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SEASONAL CHANGES IN THE CARDIOVASCULAR, RESPIRATORY AND METABOLIC RESPONSES TO TEMPERATURE AND HYPOXIA IN THE BULLFROG *RANA CATESBEIANA*

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Summary

We assessed seasonal variations in the effects of temperature on hypoxia-induced alterations in the bullfrog *Rana catesbeiana* by measuring the heart rate, arterial blood pressure, breathing frequency, metabolic rate, blood gas levels, acid–base status and plasma glucose concentration. Regardless of the season, decreased body temperature was accompanied by a reduction in heart and breathing frequencies. Lower temperatures caused a significant decrease in arterial blood pressure during all four seasons. Hypoxia-induced changes in breathing frequency were proportional to body temperature and were more pronounced during winter, less so during spring and autumn and even smaller during summer. Season had no effect on the relationship between hypoxia and heart rate. At any temperature tested, the rate of oxygen consumption had a tendency to be highest during summer and lowest during winter, but the difference was significant only at 35 °C. The $P_{a_{O_2}}$ and pH values showed no significant change during the year, but $P_{a_{CO_2}}$ was almost twice as high during winter than in summer and spring, indicating increased plasma bicarbonate levels. Lower temperatures were accompanied by decreased plasma glucose levels, and this effect was greater during summer and smaller during autumn. Hypoxia-induced hyperglycaemia was influenced by temperature and season. During autumn and winter, plasma glucose level remained elevated regardless of temperature, probably to avoid dehydration and/or freezing. In winter, the bullfrog may be exposed not only to low temperatures but also to hypoxia. These animals show temperature-dependent responses that may be beneficial since at low body temperatures the set-points of most physiological responses to hypoxia are reduced, regardless of the season.

Key words: blood pressure, heart rate, breathing frequency, acid–base status, oxygen consumption, plasma glucose, bullfrog, *Rana catesbeiana*.

Introduction

Many studies of amphibians have been motivated by the remarkable tolerance of these animals to diverse environmental conditions. The ability to inhabit an extensive environmental range is due to the capacity of amphibians to withstand long periods under unfavourable conditions (seasonal periods of drought and/or low temperature) and to condense their life cycle into active periods when conditions are less severe (Pinder *et al.* 1992). Most studies of the cardiorespiratory physiology of amphibians, however, have been performed only in active, non-aestivating animals.

Seasonal variations in the effect of temperature on heart rate have been reported both *in vitro* (Smith, 1951) and *in vivo* (Miller and Mizell, 1972; Harri and Talo, 1975). These studies revealed that the endocrine and autonomic nervous systems modulate heart rate to different extents during the different seasons of the year. For instance, Jones (1968) found that *Rana temporaria* and *Bufo bufo* showed a more pronounced diving bradycardia and a greater tendency to develop arrhythmic heart beats in winter than in summer.

The bullfrog *Rana catesbeiana*, family Ranidae, is an aquatic amphibian native to the temperate northern Unites States which, during winter, remains under water or under leaf litter (Pinder *et al.* 1992) in order to avoid freezing temperatures. In this overwintering microenvironment, the frogs may be exposed to hypoxic conditions (Tattersall and Boutilier, 1997; Stinner *et al.* 1994). Hypoxia causes tachycardia (Lillo, 1979) and tachypnoea (Kinkead and Milsom, 1994) in *Rana catesbeiana* at summer temperatures. Seasonal variations in the hypoxia-induced tachypnoea and tachycardia of the bullfrog have not been studied previously.

The purpose of the present study was to test the hypothesis that during the overwintering period the known metabolic rate reduction (Fromm and Johnson, 1955) may be accompanied by alterations in the respiratory and cardiovascular responses to

Table 1. *Minimum and maximum ambient temperature in Ribeirão Preto, São Paulo State, Brazil, measured on a daily basis*

	Season			
Temperature (°C)	Spring	Summer	Autumn	Winter
Minimum	18.08±0.22	19.54±0.17	15.69±0.33	15.61±0.20
Maximum	29.86±0.36	30.16±0.32	27.37±0.33	29.24±0.44

Values are means ± S.E.M. (*N*=91).

hypoxia. To measure such seasonal variations, we measured the effects of hypoxia on heart rate, arterial blood pressure, breathing frequency, blood gas levels, acid–base status and plasma glucose levels in *Rana catesbeiana*.

Materials and methods

Animal source and maintenance

Four groups (one for each season) of adult bullfrogs (*Rana catesbeiana*) of either sex weighing (mean ± S.E.M.) 175.90±8.16 g in spring (*N*=30, 54 % males and 46 % females), 174.99±4.31 g in summer (*N*=30, 53 % males and 47 % females), 174.37±7.31 g in autumn (*N*=31, 49 % males and 51 % females) and 180.34±3.12 g in winter (*N*=30, 46 % males and 54 % females) were obtained from a local frog farm, 2–3 weeks before experimentation throughout the year. The first animals were imported from the United States during the early 1930s, and they have been raised in Brazil ever since. At the farm, they were held at ambient temperature (Table 1) with free access to running tap water and were fed mealworms throughout the year. Average age was approximately 3 months.

Upon arrival at the laboratory, the animals were kept indoors in aquaria with free access to tap water and basking areas at 23–25 °C (the average environmental temperature throughout the year at the frog farm). All animals were force-fed chicken liver once a week until 7 days before surgery. Experiments were performed throughout the year, avoiding transition weeks between seasons (winter experiments were conducted from June 29 to September 14, spring experiments from September 29 to December 14, summer experiments from December 29 to March 14, and autumn experiments March 29 to June 14).

Anaesthesia and surgical procedures

For anaesthesia, the bullfrog was placed in a closed box saturated with ether vapour. Arterial cannulae (PE-50) filled with heparinized Ringer's solution were occlusively inserted into the femoral artery. A second catheter (PE-100), inserted into the frog's buccal cavity *via* a tight-fitting hole made in the tympanic membrane, was used to measure breathing frequency. All animals recovered promptly from anaesthesia. After surgery, the animals were left undisturbed for at least 24 h at 23–25 °C, in aquaria with free access to tap water and basking areas.

Measurements of blood pressure, heart rate, breathing frequency and O_2 consumption

Arterial blood pressure was measured by connecting the arterial catheter to a Hewlett Packard pressure transducer (HP 1280, USA) kept at the level of the frog's heart. Heart rate was determined by counting pressure pulses. Breathing frequency was recorded using a differential air-pressure transducer (Hewlett Packard, USA, model 270) connected to the buccal catheter. Signals from transducers were recorded on paper (Hewlett Packard, USA, model 7754A). Oxygen consumption was measured using a Krogh respirometer (Schmidt-Nielsen, 1970).

Analysis of blood gases and glucose

Arterial blood samples were analyzed for P_{O_2} (FAC Instruments, São Carlos, Brazil, model 204A) and pH (Metrohm, Switzerland, model 654) immediately after withdrawal. The O_2 electrode (FAC Instruments) was calibrated with pure N_2 and atmospheric air. The pH electrode (Metrohm, Switzerland) was adjusted using Radiometer (Copenhagen, Denmark) precision buffer solutions (S1510 and S1500). Electrodes were kept at the temperature of the experimental animal using a constant-temperature circulator (VWR Scientific, Niles, IL, USA, model 1160A). Blood P_{CO_2} was estimated using the Astrup technique (Astrup, 1956). Glucose concentration was determined quantitatively by enzymatic (hexokinase) analysis (Sigma, St Louis, MO, USA).

Experimental procedure

Experiments were performed on conscious, unrestrained and undisturbed frogs. During the experiments, the frogs were housed in a 1 l plastic chamber placed inside an environmental chamber (FANEM, BOD 347 cd, São Paulo, Brazil) kept at the experimental temperature of 10, 15, 25 or 35 °C. The animals were transferred to the experimental temperature 24 h before making measurements (48 h after surgery). Cloacal temperature probes confirmed that there was no difference between animal temperature and environmental chamber temperature. The animal chamber was continuously flushed with humidified room air at a rate of 1.5 l min^{-1}. The humidification flask was kept inside the environmental chamber to minimize temperature effects. Once conditions were stable in the normoxic gas, buccal and arterial blood pressures were recorded for 20 min, and arterial blood was sampled for analysis of blood gases, pH and plasma glucose level. Hypoxic gas mixtures (10, 7, 5 and 3 % inspired O_2) were then applied in random order for 60 min each (AGA, Sertãozinho, SP, Brazil). Buccal and arterial blood pressures were recorded, and 1 ml arterial blood samples were withdrawn

at the end of each experimental condition. Approximately 80 % of this volume was reinfused into the animal's circulation after blood gas measurements had been made. A small fraction (100 µl) of arterial blood was immediately centrifuged in a Ravan microcentrifuge (900 ***g***) for 2 min (model Ciclo I, São Paulo, Brazil), and plasma was frozen at −20 °C for subsequent determination of plasma glucose concentration.

Calculations and statistical analysis

Mean arterial blood pressures were estimated from the pressure pulse using the following formula: mean pressure = diastolic pressure plus (systolic pressure minus diatolic pressure)/3. Breathing frequency was obtained by counting the number of large-amplitude buccal movements, distinguished from buccal oscillations (Kinkead and Milsom, 1994). Breathing frequency, blood pressure and heart rate were calculated over 20 min periods. All values are reported as means ± S.E.M. The effects of season, temperature and hypoxia on heart rate, arterial blood pressure, breathing frequency, $P_{a_{O_2}}$, pH and plasma glucose concentration were analyzed using three-way multivariate analysis of variance (MANOVA) on log-transformed data (factors: season, temperature and percentage inspired oxygen) and using Duncan's multiple-range test. For the $P_{a_{CO_2}}$ and oxygen consumption experiments, two-way MANOVA and Duncan's multiple-range test were performed (factors: season and inspired oxygen tension; season and temperature, respectively). Values of $P<0.05$ were considered significant.

Results

Fig. 1 shows the effects of hypoxia on heart rate at different temperatures during the four seasons. Under normoxia, heart rate decreased significantly with reduced temperature. The decrease was similar in all seasons (MANOVA, $P>0.4$). Hypoxia had little effect on heart rate regardless of temperature or season. Heart rate was elevated significantly only at the lowest level of inspired O_2 at 15 and 25 °C in autumn and at 25 and 35 °C in spring. Hypoxia (below 10 %) at 35 °C was lethal, however, regardless of season.

Reduced body temperature was accompanied by decreased blood pressure in all seasons (Table 2). Season also affected blood pressure. Independently of temperature, blood pressures were significantly higher during winter than during the other seasons at most of the temperatures tested. Hypoxia caused no significant changes in blood pressure during any season or at any experimental temperature (data not shown).

Fig. 2 shows the effects of hypoxia on breathing frequency at different temperatures during the four seasons. Regardless of season, breathing frequency was reduced at lower temperatures under normoxic conditions. Similarly, the ventilatory response to hypoxia tended to become less pronounced at the lower temperatures (MANOVA, $P<0.001$). A seasonal difference (MANOVA, $P<0.05$) in the effect of hypoxia was observed: during summer, no significant increase in breathing frequency was measured at 25 °C, whereas during winter a significant effect was measured at 10 % inspired O_2 at the same temperature. Experiments performed during spring and autumn showed a significant increase in breathing frequency at intermediate values (5 and 7 % inspired O_2, respectively).

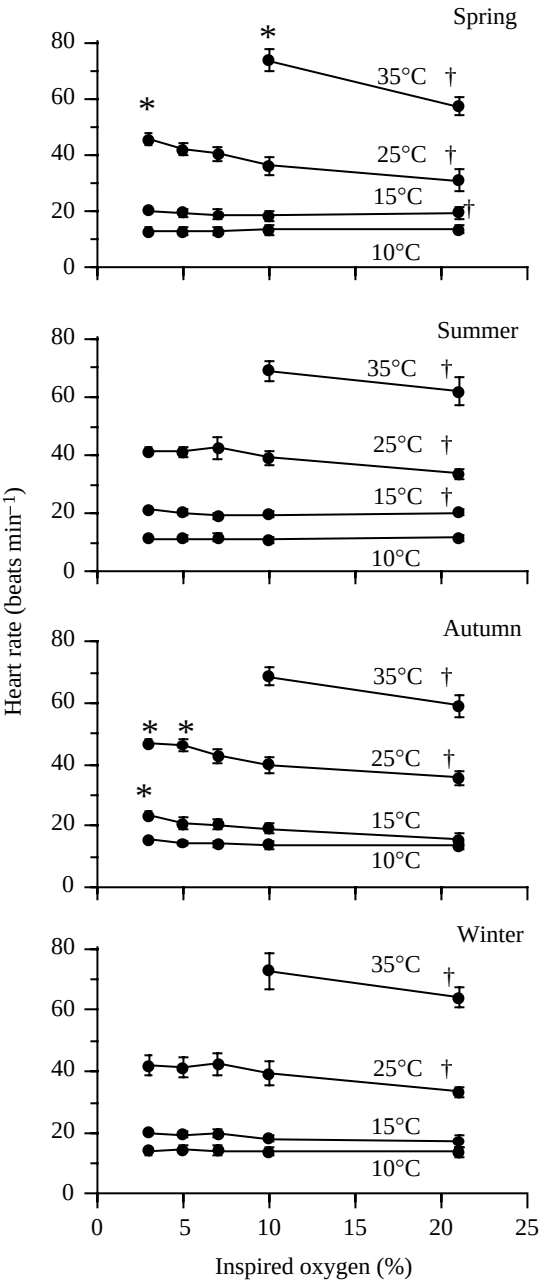

Fig. 1. Seasonal comparisons of the effect of hypoxia on heart rate at different temperatures. †A significant ($P<0.05$) effect of temperature within a season. *A significant ($P<0.05$) effect of hypoxia relative to normoxic control values, at the same temperature. Data are reported as means ± S.E.M. ($N=6$).

Table 3 shows the effects of hypoxia on blood gas levels of frogs equilibrated at different temperatures during winter. $P_{a_{O_2}}$ and pH values during spring, summer and autumn (data not shown) were similar to values in winter (MANOVA, $P>0.5$).

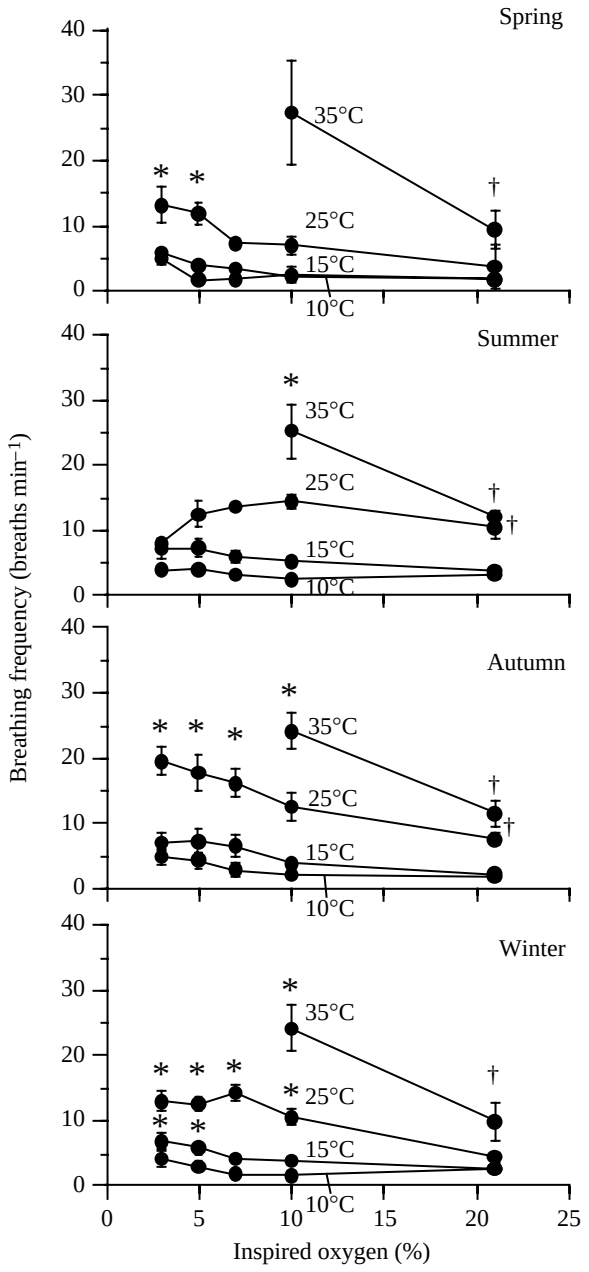

Fig. 2. Seasonal comparisons of the effect of hypoxia on breathing frequency at different temperatures. †A significant (P<0.05) effect of temperature within a season. *A significant (P<0.05) effect of hypoxia relative to normoxic control values, at the same temperature. Data are reported as means ± S.E.M. (N=6).

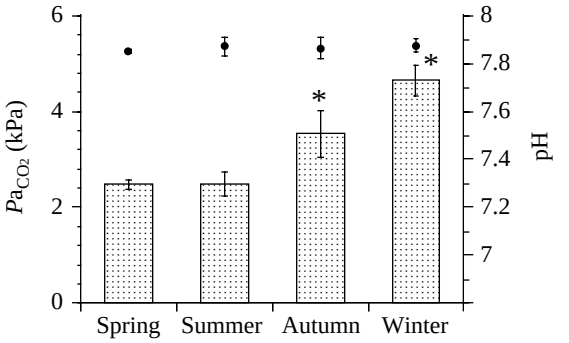

Fig. 3. Seasonal variation in the effect of temperature on Pa_{CO_2} columns, left-hand axis) and pH (circles, right-hand axis). *A significant difference from the value for spring (P<0.05). Values were obtained under normoxia at 25 °C. Data are reported as means ± S.E.M. (N=6).

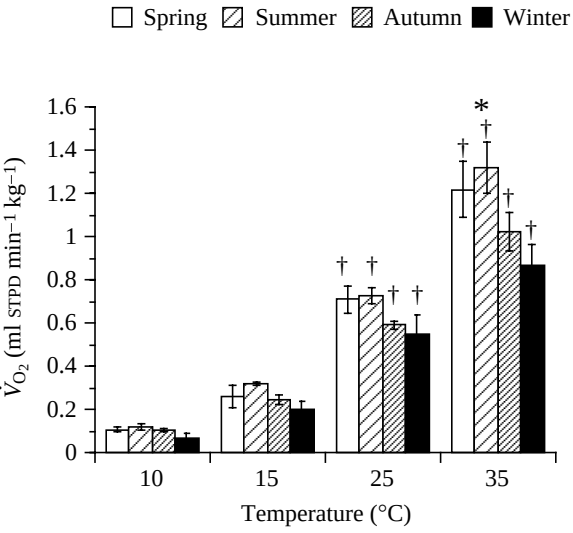

Fig. 4. Seasonal comparisons of the relationship between rates of oxygen consumption and body temperature under normoxic conditions. †A significant (P<0.05) effect of temperature within a season. *A significant (P<0.05) seasonal variation, at the same temperature. Data are reported as means ± S.E.M. (N=6).

However, Pa_{CO_2} values were significantly higher in autumn and winter than in spring and summer (Fig. 3). The effect of hypoxia on blood gas levels and pH did not change among seasons (MANOVA, P>0.2).

The effects of temperature and season on oxygen consumption are shown in Fig. 4. At any temperature tested, the rate of oxygen consumption tended to be higher during summer and lower during winter, but the difference in values between the two seasons was significant only at 35 °C.

Fig. 5 shows the relationship between plasma glucose levels and inspired oxygen level at different temperatures in each season. Hypoxia caused hyperglycaemia during all four seasons. However, the magnitude of the hypoxia-induced hyperglycaemia varied among temperatures (MANOVA, P<0.03) and seasons (MANOVA, P<0.01). During summer, exposure to hypoxia caused significant increases in plasma glucose levels at 10, 15 and 25 °C. During spring, there was an increase at 15 and 25 °C, and during winter and autumn, at 25 °C only. Under normoxic conditions, glucose levels were significantly reduced with decreasing temperatures, except during autumn. At 10 °C, glucose levels were higher in winter and autumn than in the other seasons. The slope (mean ± S.D. of residuals) from the linear regression of the relationship between

Table 2. *Seasonal variation in blood pressure of bullfrogs under normoxic conditions*

Temperature (°C)	Blood pressure (kPa)			
	Spring	Summer	Autumn	Winter
10	3.27±0.15†	2.76±0.15†	3.51±0.25	4.15±0.28
15	3.24±0.12†	3.83±0.16*	3.59±0.36†	4.29±0.36
25	3.89±0.21†	4.12±0.25*	3.93±0.21†	4.76±0.35
35	4.04±0.21*,†	4.40±0.39*,†	4.41±0.16*,†	5.59±0.23*

Values are means ± S.E.M. for six animals in each group.
*Significantly different from the value at 10 °C, in the same season (P<0.05).
†Significantly different from the winter value, at the same temperature (P<0.05).

glucose level and body temperature, under normoxia, was lowest during autumn (0.58±0.21 mg dl^{-1} °C^{-1}) and was 1.19-fold (0.69±0.20 mg dl^{-1} °C^{-1}), 1.96-fold (1.14±0.29 mg dl^{-1} °C^{-1}) and 2.59-fold (1.51±0.25 mg dl^{-1} °C^{-1}) higher during winter, spring and summer (P<0.05 for spring and summer, taking the autumn slope as reference).

Discussion

The present study provides data about the effect of temperature on cardiorespiratory and metabolic responses to hypoxia not only during the active period of the species (*Rana catesbeiana*) but also during winter, when adult bullfrogs in the wild become quieter, but are not torpid. In the field, overwintering bullfrogs (*R. catesbeiana*) have been found submerged on the bottoms of ponds in Ohio (USA) from December to February, but none of them was buried or covered with silt. Instead, they kept moving in search of areas of high oxygen content and to avoid freezing temperatures (Stinner *et al.* 1994).

Table 3. *Effect of hypoxia and body temperature on blood gases of* Rana catesbeiana *during winter*

Temperature (°C)	Inspired O_2 (%)	Pa_{O_2} (kPa)	pHa	Pa_{CO_2} (kPa)
15	21	4.56±0.41	7.94±0.07	–
	10	3.46±0.66	7.92±0.05	–
	7	2.88±0.46	7.98±0.05	–
	5	2.72±0.19*	7.98±0.04	–
	3	2.63±0.13*	7.99±0.04	–
25	21	8.68±0.79†	7.88±0.04	5.01±0.25
	10	6.46±0.46	7.95±0.06	4.43±0.35
	7	4.95±0.57*	7.95±0.05	4.33±0.28
	5	3.27±0.42*	8.00±0.05	4.17±0.36
	3	2.17±0.25*	8.01±0.06	4.05±0.41
35	21	10.57±0.58†	7.72±0.10	–
	10	7.30±0.40*	7.85±0.10	–

Values are means ± S.E.M. for six animals in each group.
*Significantly different from the value for normoxic conditions at the same temperature (P<0.05).
†Significantly different from the value at 15 °C (normoxic conditions) (P<0.05).

In the present study, we used frogs of both sexes. To our knowledge, at least in *R. pipiens*, no significant change in the concentrations of serum glucose as function of sex has been observed during summer or winter (Jungreis and Hooper, 1970; Jungreis, 1970). Moreover, Herman (1977) also found no gender difference in the effects of epinephrine and norepinephrine on plasma glucose levels and haematocrit in *R. catesbeiana*.

Cardiovascular system

A small hypoxia-induced tachycardia, which increased with increasing temperature, was observed during autumn and to a lesser extent during spring, whereas no effect of hypoxia was measured during summer or winter (Fig. 1). As a result, statistical analysis (MANOVA, P>0.4) revealed no significant effect of season on hypoxia-induced tachycardia. It has been suggested that an increased heart rate during hypoxia increases blood flow to ensure adequate oxygen uptake *via* cutaneous respiration (Boutilier *et al.* 1986).

Falling temperatures were accompanied by decreasing heart rate (Fig. 1). While temperature may act directly on pacemaker cells (Clark, 1920), it also influences cardiovascular nerves and reflexes. Courtice (1990), for instance, has reported that the effectiveness of the vagus nerve increases with decreasing temperature owing to the accumulation of acetylcholine.

We observed no seasonal variation in the effect of temperature on heart rate: the heart rate of our bullfrogs increased with increasing body temperature in a similar manner during all seasons. In agreement, Weathers (1975) observed a similar pattern for the effect of temperature on heart rate during summer and winter in *R. catesbeiana*. Moreover, Lillo (1980) demonstrated that the cardiovascular system of bullfrogs (*R. catesbeiana*) retains normal regulatory function over winter. Conversely, a clear seasonal variation in the effects of temperature on heart rate has been reported in the frogs *R. pipiens* (Miller and Mizell, 1972) and *R. temporaria* (Harri and Talo, 1975). All of these species, except *R. catesbeiana* (present study), showed a direct relationship between heart rate and temperature during summer, while during winter, a linear relationship existed only up to a core temperature of approximately 22 °C. Above

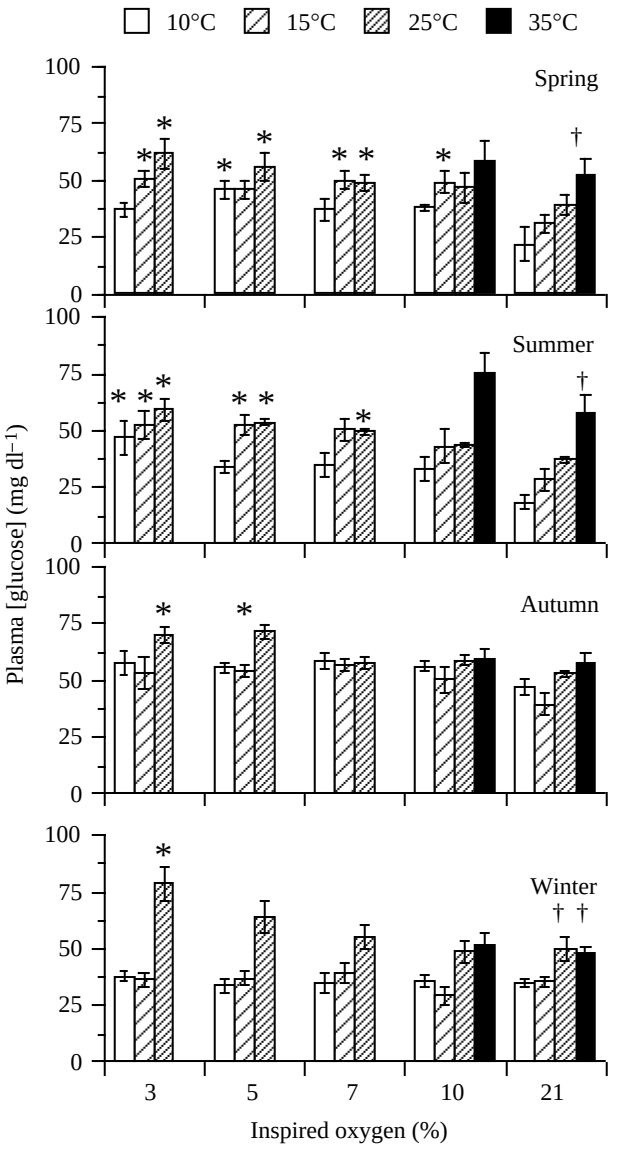

Fig. 5. Seasonal comparisons of the effects of hypoxia on glucose levels at different body temperatures. †A significant (P<0.05) effect of temperature within a season. *A significant (P<0.05) effect of hypoxia compared with the normoxic control values, at the same temperature. Data are reported as means ± S.E.M. N values, 6 (10 °C), 6 (15 °C), 7 (25 °C) and 5 (35 °C).

this temperature, heart rate remained essentially unchanged. These discrepancies may be due to interspecific differences among amphibians or to the effects of thermal acclimation. The occurrence of this thermal acclimation effect in the present study was unlikely because temperature changes little in Southeast Brazil (Table 1).

The relationship between arterial pressure and body temperature in *R. catesbeiana* was influenced by season (Table 2). Our data corroborate those of Weathers (1975), who observed that blood pressures in the bullfrog were slightly higher in winter than in summer. Higher arterial blood pressures in winter, without any change in heart rate, indicate an increase in peripheral resistance or in the stroke volume in the overwintering frogs.

Pulmonary ventilation

In most amphibians and reptiles, pulmonary ventilation increases with rising body temperature. The effect of temperature on ventilation was reported earlier for *Bufo*. Kruhøffer *et al.* (1987) were the first to report that hypoxia-induced hyperventilation in *Bufo paracnemis* is augmented in response to increased body temperature.

The present study provides the first evidence that the effect of hypoxia on breathing frequency depends on the season (MANOVA, P<0.05). Hypoxia-induced tachypnoea at 15 and 25 °C had a lower threshold during winter than during any other season (Fig. 2), suggesting that there may be a change in the threshold for the hypoxic ventilatory response. It is interesting to note that, during the summer, hypoxia had an effect only at 35 °C (under 10 % inspired O_2), in spite of the fact that frogs had higher rates of O_2 consumption during this season at all temperatures (Fig. 4). The notable effect of hypoxia on winter frogs may be due to the reduced breathing frequency under normoxic conditions (reference value used in the statistics) or to the elevation in $P_{a_{CO_2}}$ (Fig. 3). A previous study of amphibians has shown that the addition of CO_2 to the inspired gas mixture caused a shift to the right in the relationship between chemoreceptor discharge and $P_{a_{O_2}}$ (Van Vliet and West, 1992). If so, the present study would confirm that CO_2 may cause an increased O_2 drive to breathe in anurans not only during the active period (Ishii *et al.* 1985) but also during the overwintering period.

Statistical analysis (MANOVA P>0.5) revealed that the effect of temperature on resting ventilation is not affected by season (Fig. 2). In spite of this, we observed a tendency to decreased ventilation at 25 °C in winter, when $P_{a_{CO_2}}$ was higher, but this difference was not significant.

Blood gas levels

The control of breathing in *R. catesbeiana* was recently evaluated by Kinkead and Milsom (1994). They reported $P_{a_{O_2}}$, $P_{a_{CO_2}}$ and pH values that were very similar to our summer values. Our winter frogs, however, presented $P_{a_{CO_2}}$ values twice as high as summer values. An increase in $P_{a_{CO_2}}$ should cause a drop in pH unless a compensatory increase in bicarbonate concentration occurs. Similar increases in $P_{a_{CO_2}}$ have been reported for *B. marinus* (Boutilier *et al.* 1979). Under special laboratory conditions, *B. marinus* burrowed, hypoventilated and reduced its cutaneous CO_2 excretion, with a resulting twofold increase in $P_{a_{CO_2}}$. During active periods, such high $P_{a_{CO_2}}$ values would induce hyperventilation (Pinder *et al.* 1992) but, during winter, ventilation is actually reduced. At 25 °C, the breathing frequency of winter frogs under normoxia was lower than that during summer (Fig. 2). However, since pH did not change, the rise in $P_{a_{CO_2}}$ must have been completely compensated by an increase in plasma bicarbonate level, as previously reported for aestivating *Bufo* (Boutilier *et al.* 1979). This is evidence that short-term (Boutilier *et al.* 1979) and long-term (over winter,

present study) acid–base compensation might be similar among anuran amphibians.

Metabolic rate

During winter, tha rate of oxygen consumption by the bullfrogs was approximately 40.7 %, 37.7 %, 23.8 % and 34.2 % lower than during summer at 10, 15, 25 and 35 °C, respectively (Fig. 4). Previous studies have reported that the resting rate of oxygen consumption is reduced during aestivation to 25 % of the resting value in *R. pipiens* (Fromm and Johnson, 1955), to 18 % in *Scaphiopus hammondii*, to 22 % in *Scaphiopus conchii* (Seymour, 1973), to 21 % in *Lepidobrachus llanensis* (McClanahan *et al.* 1983) and to 58 % in *Neobatrachus kunapalari* (Flanigan and Guppy, 1997). Thus, metabolic depression during adverse periods seems to be a widespread strategy in amphibians from various families. This hypometabolic state may extend survival while animals rely on stored fuel supplies during unfavourable periods. Flanigan *et al.* (1991) showed that, at least in *N. pelobatoides*, the cause of the reduced rate of oxygen consumption lies in the tissues and is not induced by external factors. Metabolic depression may be initiated by a reduction in the energy demand of the cells and this, consequently, leads to reduced energy production.

Plasma glucose levels

Consistent with others report in the literature (Castro-e-Silva *et al.* 1992), plasma glucose levels of anuran amphibians in the present study varied considerably between individuals. In summer, using animals equilibrated at 25 °C, the baseline glucose level measured in the present study was consistent with values reported by Byrne and White, (1975), whereas Herman (1977) reported lower levels of approximately 10–20 mg dl^{-1}. D'Eon *et al.* (1978) attributed lower levels to the collection of blood from cannulae, a procedure that reduces disturbance to the animals during sampling. Our data, however, were also obtained from cannulated specimens. While the surgical procedure, *per se*, caused an increase in plasma glucose concentration, this effect had disappeared 2 days after surgery (see Herman, 1977). During this period, plasma glucose levels were significantly higher in females than in males, but this difference disappeared on subsequent days (Herman, 1977). Therefore, the difference in values obtained between studies is more likely to reflect biological variations.

Most studies on the glucose metabolism of amphibians were performed during the active, non-aestivating period of the species, or during overwintering by assessing the antifreeze action of glucose (Storey *et al.* 1996). The hormonal control of plasma glucose concentration has been studied. Herman (1977) demonstrated that adrenaline, besides causing hyperglycaemia, also increases haematocrit, suggesting that this hormone is important in the adaptation of the bullfrog to stressful changes in the environment. The hormones involved in the regulation of carbohydrate metabolism are expected to exhibit seasonally variable effects. Indeed, the effects of adrenaline on blood glucose and lactate levels in the autumn are double those observed in either summer or winter (Farrar and Frye, 1979). Therefore, this hormone may be important in the preparation for overwintering, increasing the capacity of the frog to remain active as temperature declines during autumn and mobilizing the extra glucose necessary for autumn migratory activity (Farrar and Frye, 1977). Consistent with this background, our results indicate that during autumn, under normoxic conditions, glucose levels tended to be the highest at the low temperatures (Fig. 5). Moreover, plasma insulin levels, at least in the frog *Rana esculenta*, seemed to have a well-defined seasonal rhythm, increasing in spring, reaching their highest value in summer and declining in autumn and winter (Schlaghecke and Blüm, 1981).

In the present study, we have shown that plasma glucose levels were affected not only by temperature but also by an intrinsic annual pattern. Indeed, plasma glucose levels showed a wide variability among seasons, mainly when the frogs were equilibrated at low body temperatures. During summer and spring, plasma glucose level was relatively low, approximately 20 mg dl^{-1} at 10 °C, whereas during autumn and winter it was twice as high (Fig. 5), in agreement with reports in *R. pipiens* (Jungreis, 1970; Jungreis and Hooper, 1970). Moreover, during spring/summer, a clear 'step-ladder' relationship between temperature and plasma glucose concentration was observed, whereas during autumn/winter the effect of temperature on plasma glucose was less evident. The starting value may be important for the interpretation of the effects of changing temperature and hypoxia on glucose levels. It appears that there was an effect when the values during normoxia were low but not when these values were already high (Fig. 5).

The effect of hypoxia on glucose levels has been evaluated in *B. cognatus* (Armentrout and Rose, 1971), *B. paracnemis* (Castro-e-Silva *et al.* 1992) and *B. marinus* (D'Eon *et al.* 1978). All of these studies reported a marked hypoxia-induced hyperglycaemia at room temperature. Castro-e-Silva *et al.* (1992) suggested that acute hypoxia-induced hyperglycaemia arose from autonomic nervous activation by means of combined cholinergic and adrenergic activation. At 10 °C, hypoxia failed to induce any increase in plasma glucose levels, except during summer at 3 % inspired O_2 (Fig. 5).

Seasonal variations in the plasma glucose levels seem to provide bullfrogs with mechanisms necessary for survival during unfavourable conditions of cold and drought. In preparation for autumn and during overwintering, plasma glucose concentration is maintained relatively elevated regardless of temperature, with an increase in intracellular osmolality to avoid dehydration and/or freezing (Storey *et al.* 1996).

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