

PHYSIOLOGICAL SIGNIFICANCE OF BEHAVIORAL HYPOTHERMIA IN HYPOXIC TOADS (*BUFO MARINUS*)

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Summary

We tested the hypotheses that hypoxic toads (*Bufo marinus*) in a thermal gradient would select a lower than normal temperature and that this behavioral response would be beneficial. Under normoxic conditions, selected body temperature was $24.2 \pm 3.6^\circ\text{C}$. When inspired O_2 was 10% or less, mean selected temperature decreased to $15.3 \pm 2.4^\circ\text{C}$. The theoretical advantages of hypoxia-induced hypothermia we tested include (1) a reduction of oxygen uptake (\dot{V}_{O_2}) by a Q_{10} effect; (2) increased arterial saturation (Sa_{O_2}), (3) a decreased ventilatory response, and (4) a decreased stress response. Gas exchange, hematocrit, hemoglobin, Sa_{O_2} , Pa_{O_2} and pH were measured at 25°C (normal preferred temperature) and 15°C (hypoxia preferred temperature) in toads breathing normoxic or hypoxic gas mixtures. During graded hypoxia at 15°C , Sa_{O_2} was significantly increased and \dot{V}_{O_2} was significantly reduced compared with 25°C . Graded hypoxia did not significantly affect \dot{V}_{O_2} at 25°C , despite evidence for increased ventilation at that temperature (increased pH and respiratory exchange ratio, RE). At 15°C , graded hypoxia had a significant effect on \dot{V}_{O_2} only at an inspired O_2 of 4%. Increased RE with hypoxia was significant at 25°C but not at 15°C . Hematocrit and [hemoglobin] rose significantly during graded hypoxia at 25°C but did not change at 15°C . Toads exposed to 10% O_2 (the value that elicits behavioral hypothermia) showed a significant respiratory alkalosis at 25°C but not at 15°C . Likewise, hypoxia caused a significant drop in Sa_{O_2} and P_{O_2} at 25°C . Cooling to 15°C during hypoxia caused a significant rise in Sa_{O_2} but no change in Pa_{O_2} . In conclusion, behavioral hypothermia is a beneficial response to hypoxia in *Bufo marinus*.

Introduction

Behavioral hypothermia in response to hypoxia occurs in each vertebrate taxon (reviewed by Wood, 1991). For amphibians, however, only an aquatic salamander (*Ambystoma tigrinum*) has been studied (Dupré and Wood, 1988). Toads (*Bufo boreas* and *Bufo marinus*) utilize behavioral hypothermia in response to food deprivation (Lillywhite *et al.* 1973), dry air (Malvin and Wood, 1991) and anemia (Wood, 1990). *Bufo marinus* also encounters hypoxia in its burrows (Zug and Zug,

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1979; Boutilier *et al.* 1979) but its behavioral response to hypoxia has not been studied. Physiological effects of hypothermia relevant to hypoxia are: (1) a left shift of the oxyhemoglobin dissociation curve and potential improvement of O₂ loading in the lungs; (2) a decreased oxygen demand (\dot{V}_{O_2}) according to the Q₁₀ effect (Krogh, 1914); and (3) a decreased ventilatory response to hypoxia (Glass *et al.* 1983; Kruhøffer *et al.* 1987; Dupré *et al.* 1989).

The effect of body temperature (T_b) on \dot{V}_{O_2} is predictable for standard metabolic rate (Q₁₀ effect). However, during hypoxia the influence of alertness, activity or catecholamines may offset the Q₁₀ effect. The effect of hypothermia on arterial O₂ saturation (S_{aO_2}) is also not readily predictable. S_{aO_2} may or may not change, depending on the degree of hypoxia, the degree of intracardiac shunting and the left shift of the oxyhemoglobin dissociation curve (Lister, 1984). These factors will depend on changes in hemodynamics and acid–base status due to metabolic, respiratory or temperature effects.

The present study tested the hypotheses that hypoxic *Bufo marinus* will select a lower than normal T_b and that this behavioral hypothermia will be beneficial. The physiological significance of behavioral hypothermia was determined with respect to metabolic rate, gas exchange, hematocrit, S_{aO_2} , acid–base balance and ventilation.

Materials and methods

Animals and preparation

Bufo marinus were obtained from Wm A. Lemberger Co. (Oshkosh, WI). Body mass of the 18 animals studied ranged from 245 to 413 g (mean = 306 ± 68 g, s.d.). They were kept in tanks with access to water in constant-temperature rooms at 25°C (the average preferred temperature) and a L:D 12 h:12 h photoperiod. They were fed crickets twice a week until 4 days before being studied. Two days before experiments, they were anesthetized with sodium brevitall (40 mg kg⁻¹), and a catheter (PE-50) was placed in the femoral artery and secured with sutures. A thermistor probe (YSI) was inserted 2 cm into the cloaca. The catheter and probe were secured to the animal with an elastic harness. Thermistor output was digitized and directed into a computer which averaged and stored temperature data every 15 min over a 24 h period.

Temperature regulation

Temperature selection was studied using a sealed chamber, 1.5 m × 0.5 m. One end was heated to approximately 40°C with a 100 W light bulb. The other end was cooled to approximately 10°C with a rubber pad under the floor. A separate experiment using a hot plate as the heat source showed that the presence of light did not influence the experimental results. Sievert and Hutchison (1988) also found no significant effect of a lighted heat source (compared with uniform light) on temperature selection of the gecko lizard. The experiments took 2 days. During day 1, toads were placed in the center of the temperature gradient. A normoxic gas

mixture (21 % O₂/79 % N₂) was pumped through the chamber at approximately 760 ml min⁻¹. Petri dishes filled with water were placed every 20 cm along the temperature gradient to provide access to water at all temperatures. On day 2, toads were placed back in the center of the gradient and a hypoxic gas mixture (7–10 % O₂, balance N₂) was pumped through the chamber.

Metabolic rate and gas exchange

Animals were placed in a 6 l desiccator jar with water under the wire mesh floor. The jar was kept in an environmental chamber (Lab Line, Inc.) at 15±1 or 25±1 °C. The catheter was fed through a small hole in the chamber, as were the gas inlet and outlets. The toads were thereby kept isolated from visual and sound disturbances. A small fluorescent light was kept on in the chamber during the studies. Metabolism was measured for 24 h, from 08:00 to 08:00 h. Flow through the chamber was 57 ml min⁻¹. For a 6 l chamber, the 99 % washout period is greater than 6 h. Only minimum values, stable for more than 6 h, were used. \dot{V}_{O_2} was corrected to STPD and reported per kilogram body mass (animals were handled until the bladder was voided before starting respirometry).

In some experiments, animal activity during graded hypoxia was televised through a hole in the chamber door with an ITC camera (ITC-40; Ikegami Tsushinki, Japan) and recorded on video tape using a Panasonic video cassette recorder. Because the measurements of metabolic rate during graded hypoxia took 6 days per toad to complete, time was a potential independent variable. Therefore, a time control experiment measured metabolic rate for 6 days at a constant inspired O₂ of 26 %.

Oxygen uptake (\dot{V}_{O_2}) and carbon dioxide production (\dot{V}_{CO_2}) were measured by flow-through respirometry using a CO₂ analyzer, model CD-3A, and O₂ analyzer, model S-3A (Applied Electrochemistry Inc., Sunnyvale, CA 94086) with an accuracy of ±0.01 % oxygen or 0.1 % of the reading, whichever was smaller, and stability of ±0.01 % oxygen in 24 h. Flow through the metabolism chamber was measured with a flow meter, Gilmont model F1100, with an accuracy of ±2 % of the reading, and corrected to ambient pressure and temperature (Gilmont and Rocanova, 1966). The respiratory exchange ratio (RE) was calculated as $\dot{V}_{CO_2}/\dot{V}_{O_2}$.

Graded hypoxia

Gas mixtures of O₂ and N₂ were produced with a model 192 precision gas mixer (Corning Medical and Scientific, Medfield, MA 02052). Graded exposure of toads to the hypoxia was carried out in the respirometer chamber at both 25 and 15 °C at the levels indicated in Table 1.

Because Albuquerque, NM, is 1600 m above sea level, 26 % inspired O₂ was used to simulate sea-level inspired P_{O₂}. The sequence of exposure went from highest to lowest O₂ fractions. For measurements of metabolic rate, the exposure duration was 24 h at each level. For blood gas analysis, the exposure was 2 h.

Table 1. *Levels of inspired oxygen and equivalent altitudes to which Bufo marinus was exposed during graded hypoxia experiments at 15 and 25°C*

	Inspired O ₂ (%)						
	26	21	12	10	8	6	4
Inspired P _{O₂} (kPa*)	21.1	16.9	9.7	8.1	6.4	4.8	3.2
Equivalent altitude (m)	0	1220	4900	6500	7800	9000	11 600

* These are approximate values. Actual values varied with temperature (15 or 25°C) and daily barometric pressure (84±0.6 kPa).

Constant hypoxia

Blood gases and pH were also measured in toads kept at the normal preferred temperature of 25°C or the hypoxia preferred temperature of 15°C, breathing either 21% O₂ or 10% O₂, the threshold level of hypoxia at which behavioral hypothermia of *Bufo marinus* is elicited. Blood was sampled at random points in the breathing cycle to obtain a range of values appropriate to periodic breathers.

Blood gas analysis and ventilation

The following variables were determined in arterial blood samples: (1) hemoglobin concentration ([Hb]) using a Radiometer OSM2 hemoximeter; (2) O₂ saturation using a Radiometer OSM2 hemoximeter (New London, OH); (3) hematocrit using a hematocrit centrifuge, Microcompur M1100 (Compur-Electronic GmbH, München), which centrifuges for 200 s at 11 500 revs min⁻¹ (5396 g); (4) blood gases and pH, using a Radiometer BMS 3 MK 2 blood micro system and PHM 73 pH/blood gas monitor (New London, OH) calibrated at 15 or 25°C with certified pH standards (Radiometer) and precision gas mixtures (Corning 192 mixer).

Because *Bufo marinus* is a periodic breather, arterial blood gases and pH show marked cyclic variations (Boutilier and Toews, 1977). To standardize comparisons between 15 and 25°C during graded hypoxia, blood was sampled 30 s after the last breath of a breathing cycle. Respiration was measured with an impedance pneumograph (no. 76407, Lafayette Inst. Co., Lafayette, IN). In the experiments comparing blood gases of animals breathing either 21% or 10% O₂ at 25° or 15°C, blood samples were taken at random times during the breathing cycle to obtain the range of values normally experienced by *Bufo marinus*.

Statistics

The graded hypoxia data were analyzed by two-way analysis of variance with factors of inspired O₂ pressure and temperature. *Post hoc* comparisons of significant analysis of variance were made using the Newman-Keuls test. The data comparing normoxic toads with toads breathing 10% O₂ were compared using paired (where appropriate) or unpaired *t*-tests. Data involving percentages

(hematocrit, arterial saturation) were found to be normally distributed (Shapiro-Wilk test) and were therefore not transformed to their arcsine before being analyzed. $P \leq 0.05$ was considered significant.

Results

Behavioral hypothermia induced by hypoxia

When inspired O_2 was reduced from 21 to 10 %, the mean selected temperature of *Bufo marinus* over 24 h was significantly reduced. Fig. 1 shows the 24 h pattern of T_b selection for seven toads during normoxia and hypoxia. The threshold for the behavioral response to hypoxia was determined by gradually reducing the inspired O_2 in the thermal gradient chamber over a 24 h period and recording T_b . As shown in Fig. 2, there is a sharp threshold at an inspired O_2 of approximately 10 %. The mean selected T_b was 24.2 ± 3.6 at 21 % inspired O_2 and $15.3 \pm 2.4^\circ\text{C}$ ($N=7$) at 8 % inspired O_2 .

Gas exchange

As shown in Fig. 3, *Bufo marinus* is an oxyregulator at 25°C , showing no significant change in \dot{V}_{O_2} from the normoxic value over the range of graded hypoxia. At 15°C , *Bufo marinus* also maintained a constant \dot{V}_{O_2} down to 4.8 kPa. However, at an inspired P_{O_2} of 3.2 kPa, \dot{V}_{O_2} was significantly increased. Because these experiments lasted up to 6 days, it was possible that a time-dependent change in \dot{V}_{O_2} was masking some effect due to hypoxia. Therefore, a time control series was conducted with six toads whose \dot{V}_{O_2} was measured under normoxic conditions for 6 days at 15°C . As shown in Fig. 4, there was no significant effect of time on \dot{V}_{O_2} .

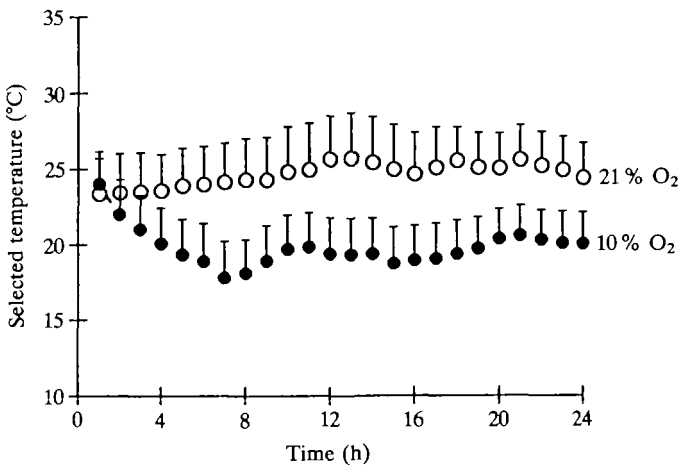


Fig. 1. Selected body temperature (mean \pm s.d.) of *Bufo marinus* ($N=7$) in a thermal gradient of $10\text{--}40^\circ\text{C}$ and breathing either 21 % (○) or 10 % (●) O_2 . The selected body temperature in animals breathing 10 % O_2 is significantly reduced ($P < 0.01$) between 6 and 24 h.

Arterial saturation, blood gases and pH

Arterial blood was sampled from unanesthetized toads at a T_b of either 25°C (normal preferred temperature) or 15°C (hypoxic preferred temperature) during graded hypoxia or when animals were breathing either 21% or 10% O_2 . Results for the latter experiment are summarized in Table 2.

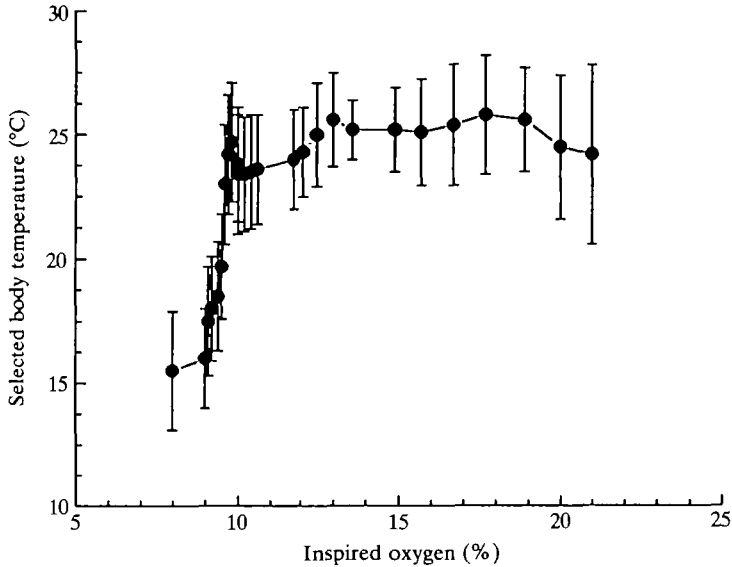


Fig. 2. Selected body temperature (mean \pm s.d.) of *Bufo marinus* ($N=7$) in a thermal gradient of 10–40°C during graded hypoxia.

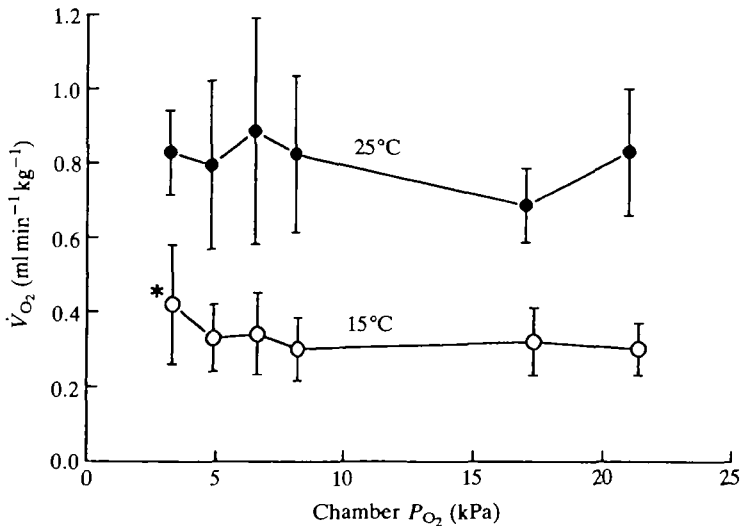


Fig. 3. Oxygen uptake (mean \pm s.d.) of *Bufo marinus* ($N=8$) at 25°C (●) and 15°C (○) during graded hypoxia. * indicates significant difference at 15°C from the value at 21% O_2 using the Newman-Keuls test.

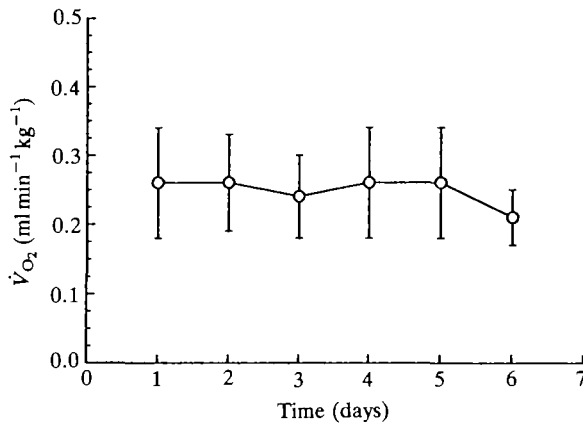


Fig. 4. Oxygen uptake (mean \pm s.d.) of *Bufo marinus* ($N=6$) at 15°C breathing 26% O₂ and measured over 6 consecutive days. Analysis of variance $P=0.8$ for the time effect. \dot{V}_{O_2} data are a time control for the experiment shown in Fig. 3.

Table 2. Arterial saturation, P_{O_2} and pH of resting *Bufo marinus* ($N=5-12$) at 15 and 25°C breathing 21 or 10% O₂

Body temperature	Arterial saturation (%)		Arterial P_{O_2} (kPa)		Arterial pH	
	21% O ₂	10% O ₂	21% O ₂	10% O ₂	21% O ₂	10% O ₂
25°C	67.4 \pm 9.2	34.8 \pm 7.5*	10.2 \pm 1.3	5.8 \pm 0.9*	7.79 \pm 0.08	7.91 \pm 0.08*
15°C	49.9 \pm 10.2†	56.2 \pm 15.3	5.8 \pm 1.8†	6.1 \pm 2.4	7.90 \pm 0.12†	7.92 \pm 0.06

Values are means \pm s.d.; * significant effects of hypoxia; † significant effects of temperature; $P<0.05$.

Sa_{O_2} was measured using two protocols. In one experiment, the blood was obtained immediately after a ventilatory cycle during graded hypoxia. These data represent maximum Sa_{O_2} at each inspired P_{O_2} and are shown in Fig. 5. At 25°C, the drops in Sa_{O_2} with progressive hypoxia are significant at each decrement of inspired P_{O_2} . At 15°C, the drops in Sa_{O_2} are also significant, except for the difference between 16.9 and 9.9 kPa. In the other experiment, arterial blood was obtained from toads at random points in their breathing cycles (=ventilatory periods and apneic periods) when they were breathing either room air or 10% O₂. Results are shown in Fig. 6A for individual toads. The scatter is large, as expected for periodic breathers like *Bufo marinus* (Boutilier and Toews, 1977). The drop in Sa_{O_2} at 25°C when toads switched from 21% to 10% inspired O₂ (25N to 25H) was from 67 to 35% ($P<0.0001$). At 15°C, the mean Sa_{O_2} of toads breathing 10% O₂ (15H) was 49.9%, significantly higher than for hypoxic toads at 25°C ($P<0.0017$). At 15°C, normoxic toads (15N) had a higher Sa_{O_2} than hypoxic toads (15H), but the difference was not significant.

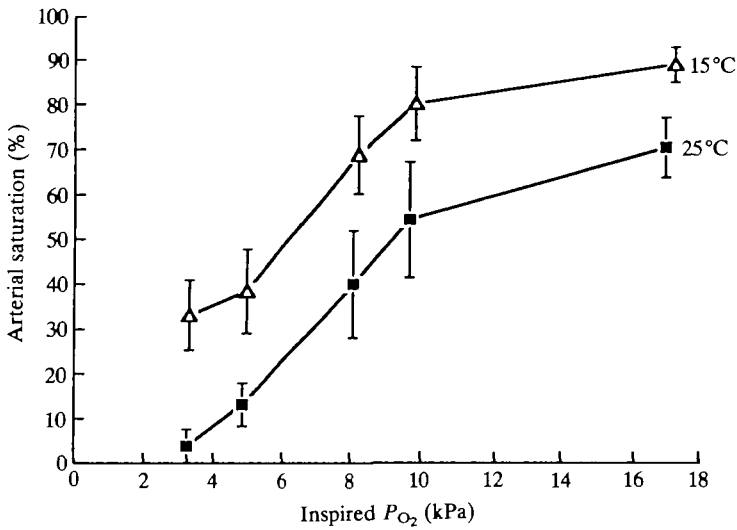


Fig. 5. Arterial saturation (mean \pm s.d.) of *Bufo marinus* at 25°C (■) and 15°C (Δ) during graded hypoxia. Blood was sampled immediately after a ventilatory period to obtain maximum saturation values; $N=5$; analysis of variance $P<0.005$ for temperature effect.

Arterial P_{O_2} for individual toads breathing 21% or 10% O_2 at 25° and 15°C are shown in Fig. 6B. As in Fig. 6A, the scatter reflects the fact that blood was sampled at random points in the breathing cycle. Hypoxia caused a significant drop in arterial P_{O_2} at 25°C ($P<0.001$) but not at 15°C. During normoxia, the reduction of T_b from 25 to 15°C (25N to 15N) caused a significant fall in arterial P_{O_2} ($P<0.001$).

As shown in Fig. 6C, arterial pH was significantly increased during hypoxia at 25°C ($P=0.006$) but not at 15°C. The effect of T_b on arterial pH was significant for normoxic toads ($dpH/dT=-0.013$; $P=0.0007$) but not for hypoxic toads.

Respiratory exchange ratio

The effect of graded hypoxia on the respiratory exchange ratio (RE) is shown in Fig. 7. Two-way analysis of variance revealed a significant effect of temperature ($P<0.003$), inspired $[O_2]$ ($P<0.001$) and their interaction ($P<0.001$) on RE. *Post hoc* tests revealed that the RE at 4% inspired $[O_2]$ was significantly higher ($P<0.01$) than that at all other inspired $[O_2]$ at 25°C. At 15°C, RE at 4% inspired $[O_2]$ was significantly higher ($P<0.01$) than the value at 21% inspired $[O_2]$ only.

Hematocrit

Graded hypoxia caused a significant increase in hematocrit at 25 but not at 15°C (Fig. 8). Hemoglobin concentration, not shown, showed the same pattern and there was no indication of altered red cell volume. A control experiment where

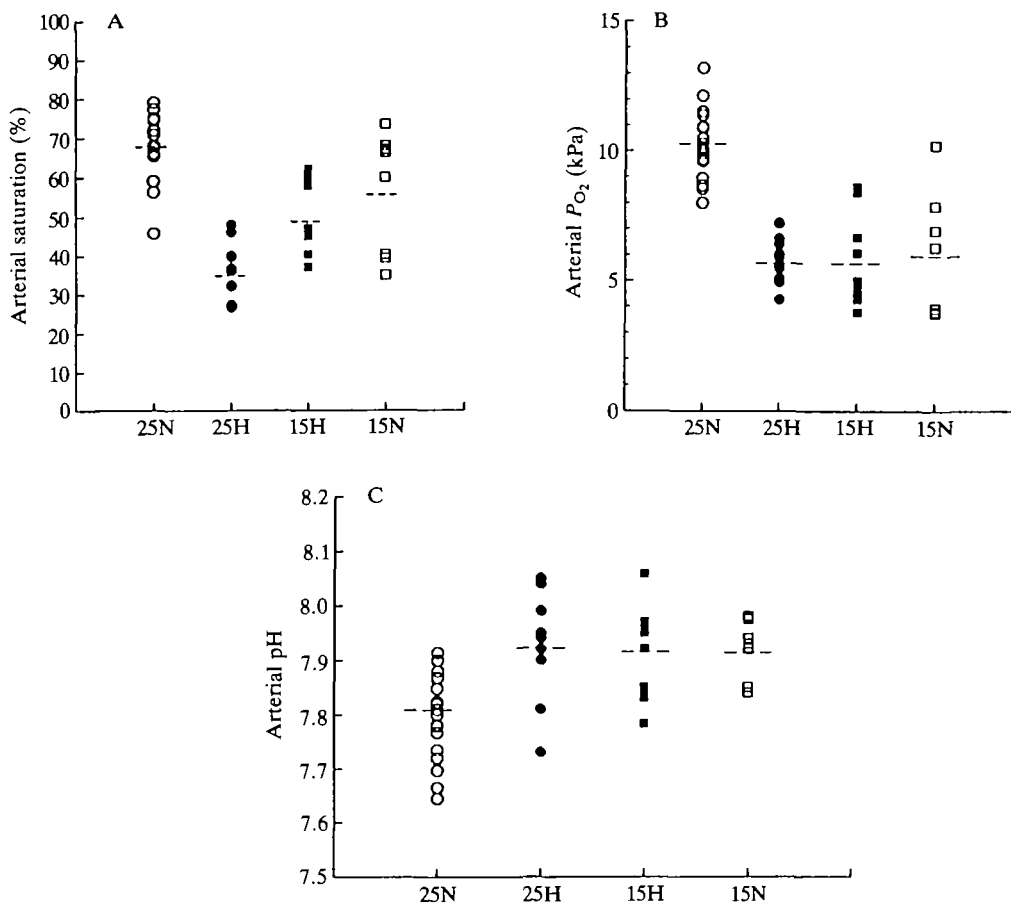


Fig. 6. Arterial saturation (A), arterial P_{O_2} (B) and arterial pH (C) of *Bufo marinus* breathing 21% (N) or 10% O_2 (H) at 25 or 15°C. Blood was sampled at random times in breathing cycles. Dashes show mean values.

blood samples were taken every hour for 5 h from toads breathing 21% O_2 showed no significant change in hematocrit (Fig. 8, inset).

Discussion

Behavioral hypothermia induced by hypoxia

Bufo marinus shows the same behavioral response to hypoxia seen previously in other ectotherms and homeotherms, i.e. a threshold level of hypoxia (approximately 10%) below which the selected T_b is significantly reduced (Wood, 1991). For *Bufo marinus*, the selected T_b decreases by 10°C during exposure to 8% inspired O_2 . As shown in Fig. 2, *Bufo marinus* does not have a diel cycle of selected T_b , unlike the pattern in most ectotherms (e.g. Sievert and Hutchison, 1988).

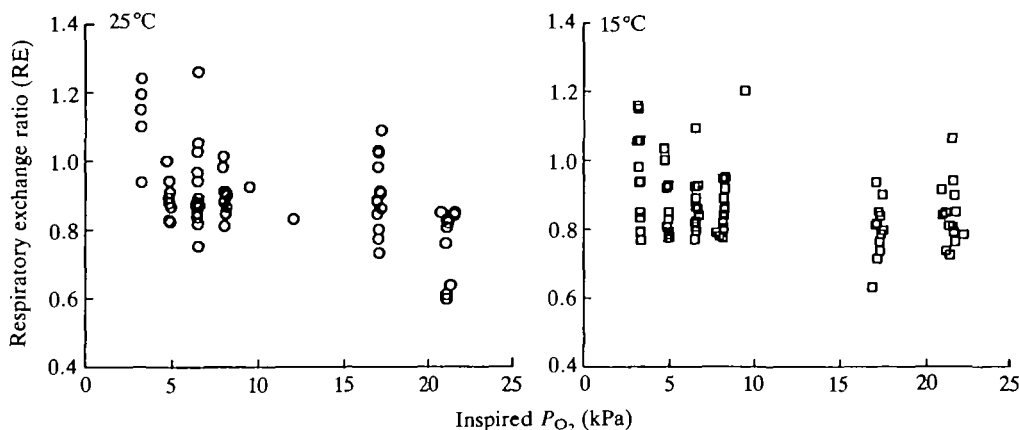


Fig. 7. Respiratory exchange ratio of *Bufo marinus* during graded hypoxia at 25 and 15°C; $N=18$; Two-way analysis of variance revealed significant effects of inspired P_{O_2} ($P=0.001$), temperature ($P=0.003$) and their interaction ($P=0.001$).

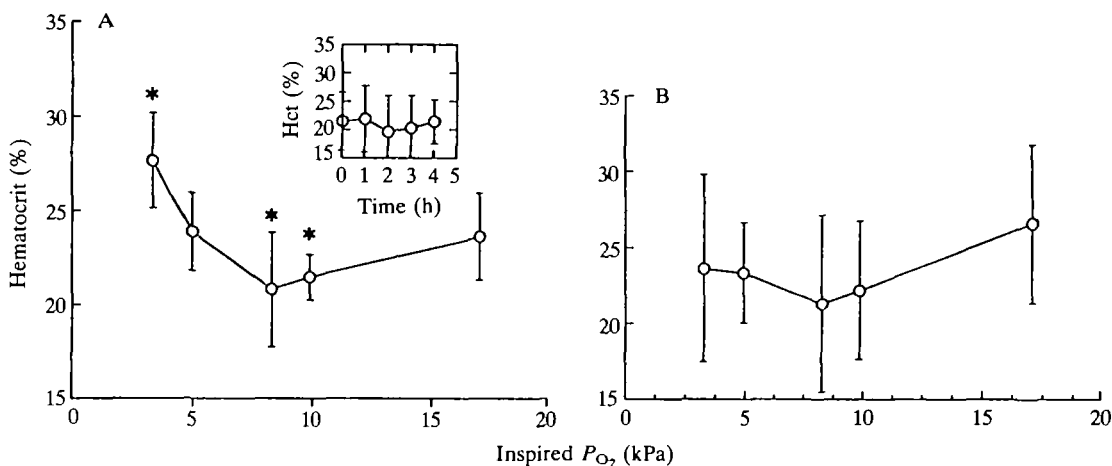


Fig. 8. Hematocrit of *Bufo marinus* during graded hypoxia at 25 (A) and 15°C (B). The time control experiment at 25°C is in upper inset. * indicates a significant difference ($P<0.05$; Newman-Keuls test) from the normoxic value.

Gas exchange

The predicted decrease in \dot{V}_{O_2} based on the drop in temperature was realized with a Q_{10} over the range of inspired P_{O_2} values of approximately 2.3. Pörtner *et al.* (1991) measured \dot{V}_{O_2} in *Bufo marinus* during graded hypoxia at 20°C. Their normoxic value of \dot{V}_{O_2} is $0.44 \text{ ml min}^{-1} \text{ kg}^{-1}$, in close agreement with the present values corrected to 20°C. At 21% inspired O_2 , the present Q_{10} was 2.07, predicting a \dot{V}_{O_2} at 20°C of $0.43 \text{ ml min}^{-1} \text{ kg}^{-1}$. Pörtner *et al.* (1991) also found that \dot{V}_{O_2} increased significantly at an inspired P_{O_2} of 2.8 kPa. This was associated with

an increase in lactic acid levels. They suggested that this increase in \dot{V}_{O_2} , instead of the expected decrease at the 'critical' P_{O_2} , may reflect a stress response mediated by catecholamine release (Boutilier and Lantz, 1989).

In the present study, video analysis of toads during graded hypoxia, while not quantified, showed increased activity as well as exaggerated breathing at 4% inspired O_2 . These factors could also contribute to the higher \dot{V}_{O_2} . At 25°C, the trend towards higher values of \dot{V}_{O_2} during hypoxia was not significant and the variance of the data was much higher. Some animals showed a marked increase in \dot{V}_{O_2} when P_{O_2} was below 6.7 kPa, while others showed no change or a decrease. Video recordings of toads at 25°C and 4% inspired O_2 showed that some toads moved more, that ventilation was depressed in some toads (as reported previously for *Bufo marinus*, Boutilier and Toews, 1977) and that some of the toads died (data on their \dot{V}_{O_2} are not included in Fig. 3). Conversely, all of the toads exposed at 15°C survived 24 h of 4% inspired O_2 .

Arterial saturation, P_{O_2} and pH

The effect of T_b on Sa_{O_2} and P_{O_2} under normoxic conditions is consistent with previous findings for *Bufo marinus* and most other ectotherms. Arterial P_{O_2} was lower at 15°C than at 25°C, as predicted for constant Sa_{O_2} from models and data for animals with an intracardiac shunt (Wood, 1984). Normoxic Sa_{O_2} also decreased from 67 to 54% as T_b dropped from 25 to 15°C ($P=0.0001$). The reason for the large decrease in Sa_{O_2} with increased T_b in the present study is not clear, except that resting toads at 15°C may allow their Sa_{O_2} to decline to lower values between breathing periods than they do at 25°C. When blood was sampled immediately after a breathing period, the opposite results were obtained, i.e. Sa_{O_2} was higher at 15°C (Fig. 5). Boutilier *et al.* (1987) measured arterial blood gases and Sa_{O_2} of *Bufo marinus* at 10, 20 and 30°C using samples obtained during ventilatory periods. They also found a decrease in Sa_{O_2} of approximately 77–70% between 15 and 25°C and a significant increase in Pa_{O_2} . Clearly, there is a different pattern of temperature effects depending on the phase of the ventilatory cycle in which blood is sampled. Despite this, it is clear from Figs 5 and 6 that behavioral cooling to 15°C during hypoxia greatly improves arterial oxygenation.

The effect of hypoxia on arterial pH at 25°C indicates a respiratory alkalosis due to hypoxia-induced hyperventilation. The absence of a significant effect of hypoxia on pH at 15°C is consistent with data showing a reduced ventilatory response to hypoxia at lower temperatures in turtles (Glass *et al.* 1983), lizards (Dupré *et al.* 1989) and the toad *Bufo paracnemius* (Kruhøffer *et al.* 1987). Consistent with the lack of an effect of 10% inspired O_2 on arterial pH at 15°C (Fig. 6C), the increase in RE at 10% inspired $[O_2]$ was also not significant (Fig. 7), indicating that there was no hyperventilation.

Arterial pH of *Bufo marinus* at 25°C is 7.79 ± 0.08 , similar to the value reported by Howell (1970) of 7.71 at 28°C. The effect of body temperature on arterial pH is significant under normoxic conditions. The temperature coefficient ($d \log pH / dT =$

-0.013) is similar to that reported for *Bufo marinus* (-0.014) by Boutilier and Heisler (in Heisler, 1986) and by Boutilier *et al.* (1987).

The degree of hypoxia used in the present study was designed to elicit a stress response and not to mimic levels of hypoxia encountered routinely in nature. However, *Bufo marinus* does burrow voluntarily. Boutilier *et al.* (1979) showed that during a voluntary 6-day burrow in sand, the P_{O_2} of *Bufo marinus* at 25°C declined from approximately 12 kPa to 8 kPa. This is approximately the same range of values obtained by us sampling arterial blood at random times during the ventilatory cycle (Fig. 6B). Thus, this periodic breather allows its arterial P_{O_2} to fall to approximately 8 kPa during its apneic periods at 25°C. During hypoxic exposure at 25°C, maximum arterial P_{O_2} was approximately 7.6 kPa, only slightly less than values tolerated during normoxia.

Respiratory exchange ratio

An increase in RE with temperature, as we found in *Bufo marinus*, has been previously reported for frogs and tortoises (Dontcheff and Kayser, 1937) and snakes (Stinner, 1982). Possible mechanisms include the release of stored CO_2 in the body fluids and a shift to carbohydrate metabolism with rising temperature. The increase in RE with progressive hypoxia also relates to release by hyperventilation of CO_2 originally stored as bicarbonate.

Hematocrit

The increase in hematocrit during hypoxia at 25°C but not 15°C is additional evidence for reduction of stress due to hypothermia. Previous studies of hypoxic amphibians have shown that hemoconcentration is secondary to fluid shifts out of the plasma into interstitial fluid. The mechanism is not known but the effect may be due to increased blood pressure or increased tissue lactate concentration and osmotic flux of fluid from plasma to tissues (Boutilier *et al.* 1986). Pörtner *et al.* (1991) showed a significant increase in plasma and tissue lactate levels in *Bufo marinus* at 20°C when inspired P_{O_2} was reduced below 4–4.9 kPa.

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