

**Electrocardiography of the Normal
Inland Bearded Dragon
(*Pogona vitticeps*)**

**Submitted in part fulfilment of the requirements for the
Royal College of Veterinary Surgeons (RCVS) Diploma in
Zoological Medicine**

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Abstract

Electrocardiograms (ECGs) were obtained from fifty two healthy inland bearded dragons (*Pogona vitticeps*) in order to establish a repeatable, well-tolerated, non-invasive technique for recording ECGs and establish reference ranges for the normal captive inland bearded dragon. The following reference ranges were calculated. Heart rate was 24-170 beats per minute; P wave duration was 30-100mS and amplitude was 0.01-0.06mV. The P-R interval was 75-243mS, SV wave duration was 30-125mS and amplitude was 0.01-0.07mV, the SV-R interval was 130-440mS and the duration of the QRS complex was 60-120mS. The amplitude of the R wave was 0.08-0.57mV; the amplitude of the S wave was 0.01-0.13mV. The Q-T interval was 120-980mS. The amplitude of the T wave was 0.01-0.14mV. Mean electrical axis was $+60^{\circ}$ to $+110^{\circ}$. Heart rate was significantly increased and the Q-T interval was significantly decreased in gravid females. Evidence of variation due to sex and snout-vent length was not found to be significant but there was a significant reduction in heart rate with increasing bodyweight. The ECG recording technique used in this study for dragons was well tolerated and shown to be repeatable.

Introduction

Cardiac disease in reptiles is under researched ante-mortem but retrospective pathological studies report an incidence of cardiovascular problems between 6-13% (Reavill and Schmidt 2009; Garner 2009). A retrospective pathological review of diseases in lizards of the family agamidae by Reavill and Schmidt (2009) revealed an incidence of cardiovascular pathology of 11% in *Uromastyx spp.*, 13% in water dragons (*Physignathus cocincinus*) and were described as frequent in bearded dragons (*Pogona vitticeps*). Commonly reported lesions were soft tissue mineralization or systemic infection with bacteria and fungi. A retrospective pathological review of diseases in geckos (Garner 2009) revealed an incidence of cardiovascular pathology in 6% of submissions. Clinical cases of cardiovascular disease are reported in the literature, usually as individual case reports where the diagnosis has been obtained post-mortem, Clippinger *et al* (2000), Frye (1991a), Hruban *et al* (1992), Innis (2006), Jacobson *et al* (1979), Jacobson and Kollias (1986), Obendorf *et al* (1987), Rush *et al* (1999) and Schuchman and Taylor (1970).

The lack of studies documenting normal species-specific cardiac parameters for radiography, echocardiography and electrocardiography makes the diagnosis of cardiovascular disease in the reptile patient difficult. The lack of literature would be expected to be associated with the difficulty in recognising the clinical signs of cardiovascular disease in reptiles, a group recognised to mask illnesses until they are advanced (Wellehan and Gunkel 2004). The low metabolic rate and inactive lifestyle of many captive reptiles allows cardiovascular disease to become advanced before clinical signs are identifiable by either the owner or clinician.

Clinical signs of reptile cardiovascular disease are frequently non-specific and rarely, if ever, pathognomic (Murray 2006). Swelling in the area of the heart, pleural or peripheral oedema, ascites, cyanosis and ecchymoses are suggestive of an underlying cardiovascular disease. Other nonspecific clinical signs, including generalized weakness, inactivity, exercise intolerance, anorexia, weight loss, change in skin colour, and sudden death are also reported in reptiles suffering from cardiovascular problems (Murray 2006).

Various aetiologies and presentations of cardiac-associated disease have been described in reptiles. Reptile cardiac disease may be primary including idiopathic cardiomyopathy, congenital defects and degenerative disease or secondary to metabolic or nutritional diseases

(Murray 2006). Systemic infectious and parasitic diseases may also affect the cardiovascular system and thus manifest clinical signs referable to this system (Murray 2006). Infectious causes of parasitic, bacterial and viral origin have been described as well as cases of nutritional deficiency (vitamin E), atherosclerosis, arteriosclerosis, congenital defects, aneurism and neoplastic disease.

Inadequate husbandry is the most likely common predisposing factor for the development of cardiovascular pathology in captive reptiles resulting in chronic stress, immune suppression and malnutrition (Paré *et al* 2006). Bacterial septicaemia appears common in reptiles and predisposes to secondary endocarditis and myocarditis; organisms isolated include *mycoplasma sp.* in captive American alligators, *Alligator mississippiensis* (Clippinger *et al* 2000), *Escherichia coli* in a green iguana, *Iguana iguana* (Innis 2000), *Salmonella arizona* and *Corynebacterium sp.* in a Burmese python, *Molurus bivittatus* (Jacobson *et al* 1991), *Chlamydia sp.* in puff adders, *Bitis arietans* (Jacobson *et al* 1989a), *Flavobacterium meningosepticum* in a Barbour's map turtle, *Graptemys barbouri* (Jacobson *et al* 1989b), *Mycobacterium sp.* in a Frilled Lizard, *Chlamydosaurus kingi* (Murray 2006), *Vibrio damsela* in a leatherback sea turtle, *Dermochelys coriacea* (Obendorf 1987), and *Salmonella enterica arizonae* in a Dumerili's boa, *Acrantophis dumerili* (Schilliger *et al* 2003); bacterial endocarditis was suspected in a Burmese python (*Python molurus bivittatus*) with sinoatrial and atrioventricular valvular deficiencies but an organism was not cultured (Schilliger *et al* 2010b).

Adenovirus-like particles have been identified in a savannah monitor (*Varanus exanthematicus*) with myocarditis (Jacobson and Kollias 1986) and in a rosy boa (*Lichanura trivirgata*) with hepatitis and endocarditis (Schumacher *et al* 1994).

Adult filarid nematodes of the genera *Oswaldofilaria*, *Foleyella* and *Macdonaldius* are capable of living in the vascular system where they release microfilaria into the circulation which can cause ischaemic necrosis if they obstruct peripheral capillaries (Murray 2006). Adult digenetic spirorchid flukes can be found in the heart and great vessels of a variety of reptilian species, though turtles are most commonly affected (Jacobson 1986). Adults may cause some focal endothelial hyperplasia though their eggs may be of more clinical concern if they occlude terminal vessels in major organs such as gastrointestinal tract, spleen, liver,

heart and kidneys (Jacobson 1986). Cardiovascular lesions may include mural endocarditis, arteritis, and thrombosis, and are frequently associated with aneurism formation (Kik and Mitchell 2005).

Nutritional disease may have direct and substantial effects on the cardiovascular system. Delayed cardiac muscular repolarisation, as shown by increased S-T and Q-T intervals in the electrocardiogram (ECG) in mammals with hypocalcaemia probably occurs in reptiles as well (Murray 2006). Secondary nutritional hyperparathyroidism is one of the most common diseases reported in herbivorous and insectivorous reptiles offered a calcium-deficient diet (Kik and Mitchell 2005). Aortic rupture associated with mineralisation of the aorta attributed to secondary nutritional hyperparathyroidism is reported in a Chinese water dragon, *Physignathus concincinus* (Kik and Mitchell 2005). Diets high in calcium and vitamin D3 have also been linked to calcification of the tunica media of large blood vessels (Murray 2006). Obesity resulting in hyperlipidaemia and cardiovascular disease is reported in rattlesnakes (Bauer and Jacobson 1989) and atherosclerosis in a central bearded dragon (*Pogona vitticeps*) presenting with a pericardial effusion was attributed to an inappropriate diet consisting predominantly of mealworms (*Tenebrio molitor*), king mealworms (*Zophobas morio*) and wax moth larvae (*Galleria mellonella*) (Schilliger *et al* 2010a). A diet of soft foods was implicated as the cause of periodontal disease in a population of captive bearded dragons, (*Pogona vitticeps*) resulting in septicaemia and hepatic thrombosis from jaw osteomyelitis (Redrobe and Frye 2001).

Cardiac disease caused by vitamin E deficiency has been described in a variety of reptilian species (Frye 1991b). Possible lysosomal storage disease was attributed to the cause of cardiomegaly in a Deckert's rat snake (*Elaphe obsoleta deckerti*) with inclusion bodies in the heart and several other organs (Jacobson *et al* 1979).

Published reports describing cardiac and/or vascular pathology as the primary cause of illness or death in reptiles included carotid artery and/or aortic aneurism in several bearded dragons (Barten *et al* 2006 and Sweet *et al* 2009) and in a Burmese python, *Python molurus* (Rush *et al* 1999), arteriosclerosis in a green iguana, *Iguana iguana* (Schuchman *et al* 1970), cardiomyopathy in a king snake, *Lampropeltis calligaster rhombomaculata* (Barten 1980 and a Black king snake, *Lampropeltis niger* (Wagner 1989) and myocardial abscess and haemopericardium in a green iguana, *Iguana iguana* (Innis 2000).

Cardiac valvular insufficiency is reported in a Burmese python, *Python molurus* (Schilliger *et al* 2010b) and a boa constrictor, *Boa constrictor* (Kik and Mitchell 2005). Two juvenile ball pythons (*Python regius*) undergoing blood pressure experiments were found to have bifid ventricles (Jensen and Wang 2009) and a green iguana (*Iguana iguana*) was diagnosed with aortic stenosis and atrioventricular dilatation (Clippinger 1993).

Pericardial effusion associated with fibrinous epicarditis and atrial dilatation has been reported in a spur-thigh tortoise (*Testudo graeca*) presented with post-hibernation anorexia and peripheral and pulmonary oedema (Redrobe and Scudamore 2000).

Primary myocardial neoplasms reported in the literature include haemangioma in a corn snake, *Pantherophis guttatus* (Stumpel *et al* 2012), haemangiosarcoma in a copperhead snake (*Agkistrodon contortrix*) and rhabdomyosarcoma in a black king snake, *Lampropeltis getula nigrata* (Catão-Dias and Nichols 1999).

Individual case reports and case series where the heart is identified as being affected but is not the primary target organ include lymphoma in *Uromastyx sp.* (Greek 2005), *Uromastyx aegyptius* (Gyimesi *et al* 2005) and in a loggerhead turtle, *Caretta caretta* (Oros *et al* 2001); leukaemic cells were identified in the epicardium and myocardium of a Honduran milk snake (*Lampropeltis triangulum hondurensis*) and a broad banded copperhead, *Agkistrodon latiscincus* subjected to post-mortem examination (Hruban *et al* 1992). Innes (2006) described cardiac pathology in 3 out of 17 Impressed tortoises (*Manouria impressa*) subjected to a post-mortem examinations following euthanasia (4 cases) and death from natural causes (14 cases). Infectious agents reported include herpes virus infection in Hermann's tortoises, *Testudo hermanni* (McArthur 2001) and a marginated tortoise, *Testudo marginata* (Hunt 2006), *Flavobacterium meningosepticum* infection in a Barbour's map turtle (Jacobson *et al* 1989b), chlamydiosis in green sea turtles, *Chelonia mydas* (Homer *et al* 1994) and systemic microsporidiosis in a bearded dragon, *Pogona vitticeps* (Cole 2001).

The approach to the investigation of cardiovascular disease in reptiles is similar to that in mammalian species such as the dog and cat. Auscultation is of value to record heart rate and rhythm and cardiac murmurs which have been described in reptiles with cardiac diseases (Clippinger 1993; Rishiniw and Carmel 1999). Radiography, echocardiography and electrocardiography are the mainstay of investigation of cardiovascular disease in mammalian

species and may be applied to reptiles with some limitations. A standardized approach to two-dimensional echocardiography has been proposed in snakes (Snyder *et al* 1999). In reptile species where the heart is located cranially within the pectoral girdle such as the bearded dragon however, echocardiography is limited due to surrounding bony structures, especially the sternum (Mathes and Wachsmann 2011); radiography has similar limitations (Murray 2006) (see **Figures 1-3**).

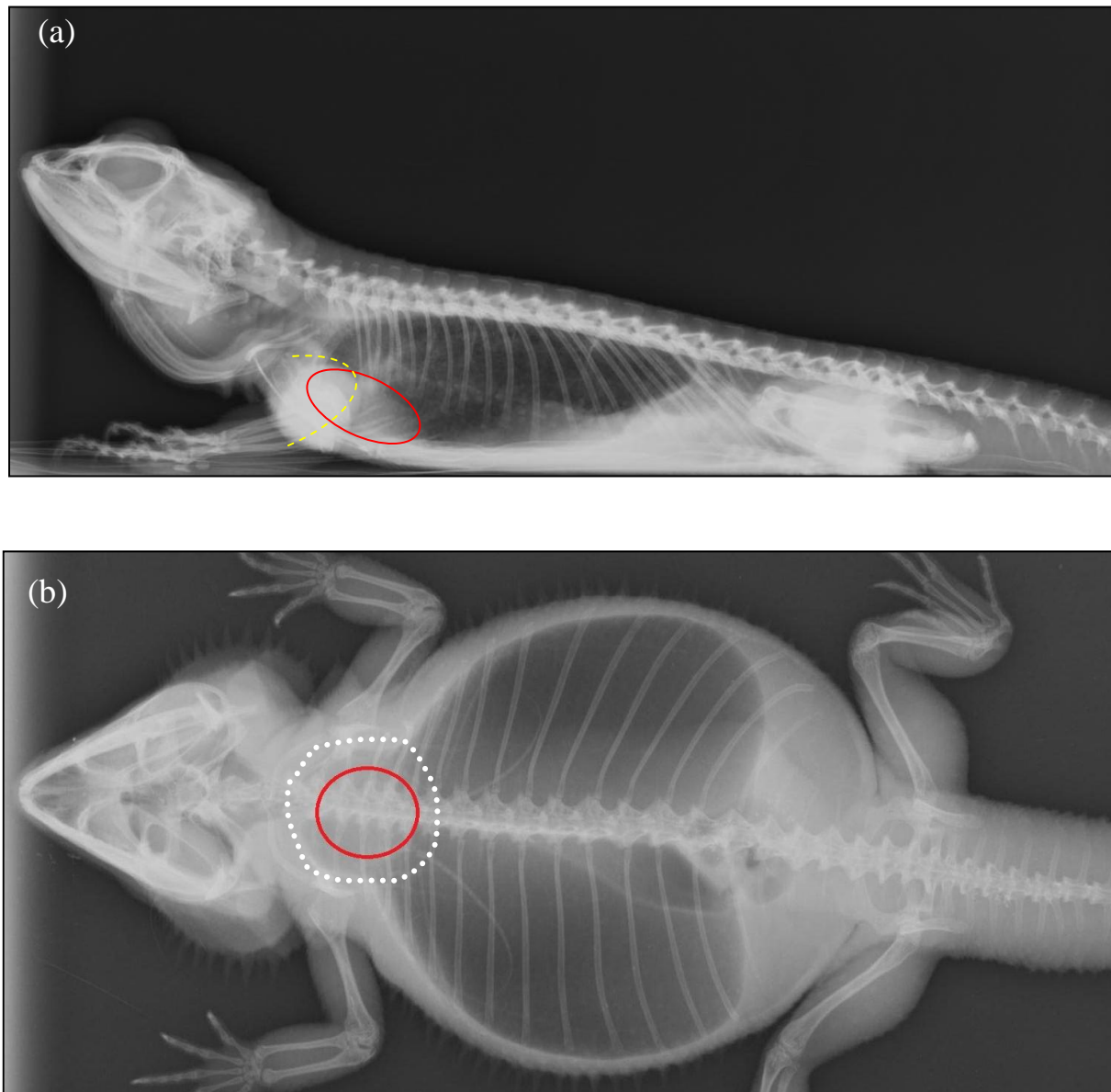


Figure 1: Radiograph of a healthy bearded dragon (*Pogona vitticeps*) demonstrating superimposition of structures overlying the heart. Horizontal-beam lateral view (a), dorsoventral view (b).

Solid red line = heart, yellow dotted line = forelimb, white dotted line = outline of sternum

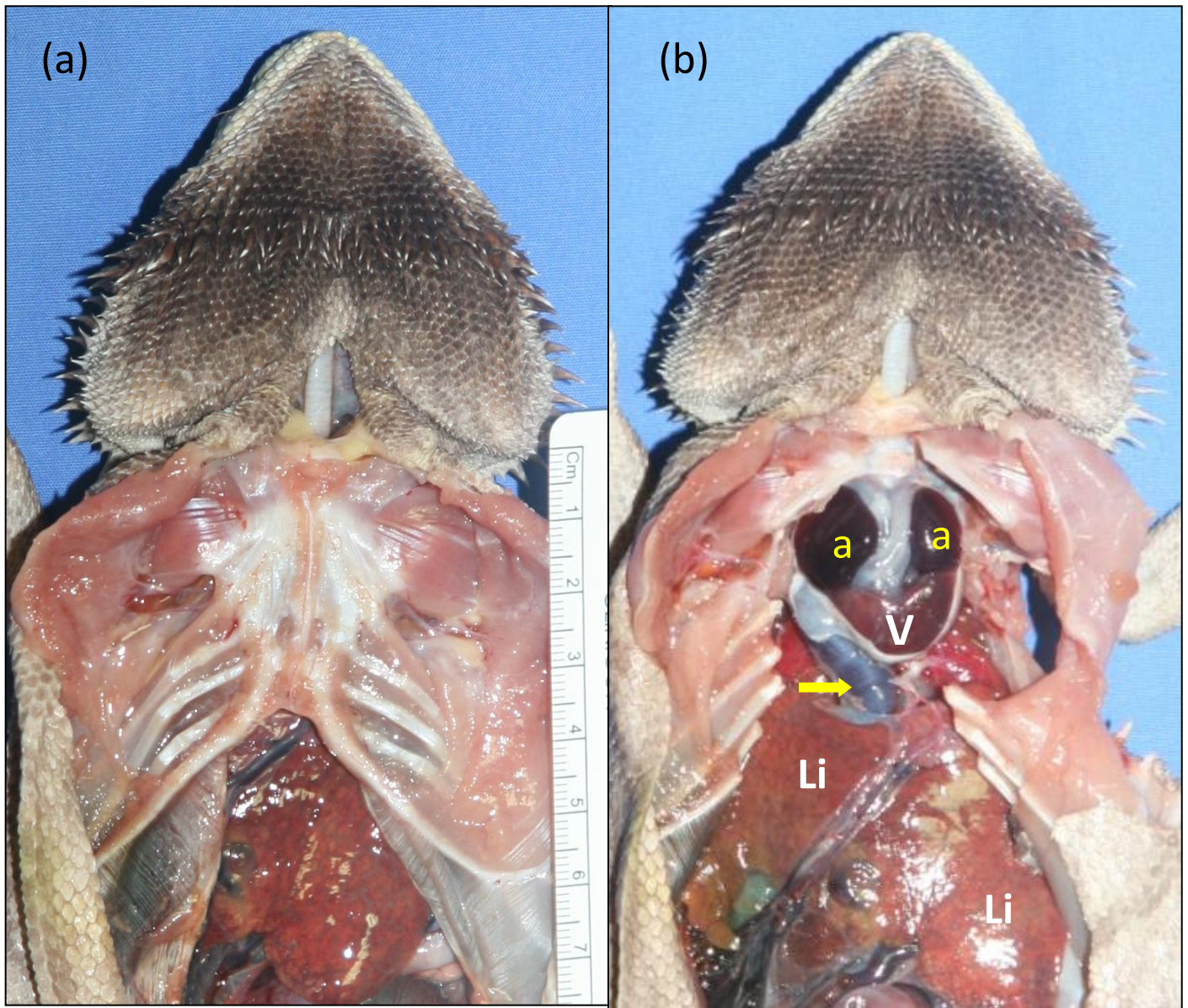


Figure 2: Ventral view of a bearded dragon (*Pogona vitticeps*) demonstrating heart and overlying structures;

(a) Skin and pectoral musculature reflected to reveal sternum.

(b) Sternum and pericardium removed to reveal heart

Atria (a), ventricle (V), liver (Li), post-caval vein (yellow arrow)

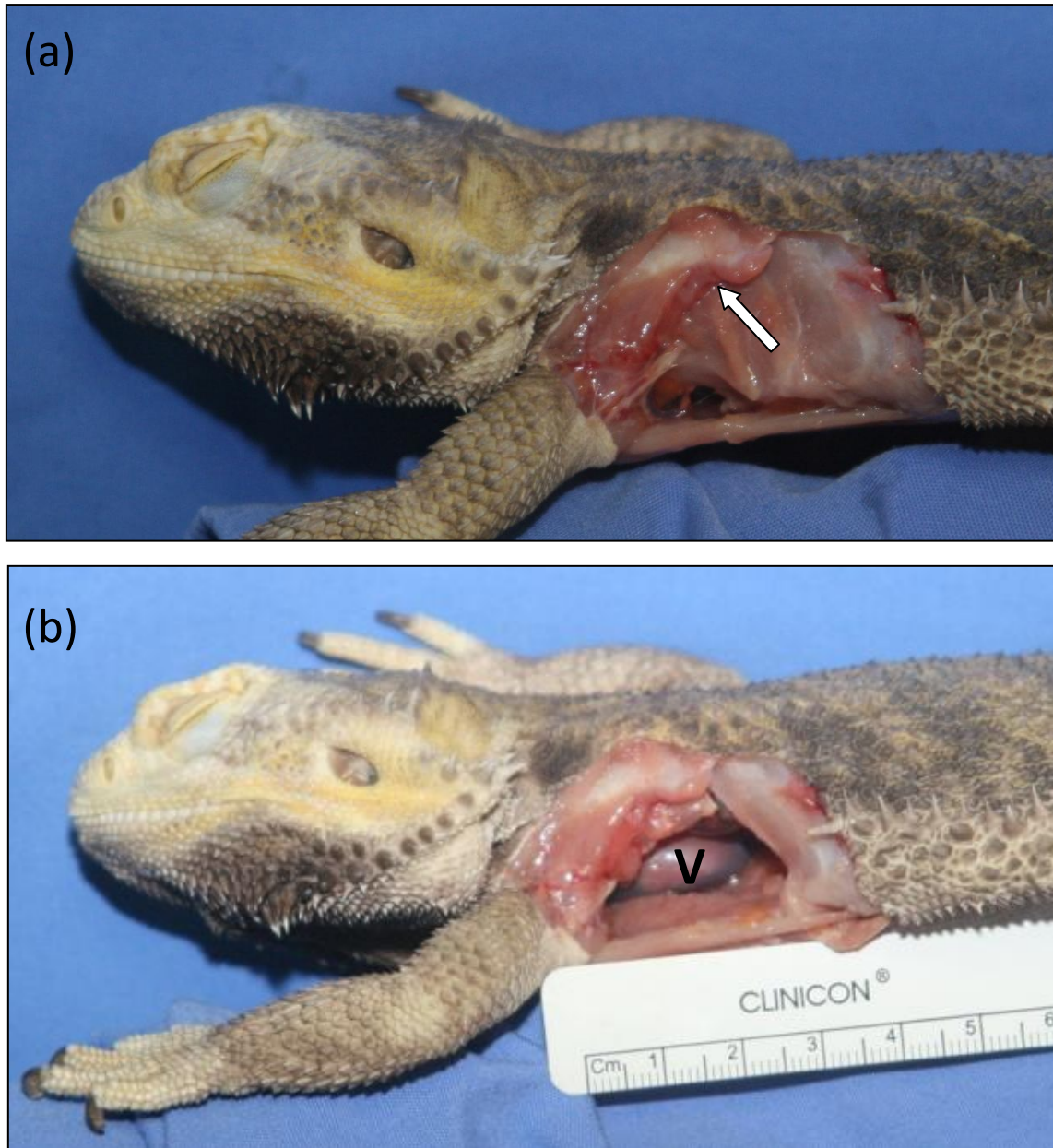


Figure 3: Lateral view of a bearded dragon (*Pogona vitticeps*) demonstrating the position of the heart and overlying structures;

- (a) Skin and some shoulder musculature removed to reveal ribs and scapula (white arrow)
- (b) Ribs removed to reveal ventricle of heart (V); the heart base is obscured by the forelimb

Described limitations for electrocardiography include low potentials, interference from muscle activity obscuring waveforms, limited sensitivity associated with available equipment and a lack of published reference ranges (Girling and Hynes 2004, Kik and Mitchell 2005 and Murray 2006). Where reference ranges do exist they may not be reproducible because lead placement and temperature have not been described or made clear (McDonald 1976). Even slight alterations in lead placement may alter an ECG; for that reason McDonald (1976) states that electrode positions used should be precisely identified.

In cardiovascular disease cases diagnosed ante-mortem echocardiography and radiographic studies were the modalities usually employed (Innis 2000, Jacobson *et al* 1991, Redrobe and Scudamore 2000, Schilliger *et al* 2010a and 2010b and Stumpel *et al* 2012) with the frequency of arrhythmias or electrocardiographic abnormalities poorly documented. Clinical application of electrocardiography is infrequently documented in the literature; Clippinger (1993) found widened QRS complexes and a lengthened Q-T interval in a green iguana (*Iguana iguana*) with aortic stenosis and atrioventricular dilatation; Rishniw and Carmel (1999) demonstrated tall, wide QRS complexes in a carpet python with atrioventricular valve insufficiency and Kik and Mitchell (2005) report an increased P-R interval and decreased Q-T interval in a boa constrictor (*Boa constrictor*) with atrioventricular valve insufficiency.

Electrocardiography is an atraumatic, inexpensive and useful technique for gaining information about the heart in dogs and cats (Tilley 1992). Recording of electrocardiograms (ECGs) requires limited expertise in comparison with echocardiography.

The normal ECG has been fully described in only a few reptiles including the green iguana, *Iguana iguana* (Albert *et al* 1999), brown tree snake, *Boiga irregularis* (Anderson *et al* 1999), desert tortoise, *Gopherus agassizii* (Belcher *et al* 2009), leatherback sea turtle, *Dermochelys coriacea* (Harms *et al* 2007), American alligator, *Alligator alligator* (Heaton-Jones and King 1994), red-eared slider, *Trachemys scripta elegans* (Holz and Holz 1995) and Gomeran Giant Lizard, *Gallotia bravoana* (Martinez-Silvestre *et al* 2003); Mullen (1967) describes ECG data for 50 species of the order squamata.

The reptile ECG is similar to that of the mammal; there is the P wave (depolarisation of atrial tissue), the QRS complex (ventricular depolarisation) and the T wave (ventricular repolarisation) (McDonald 1976). A sinus venosus wave (SV wave) has been described

originating in the upper portion of the caudal vena cava and sinus venosus and corresponds to the depolarisation of the sinus venosus (Mullen 1967). Atrial contraction proceeds from the sinus venosus to the left and caudally, ventricular contraction is initiated at the base of the ventricle and proceeds towards the apex with a tendency to travel to the left; ventricular repolarisation is from base to apex (Valentinuzzi et al 1969) (**Figure 4**).

The SV wave is documented as preceding the P wave by McDonald (1976), Cook and Westrom (1979), and Martinez-Silvestre *et al* (2003) but Mullen (1967) states that it precedes the T wave suggesting there may be species variability and/or different origins of the wave. Mullen (1967) recorded the SV wave in approximately 25% of snakes and less than 10% of lizard species but Girling and Hynes (2004), state that the SV wave is rarely encountered. The disparity may be explained by the low amplitude of the SV wave, typically 0.1mV or less (Mullen 1967), species variability or differing recording equipment and/or electrode placement. Being of low amplitude, the SV wave may be obscured by background interference associated with skeletal muscle activity (McDonald 1976).

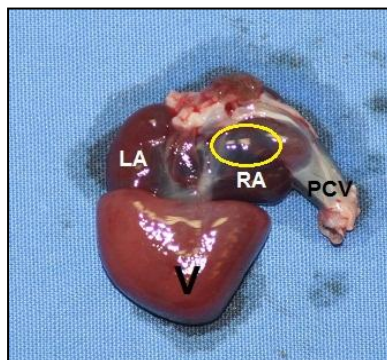


Figure 4a: Heart from a bearded dragon (*Pogona vitticeps*); dorsal view demonstrating the position of the sinus venosus dorsal to the right atrium

Left atrium (LA), right atrium (RA), ventricle (V), sinus venosus (yellow oval), post-caval vein (PCV)

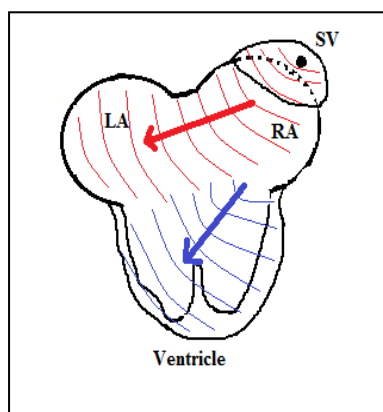


Figure 4b: Depolarisation waves in the squamata heart from the dorsal view.

Atrial depolarisation (red arrow), ventricular depolarisation (blue arrow), sinoatrial node (●), sinus venosus (SV), right atrium (RA), left atrium (LA)

Adapted from Cook and Westrom (1979)

In those lizards with a heart located at the level of the pectoral girdle (e.g. skinks, iguanas, chameleons, bearded dragons and water dragons) the use of electrodes placed in the cervical region, rather than on the forelimbs is preferable (Mullen 1967 and Murray 2006).

The reptilian heart rate is dependent on a number of non-cardiac factors and varies with size, metabolic rate, season, respiratory rate, physiological conditions, sensory stimuli but especially with body temperature (Mader and Wyneken 2000; Murray 2006). The heart rate is dependent on the body temperature and the intervals such as P-R segment and Q-T segment are dependent on the heart rate (White 1976). Clinically relevant parameters for the ECG of the American alligator (*Alligator mississippiensis*) collected at 25°C have been published and demonstrated ECG parameters to be dependent on body temperature, body size, age and state of excitement (Heaton Jones and King 1994).

Sedgwick (1991) suggested that the heartbeat frequency (hbf) in reptiles maintained within their preferred optimum temperature zone can be predicted by the following formula:

$$\mathbf{hbf = 33.4 (Wt_{kg})^{-0.25}}$$

Wt_{kg} = weight in kilograms; 33.4 is a constant

The formula uses allometric scaling to predict heart rate and can help determine, in conjunction with ECG waveform and interval measurements, whether an ECG represents normality or a pathological condition.

The aim of this study was to establish a well-tolerated, repeatable technique for obtaining ECG recordings from healthy inland bearded dragons (*Pogona vitticeps*) using the standard limb lead system and to establish a normal reference range.

Materials and methods:

Animals

Fifty two healthy, captive inland bearded dragons (*Pogona vitticeps*) from a large breeding establishment were studied. There were twenty two males and thirty females of which six were gravid. Age ranged from 4 to 36 months (gravid females were all 30 months) and bodyweight from 66g to 517g. Forty nine of the dragons were of normal skin type and three were genetic mutations with reduced or absent scales (two 'leatherbacks' and one 'silkback') (**Figure 5**); Two dragons (one male and one female) were undergoing ecdysis.

Husbandry was deemed appropriate for the species; adults were kept in small groups of between three and 6 individuals, and juveniles in groups of 5 to 10 individuals in open-topped enclosures. A daytime temperature gradient of 26 – 32°C was provided with a temperature drop of 5°C overnight. An elevated daytime basking area of 45°C was provided by a full spectrum reptile lamp (Arcadia[®] D3 UV basking light). A photoperiod of 12hrs daylight and 12 hours darkness was provided. Diet consisted of gut-loaded crickets dusted in calcium carbonate and ad-lib fresh greens daily. Constant access to a water dish was provided; in addition the dragons were given a warm-water bath once or twice weekly.

A physical examination was performed on each individual to ensure the individuals were healthy. Cardiovascular examination consisted of heart rate and rhythm using crystal doppler and stethoscope. Only animals without evidence of systemic and heart disease were included in the study. The ECGs were carried out as part of a routine health check.

Cloacal temperature was measured using a digital probe (SureTemp Plus[®]; Welch Allyn) inserted per cloaca to the level of the pelvis, bodyweight was recorded using a digital scale (Kern Economic Precision Balance –PCB 1000-1) and snout vent length was measured using a standard centimetre ruler with the dragon held in full extension. Ambient air temperature was measured using a digital probe (810-210; etiltd.com). Snout-vent length, cloacal temperature and bodyweight were recorded immediately after the ECG recording to minimise the effects of stress on the recording.



Figure 5: Different ‘breeds’ of bearded dragon used in the study

Normal type (top),

Leatherback (middle)

Silkback (bottom)

Materials and Methods

Electrocardiography – Technique

Atraumatic, crocodile clip electrodes (Vetronic Services Ltd), were applied to the skin using a modified Einthoven lead system as described for squamata by Mullen (1967), Valentinuzzi *et al* (1969) and Albert *et al* (1999). Two different configurations were evaluated on each individual. In the first configuration (lead configuration 1) the cranial electrodes were placed in the lateral cervical region on a line ventral to the ear scale (red lead on the right, yellow lead on the left) and the caudal electrodes were placed on the lateral body wall at a point just caudal to the elbow when the elbow was held adducted and flexed against the body wall, immediately below the row of lateral spines (green lead on the left, black on the right) (**Figures 6 and 9**). In the second configuration (lead configuration 2) the cranial electrodes were placed as in configuration 1 but the caudal electrodes were placed on the lateral body wall at a point just cranial to the stifle when the stifle has held adducted and flexed against the body wall, directly over the lateral spines (**Figures 7 - 9**).

The animal was allowed to settle and assume a relaxed posture once the clips had been applied. Coupling gel (ECG Gel; Konix[®]) was applied to the skin five minutes before the clips were applied to improve skin contact and eliminate air spaces by allowing the gel to penetrate the keratin skin layer.

Recording of the ECG commenced 30 seconds after the lizard had settled into a relaxed position and had become inactive. One minute of six-lead (bipolar I, II, III and augmented unipolar aVR, aVL, aVF) ECG recordings were taken with both lead configurations from each individual using a digital ECG recorder (CardioStore; Vetronics Services Ltd). Lead configuration 1 (LC1) was applied before lead configuration 2 (LC2) in half of the subjects and after LC2 in the other half of the subjects in an alternate manner.

A total of ninety-eight ECG traces (49 traces each of LC1 and LC2) were obtained from the fifty-two individual bearded dragons; three individuals would not tolerate application of LC1 and another three would not tolerate application of LC2. No form of physical restraint, sedation or anaesthesia was used. Dragons typically adopted a resting posture with head and neck raised and the forelimbs extended (**Figures 6 - 9**) though three individuals adopted a lying posture (**Figure 10**).

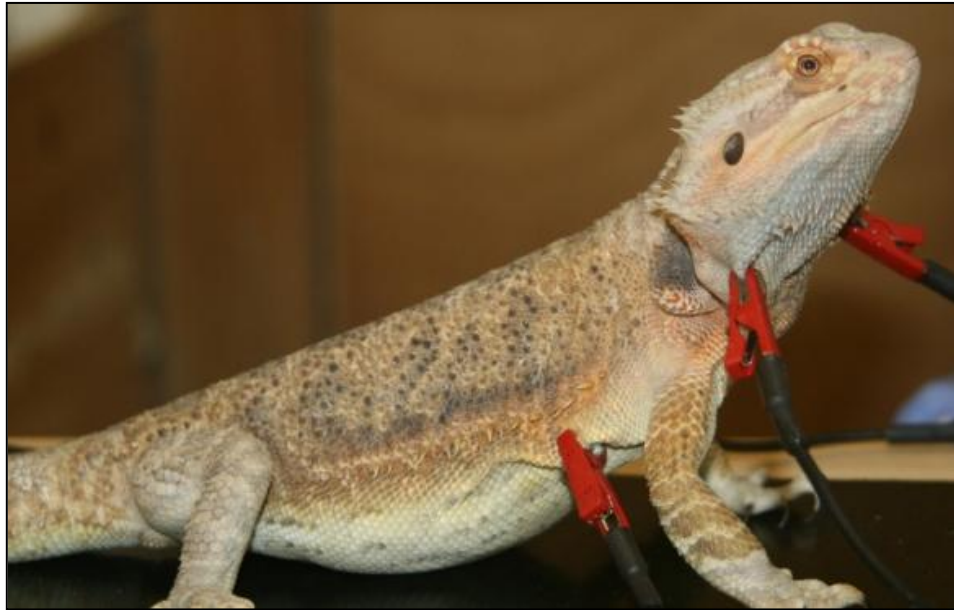


Figure 6: ‘Lead configuration 1’ - subject is adopting the typical resting posture. Cranial electrodes are attached to the cervical skin, ventral to the tympanic scale (red electrode on right, yellow electrode on left); caudal electrodes are attached to the skin immediately ventral to the lateral spines at the point just caudal to where the elbow would touch the body when held in adduction (green electrode on left and black electrode on right).



Figure 7: ‘Lead configuration 2’ - subject is adopting the typical resting posture. Cranial electrodes are attached to the cervical skin, ventral to the tympanic scale (red electrode on right, yellow electrode on left); caudal electrodes are attached to the skin overlying the lateral spines at the point just cranial to where the stifle would touch the body when held in adduction (green electrode on left and black electrode on right).



Figure 8: Dorsal view of a subject in the typical resting posture with the electrodes placed in 'lead configuration 2'



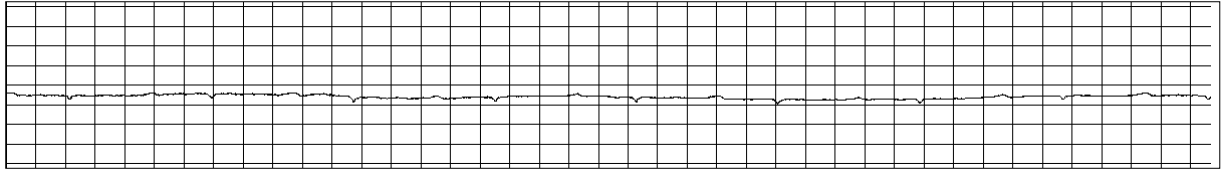
Figure 9: Cranial view of the subject in figure 8.



Figure 10: ‘Lead configuration 1’ - subject is adopting a lying posture. Cranial electrodes are attached to the cervical skin, ventral to the tympanic scale (red electrode on right, yellow electrode on left); caudal electrodes are attached to the skin immediately ventral to the lateral spines at the point just caudal to where the elbow would touch the body when held in adduction (green electrode on left and black electrode on right).

Post-recording digital enhancement and enlargement of the ECG using the CardioStore software improved accuracy and reduced errors when measuring small complexes (**Figures 11 and 12**). All recordings were filtered for electrical interference (50 Hz).

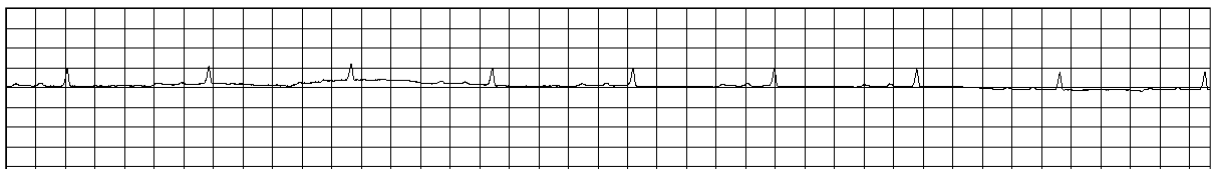
Lead I



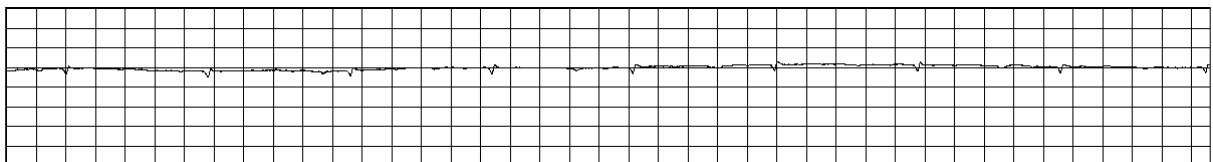
Lead II



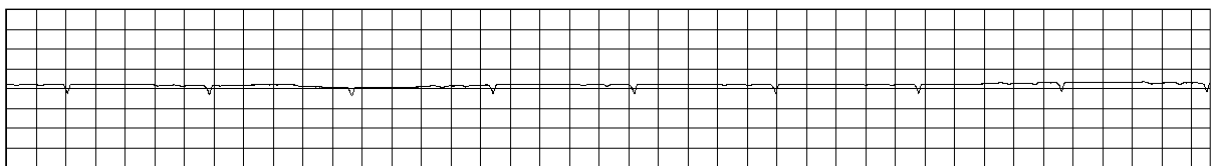
Lead III



aVR



aVL



aVF

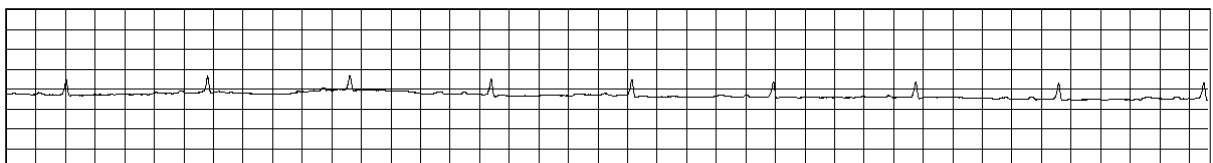
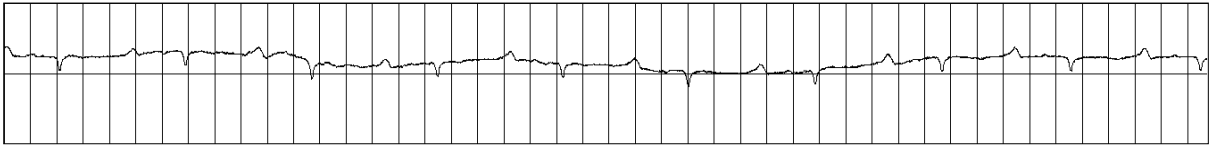
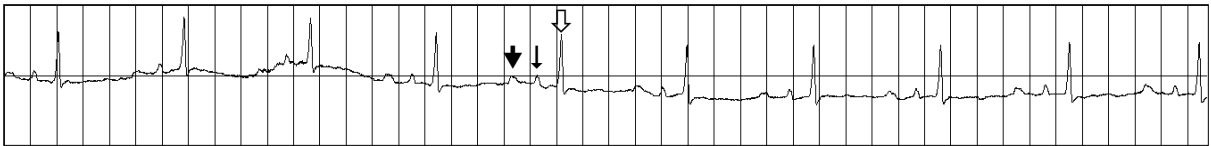


Figure 11a: An example of a printed ECG trace from a digital recording of a healthy bearded dragon (*Pogona vitticeps*) as seen with no manipulation; waveforms are of low amplitude and difficult to analyse accurately, in some areas the trace is almost obscured by the grid lines. Heart rate = 65bpm (Recorded at 25mm/sec and 0.5mV/cm). ↓ = P wave; ↓ = R wave; ▼ = T

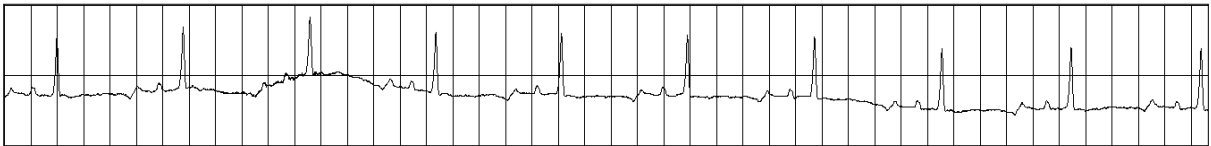
Lead I



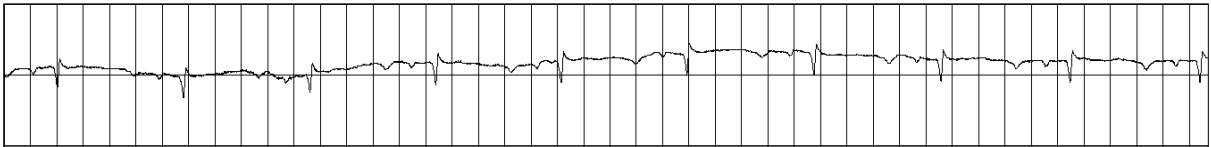
Lead II



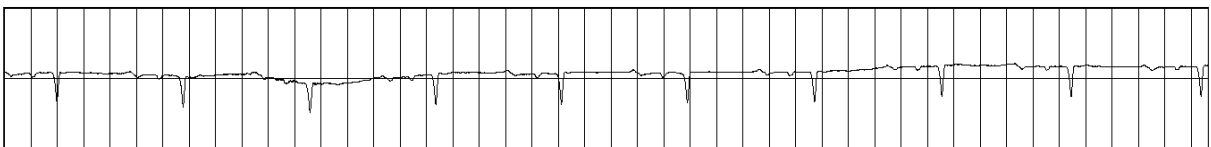
Lead III



aVR



aVL



aVF

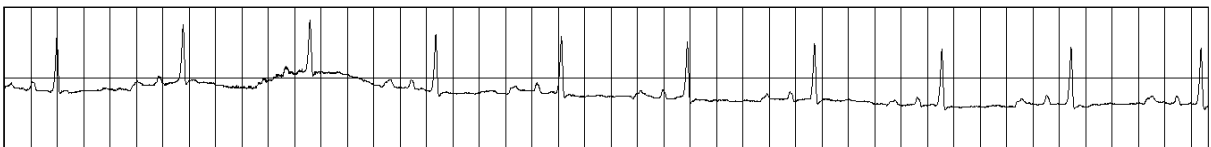


Figure 11b: The same recording as in **Figure 11a** has been digitally expanded to allow easier identification and measurement of the waveforms; ↓ = P wave; ⇓ = R wave; ▼ = T wave; SV waves are not distinguishable on this trace

Lead II

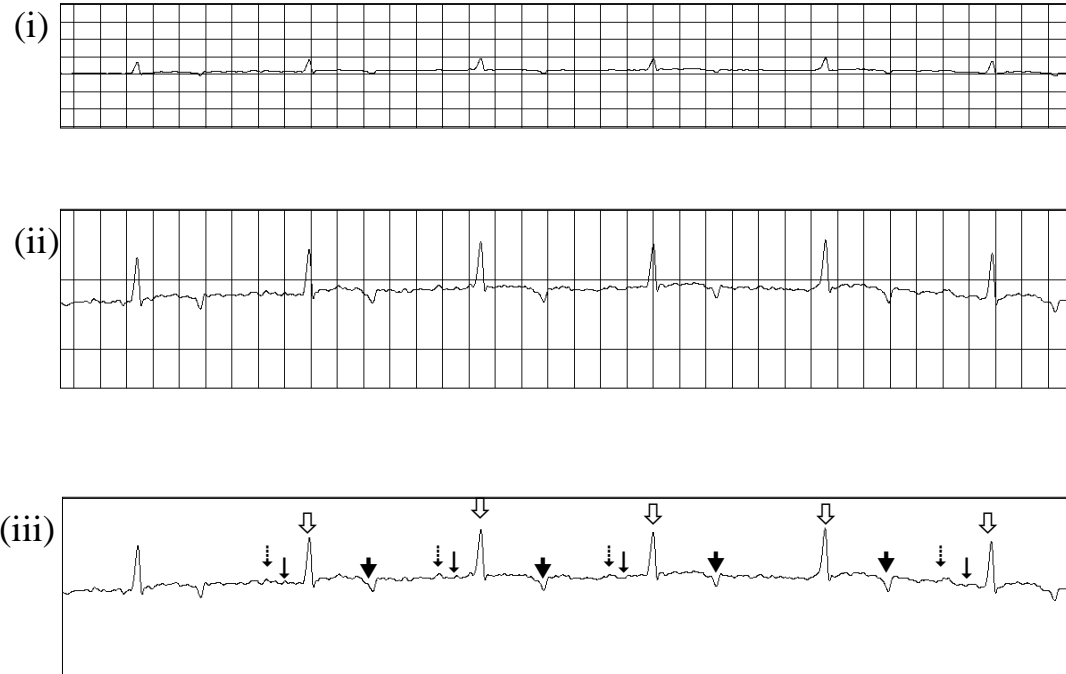


Figure 12: Lead II ECG trace (25mm/sec, 0.5mV/cm) of a healthy bearded dragon (*Pogona vitticeps*):

- (i) Unaltered trace with ECG gridlines
- (ii) Digitally enhanced trace (i) with gridlines
- (iii) Same trace as (ii) with gridlines removed

Without digital expansion only the R and T waves are discernible. In trace (iii) SV and P waves are discernible but only in 4 of the 5 complexes shown

↓ = SV wave; ↓ = P wave; ↑ = R wave; ↓ = T wave; Heart rate = 45bpm

Electrocardiography – Measurements

Measurements from lead II were made from 5 successive cycles and the mean calculated. Where possible, the following parameters were measured: heart rate, R-R interval, P wave amplitude and duration, P-R interval, SV wave amplitude and duration, SV-R interval, QRS duration, R wave amplitude, S wave amplitude, Q-T interval and T wave amplitude. Due to the difficulty in identifying the beginning and end of the T wave in most instances, the T wave duration was not measured and the Q-T interval was taken from the beginning of the QRS complex to the peak of the T wave. Q waves were difficult to identify from the beginning of the R wave and were not reliably measured; where no Q wave was identified, the beginning of the QRS complex was taken to be from the first deflection from the baseline after the P wave. Measurements taken are illustrated in **figure 13**.

The frontal mean electrical axis (MEA) was determined where possible by identifying an isoelectric lead, otherwise it was calculated from the sum of the amplitude of positive and negative deflections in lead I and III as described by Tilley (1992).

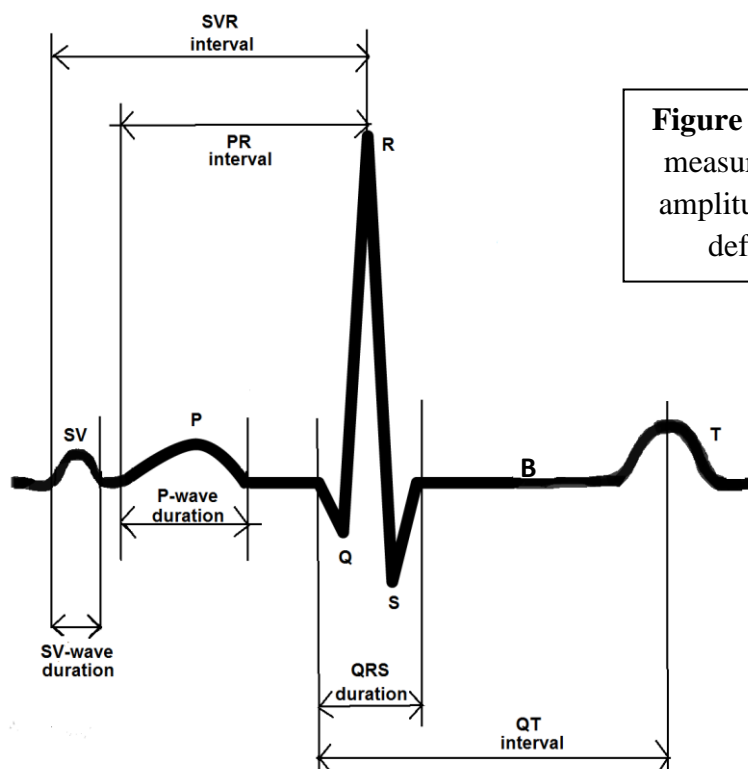


Figure 13: ECG complex demonstrating measurements used in the study. Wave amplitudes were taken as the maximum deflection from the baseline (B)

Materials and Methods

Statistical Analysis

The continuous data for weight, heart rate and derived parameters from the ECG were first checked for normality or otherwise of their distribution. If normally distributed, then the Mean \pm standard deviation from the mean (SD) is presented, if skewed, then the median \pm interquartile range (IQR; 25-75%) is presented. Data were analysed by Analysis of Variance (ANOVA). Heart rate was significantly influenced by reptile weight, which was significantly confounded with age and gender. Since heart rate has a significant impact upon derived parameters of cardiac function such as ECG intervals, then these data were only analysed after adjustment for these factors by including them as co-variates in the ANOVA model. Where multiple data points were obtained from an individual and compared by ANOVA (e.g. values from LC1 and LC2) then the individual was included in the statistical model as a random effect (to account for reduced variance) and data were analysed by a General Linear Mixed Model (GLMM; Genstat v14, VSNi, UK). Correlation was performed in Graphpad Prism 6, and the resulting fit is presented with 95% confidence interval bands and the associated R-square and P-value. Statistical significance was accepted at $P < 0.05$.

Results

	Mean or [Median]	Range	Standard Deviation or [IQR]
Weight (g)	335	66 - 517	140
Age (months)		4 – 30	
Snout-vent length (cm)	18.9	11.5 – 23.0	3.0
Cloacal temperature (°C)	32.7	27.7 – 37.9	2.0
Ambient Temperature (°C)		26 & 35	
Heart Rate (beats/minute)	90	24 - 170	39
R-R interval (mS)	[723]	353-2520	[533-1020]
P wave duration (mS)	56	30 - 100	13
P wave amplitude (mV)	0.03	0.01 – 0.06	0.01
P-R interval (mS)	145	75 - 253	38
SV wave duration (mS)	[57.5]	30 - 125	[50-67]
SV wave amplitude (mV)	0.03	0.01 – 0.07	0.01
SV-R interval (mS)	243	130 – 440	62
QRS duration (mS)	85	60 - 120	15
R wave amplitude (mV)	0.23	0.08 – 0.57	0.11
S wave amplitude (mV)	0.04	0.01 -0.13	0.02
Q-T interval (mS)	355	120 - 980	139
T wave amplitude (mV)	0.04	0.01 – 0.14	0.02
MEA		+60 - +110	

Table 1:
Electrocardiographic measurements of the bearded dragon
(*Pogona vitticeps*)

Recorded at 25mm/sec and 0.5mV/cm.

Post-recording digitally enhancement was used to improve accuracy

P, R, S and T waves were identified on all traces in both lead configuration 1 (LC1) and lead configuration 2 (LC2). Both P and R waves were positive in lead II, S waves were negative in lead II but T waves were variable and could be positive or negative in lead II even in the same trace. Q waves were difficult to identify from the beginning of the R wave and were not reliably measured. The SV wave was identified in 80 of the 98 traces; it occurred after the T wave and before the P wave and was always positive in lead II. R waves were positive in leads III and aVF and negative in leads I, aVR and aVL. P waves and SV waves were positive in leads I, III and aVF and negative in leads aVR and aVL.

Q-T interval, SV wave duration and SV wave amplitude differed between LC1 and LC2; the average standard error of difference on a variance scale was 32, 4 and 0.005 respectively.

The amplitudes of the P, R and S wave of the ECG were significantly lower at an ambient temperature of 35°C, relative to 26°C (**Figure 14**). Heart rate was positively correlated with cloacal temperature (**Figure 15**) and as cloacal temperature increased, the P-R (**Figure 16a**), SV-R (**Figure 16b**) and Q-T (**Figure 16c**) intervals decreased. At each ambient temperature, and at each age, gender did not affect the measurement of heart rate or any derived parameter from the ECG, but the heavier the animal the lower the heart rate (**Figure 17**). Snout-vent length did not have an effect on parameters when age, weight and sex were taken into account.

Gravid females at 30 months of age had a significantly higher mean heart rate = 104bpm (range 68-125) compared with non-gravid females of similar size and age, heart rate = 53bpm (range 38-91; **Figure 18**). The Q-T interval was also shorter in gravid females (298 ± 44 vs. 530 ± 88 ms; $P = <0.001$, students t-test).

Reduction in cutaneous scales (leatherback) and a lack of scales (silkback) did not appear to have an effect on the ECG parameters but the small number of dragons with these genetic mutations (two leatherbacks and one silkback) in the study precluded accurate statistical analysis. Ecdysis did not appear to have an effect on ECG parameters but only two dragons (one female and one male) in the study were undergoing ecdysis which precluded accurate statistical analysis. Results from the three dragons which adopted a lying posture were very similar to those adopting the typical upright posture though the small sample size precluded accurate statistical analysis

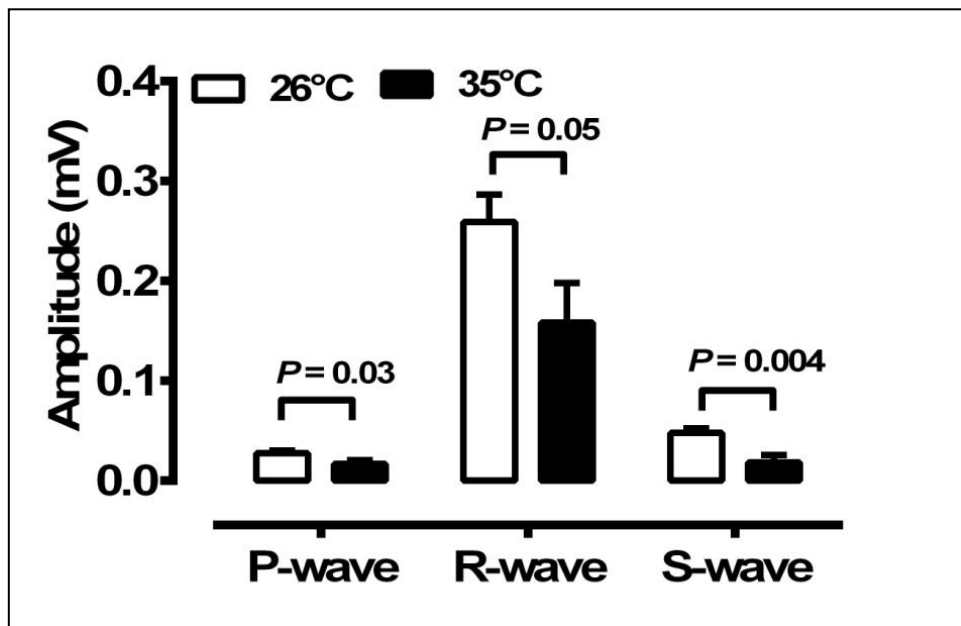


Figure 14: Bar graph to show reduction in P wave, R wave and S wave amplitudes when measured at an ambient temperature of 35°C vs. 26°C; Statistical significance was considered acceptable at $P=0.03$, $P=0.05$ and $P=0.004$ respectively, as analysed by ANOVA

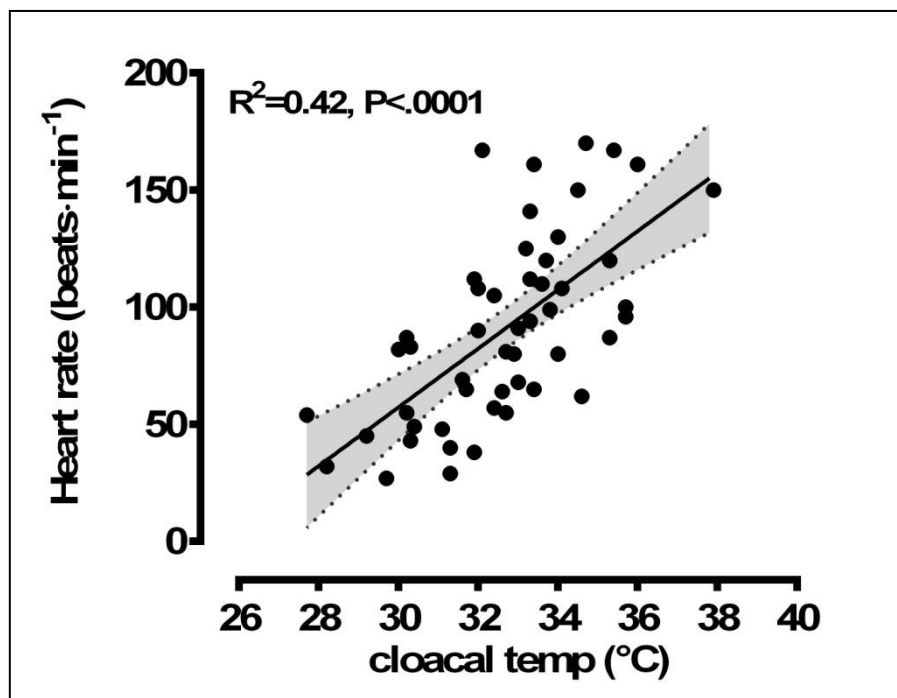


Figure 15: Scatterplot to show the significant relationship between cloacal temperature and heart rate; $P<0.0001$, as analysed by ANOVA

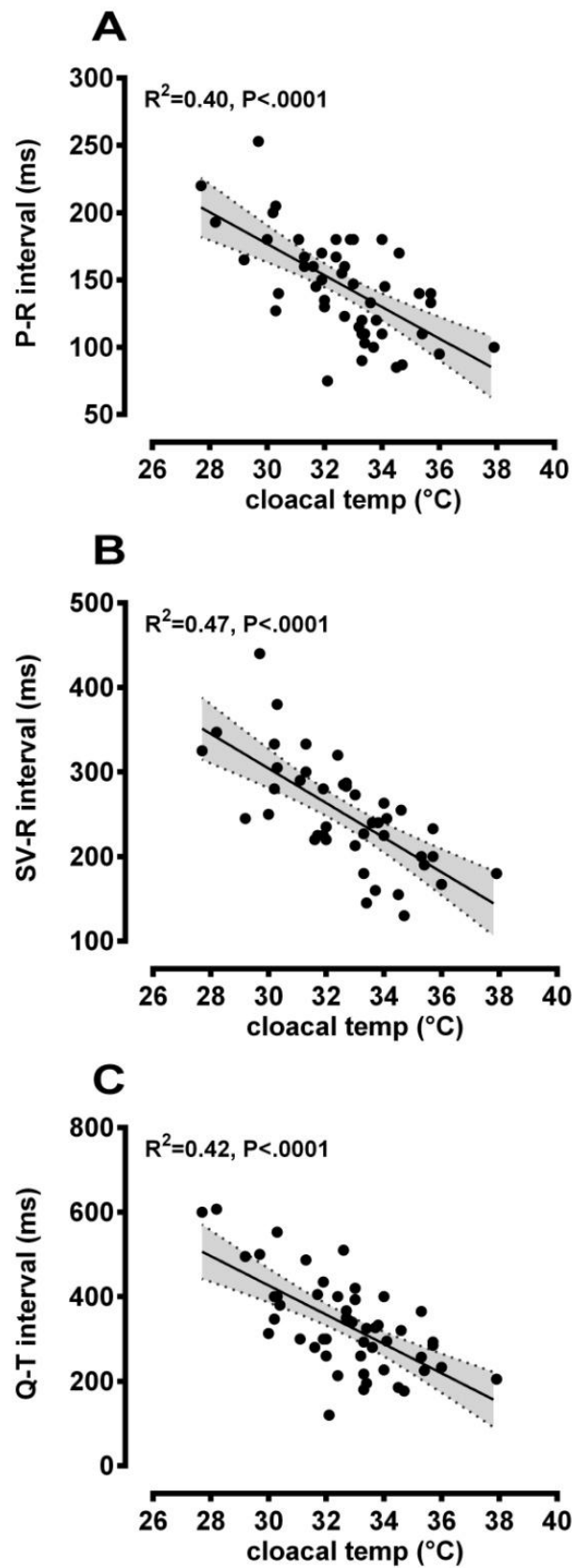


Figure 16: Scatterplots to show the significant relationship between cloacal temperature and P-R interval (A), SV-R interval (B) and Q-T interval (C); $P<0.0001$, as analysed by ANOVA

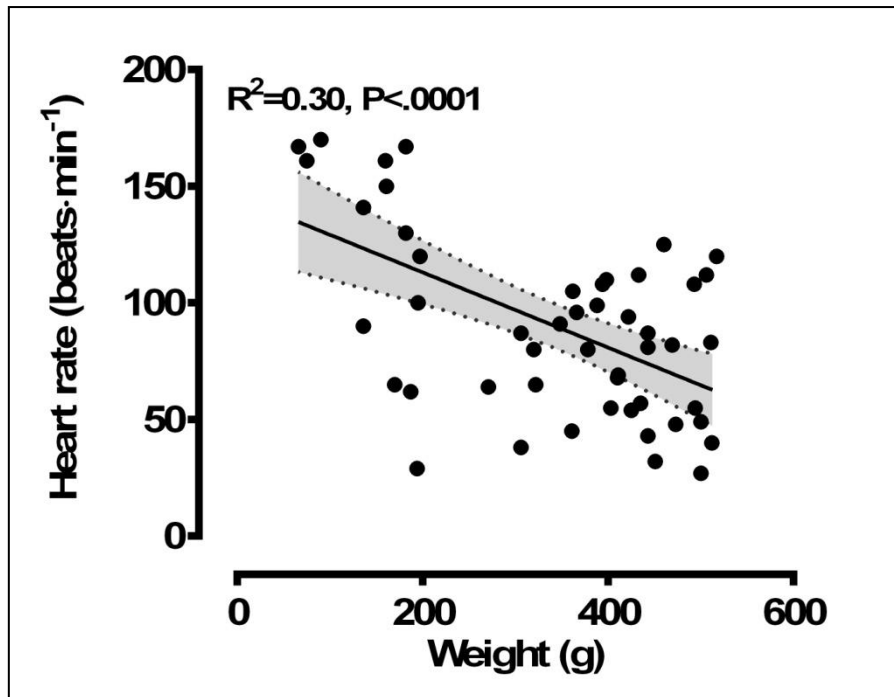


Figure 17: Scatterplot to show the significant relationship between body weight and heart rate; $P<0.0001$, as analysed by ANOVA

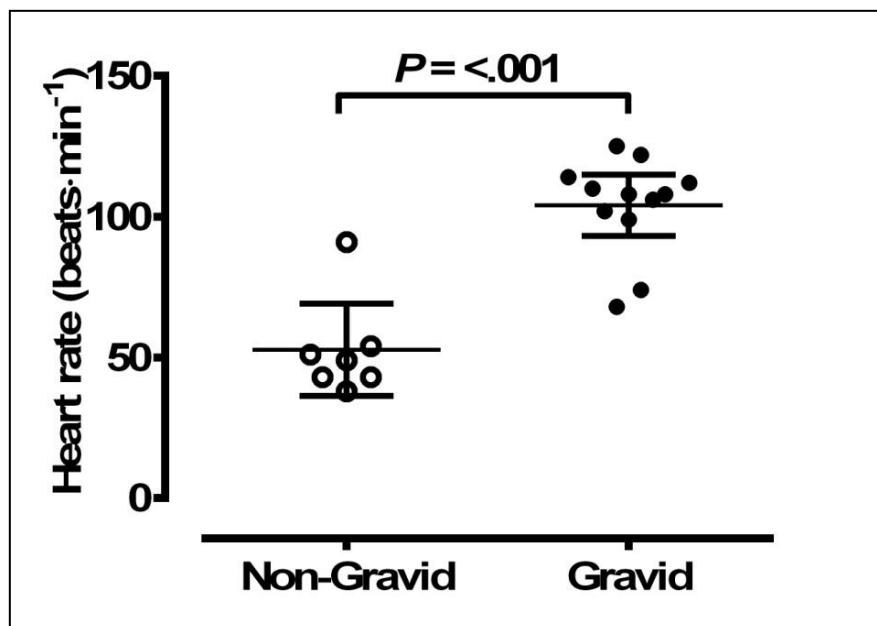


Figure 18: Dot-plot with mean \pm 95% confidence intervals to show the significant heart rate increases in gravid female compared with non-gravid, female bearded dragons; $P<0.001$

Discussion

Overall there was good agreement between LC1 and LC2 with respect to wave amplitudes and wave intervals. A statistically significant difference in the Q-T interval, SV wave duration and SV wave amplitude between LC1 and LC2 was noted though the difference in measureable terms was very small and unlikely to be of clinical significance.

A sinusoidal wave respiratory pattern was occasionally observed in LC1 but was not observed in LC2 on the same individual (**Figure 19**). The sinusoidal pattern is due to movement of the caudal electrodes during respiration; the caudal electrodes in LC2 tended not to be affected by respiratory movements being placed more caudally than in LC1. The sinusoidal trace was more difficult to analyse since waveforms, particularly the P wave, T wave and SV wave were distorted. Heaton-Jones and King 1994 recorded a similar sinusoidal wave pattern in the American alligator (*Alligator mississippiensis*) and noted that the T wave tended to be obscured. LC2 recordings may therefore be preferable to LC1 recordings especially if the animal has notable respiration to avoid distortion of the waveforms.

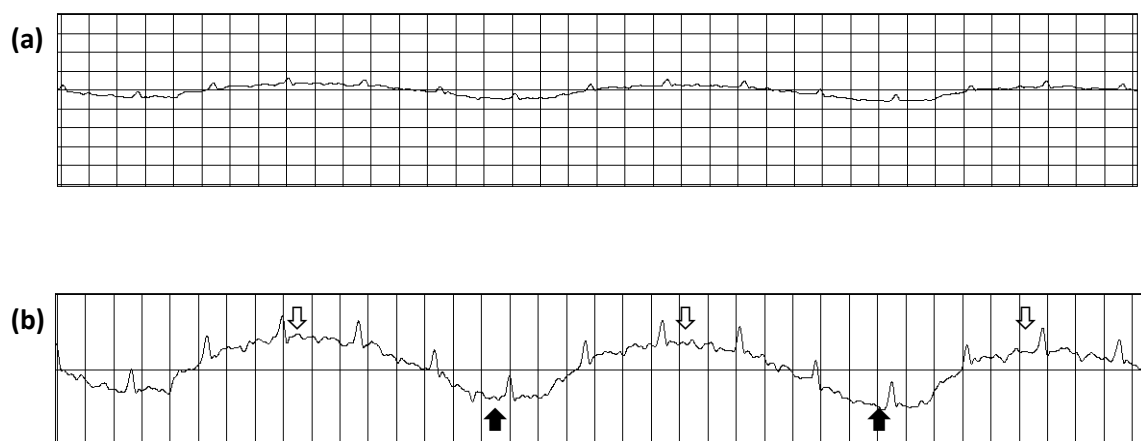


Figure 19: Sinusoidal wave respiratory pattern from a lead II recording in LC1; shown with (b), and without (a) digital enhancement

↓ = Inspiration; ↑ = expiration

T wave morphology was very variable between different ECGs but could also be variable within the same ECG as described in other studies (Valentinuzzi, 1969 and McDonald and Heath, 1971, Martinez-Silvestre and Pether, 2003) and noted by McDonald (1976) who describes three typical forms of T wave. Variability of the T wave and difficulties in identifying the beginning and end of the T wave hampered accurate measurement of the Q-T interval and T wave duration such that the latter could not be reliably measured; in order to improve repeatability and accuracy, the maximum deflection from the baseline of the T wave was used as the endpoint of the T wave as described by McDonald (1976).

The SV wave was identified in 82% of traces and 87% of the bearded dragons; in some animals an SV wave could only be identified on one of the two lead configurations though neither lead configuration was more likely to show an SV wave. When the SV wave was detected it occurred after the T wave and before the P wave as described by McDonald (1976), Cook and Westrom (1979), and Martinez-Silvestre *et al* (2003), but contrary to Mullen (1967) who stated the SV wave precedes the T wave. In some cases the SV wave was difficult to separate from the preceding T wave particularly at higher heart rates.

Heart rates noted in this study (24 -170; mean = 90) were significantly higher than those recorded for the Gomeran giant lizard, *Gallotia bravoana* (35 – 60bpm; mean = 44) by Martinez-Silvestre *et al* (2003) which is of similar size to the bearded dragon and was maintained at similar temperatures to the bearded dragons in this study; core body temperatures were significantly lower however, and would result in the lower heart rates reported. Heart rates in this study were similar to those reported in lizards by Mullen (1967) with a similar range of core body temperatures to those in this study.

As noted in previous studies by Mullen (1967) and Jacob and McDonald (1975) heart rates were significantly increased with increasing cloacal temperature and heart rate tended to decrease with increasing bodyweight. Jacob and McDonald (1975) noted a significant positive correlation between heart rate and snout-vent length in the snake, *Elaphe obsoleta*; in this study snout-vent length did not have an effect on heart rate. Sex did not affect heart rate at any age with the exception of gravid females which had significantly higher heart rates. The increased in heart rate in gravid females was likely due to the increased metabolic demands to produce and lay eggs.

The heart beat frequency prediction formula described by Sedgwick (1991) tended to underestimate heart rates in this study and should therefore be used with caution in bearded dragons. One possible explanation is that the bearded dragon has a higher than average metabolic rate amongst reptiles. Mullen (1967) stated that at any given temperature between 22°C and 30°C, the heart rate of lizards is significantly higher than that of snakes (Mullen 1967), which the above formula does not account for using only a single constant for all reptile species.

The reduction in P-R, SV-R and Q-T intervals with increasing cloacal temperatures is due to the increasing heart rate and they are inextricably linked as noted in other studies. P wave, R wave and S wave amplitudes were significantly lower at 35°C compared with 26°C illustrating the importance of a consistent ambient temperature when comparing ECGs between individuals.

It was theorised prior to the study that changes in skin anatomy could affect the conduction of electrical impulses through the skin and in turn affect wave amplitudes. Skin type and ecdysis did not appear to affect the ECG although the number of individuals with reduced or absent scales, or were undergoing ecdysis at the time of the study were too small to allow accurate statistical analysis; further studies on a larger sample size are required to establish whether this is true.

Murray (2006) states the mean electrical axis (MEA) is difficult if not impossible to determine in reptiles due to low potentials and is not of clinical importance in part due to inconsistent placement of electrodes. MEA has been calculated for the American alligator, *Alligator mississippiensis* (+69 - +97°) (Heaton-Jones and King 1994), the Gomeran giant lizard, *Gallotia bravoana* (+45 - +135) (Martinez-Silvestre and Pether 2003) and the green iguana, *Iguana iguana* (+64 - +91) (Albert *et al* 1999). In this study electrodes were placed in a consistent manner and the MEA was relatively easy to determine (+60 - +110) and was similar to that reported in the Gomeran giant lizard; R waves in leads I and III were generally easily identified and measured (with digital enhancement) and in many cases lead aVL was isoelectric.

Post-recording digital enhancement of the ECG used in this study greatly improved the ability to identify waveforms and perform accurate measurements. The improved accuracy

may explain the increased observation of the smaller wave forms (SV wave, P wave and Q wave) compared to other similar studies.

Murray (1996) states that as the husbandry, nutrition and medical management of reptiles continues to improve, the reported incidence rate of geriatric cardiovascular disease is certain to increase. Given the current difficulty in diagnosing cardiac disease in reptiles, in particular those species with hearts positioned in areas difficult to observe using conventional radiography and echocardiography, electrocardiography is a diagnostic procedure that has the potential to improve evaluation of the reptilian cardiovascular system. Further studies are required to evaluate how in particular, pathological conditions, both cardiac and non-cardiac affect the normal ECG. ECG parameters for the healthy bearded dragon are presented at an ambient temperature of 26°C and 35°C and cloacal temperature range of 27.7°C to 37.9°C

Conclusion

This study established a protocol for a repeatable, non-invasive technique for ECG recording in the inland bearded dragon (*Pogona vitticeps*) and provides a normal reference range.

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Appendix

Reported Lead II ECG Values

for

Selected Reptile Species

Species	American alligator (<i>Alligator mississippiensis</i>) ^a	Brown tree snake (<i>Boiga irregularis</i>) ^b	Desert tortoise (<i>Gopherus agassizii</i>) ^c
Weight (kg)	Juveniles	0.070 – 0.915	adults
Ambient Temperature (°C)	22 - 24	22.5 - 26	26.6
Body Temperature (°C)	22.1 – 28.2	24.1 - 28.7	NR
Restraint	Blindfold	Propofol	Sedated
Electrode Type	Percutaneous wire	Oesophageal	Needle
Recording (mm/sec)[mm/mV]	(25) [20]	NR	NR
Recumbency	Ventral	Dorsal	Ventral
Heart Rate (bpm) [mean]	33 – 42	56 - 73[67]	10 – 30
R-R interval (sec) [mean]	1.71	0.82 - 1.06 [0.90]	NR
SV amplitude (mV)	NR	NR	NR
SV duration (sec)	NR	NR	NR
P amplitude (mV)	NR	-0.25- 0.195 [-0.0036]	NR
P duration (sec)	NR	0.017 - 0.072 [0.044]	NR
P-R interval (sec)	NR	0.176 - 0.248 [0.202]	0.38 - 1.5
R amplitude (mV) [mean]	0.25	0.082 - 1.3 [0.515]	0.03 - 0.18
QRS duration (sec) [mean]	0.10	0.052 - 0.12 [0.0874]	0.04 - 0.2
Q-T interval (sec) [mean]	1.18	0.52 - 0.76 [0.65]	NR
S-T interval (sec) [mean]	NR	0.24 - 0.48 [0.35]	NR
S amplitude (mV)	0.16	NR	NR
T duration (sec)	0.33	0.1 - 0.24 [0.15]	NR
T amplitude (mV)	0.13	0.038 - 0.683 [0.392]	NR
Q amplitude (mV)	0.09	NR	NR
MEA	69 - 97	NR	NR

(a) Heaton-Jones and King (1994); (b) Anderson *et al* (1999); (c) Belcher *et al* (2009); NR = not recorded

Species	Gomoran giant lizard (<i>Gallotia bravoana</i>) ^d	Green iguana (<i>Iguana iguana</i>) ^e	Leatherback Sea Turtle (<i>Dermochelys coriacea</i>) ^f	Red-eared slider (<i>Trachemys scripta elegans</i>) ^g
Weight (kg)	0.105 – 0.244	0.227-1.69	242 - 324	0.45 - 1.81
Ambient Temperature (°C)	20 - 21	NR	26.4 28.6	22 - 23
Body Temperature (°C)	18 - 24	NR	30.3 -31.6	kept at 23 - 25°C before study
Restraint	Head and eyes covered	NR	Medetomidine and ketamine	Ketamine +/- xylazine or midazolam
Electrode Type	Alligator clips	NR	Needle	Alligator clips, no gel
Recording (mm/sec)[mm/mV]	(25) [10]	NR	NR	(25) [40]
recumbency	Ventral	NR	Ventral	Ventral
Heart Rate (bpm) [mean]	35 – 60 [44]	38 - 68	14.7 – 20.8 [17.9]	16 - 37 [25]
R-R interval (sec) [mean]	1.05 - 1.78 [1.43]	NR	2.88 – 4.08 [3.4]	NR
SV amplitude (mV)	0.04 - 0.2 [0.12]	NR	NR	NR
SV duration (sec)	0.08 - 0.2[0.13]	NR	NR	NR
P amplitude (mV) [mean]	0.05 - 0.1 [0.08]	0.06-0.16	0.055 – 0.12 [0.096]	0.013-0.063 [0.03]
P duration (sec)	0.08 - 0.1 [0.09]	NR	0.16 – 0.25 [0.20]	0.05-0.16 [0.12]
P-R interval (sec)	0.1 - 0.18 [0.15]	0.19 - 0.43	0.80 – 1.17 [1.00]	0.44 - 0.56 [0.51]
R amplitude (mV) [mean]	0.1 - 0.18 [0.15]	0.2 - 0.54	0.34 – 0.50 [0.45]	0.15 - 0.35 [0.25]
QRS duration (sec) [mean]	0.05 - 0.1 [0.08]	0.04 - 0.14	0.47 – 0.55 [0.51]	0.10 - 0.18 [0.15]
Q-T interval (sec) [mean]	0.1 - 0.32 [0.21]	0.39 - 0.85	0.94 – 1.26 [1.14]	1.06 - 2.28 [1.41]
S-T interval (sec) [mean]	0.02 - 0.2 [0.14]	0.25 - 0.57	0.32 – 0.54 [0.40]	0.76 - 1.48 [1.05]
S amplitude (mV)	0.02 - 0.05 [0.03]	0.01 - 0.11	0.36 – 0.60 [0.48]	NR
T duration (sec)	0.1 - 0.15 [0.12]	0.07 - 0.17	0.20 – 0.36 [0.26]	NR
T amplitude (mV)	0.03 - 0.14 [0.07]	0.05 - 0.17	0.09 – 0.20 [0.15]	0.025 - 0.15 [0.07]
Q amplitude (mV)	NR	NR	NR	NR
MEA	45-135 ^o	64 - 91	NR	NR

(d) Martinez-Silvestre and Pether (2003); (e) Anderson *et al* (1999); (f) Harms *et al* (2007); (g) Holz and Holz (1995); NR = not recorded