Physiological Basis for Prolonged Submergence in Hibernating Garter Snakes Thamnophis sirtalis: Evidence for an Energy-sparing Adaptation

Jon P. Costanzo
Department of Zoology, Miami University, Oxford, Ohio 45056

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Abstract
Garter snakes (Thamnophis sirtalis) submerge in a water-filled hibernaculum in Wisconsin where they remain for up to 5.5 mo during winter. Since ice cover may inhibit air breathing for long periods, this study examined the role of nonpulmonary ventilation in the overwintering biology of these snakes. During submerged hibernation, mean standard rates of oxygen consumption and cardiac activity were depressed by 54.8% and 77.0%, and O₂ pulse (O₂ consumed per heart stroke) increased 1.8-fold, relative to laboratory hibernation in air under otherwise similar conditions (5°C, total darkness). Owing to metabolic, cardiovascular, and behavioral adjustments to submergence in cold water, snakes were able to remain in oxygen balance during winter; lactate analyses indicated that, in normoxic water (P₀₂ ≈ 110 mmHg), cutaneous diffusion of oxygen was adequate for aerobic metabolism. Calculated energy consumption in the average (52.6 g) snake during a typical, 165-d winter in the Wisconsin den was 55.2% less in water (3.13 kcal) than in air (6.98 kcal). Since spring reproductive activities of garter snakes inhabiting high latitudes are supported by stored nutrients, this energy-sparing adaptation may significantly increase individual fitness.

Introduction

Among reptiles, ecological aspects of hibernation are perhaps best known for the ophidians (review in Gregory 1982). Snakes, with few exceptions, are unable to construct dens and must therefore make use of preexisting features of the physical environment to survive winter conditions.

Moisture availability is an important feature of snake dens that has received relatively little attention in the literature. The association between
hibernating snakes and water in dens (e.g., substrate moisture, standing water, water table, water vapor, etc.) varies considerably. For example, snakes overwintering in a cave avoided small pools (Drda 1968), whereas those using an ant mound maintained their bodies (but not their heads) below the water table (Cridle 1937). Snakes hibernating in a Missouri cave submerged occasionally, except for their snouts (Sexton and Hunt 1980).

Prolonged, complete submergence of hibernating snakes has rarely been documented. However, the contention that this behavior often occurs is substantiated by several reports. Owens (1949) described the spring emergence of blue racers (Coluber constrictor) and rat snakes (Elaphe obsoleta) from a water-filled cistern in Missouri. The snakes were 5-15 cm underwater, but periodically surfaced to breathe. In Michigan, flooded crayfish burrows excavated in winter by Carpenter (1953) held garter snakes (Thamnophis sirtalis) and water snakes (Nerodia sipedon) but contained little or no air. These snakes presumably would have remained completely submerged until spring. Fox snakes (Elaphe vulpina) apparently hibernate underwater in cisterns for extended periods in Wisconsin (Zarembo 1978; Costanzo 1986).

Aspects of prolonged winter submergence were recently reported (Costanzo 1986) in a detailed study of a communal snake hibernaculum in Portage County, Wisconsin. An abandoned farm well that served as a winter refuge for 200-300 garter snakes, became partly flooded in late summer. Upon entering the den in October, garter snakes (and fox snakes) occupied underwater sites where, to counter buoyancy, they anchored themselves in place among structural rocks. This curious behavior seemed facultative since, at the time of ingress, physical and thermal conditions within the den were uniform and adequate air space was available. Periodic observations (lasting up to 2 h) made during fall and spring, over the course of several years, showed that snakes nearest the surface gulped air occasionally, but those positioned deep underwater (up to 80 cm) did not. Since ice usually forms at the water surface during winter, pulmonary respiration is probably inhibited in all submerged snakes. Although it has not been measured directly, the duration of ice cover in the Wisconsin den is undoubtedly influenced by water depth, precipitation, and prevailing ambient temperatures. Winters in central Wisconsin are severe; thus, the potential for extended apnea is correspondingly high; however, winter mortality in this den population is remarkably slight.

This scenario poses some interesting questions regarding the role of non-pulmonary gas exchange in the winter life histories of snakes that use flooded dens. Accordingly, the present study was undertaken to characterize the physiological basis for this phenomenon.
Material and Methods

Experimental Animals and Maintenance

On October 7, 1986, eastern garter snakes (Thamnophis sirtalis) were collected at a communal hibernaculum in Portage County, central Wisconsin (44°N, 90°W). Autumnal conditions were simulated by exposing snakes to cyclic thermal and photo regimes (20°C, 12-h photophase; 5°C, 12-h scotophase), including dawn and dusk, for 3 wk. Water was always available, but food was withheld.

On November 1, 37 snakes were placed in a laboratory hibernaculum constructed with a wooden frame and pegboard walls. A Styrofoam container in the bottom of the hibernaculum, which was filled with dechlorinated water to a depth of 25 cm, permitted snakes to submerge completely. Environmental conditions were adjusted gradually over 2 wk and thereafter maintained at 5° ± 1°C, DD (total darkness), until the experiment ended on April 25, 1987.

General Testing Protocol

Oxygen consumption (V\textsubscript{O2}) and standard heart rates (SHR) of hibernating snakes were measured between January 5 and April 25, 1987. Each animal was sequentially tested once in four situations: V\textsubscript{O2} in water, SHR in air, SHR in water, and V\textsubscript{O2} in air. For submergence experiments, snakes were taken directly from the water compartment in the hibernaculum. Measurements in air were made after snakes were habituated in dry plastic boxes for 7–14 d.

Testing was conducted at 5° ± 1°C, DD, and without regard to body size or sex. The V\textsubscript{O2} measurements of snakes (mean mass = 53.2 ± 6.6 g; N = 14) in water were made from 1630 hours to 1000 hours the next day, following a 2.5-h habituation period. The V\textsubscript{O2} measurements of snakes (mean mass = 53.7 ± 7.0 g; N = 13) in air were made during a 3-h trial (starting at 0900 hours) subsequent to a 16.5-h habituation period. Following a similar habituation protocol, SHRs of snakes (mean mass = 52.6 ± 6.2 g; N = 15) tested in both media were measured during 10-min periods starting at 0900 hours.

Oxygen Consumption in Water

A 20-L plastic carboy (28 cm in diameter), modified by its top's being removed, served as the testing chamber. A peristaltic pump circulated dechlorinated chamber water (7,000 mL) via Tygon plastic tubing through a flowmeter and flow cell fitted with a Beckman dissolved oxygen (DO) probe.
Submerged Hibernation in Garter Snakes

Snakes were prevented from surfacing by being confined to a hardware-cloth cage within the chamber. Mineral oil poured on the water formed a 0.8-cm-thick barrier to gas diffusion. Control experiments, using the respirometer without animal, indicated that chamber DO remained stable for at least 20 h. Observations of dye distribution showed that the water was well mixed at the flow rate (20 mL/min) used; complete turnover occurred within 6 h. Typical pretrial values for chamber water pH and dissolved gases (measured with a Hach kit, Hach Chemical Co., Ames, Iowa) were pH 7.6, $\text{CO}_2 = 8.8 \text{ mg/L (P}_{\text{CO}_2} = 2.5 \text{ mmHg})$, and $\text{DO} = 9.7 \text{ mg/L (P}_{\text{O}_2} = 117.6 \text{ mmHg})$. Estimates of $\dot{V}_{\text{O}_2}$ were made by converting the change in DO to its equivalent volume at STP. During trials, chamber DO was typically reduced by ca. 0.7 mg/L (8.5 mmHg).

**Oxygen Consumption in Air**

Snakes were placed, unrestrained, in an airtight, 693-mL plastic cylinder fitted with two gas ports. Baseline $\text{O}_2$ content of air drawn through the chamber at 50 mL/min during habituation was measured using an Ametek $\text{O}_2$ analyzer (model S-3A/II). The test chamber was subsequently sealed for 3 h, following which the $\text{O}_2$ concentration, with respect to baseline, was determined. An identical chamber, without animal, served as a calibration reference. Water vapor and $\text{CO}_2$ were removed from gas samples prior to analysis, using Drierite and Ascarite. Snake $\dot{V}_{\text{O}_2}$ (at STPD) was calculated from the change in $\text{O}_2$ content (typically ca. 0.25%) from the total volume of chamber gas. To estimate the latter, the mass of the subject was subtracted, using 1.0 g/mL as the specific density of snake tissue.

**Standard Heart Rates**

The SHRs were measured in air (inside dry plastic boxes) and water (submerged, as in $\dot{V}_{\text{O}_2}$ trials) using three ultrafine needle electrodes positioned subdermally on the right, dorsolateral aspect of the snake’s body, with two leads bracketing the heart.

Electrocardiograms were recorded on a Grass polygraph (model 7D) within 3 min after the animal had been placed in the appropriate testing situation, and again after a 16.5-h habituation period. The SHRs were calculated from the mean time interval between successive $R$-wave peaks on the (posthabituation) trace and converted to beats per minute (bpm).
Lactate Determinations

Whole-body lactate concentrations were determined for snakes kept in water (mean mass = 37.4 ± 2.1 g; N = 5) and air (mean mass = 32.6 ± 2.6 g; N = 5). Within 2 wk of being quick-frozen (−70°C), they were homogenized for 5 min in a blender with cold, 0.31 M trichloroacetic acid. An aliquot (10%) of the 300-mL homogenate was centrifuged in the cold at 15,000 × g for 20 min; the resulting supernatant was again centrifuged. Supernatant fractions were passed through a 0.45-μm millipore filter prior to analysis with a Sigma lactate test kit (825-UV). For comparative purposes, garter snakes (N = 3) from a laboratory colony maintained at 22°C were killed and assayed identically.

Statistical Analyses

Physiological parameters measured from the same snakes in air and water were compared using Student’s t-test for dependent measures. Linear regression was used to evaluate the relationship between body mass and physiological rate functions. Lactate values for snakes hibernating in air and water were compared using Student’s t-test for independent samples. Statistical procedures followed Sokal and Rohlf (1973); significance was judged, a priori, at P < .05. Mean values are reported ±1 SEM.

Results

Mass loss over the 175-d study period averaged 7.0% ± 0.8% of initial values but occurred only when snakes were kept in air. Three snakes died during the study (all while hibernating in air), and dehydration may have been a causative factor; water contents of these animals at death (64.4%–68.6% of fresh mass, measured by drying at 40°C to constant mass) were well below 75%, the expected value for hydrated Thamnophis sirtalis during winter (Costanzo 1985). Individual values of body mass, measured before and after experimentation, were averaged for computational purposes.

Periodic observations made during the habituation phases of VO2 and SHR trials indicated that snakes in air remained alert, moved occasionally, and showed typical resting body postures. In contrast, submerged snakes were motionless and limp and otherwise appeared lifeless.

Garter snakes responded to preparatory handling by increasing locomotor activity, but all subjects quickly resumed a quiescent state. Adequate habitu-
ation time was allowed prior to sampling; hence, values for \( \dot{V}O_2 \) and cardiac activity reported herein should represent standard rates.

Mean oxygen-consumption rates were significantly \( (P < .001) \) higher when snakes were tested in air \((372 \pm 23 \mu L/h)\) than in water \((166 \pm 6 \mu L/h; \text{fig. 1})\). Low correlation of determination values \((r^2 = 0.033 \text{ in air}; r^2 = 0.001 \text{ in water})\) indicated that, in both media, \( \dot{V}O_2 \) was independent of body mass.

Relative to SHR's, preparatory handling elevated cardiac activity in snakes tested in air about 35%. Periodic observations indicated that, following initial submergence, cardiac activity in snakes tested in water declined slowly (fig. 2), although SHR's were likely attained prior to the final sampling.

Cardiac arrhythmia was observed often in snakes tested in both air and

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**Fig. 1.** Oxygen consumption rates of garter snakes hibernating at 5° C, DD, in air and water, relative to body mass.

**Fig. 2.** Representative time course of heart rate depression following the onset of submergence (at time zero) in a 38-g garter snake tested at 5° C, DD. Mean values (points) were calculated from R-R time intervals for each min and are shown ±1 SEM (vertical bars).
water; however, it was much more pronounced in the latter group. The mean coefficient of variation in R-R time intervals for submerged snakes (21.1% ± 3.9%) was statistically (P = .002; N = 15) distinguishable from that (6.7% ± 2.2%) for snakes tested in air. In an extreme case, R-R time intervals measured for a submerged snake ranged from 10 to 541 s.

The SHRs of snakes were significantly (P < .001) higher when tested in air (fig. 3); paired sample means were 6.0 ± 0.3 bpm in air and 1.4 ± 0.1 bpm in water. Body mass had little influence on SHRs of snakes tested in air (r² = 0.000) and water (r² = 0.141). Mean O₂ pulse was 2.53 ± 0.37 mL g⁻¹ beat⁻¹ × 10⁻⁵ in air and 4.53 ± 0.68 mL g⁻¹ beat⁻¹ × 10⁻⁵ in water.

There was no difference (P = .301) in mean lactate concentration between air (0.52 ± 0.05 mg/g) and water (0.49 ± 0.04 mg/g) hibernating groups. Values for *T. sirtalis* acclimated to 22°C (0.43 ± 0.03 mg/g) were similar to those of hibernating snakes.

**Discussion**

Seymour (1982, p. 24) defined forced dives as "... artificial extensions of voluntary dives or immediate involuntary submersions of restrained animals." It is currently accepted (e.g., Gatten 1984) that the results of such "forced-dive" experiments are often distinct from those obtained from free-diving animals; hence, they should be interpreted cautiously. Since the garter snakes in this study were unrestrained and since they volitionally submerge in cold water for extended periods in natural (Costanzo 1986) and simulated (Costanzo 1985; personal observation) hibernacula, behavioral
and physiological responses of *Thamnophis sirtalis* under these experimental conditions may closely approximate those in nature.

**O₂ Consumption**

Metabolic rates of *T. sirtalis* measured in air at 5°C, DD, are similar to those reported for *T. s. parietalis* from Manitoba, Canada (Aleksiuk 1976). The scaling exponents \((b = 0.09 \text{ in air, } b = -0.01 \text{ in water})\) for the relationship between log-transformed \(\dot{V}_O_2\) at 5°C and body mass is, however, well below the expected value \((b = 0.66; Dmi'el 1986)\) for snakes at warmer temperatures. This puzzling result may be related to the low test temperature used in this study but may also be an effect of the high variability in \(\dot{V}_O_2\) and/or the small range in body mass (28–102 g) used in this study. Unfortunately, allometric exponents have not previously been reported for this relationship at low body temperatures.

**Lactate**

The mean lactate concentration of *T. sirtalis* hibernating in air (0.52 mg/g) and water (0.49 mg/g) were similar and only slightly greater than values reported for resting garter snakes at warmer temperatures (e.g., 0.31 mg/g in *T. sirtalis*—Pough 1977; 0.31 mg/g in *T. elegans*—Feder and Arnold 1982). Since lactate concentrations in active reptiles exceed these by many-fold (Feder and Arnold 1982), values determined in this study are probably indicative of resting levels.

The absence of a strong anaerobic component in the energy metabolism of submerged *T. sirtalis* is in striking contrast to the results of forced-submersion experiments with reptiles at warmer temperatures (Seymour 1982). Also, a submerged *Chrysemys picta* bears extremely high lactate loads (>5.6 mg/g) during winter with no apparent ill effects (Gatten 1981); this is adaptive since these turtles hibernate in anoxic mud. Under the more favorable conditions (i.e., \(P_O_2 \approx 110 \text{ mmHg}\)) of this study, however, cutaneous gas exchange apparently sustained aerobic metabolism.

**Heart Rates**

The mean SHR for *T. sirtalis* tested in air, at 5°C, (6.0 ± 0.3 bpm), compares favorably with rates reported (e.g., Risher 1984) for other reptiles. There was no evidence that SHR was related to mass; however, studies of other reptiles (Hutton et al. 1960; Bethea 1972) indicate that this relationship is
evident only at warm body temperatures and with a large range in body mass. Thus, the lack of correlation in this study was not unexpected.

Bradycardia was a prominent adjustment to submergence as mean SHRs of snakes tested in air and water differed by 77%. Cardiovascular control in submerged reptiles is complex and poorly understood; probably vagal stimuli include water entering the nares, changes in pulmonary volume, hypoxemia, and even volition (Seymour 1982).

Cardiac arrhythmia in diving reptiles has been reported often, although seldom quantified. The mean coefficient of variation (CV) in R-R time intervals for *T. sirtalis* tested in air (6.7%) is in accord with data for five turtle species (5.1%–16.6%) tested under similar conditions (Risher 1984). For the marine iguanid *Amblyrhynchus cristatus*, the CV in R-R time intervals in water (40%) exceeded that in air by a factor of 9.3 (Bartholomew and Lasiewski 1965). Similarly, arrhythmia in this study was more pronounced (3.2-fold) when garter snakes were submerged.

The cardiac responses of winter-submerged *T. sirtalis* and reptiles forcibly submerged in warmer water differ in that SHRs of reptiles in the latter case are attained more quickly (e.g., 8–180 min in *Chrysemys picta*—White and Ross 1966; ca. 4 min in *Nerodia taxispilota*—Irvine and Prange 1976). In contrast, the HR of one *T. sirtalis* (fig. 2) decreased 38% within 8 min of submergence but, thereafter, the average rate of decrease (1.25 beats/h) was much less. This suggests that cardiovascular adjustments to submergence under winter conditions are more gradual, perhaps owing to the lower temperature.

**O₂ Pulse**

The VO₂ and HR data, used to calculate O₂ pulse, were obtained sequentially rather than simultaneously to avoid undue stress of the hibernating snakes. The average, 1.8-fold increase in O₂ pulse in water is due to disproportionate metabolic and cardiac adjustments during submergence, as mean VO₂ and SHR decreased 54.8% and 77.0%, respectively. This finding agrees with the higher O₂ extraction coefficients reported (Seymour 1982) for diving reptiles during prolonged apnea. This result is adaptive since submerged snakes consume more O₂ per heart stroke, and, therefore, energy expenditure and cardiovascular stress during dormancy may be reduced.

**Eco-Physiological Aspects of Submerged Hibernation**

The variable capacity for cutaneous O₂ uptake among reptiles is related to habitat (data presented in Gatten 1984; Feder and Burggren 1985) and prob-
ably reflects differential distributions of capillary beds within the integument (Rosenberg and Voris 1980). Nonpulmonary O₂ uptake from air or water accounts for a considerable fraction of the total VO₂, measured between 20° and 29°C, in some turtles (3%-32%) and squamates (2%-38%) including, notably, the terrestrial forms *Lacerta*, *Sauromalus*, and *Constrictor* (table in Feder and Burggren 1985). Seymour (1982) surmised that cutaneous exchange might completely sustain reptiles submerged in well-oxygenated water if metabolic demands were reduced sufficiently.

Survival of apneic snakes during prolonged, winter submergence may well depend on their ability to meet oxygen requirements via nonpulmonary ventilation. This is likely facilitated by reduced metabolism (caused by low ambient temperature and the observed reduction in basal activity) and by some of the typical physiological responses to diving. For example, cardiac activity in diving reptiles is reduced owing to increased vagal stimulation, and right-to-left intracardiac shunting (or even complete pulmonary bypass—Seymour 1982) maximizes the transcutaneous O₂ tension gradient, thereby aiding gas exchange. Diffusion is also enhanced by the extremely low arterial Po₂ (e.g., <20 mmHg) accompanying prolonged apnea during submergence in some primarily lung-breathing reptiles (Feder and Burggren 1985).

Environmental factors undoubtedly determine whether submerged snakes obtain enough oxygen to remain aerobic during winter. In theory, maximal diffusion rates are achieved only if the medium is well stirred and saturated with air and the integument is well ventilated (Feder and Burggren 1985). Interestingly, garter snakes submerged in the Wisconsin den occasionally move slowly about (Costanzo 1986), possibly facilitating gas exchange by flushing stagnant water from the skin. Reptiles are relatively insensitive to atmospheric Po₂ over a wide physiological range, but nonpulmonary uptake must be depressed to critical rates at very low ambient tensions. On one occasion, den water contained little DO (ca. 35 mmHg—Costanzo 1986) in early April, the end of the hibernation. Whether submerged *T. sirtalis* remain aerobic under these conditions remains to be determined. Fortunately, the ice cover has usually melted by this time; thus, pulmonary exchange at the water surface is not impossible. Systematic measurements of the gas tensions in den water during winter have not yet been made.

By submerging rather than remaining in air, *T. sirtalis* evidently conserves a substantial amount of energy during winter. Theoretically, using an oxygenic coefficient of 4.825 cal/mL O₂ (Guyton 1976) and Vo₂ data gathered in this study, a 52.6-g snake submerged in 5°C water would consume 3.13 kcal (13.10 kJ) during 165 d, the usual period of natural hibernation in central
Wisconsin. If the same snake remained in air (obligatory behavior in dry dens), 6.98 kcal (29.22 kJ) would be expended. Thus, submergence results in a 55.2% reduction of the total winter energy expenditure. This energy budget (0.36 cal g\(^{-1}\) d\(^{-1}\)) is comparable to that (0.42 to 0.45 cal g\(^{-1}\) d\(^{-1}\)) calculated for submerged \(T. \text{sirtalis}\) from changes in energy content (Costanzo 1985). Since mating immediately follows spring emergence (and precedes feeding) in northern \(T. \text{sirtalis}\) populations (Gregory 1982), snakes rely solely on stored nutrients to fuel reproductive activities. Accordingly, this savings may significantly increase individual fitness.

**Conclusions**

In addition to the marked reduction in energy expenditure apparent in this study, winter submergence provides a stable thermal regime, protection from lethal temperatures, and prevents desiccation-induced mortality (Costanzo 1986; unpublished data). Garter snakes probably are not as well suited for cutaneous ventilation as are the more aquatic turtles and snakes. However, the metabolic, cardiovascular, and behavioral adjustments to submergence, in normoxic water and under hibernative conditions, apparently enable Wisconsin \(T. \text{bannophis sirtalis}\) to remain in \(O_2\) balance despite complete reliance on cutaneous diffusion. Garter snakes (and probably others) inhabiting northern latitudes seem preadapted to this behavior, since oxygen requirements under winter conditions are initially depressed by thermal, photo, and seasonal influences (Aleksiuk 1976). Further investigation of the eco-physiological, geographical, and taxonomic limits of this adaptation will be rewarding.

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