27 kJ). However, when dormant, *C. australis* reduced respiratory expenditure by depressing metabolism to 35% of that when resting under optimal wet season conditions. Furthermore, the large amount of energy ingested in the wet season (daily available energy 22.6 kJ) offset the dry season daily energy deficit. Unlike *C. australis*, the daily available energy for *L. caerulea* and *L. dahlii* did not vary between seasons. Although *L. caerulea* and *L. dahlii* ingest energy year-round, the energy ingested in the wet season was greater than that in the dry season. Nonetheless, the low dry season body temperatures of these two species influenced energy expenditure. Thus, in contrast to *C. australis*, the dry season reduction in respiratory expenditure for *L. caerulea* and *L. dahlii* resulted from seasonal differences in body temperature rather than from metabolic depression.
6.2 Introduction

Energy flow models have been used to examine the variation in energy acquisition and allocation in ectotherms at different temperatures (Fitzpatrick 1973, Bobka et al. 1981), in species living in widespread geographical regions (Beaupre 1996, Christian et al. 1998, Christian et al. 1999, Angilletta 2001), in response to highly seasonal variation (van Marken Lichtenbelt and Wesselingh 1993, Christian and Green 1994, Christian et al. 1996, Christian et al. 2003), and in direct relationship with species’ ecology (Kitchell and Windell 1972). Yet, few studies have modelled the energy flow in amphibians (Fitzpatrick 1973). To date, no studies have modelled the energy flow of ecologically different species of frog that live in the wet-dry tropics of northern Australia, which experience markedly seasonal rainfall (Ridpath 1985, Taylor and Tulloch 1985, Cook and Heerdegen 2001).

Energy flow models account for energy ingested as food that is transformed into chemical energy and expended on various physiological processes in the body tissues (Brody 1964, Brafield and Llewellyn 1982, Blaxter 1989). The physiological processes of energy utilisation are varied and can be complicated by external environmental variables, in particular, animal thermodynamics (Tracy 1976, Brafield and Llewellyn 1982, Lillywhite et al. 1998). The effect of temperature on standard metabolic rate (SMR), resting metabolic rate (RMR) (Preest et al. 1992, Navas 1996, Bedford and Christian 1998) and the specific dynamic action (SDA) of digestion (Powell et al. 1999, Busk et al. 2000, Secor and Faulkner 2002, Zaidan and Beaupre 2003) can influence the flow of energy. I use a simple equation that is based on a temperature-specific relationship. Since energy can neither be created nor destroyed, but can be transformed into one form or another (Brafield and Llewellyn 1982), the equation I use assumes that energy expenditure is equal to the sum of energy used for production (i.e. growth and fat stores), respiration (loss of heat to the environment) and that lost in feces.

During optimal wet season conditions, the cocoon-forming frog, Cyclorana australis, consumes three and four fold more energy than the two non-cocoon forming frogs,
Litoria caerulea and Litoria dahlii, respectively (Chapter Three). However, during the dry season, C. australis aestivate underground and do not consume food for six to seven months (Chapter Two). In contrast, L. caerulea and L. dahlii continue to forage during dry season conditions, although activity is significantly reduced during adverse dry periods (Chapter Two). During dormancy, C. australis decrease energy expenditure by physiologically depressing metabolism to 35% of normal resting values (Chapter Five). However, L. caerulea and L. dahlii do not depress metabolism in the field (Chapter Five). The purpose of this study was to examine the daily energy flow of ingested energy in the field in both the wet and dry seasons for each of these species. I hypothesised that, during the dry season, C. australis would incur an energy deficit because no energy is ingested when dormant. However, the large amount of energy ingested by this species in the wet season would compensate for the dry season energy deficit. Furthermore, I hypothesised that energy flow would not differ between wet and dry seasons for both L. caerulea and L. dahlii because these two species ingest energy year-round.

Daily energy flow was modelled for hypothetical free-ranging C. australis, L. caerulea and L. dahlii in both the wet and dry seasons. Seasonally dependent energy flow models were based on laboratory measurements of SMR, RMR and SDA over a range of temperatures (15, 20, 25 and 30°C) that used known quantities of energy ingested and egested. Laboratory measurements were combined with field measurements of body temperature ($T_b$) and fecal mass to estimate the SMR, RMR and SDA of each species in the field, and consequently model the daily energy flow of estimated ingested energy in the wet and dry seasons.
6.3 Materials and methods

6.3.1 Experimental protocol

_Cyclorana australis, L. caerulea and L. dahlii_ were captured by hand in the wet (December-February) and dry (June-August) seasons and taken back to a laboratory at Charles Darwin University. For three days following capture and prior to experiments on respiratory metabolism, frogs were housed individually in a temperature controlled cabinet (25°C ± 1.0) and provided water _ad libitum_ using wet paper towel, but food was withheld to exclude the effect of digestion on oxygen consumption (Seale 1987).

Prior to respiratory measurements, frogs were weighed to the nearest 0.01 g and placed individually into respiratory chambers (dimensions are described in Chapter Five) on wet paper towel, to prevent dehydration, and acclimated to the experimental temperature inside the temperature control cabinet for 48 hours. In all respiratory experiments, oxygen consumption was measured using a continuous airflow respirometry system for indirect calorimetry of energy consumption (Brafield and Llewellyn 1982, Vleck 1987, Walsberg and Hoffman 2005, 2006). The respirometry system used to measure oxygen consumption is described in detail in Chapter Five. Briefly, three frogs were monitored concurrently. Frog chambers were sampled consecutively for one hour each by systematically switching between chambers, with baseline air measured every 60 minutes. Airflow averaged 0.042 L min⁻¹ (± 2.2 SD) during experiments. The oxygen consumption of frogs was calculated using the equations of Withers (1977, 2001). Values of oxygen consumption were converted to units of energy using the energy equivalent, 20.08 kJ L⁻¹ O₂ (Benabib and Congdon 1992).
6.3.2 Measurements of SMR and RMR

The oxygen consumption of frogs collected during the wet and dry seasons was measured at each of the experimental temperatures: 15, 20, 25 and 30°C. The temperature sequence for respiratory measurements was randomised. Measurements of SMR and RMR were taken over 24 h. SMR (whole animal) was measured during the normal period of inactivity when frogs were asleep (12 h daylight period: 0600-1800 hours) and RMR (whole animal) was measured during the normal period of activity (12 h night time period: 1800-0600 hours), and expressed as kJ h\(^{-1}\).

*Cylorana australis* are normally dormant underground during the dry season, and therefore, for a seasonal comparison, aestivating frogs were compared with wet season frogs. A group of *C. australis* was collected in the late-wet season (March–May) and induced into dormancy in laboratory conditions, as described in Chapter Five. Briefly, frogs were held individually inside the temperature controlled cabinet for 30 days without food and water, in dark and dry conditions at 25°C (± 1.0). Following 30 days of induced dormancy, *C. australis* were kept in the same conditions, but acclimated to each experimental temperature for 48 hours prior to respiratory measurements, and subsequently measurements of SMR and RMR were taken as above.

6.3.3 Measurements of SDA

At the end of measurements of SMR and RMR, frogs were reweighed and then force fed crickets that had a wet mass 6-7% of their initial body mass. *Cylorana australis* (37.3 g ± 8.6 SD) ingested 2.4 g (± 1.4 SD) of crickets, *L. caerulea* (36.3 g ± 11.2 SD) ingested 2.3 g (± 0.7 SD) and *L. dahlii* (16.7 g ± 6.4 SD) ingested 1.1 g (± 0.1 SD) before being placed back into respiratory chambers and measurements resumed at the respective experimental temperature. The energy content of crickets averaged 19.3 kJ g\(^{-1}\) (± 2.9 SD) (determined using methods for bomb calorimetry, as described in Chapter Three).
After each measurement of respiration was taken, wet paper towels were refreshed, and feces were collected to determine their energy content. Respiratory measurements continued until metabolism re-established baseline SMR (determined using statistical $t$-tests).

The digestion period ($D$) is defined as the duration of oxygen consumption elevation above baseline SMR following a meal, and was measured as the total number of days. Specific dynamic action (SDA) is a measure of the amount of energy used during manipulation, digestion and assimilation of food (Robert and Thompson 2000, Iglesias et al. 2003), but energy used during manipulation of food was not measured in this present study. SDA is measurable as the difference between pre-feeding and post-feeding oxygen consumption (Wang 2001). Methods used to measure SDA follow Iglesias (2003). Briefly, the magnitude of the SDA was calculated as the area under the curve produced over the digestion period, minus the area under the curve produced over the same period, but when resting. Values for SDA are expressed in kJ.

### 6.3.4 Temperature dependent energy flow

Models described the acquisition of energy from one meal of crickets and the allocation of energy over the digestion period. Energy flow was produced for each experimental temperature ($15$, $20$, $25$ and $30^\circ C$) using the energy equation:

\[
I = P + R + E
\]

that is adapted from Brafield and Llewellyn (1982), Seale (1987) and Blaxter (1989), where $I =$ ingested, $P =$ production, $R =$ respiratory and $E =$ egested energy. The energy in urine was excluded from the energy flow model because it is considered to be negligible (Smith 1976). Ingested and egested energy were determined using semi-micro bomb calorimetry, as described in Chapter Three, but briefly, I measured the energy content of feces egested following ingestion of a known mass and energy content of
crickets. Respiratory energy is defined as the energy lost to the environment as heat during aerobic and anaerobic respiration (Seale 1987). SMR and RMR over the digestion period (D), and SDA were included in R. However, R is underestimated because it does not include energy used during other activities and from anaerobic metabolism, and thus assumes that frogs remain inactive (Brafield and Llewellyn 1982).

P was calculated by rearranging equation 1:

\[ P = I - (R + F) \]  

(2)

P is expressed in terms of energy available for tissue production and for activity, since R does not include activity respiration. Tissue production is the energy that contributes to growth and includes the increase in lean biomass until maturity, the synthesis of energy stores (fats and lipids), tissue maintenance, mucus production, maturation of gametes and the cost of replacing shed and damaged integument (Seale 1987). However, variables for P were not measured in this present study, and thus P is inclusive of these many sub components and described hereafter as available energy. All components of energy flow are expressed in kJ.

In addition to energy flow models, assimilated energy (A) was calculated using the equation:

\[ A = I - E \]  

(3)

6.3.5 Measurements of field body temperature

Frog body temperature (\(T_b\)) was measured using field sampling and radio telemetry, as described in Chapter Two. Briefly, field sampling was used to measure the \(T_b\) of frogs when encountered in the field at night, using an infrared thermometer. Radio telemetry, using surgically implanted temperature sensitive transmitters (Sirtrack; Hawkes Bay, New Zealand), was used to measure the \(T_b\) of frogs when inactive in the field during the
day and night. Individuals were measured once during field sampling, but radio telemetry measured the same individuals once every 3-10 days in the field, at randomly selected times of the day to maintain independence of observations (Swihart and Slade 1985). Day and night data from radio telemetry were averaged for individuals in each season. Night T\textsubscript{b} averages were combined with random sampling data to provide means for the wet and dry seasons.

6.3.6 Seasonally dependent estimated daily energy flow

The wet and dry season daily energy flow of hypothetical free-ranging frogs consisted of estimated energy acquisition and allocation using equation 1. Ingested energy was estimated using the feces egested by frogs captured from the field. The energy content of feces was determined using bomb calorimetry (Chapter Three). Energy egested in the field was substituted into equations derived by regressing known quantities of energy ingested with energy egested in controlled laboratory conditions (Chapter Three) to provide an estimate of energy ingested. Respiratory energy included the estimated wet and dry season SMR and RMR of frogs at their respective day and night field T\textsubscript{b}, and the estimated SDA of frogs at their mean daily, wet and dry season body T\textsubscript{b}. To estimate respiratory energy, means for field T\textsubscript{b} were substituted into equations derived by regressing respiration energy (SMR, RMR and SDA) with temperature under controlled laboratory conditions. Available energy (P) was subsequently calculated using equation 2. All components of the daily energy flow equations are expressed as kJ.

Energy flow is based on measurements taken under laboratory conditions that combine only some measurements of frogs under field conditions (T\textsubscript{b} and egested energy). Equations assume that the size of the meals ingested in the field does not differ from that in the laboratory, and consequently SDA would be similar at a given temperature. In addition to this, the equations assume that only crickets were ingested in the field. Costs associated with various activities such as reproduction, shedding, and production costs (tissue maintenance, growth and fat accumulation) (Smith 1976) have not been
quantified. Therefore, similar to temperature dependent energy flow, P is inclusive of these sub components.

6.3.7 Statistical analyses

Oxygen consumption data were analysed in 30-minute blocks corresponding with the period of lowest oxygen consumption during the normal period of inactivity (SMR) and during the normal period of activity (RMR), and in 24-hour blocks over the digestion period. The area under the curve that was produced over the SDA period was calculated using the Simpson’s approximation (Alldis 1986). All data were normalised using log-transformation, so that parametric tests could be conducted (Zar 1996) in the program SuperAnova (Abacus Concepts Inc.). Repeated measures analysis of variance (ANOVA) was used for experiments in which repeated measurements were taken of individuals, but at different experimental temperatures. Body mass was used as a covariate in analyses (ANCOVA). Logarithmic regression was used to express the relationship between oxygen consumption (SMR, RMR and SDA) and temperature (Andrews and Pough 1985) in the program StatView 5.0 (SAS Institute Inc.). The least squares means of non-transformed data (adjusted for frog body mass, ANCOVA) were used in tables and in graphs (Packard and Boardman 1999). Differences among means were considered statistically significant when $P < 0.05$. Pair-wise Bonferroni Dunn $t$-tests were used as a post-hoc determination of differences among means following ANCOVA.
6.4 Results

6.4.1 The effect of temperature and season on SMR and RMR

The energy consumption of resting (SMR and RMR) frogs was positively correlated with increasing temperature (Fig. 6.1). The energy consumption of frogs at 15°C was significantly less than at 25 and 30°C ($P < 0.05$ for each species in each season for SMR and RMR), but energy consumption generally did not differ between 15 and 20°C. The energy consumption of resting frogs at 30°C was significantly greater than at 15 and 20°C ($P < 0.05$ for each species in each season for SMR and RMR), but energy consumption generally did not differ between 25 and 30°C. Furthermore, the energy consumption of frogs generally did not differ between 20 and 25°C.

SMR was significantly less than RMR for all three species ($C. australis$, $P = 0.015$; $L. caerulea$, $P = 0.04$; and $L. dahlii$, $P = 0.02$). However, both SMR and RMR did not differ between the wet and dry seasons for $L. caerulea$ and $L. dahlii$ ($P > 0.05$ at each temperature), but for $C. australis$, energy consumption in the dry season was significantly less than that in the wet season ($P < 0.05$ for SMR and RMR at each temperature). Thus, regressions (logarithmic) of SMR and RMR with temperature in the wet and dry seasons were treated separately to yield the following equations for $C. australis$:

- **Wet season**
  - SMR = $-0.067 + 0.026 \times \ln(\text{temperature})$ ($P < 0.001$)
  - RMR = $-0.078 + 0.031 \times \ln(\text{temperature})$ ($P < 0.001$)

- **Dry season**
  - SMR = $-0.034 + 0.013 \times \ln(\text{temperature})$ ($P < 0.001$)
  - RMR = $-0.028 + 0.012 \times \ln(\text{temperature})$ ($P < 0.001$),

but regressions of SMR and RMR were not separated by season for $L. caerulea$:

- SMR = $-0.191 + 0.073 \times \ln(\text{temperature})$ ($P < 0.001$)
- RMR = $-0.255 + 0.098 \times \ln(\text{temperature})$ ($P < 0.001$)

and for $L. dahlii$:

- SMR = $-0.119 + 0.044 \times \ln(\text{temperature})$ ($P < 0.001$)
RMR = -0.12 + 0.047 x ln(temperature)  \hspace{1cm} (P < 0.001).

![Figure 6.1](image_url)

Figure 6.1. The least squares means (untransformed, but adjusted for body mass using ANCOVA) SMR and RMR, respectively of *Cyclorana australis* (A, B), *Litoria caerulea* (C, D) and *Litoria dahlii* (E, F), as a function of body temperature in the wet and dry seasons. Y-error bars represent standard error and each temperature has a sample size of 6, for each species. Curves represent the logarithmic relationship between energy consumption and temperature (Andrews and Pough 1985).

The energy consumption of *C. australis* was generally lower than that of *L. caerulea* and *L. dahliii* at most measured temperatures during the wet and dry seasons, but the energy consumption of *C. australis* did not differ from *L. caerulea* and *L. dahliii* at 15 and 30°C in the wet season. Energy consumption did not differ between *L. caerulea* and *L. dahliii* at most temperatures, but in the dry season at 30°C, the energy consumption of *L. caerulea* was greater than that of *L. dahliii* (P = 0.002).
6.4.2 Specific dynamic action

The energy consumption of frogs following feeding (SDA), shows a strong positive relationship with increasing temperature, as shown in Figure 6.2 and as expressed in the following regressions (logarithmic):

- **C. australis**  
  \[ SDA = -36.22 + 14.32 \times \ln(\text{temperature}) \quad (P < 0.001) \]

- **L. caerulea**  
  \[ SDA = -26.03 + 10.09 \times \ln(\text{temperature}) \quad (P < 0.001) \]

- **L. dahlii**  
  \[ SDA = -16.76 + 6.53 \times \ln(\text{temperature}) \quad (P < 0.001) \]

Although the energy consumption of frogs was generally greatest at 30°C and lowest at 15°C, SDA at 20°C did not differ from that at 25°C for *L. caerulea* and *L. dahlii* (*P* = 0.205 and *P* = 0.115, respectively). SDA did not differ among species at each experimental temperature.

**Figure 6.2.** The least squares means (untransformed, but adjusted for body mass using ANCOVA) energy consumption (above SMR and RMR) of *Cyclorana australis*, *Litoria caerulea* and *Litoria dahlii* following a meal (SDA), as a function of body temperature. Y-error bars represent standard error, and sample size equals 6 for each species, at each temperature. Curves represent the logarithmic relationship between energy consumption and temperature (Andrews and Pough 1985).
Table 6.1. Energy flow of *Cyclorana australis*, *Litoria caerulea* and *Litoria dahlii* at four experimental temperatures under laboratory conditions. B\textsubscript{Mass} = frog body mass; D = period that energy consumption remained elevated above resting metabolism following a meal (SDA); I = ingested energy; E = egested energy; A = assimilated energy (I–E); R = respiratory energy (((SMR*12 hours)+(RMR*12 hours))*D + SDA), where SMR and RMR = standard and resting energy (kJ h\(^{-1}\)), respectively, and SDA = specific dynamic action (kJ). P = production, calculated using Brafield and Llewellyn’s (1982) equation, P = I – (R + E). Means are the least squares of untransformed group data, but adjusted for body mass (ANCOVA), ± standard deviation, and n = the number of frogs sampled. The P–value is the probability of measured variables differing among temperatures (ANCOVA) and italic P–values are significant at the 5% significance level. Superscript letters indicate similarities among temperature groups using the Bonferroni Dunn post-hoc test.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>P–value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclorana australis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B\textsubscript{Mass} (g)</td>
<td>42.6 ± 9.5</td>
<td>39.0 ± 9.0</td>
<td>37.0 ± 9.8</td>
<td>37.4 ± 7.5</td>
<td>0.740</td>
</tr>
<tr>
<td>D (days)</td>
<td>8.7 ± 0.5(^{c})</td>
<td>5.2 ± 0.4(^{b})</td>
<td>4.2 ± 1.0(^{a})</td>
<td>4.2 ± 0.7(^{a})</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>I (kJ)</td>
<td>44.4 ± 8.0</td>
<td>46.4 ± 11.2</td>
<td>51.5 ± 9.6</td>
<td>46.2 ± 9.3</td>
<td>0.326</td>
</tr>
<tr>
<td>E (kJ)</td>
<td>5.9 ± 0.6</td>
<td>6.0 ± 0.9</td>
<td>6.4 ± 0.7</td>
<td>6.0 ± 0.7</td>
<td>0.360</td>
</tr>
<tr>
<td>A (kJ)</td>
<td>38.5 ± 7.4</td>
<td>40.4 ± 10.3</td>
<td>45.1 ± 8.9</td>
<td>40.2 ± 8.6</td>
<td>0.320</td>
</tr>
<tr>
<td>R (kJ)</td>
<td>5.4 ± 0.4(^{a})</td>
<td>6.6 ± 0.5(^{b})</td>
<td>10.3 ± 1.1(^{c})</td>
<td>15.8 ± 0.8(^{d})</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>P (kJ)</td>
<td>33.2 ± 7.5(^{c})</td>
<td>33.8 ± 10.4(^{bc})</td>
<td>34.9 ± 9.5(^{b})</td>
<td>24.4 ± 8.6(^{a})</td>
<td>0.006</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

| Litoria caerulea |     |     |     |     |         |
| B\textsubscript{Mass} (g) | 41.2 ± 11.8 | 34.3 ± 11.1 | 32.2 ± 11.0 | 33.1 ± 11.3 | 0.551 |
| D (days) | 8.6 ± 0.6\(^{c}\) | 4.3 ± 0.5\(^{b}\) | 3.6 ± 0.6\(^{ab}\) | 3.1 ± 0.8\(^{a}\) | < 0.001 |
| I (kJ) | 46.3 ± 9.6 | 46.7 ± 13.3 | 41.5 ± 9.1 | 49.7 ± 14.0 | 0.690 |
| E (kJ) | 10.8 ± 2.3 | 10.9 ± 3.2 | 9.7 ± 2.2 | 11.7 ± 3.3 | 0.691 |
| A (kJ) | 35.5 ± 7.3 | 35.8 ± 10.2 | 31.8 ± 7.0 | 38.1 ± 10.7 | 0.689 |
| R (kJ) | 5.3 ± 0.2\(^{a}\) | 8.0 ± 0.7\(^{b}\) | 7.0 ± 0.8\(^{b}\) | 13.8 ± 1.1\(^{c}\) | < 0.001 |
| P (kJ) | 30.2 ± 7.5 | 27.8 ± 10.2 | 24.7 ± 7.2 | 24.4 ± 11.2 | 0.579 |
| n | 6 | 6 | 5 | 6 | |

| Litoria dahlii |     |     |     |     |         |
| B\textsubscript{Mass} (g) | 18.4 ± 7.6 | 15.9 ± 5.5 | 15.6 ± 5.0 | 14.6 ± 6.1 | 0.783 |
| D (days) | 3.8 ± 0.4\(^{b}\) | 3.2 ± 0.4\(^{ab}\) | 2.6 ± 0.5\(^{a}\) | 2.7 ± 0.5\(^{a}\) | 0.001 |
| I (kJ) | 17.8 ± 10.3\(^{a}\) | 15.8 ± 5.3\(^{a}\) | 19.4 ± 5.5\(^{a}\) | 26.1 ± 10.3\(^{a}\) | 0.037 |
| E (kJ) | 1.3 ± 0.5\(^{a}\) | 1.2 ± 0.2\(^{a}\) | 1.3 ± 0.2\(^{a}\) | 1.6 ± 0.5\(^{a}\) | 0.029 |
| A (kJ) | 16.5 ± 9.8\(^{a}\) | 14.7 ± 5.0\(^{a}\) | 18.1 ± 5.3\(^{a}\) | 24.5 ± 9.9\(^{a}\) | 0.040 |
| R (kJ) | 2.1 ± 0.5\(^{a}\) | 2.6 ± 0.5\(^{b}\) | 4.1 ± 0.8\(^{b}\) | 5.3 ± 1.0\(^{b}\) | < 0.001 |
| P (kJ) | 17.1 ± 9.9\(^{a}\) | 16.5 ± 5.0\(^{a}\) | 15.0 ± 5.8\(^{a}\) | 13.8 ± 10.3\(^{a}\) | 0.022 |
| n | 6 | 6 | 7 | 9 | |
6.4.3 SDA period

The number of days that the metabolic rate remained elevated above resting following a meal (SDA) was significantly greater at 15°C than that at 20, 25 and 30°C (Table 6.1), but generally did not differ among 20, 25 and 30°C for each species (Table 6.1). The period that metabolic rate remained elevated above resting following feeding was significantly shorter for *L. dahlii* than for *C. australis* and *L. caerulea* at 15, 20 and 30°C (*P* < 0.05 at each temperature among species), but at 25°C, the SDA period did not differ among species (*P* = 0.149). The number of days that SDA remained elevated generally did not differ between *C. australis* and *L. caerulea* (*P* > 0.05).

6.4.4 Temperature dependent energy flow

Temperature dependent energy flow is presented for each species of frog in Table 6.1. As a consequence of energy ingested, *L. dahlii* assimilated and egested more energy at 30°C than at 15, 20 and 25°C. However, energy ingested and assimilated did not differ among temperatures for *C. australis* and *L. caerulea*. The metabolic rate (R) of species differed among temperatures. Respiratory energy was highest at 30°C and lowest at 15°C and contributed to the overall differences in available energy (P). While P was generally lowest at 30°C and highest at 15°C, available energy varied among temperatures for *C. australis*, but not significantly for *L. caerulea* or *L. dahlii*.

Assimilated energy (A) differed among species at 25°C (*P* = 0.026), but not at 15, 20 and 30°C (*P* > 0.05). At 25°C, the assimilation energy of *L. dahlii* was less than that of *C. australis* and *L. caerulea*, and the assimilation energy of *L. caerulea* was less than that of *C. australis*. Metabolic rate (R) differed among species (*P* < 0.05 at all temperatures). The respiratory energy of *L. dahlii* was less than that of *C. australis* and *L. caerulea* at all experimental temperatures, and the respiratory energy of *L. caerulea* was less than that of *C. australis* at only 25°C. However, P did not differ among species at any of the experimental temperatures (*P* > 0.05).
6.4.5 Field body temperatures and estimated resting energy of free-ranging frogs

The day and night field $T_b$ of frogs differed significantly between seasons (Table 6.2). Generally, daytime $T_b$ was greater than night $T_b$ in both the wet and dry seasons, except for *C. australis* in the dry season, when night $T_b$ was greater than day $T_b$. Generally, wet season $T_b$ was greater than dry season $T_b$, although the night time $T_b$ of *C. australis* was higher in the dry season than in the wet season. The range in $T_b$ varied among species between seasons and between the day and night. The range in $T_b$ for *C. australis* (5°C difference) was comparatively less than that for *L. caerulea* (8°C difference) and *L. dahlii* (9°C difference), but the upper and lower mean estimates of $T_b$ for *C. australis* were higher than those for *L. caerulea* and *L. dahlii* (Table 6.2).

The estimated daytime resting energy (SMR) of species was only marginally lower than night time resting energy (RMR) in both the wet and dry seasons (Table 6.2). This is because SMR is lower than RMR, even though $T_b$ in the day was greater than at night, in most cases. Similarly, the wet season resting energy of frogs was considerably higher than dry season resting energy, as a consequence of seasonal differences in metabolism (for *C. australis* only) and seasonal differences in $T_b$ (*L. caerulea* and *L. dahlii*). The dry season resting energy of *C. australis* was 65 and 50% of wet season day and night resting energy, respectively. The dry season daytime resting energy of *L. caerulea* and *L. dahlii* was 55 and 53% of wet season resting energy, respectively, and the dry season night time resting energy of *L. caerulea* and *L. dahlii* was 67 and 57% of wet season resting energy, respectively.
Chapter Six: Energy Flow

Table 6.2. The mean body temperature ($T_b$) and the estimated resting energy expenditure (SMR and RMR) of free-ranging Cyclorana australis, Litoria caerulea and Litoria dahlii during the day (0600-1800 hours) and night (1800-0600 hours), in the wet and dry seasons. The energy expenditure of resting frogs was estimated by substituting $T_b$ into equations that were derived from regressing temperature with respiratory energy (kJ h$^{-1}$) under laboratory conditions, and multiplied by 12 (hours) to provide a total (kJ). Daytime respiration corresponds with standard metabolic rate (SMR), and that at night with resting metabolic rate (RMR). The number of frogs in which $T_b$ was sampled = n. Values for $T_b$ are means, ± standard deviation. $P$–values are the probability of $T_b$ varying between night and day in the wet and dry seasons (2-Factor ANOVA). Italic $P$–values are significant at the 5% significance level, and superscript letters indicate similarities among $T_b$ groups by using Bonferroni Dunn post-hoc $t$-tests.

<table>
<thead>
<tr>
<th></th>
<th>Wet Season</th>
<th>Dry Season</th>
<th></th>
<th></th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day (SMR)</td>
<td>Night (RMR)</td>
<td>Day (SMR)</td>
<td>Night (RMR)</td>
<td></td>
</tr>
<tr>
<td><strong>Cyclorana australis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_b$ (°C)</td>
<td>32.2 ± 2.0$^c$</td>
<td>27.1 ± 0.6$^a$</td>
<td>28.7 ± 2.5$^b$</td>
<td>29.4 ± 2.0$^b$</td>
<td>$&lt; 0.001$</td>
</tr>
<tr>
<td>Resting (kJ)</td>
<td>0.28</td>
<td>0.29</td>
<td>0.11</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>17</td>
<td>4</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td><strong>Litoria caerulea</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>$T_b$ (°C)</td>
<td>29.2 ± 1.4$^b$</td>
<td>28.5 ± 1.5$^b$</td>
<td>22.7 ± 3.5$^a$</td>
<td>21.1 ± 3.9$^a$</td>
<td></td>
</tr>
<tr>
<td>Resting (kJ)</td>
<td>0.66</td>
<td>0.88</td>
<td>0.44</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>27</td>
<td>4</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td><strong>Litoria dahlii</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.016</td>
</tr>
<tr>
<td>$T_b$ (°C)</td>
<td>31.4 ± 1.8$^c$</td>
<td>30.4 ± 1.0$^b$</td>
<td>22.2 ± 1.2$^a$</td>
<td>22.5 ± 1.4$^a$</td>
<td></td>
</tr>
<tr>
<td>Resting (kJ)</td>
<td>0.39</td>
<td>0.49</td>
<td>0.21</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>16</td>
<td>6</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

6.4.6 Seasonally dependent daily energy flow

Estimated wet and dry season daily energy flow is presented for hypothetical free-ranging frogs following ingestion of a meal in Table 6.3. The estimated available energy (P) for *C. australis* was markedly higher in the wet season than in the dry season. This results from no energy being assimilated in the dry season when *C. australis* are normally dormant underground (i.e. dry season I = 0). For *L. caerulea* and *L. dahlii*, estimated energy assimilation was higher and quicker in the wet than in the dry season. However, available energy generally did not differ between the wet and dry seasons for these two species, even though respiratory energy was highest in the wet as a consequence of $T_b$. 128
Table 6.3. Estimated daily energy flow of hypothetical free-ranging *Cyclorana australis*, *Litoria caerulea* and *Litoria dahlii* following a meal in the wet and dry seasons. Energy flow includes field measurements of body temperature (T<sub>b</sub>), B<sub>Mass</sub> = frog body mass and E = egested energy. Laboratory estimates of D = the period that energy consumption remained elevated above resting metabolism following a meal (SDA), I = ingested energy and R = respiration energy ((SMR+RMR) + SDA/D) were also included. SMR and RMR = standard and resting energy (Table 6.2; kJ), respectively, and SDA = specific dynamic action (kJ) were determined by substituting T<sub>b</sub> into regression equations derived under laboratory conditions. A = assimilated energy (I–E); P = available energy, calculated using Brafield and Llewellyn’s (1982) equation, P = I – (R + E). *Cyclorana australis* are normally dormant during the dry season, therefore values for I and E = zero, and * in the dry season is the same as that measured in the wet season. Values for T<sub>b</sub>, body mass, I (estimated) and E are means of untransformed data, ± standard deviation, and n = the number of frogs from which faecal samples were collected.

<table>
<thead>
<tr>
<th></th>
<th>Wet</th>
<th>Dry</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cyclorana australis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;b&lt;/sub&gt;</td>
<td>29.7 ± 3.6</td>
<td>29.1 ± 2.1</td>
</tr>
<tr>
<td>B&lt;sub&gt;mass&lt;/sub&gt; (g)</td>
<td>40.4 ± 9.6</td>
<td>*40.4 ± 9.6</td>
</tr>
<tr>
<td>D (days)</td>
<td>3.7</td>
<td>3.8</td>
</tr>
<tr>
<td>I (kJ)</td>
<td>30.2 ± 13.7</td>
<td>0</td>
</tr>
<tr>
<td>E (kJ)</td>
<td>3.7 ± 4.2</td>
<td>0</td>
</tr>
<tr>
<td>A (kJ)</td>
<td>26.5</td>
<td>0</td>
</tr>
<tr>
<td>R (kJ)</td>
<td>3.9</td>
<td>0.27</td>
</tr>
<tr>
<td>P (kJ)</td>
<td>22.6</td>
<td>-0.27</td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td><strong>Litoria caerulea</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;b&lt;/sub&gt;</td>
<td>28.3 ± 2.0</td>
<td>21.6 ± 2.0</td>
</tr>
<tr>
<td>B&lt;sub&gt;mass&lt;/sub&gt; (g)</td>
<td>47.1 ± 17.9</td>
<td>47.8 ± 18.0</td>
</tr>
<tr>
<td>D (days)</td>
<td>2.9</td>
<td>4.6</td>
</tr>
<tr>
<td>I (kJ)</td>
<td>12.1 ± 6.6</td>
<td>8.4 ± 4.3</td>
</tr>
<tr>
<td>E (kJ)</td>
<td>3.4 ± 1.5</td>
<td>2.3 ± 2.0</td>
</tr>
<tr>
<td>A (kJ)</td>
<td>8.7</td>
<td>6.1</td>
</tr>
<tr>
<td>R (kJ)</td>
<td>4.2</td>
<td>2.1</td>
</tr>
<tr>
<td>P (kJ)</td>
<td>4.5</td>
<td>4.1</td>
</tr>
<tr>
<td>n</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td><strong>Litoria dahlii</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;b&lt;/sub&gt;</td>
<td>30.9 ± 3.5</td>
<td>23.4 ± 2.5</td>
</tr>
<tr>
<td>B&lt;sub&gt;mass&lt;/sub&gt; (g)</td>
<td>14.4 ± 6.0</td>
<td>17.5 ± 4.5</td>
</tr>
<tr>
<td>D (days)</td>
<td>2.6</td>
<td>3.1</td>
</tr>
<tr>
<td>I (kJ)</td>
<td>7.2 ± 6.3</td>
<td>5.3 ± 4.5</td>
</tr>
<tr>
<td>E (kJ)</td>
<td>1.0 ± 0.9</td>
<td>0.6 ± 1.1</td>
</tr>
<tr>
<td>A (kJ)</td>
<td>6.2</td>
<td>4.0</td>
</tr>
<tr>
<td>R (kJ)</td>
<td>3.1</td>
<td>1.8</td>
</tr>
<tr>
<td>P (kJ)</td>
<td>3.2</td>
<td>2.9</td>
</tr>
<tr>
<td>n</td>
<td>14</td>
<td>16</td>
</tr>
</tbody>
</table>
The estimated available energy for *C. australis* in the wet season was considerably greater than for *L. caerulea* and *L. dahlii* because of markedly higher ingested energy (and thus assimilated energy). In contrast to the wet season, *C. australis* incurred an energy deficit (P) in the dry season, and the available energy for *L. caerulea* and *L. dahlii* was markedly greater than for *C. australis*. 
6.5 Discussion

The estimated daily energy flow of each hypothetical free-ranging species of frog differed between the wet and dry seasons (Table 6.3). The calculated energy available for production and other respiratory activities (P) was considerably greater in the wet season than in the dry season for *C. australis*, but generally did not differ between seasons for *L. caerulea* and *L. dahlii*. Furthermore, the wet season available energy of *C. australis* was considerably greater than that of *L. caerulea* and *L. dahlii*, but in the dry season, the calculated available energy of *L. caerulea* and *L. dahlii* was greater than that of *C. australis*. The differences in energy flow between seasons and among species are a consequence of differences in assimilated energy and respiratory energy, and the influence of body temperature on these variables.

In the wet season, *C. australis* assimilated three and four times more energy in the field than *L. caerulea* and *L. dahlii*, respectively, but in the dry season *C. australis* assimilated no energy. The ability of *C. australis* to assimilate large amounts of energy in the wet season is comparable to that of desert dwelling species (Seymour and Lee 1974, van Beurden 1980, Loveridge and Withers 1981, Flanigan and Guppy 1997, Withers and Thompson 2000). As an extreme example, the desert dwelling American frog, *Scaphiopus couchi*, consumes 55% of its body weight in one sitting following rain, which provides enough energy to last at least one year (Larsen 1992). However, as a consequence of not ingesting energy in the dry season, *C. australis* incurred a daily energy deficit (-0.27 kJ) that resulted from respiratory costs. Nonetheless, the ability to consume considerable amounts of energy during the wet season enables *C. australis* to offset the dry season energy deficit.

Seasonal differences in resting energy (SMR and RMR) influenced the respiratory expenditure (R) of *C. australis*. Dry season resting energy expenditure was 35% of that in the wet season. This reduction in respiratory costs resulted from metabolic depression (Chapter Five). The depressed metabolism of *C. australis* is similar to other seasonally dormant species of frog (Seymour 1973, Whitford and Meltzer 1976, McClanahan et al.)
Australian desert-dwelling species of *Neobatrachus* depress metabolism to 15-50% of normal resting levels (Withers 1993, Flanigan and Guppy 1997), and other species of *Cyclorana* reduce metabolism to 20-60% of normal resting levels (van Beurden 1980, Withers 1993, Withers and Thompson 2000). This reduction in respiratory energy expenditure during dormancy allows some species to remain dormant for very long periods, for example, up to five years for *Scaphiopus hammondii* (Seymour 1973). For a hypothetical free ranging *C. australis* (40 g), a miniscule respiratory cost over a 6-month dormancy period (49.1 kJ) would be incurred compared to the six month wet season residual energy (4113.2 kJ). Thus, similar to *S. hammondii*, wet season residual energy would allow *C. australis* to remain dormant for very long periods, depending on other production costs, such as activity, reproduction and growth.

The energy available for *L. caerulea* and *L. dahlii* varied marginally between seasons. More energy was ingested in the wet season than in the dry season, but the dry season resting energy expenditure of *L. caerulea* and *L. dahlii* was 37% and 40%, respectively, of that in the wet season, which is comparable to that of *C. australis* (Table 6.2). Unlike *C. australis*, *L. caerulea* and *L. dahlii* do not use metabolic depression in the field. However, the dry season body temperatures of *L. caerulea* and *L. dahlii* were significantly lower than in the wet season (Table 6.2). Subsequently, the reduced energy expenditure resulted from the logarithmic relationship between body temperature and respiratory metabolism (Andrews and Pough 1985). In addition to this, the temperature dependence of digestion (Chapter Three) also influenced the dry season respiratory expenditure of *L. caerulea* and *L. dahlii*, producing estimations of 50% and 42% of wet season values respectively.

The effect of temperature on seasonal metabolism in *L. caerulea* and *L. dahlii* is similar to that found in other species of ectotherms that do not show metabolic depression in the field. For example, the Australian bluetongue lizard, *Tiliqua scincoides*, expends significantly less energy in the dry season (Christian et al. 2003). Of the energy saved during this season, 66% is the consequence of reduced activity and the other 34% is the consequence of reduced body temperature. Wet and dry season body temperatures were
similar for *C. australis* because the burrows used during aestivation were insulated from the low fluctuating dry season air temperatures by a soil barrier (Chapter Two). Thus, in contrast to *C. australis*, the reduction in respiratory expenditure for *L. caerulea* and *L. dahlii* resulted from seasonal differences in body temperature rather than from metabolic depression.

In the wet season, the respiratory energy expenditure of hypothetical free-ranging frogs was similar among species. This is because resting costs (SMR and RMR) and SDA at 30°C (average wet season body temperature) did not differ among species under laboratory conditions. However, in the dry season, the respiratory expenditure of *L. caerulea* and *L. dahlii* was greater than that of *C. australis*. *Litoria caerulea* and *L. dahlii* assimilate energy year-round, but *C. australis* feed only when active in the wet season. Therefore, *L. caerulea* and *L. dahlii* expended energy during digestion (SDA) year-round, but *C. australis* expended energy during digestion in the wet season only, thus further reducing dry season respiratory costs when dormant.

The seasonal energy flow of three ecologically different species of frog has provided a coarse estimate to compare and examine the physiological consequences of the wet and dry season conditions in northern Australia. The seasonally dependent daily energy flow of hypothetical frogs in the field, are neither complete nor entirely accurate. In particular, locomotory activity and activities associated with reproduction were not included. Thus, the daily available energy for frogs was overestimated. Nonetheless, the results obtained clearly indicate that different strategies are used by ecologically distinct species of frog to cope with the same environmental conditions.
6.6 Bibliography


Chapter Seven: Synthesis and Conclusions

Northern spadefoot toad
*Notaden melanoscaphus*
Chapter Seven: Synthesis and conclusions

The wet-dry tropics in the north of Australia experience both seasonal drought and flooding (Cook and Heerdegen 2001). Yet, over 30 species of frog living in this region are adapted to these dramatic changes in climatic conditions (Cogger 2000). In this dissertation three species were used to present case studies for three very different life histories (burrowing, terrestrial and aquatic). The physiological capacities of the selected species of frog appear to differ with respect to their ecological habits. This is despite of the fact that water and temperature have significant implications for the physiology of each species, and despite the fact that they all live in the same seasonal tropical climate.

The dissertation covers research that compares the behavioural ecology, digestive and respiratory physiology, gastrointestinal flexibility and energy flow of frogs between the wet and dry seasons. In general I hypothesised that the measured response of the burrowing frog would be more extreme than the terrestrial and aquatic frog to match differences among life histories.

The giant burrowing frog, *Cyclorana australis*, lives only in the tropical climatic zone of Australia. However, the traits of this species are similar to those of frogs living in desert climatic zones (Withers and Thompson 2000). *Cyclorana* living in both regions are dormant for prolonged periods, their activity is confined to rainy periods, they are fossorial and cocoon forming, they significantly adjust both digestive and respiratory physiology when dormant, and they assimilate large meals when active (van Beurden 1980, Larsen 1992). The high-energy intake and low energy expenditure of *C. australis* during active periods offset the negative energy balance during periods of inactivity. This annual pattern of energy expenditure is similar to that of related species of frog that live in arid regions (Seymour 1973).

*Litoria caerulea* and *Litoria dahlii* experience the same seasonal tropical climate as *C. australis*. However, unlike *C. australis*, the traits of *L. caerulea* and *L. dahlii* are more similar to those of frogs living in temperate climatic zones rather than to those of desert-dwelling frogs. *Litoria caerulea* and *L. dahlii* can be active throughout the year, but they use retreat sites at night more frequently in the dry season than in the wet season.
Consequently, the digestive and respiratory physiology of these two species is affected little by the seasonal conditions of the wet-dry tropics, and their gastrointestinal tract shows no seasonal changes, with respect to morphology and histology. Interestingly, however, these two species do moderately adjust their respiratory physiology in response to dormancy in laboratory conditions, which is similar to some temperate zone frogs. This ability may be utilised when conditions are more severe, such as when the wet season arrives late or is short. The low, but frequent, energy intake and high-energy expenditure of *L. caerulea* and *L. dahlii*, relative to that of *C. australis*, offsets wet and dry season energy flow, and thus these two species are able to endure dry season conditions.

The major ecological difference among these three species is that *C. australis* spends 6-7 months dormant underground, whereas *L. caerulea* and *L. dahlii* remain relatively active year-round. *Cyclorana australis* is able to spend long periods dormant because it forms a cocoon, which is in contrast to *L. caerulea* and *L. dahlii*. Cocoon formation is effective in markedly reducing water loss (Christian and Parry 1997). *Litoria caerulea* and *L. dahlii* are able to exploit conditions opportunistically when favourable. *Litoria caerulea* retreat to tree hollows when conditions are unfavourable. The high humidity inside tree hollows offers respite from ambient dry season conditions, and low body temperatures reduce energy expenditure. For *L. dahlii*, its aquatic habitats generally persist during the dry season when conditions are least favourable. Aquatic habitats generally dry out in the late-dry season when temperature and humidity are similar to those in the wet season. Thus, *L. dahlii* has only a few months in which their preferred habitat may be devoid of water resources.

The ecological differences among the three species of frogs provide different constraints on their physiology. Yet each species is able to solve the same problem, with respect to drought-like conditions, with different solutions. Although water and temperature directly influence the physiology of all three frog species, the physiological adaptations of each species were primarily related to their ecology.
Chapter Seven: Synthesis and conclusions

Management implications

The frogs in the wet-dry tropics of the Northern Territory are not considered threatened, with respect to their conservation status. However, regional Darwin has only recently been urbanised, relative to other regions of Australia, and our understanding of the ecology of native species of frogs in the wet-dry tropics is incomplete. One advantage of Darwin’s relatively recent foundation is that we can avoid the negative impacts of poor land management decisions that have been made by comparatively more established states and territories. Unfortunately, however, the tropical climatic zone has its own unique set of issues that do not occur elsewhere in Australia, but which may be related to problems faced in other tropical climatic zones in the world.

Fire plays an integral part in the savanna landscapes of the wet-dry tropics. Indigenous people have used fire practices for land management for centuries, and, more recently, non-Indigenous people use fire extensively. However, fire practices are changing over time, and, as a consequence, fire may be permanently altering landscapes. Little is known about the impacts of fire on frog populations (Driscoll and Roberts 1997) or the implications of fire for frog physiology. Fire was evident at most study sites during this research. For *C. australis*, fire occurred late in the dry season when frogs were aestivating in burrows underground and presumably insulated from the heat of the fire. Although no fires were noted at the study site of *L. caerulea*, this species lives in habitats that are prone to fire (open woodland and savanna). For *L. dahlii*, aquatic refuge sites often dry out in the late-dry season. Early dry season burns potentially do not threaten the status of *L. dahlii* when their aquatic habitats persist. However, late-dry season burns may impact upon this species when their aquatic habitats are dry.

Recently, the introduced toad, *Bufo marinus*, arrived in Darwin from Queensland. The impacts of this toad on native fauna are unclear (van Dam et al. 2002). However, *B. marinus* has the potential to affect native species of frog by competing for resources at all stages of development. Furthermore, the toxic secretions produced by the parotoid glands of this toad can be unappetising for many species of fauna and lethal for others.
(Covacevich and Archer 1975, Ingram and Covacevich 1990, Phillips et al. 2003), which can upset the balance of the entire food chain. Frogs may not only be potential prey items for the toad, but many native species of frogs eat other frogs, which may result in some deaths. This dissertation provides some base-line data of frog populations at various locations around Darwin before the arrival of *B. marinus*. This information may provide some indication of any effects of *B. marinus* on native frog species when they become established in Darwin.

Other natural resource management issues include the effects of habitat loss by development (roads, urban landscapes and agricultural industries), and the effects of chemical run off from mismanaged land practices (mining and agriculture) on wetland habitats, with respect to water quality (Hazell 2003). The preservation of natural habitat is important for the future of native frogs that are inseparably linked with other fauna species. The interrelationships between the physiology and ecology of frogs that have been examined in this study increase our understanding of how some species interact with their environment. It is hoped that this knowledge assists in providing effective strategies for the future management of frogs in their natural environment.

*Future directions.*

As with many other studies, the leading research questions tend to create more questions rather than provide all the answers. While this dissertation provides a framework from which to examine how three ecologically distinct species of frogs interact with their environment, this research develops only some aspects of frog eco-physiology in the wet-dry tropics. Obviously the number of species examined needs to be increased to provide more general comparisons among frogs with different life histories. Of interest and complementary to this research would be further research on the seasonal physiological adjustments of non-cocoon forming, burrowing species, of other terrestrial species of *Hylidae*. In addition to this, determining refuge sites and examining seasonal movements (sit and wait, migratory behaviour) may improve our understanding of how
some species endure dry season conditions. A better understanding of the microclimatic conditions of dry season refuge sites would provide important information that has implications on frog physiology (Huey 1991). Given the differences between some of the laboratory and field results, with respect to seasonal metabolism, it would be interesting to study *L. caerulea* under more extreme seasonal conditions, with respect to rainfall, humidity and temperature, such as further inland in the grassland climatic zones of Australia. The effects of fire on the frogs would be interesting from an eco-physiological perspective, but in addition to this, the impacts of mining and development on frog populations are also potentially important for the effective management of frogs in the wet-dry tropics.
7.1 Bibliography


