Diving energetics and temperature regulation of the star-nosed mole (*Condylura cristata*) with comparisons to non-aquatic talpids and the water shrew (*Sorex palustris*)

by

IAN W. McINTYRE

A Thesis Submitted to the Faculty of Graduate Studies
In Partial Fulfillment of the Requirements
for the Degree of

MASTER OF SCIENCE

Department of Zoology
University of Manitoba
Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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Abstract

The dive performance, oxygen storage capacity, partitioning of body oxygen reserves and thermoregulatory competence of one of the world's smallest mammalian divers, the star-nosed mole, *Condylura cristata*, were investigated. For comparative purposes, I also examined the metabolism and O_2 stores of the coast mole, *Scapanus orarius*, and indices of voluntary dive behaviour in the water shrew, *Sorex palustris*. The mean dive duration of star-nosed moles was 9.2 ± 0.24 s with a maximum recorded dive time of 47 s. The ratio of submergence time to surface recovery time averaged 0.21 ± 0.10 and did not vary with length of dive (r^2 = 0.001, P = 0.37). Mean lung volume of star-nosed moles (8.09 ml $100g^{-1}$) was 1.74 times the predicted allometric value and exceeded that of coast moles, *Scapanus orarius*, by 40% (P = 0.0001). The overall mean myoglobin (Mb) concentration of skeletal muscles of star-nosed moles (13.57 ± 0.40 mg g wet $tissue^{-1}$) was 16.3% higher than values recorded for the coast mole (11.36 ± 0.34 mg g wet $tissue^{-1}$, P = 0.0008). Mb content of forelimb skeletal muscle exceeded hindlimb values in both mole species. Mean skeletal muscle Mb content was higher in adult, compared to juvenile star-nosed moles (P < 0.0001). Total body O_2 stores of adult star-nosed moles (34.0 ml kg^{-1}) and coast moles (29.4 ml kg^{-1}) were high, relative to other terrestrial mammals. The basal metabolic rate (BMR) of star-nosed moles (2.35 ± 0.005 ml O_2 g^{-1} h^{-1}) was 2.1 X the allometric value for eutherian mammals and was nearly twice that of the coast mole (P > 0.0001). However, the maximum rate of O_2 consumption of star-nosed moles

immersed in water (8.53 ml O_2 g^{-1} h^{-1}) was only 14.7% higher than the peak rate predicted from allometry. Based on an observed mean diving metabolic rate of 5.38 ± 0.35 ml O_2 g^{-1} h^{-1}, the calculated aerobic dive limit (ADL) of star-nosed moles was 22.8 s in adults and 20.7 s in juveniles. Only 2.9% of voluntary dives by adult and juvenile star-nosed moles exceeded their respective calculated ADLs. This finding suggests that star-nosed moles rarely exploit anaerobic metabolism while diving, a conclusion supported by the low buffering capacity and glycogen content of the skeletal muscles of these diminutive insectivores.

Mean body temperature (Tb) varied positively with Tw, but was independent of air temperature. Water temperature (Tw) influenced both dive duration (P = 0.0001) and diving frequency (P = 0.004). During 20-min immersion trials, star-nosed moles cooled at all Tws < 30 °C, and elimination of the air boundary entrapped in the pelage increased carcass cooling rate by 40% for star-nosed moles (P > 0.0001) and 38.4 % for water shrews, *Sorex palustris*, (P > 0.0001). Specific gravity estimates for the water shrew exceeded those for the star-nosed mole and the coast mole, but all 3 species were positively buoyant. The thermal conductance of live star-nosed moles in 20 °C water exceeded values previously reported for semi-aquatic mammals, suggesting that this mole possesses limited thermoregulatory ability when immersed in cold-water.

Acknowledgements

I have found my Masters program to be a particularly rewarding period in my life. I attribute this mainly to the members of our laboratory and the Department of Zoology as a whole.

First and foremost, I extend sincere thanks to my advisor, Dr. R. A. MacArthur for providing an environment that fosters significant personal and professional growth. Bob believed in me when no one else would and I will always cherish his contribution to my education. In short, it was an honour and a privilege to work with Bob, and I look forward to continued collaborations in the future. I thank Dr. K. L. Campbell, the undisputed "King of Moles", for finally giving moles the attention they deserve. Kevin's enthusiasm is a beacon that I personally think will inspire the next generation of zoologists in our department. Most importantly, I thank him for his encouragement and friendship.

The foundation for any project resides in the efforts of those individuals that researchers and their students rely on for technical support. I gratefully acknowledge B. Dean "Hacksaw" Jeske who exhibited an extraordinary level of patience, dedication and hard work during all phases of this project, which I attribute to his general strength of character. I also thank my committee members Dr. T. J. Wiens and Dr. G. Giesbrecht, for providing thoughtful comments and suggestions, which I feel greatly improved the final version of this thesis. The contribution of Dr. Dennis Murphy and Jeff Babb of Statistical Advisory Service in designing the Split-plot analysis is gratefully acknowledged. S. Tim Sheehan provided generous "mole-whispering" assistance during fieldwork conducted in British Columbia. Mike Goodyear also provided valuable assistance in the field and laboratory, especially as it pertained to omelet preparation and haberdashery.

Special thanks are also extended to Allyson Hindle and my teaching supervisor, Diane MacArthur for their friendship, advice and general willingness to laugh at my jokes. I thank Jack Dubois, Dr. Jim Duncan, Heather Hinam, and Glen Fines for logistical assistance involving specimen donation and animal collection. Roger Pearce, of Piney Mb. kindly allowed us to trap and live on his mosquito-infested land. I thank Dr. E. Huebner and Dr. S. Sealy for their enthusiasm and advice. To my fellow graduate students: Chris "Bison" [a] Bjornsson, Christine Abrahams, Bruce Adams, Tonia Robb, Fred Laberge, Ernie Watson, Todd Underwood, Celia McLaren, Doug Watkinson, Susanna Wiens, Mike Johnson, James Plohman, Joe Carney and Azi Mhanni, I extend my sincere thanks for your friendship and kindness. To: Skye, Mike, Craig Y., Todd, Dr. John A., John F. and Craig W. I thank you for tolerating my frequent absence from the many parties and beer bashes, a deficit that I will soon remedy! To my family: Elaine, Fiona, Bill, Betty, Lorne, Shelley and Sri I extend my love and respect, for which you have always been so willing to give in return. Finally, I thank the person who supported me more than anyone else throughout this entire process, my girlfriend, Jeanna, without your love none of this would be possible.

Also, thanks to readers like you!

[a] Bisons have large brains that are especially beneficial when used to advise mole-men, since we know that brain mass tends to scale to body mass.
i.e.: Brain mass (kg) = $0.0093M^{0.73}$, where M = body mass in kg (Stahl, 1965).

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Abbreviations used in Text

ADL	aerobic dive limit
β	Slyke (a unit of buffering capacity – see p. 16)
BADL	behavioural aerobic dive limit
BMR	basal metabolic rate
CADL	calculated aerobic dive limit
C	whole-body thermal conductance
DMR	diving metabolic rate
EHL	evaporative heat loss
FeO_2	fractional oxygen content of expired gas
Hct	hematocrit
Hb	hemoglobin
HIF	heat-increment of feeding
Hm	specific heat of mammalian tissue
IEC	International Equipment company
ISFET	ion selective field effect transistor
K	Meeh factor (see p. 48)
LCT	lower critical temperature
MAD-1	motion activity detector
Mb	myoglobin
mya	million years ago
orts	uneaten food rations
RMR	resting metabolic rate
SA	surface area
STPD	standard temperature pressure (dry)
Ta	ambient temperature
Tb	body temperature
TCA	trichloroacetic acid

TNZ	thermoneutral zone
Tw	water temperature
VO_2	oxygen consumption
vol. %	volume percent

General Introduction

The insectivores comprise the most ancient order of mammals and one can only marvel at the remarkable evolutionary success of this group. Energetic considerations should, in theory, impose strict limitations on insectivore behaviour. However, the members of this group engage in some of the most energetically taxing activities performed by animals, including flight, burrowing and, in the case of semi-aquatic forms, diving. Despite the rich diversity of forms, our understanding of the thermal biology and general physiology of semi-aquatic insectivores in particular continues to lag behind that of other representative mammalian fauna.

Research to date has clearly established that cold-water immersion poses major physiological challenges to endothermic divers, both in terms of thermoregulating and maintaining an O_2-based metabolism during underwater foraging. Of particular interest are the smallest mammalian divers, the star-nosed mole, *Condylura cristata*, and the North-American water shrew, *Sorex palustris*, neither of which has been adequately studied by comparative physiologists. The small body size of insectivores should dictate a high mass-specific basal metabolic rate (BMR), yet severely limit their ability to enhance insulation, as extensive pelage would be cumbersome on land. Attempts to decrease heat loss by minimizing thermal conductance (C) would be especially beneficial to animals of small body size. Conversely, environmental constraints like a confined burrow microenvironment of a fossorial species such as the coast mole, *Scapanus orarius*, dictate a need for elevated C and

a diminished BMR in order to avoid hyperthermia. While both the star-nosed mole and the coast mole appear to have adapted to the effects of burrowing-induced hypoxia associated with subterranean habitation (Schaffer and Sadleir, 1979), it is informative to know to what extent diving has modified the O_2 stores of the star-nosed mole. These comparisons are made more interesting by the dramatic differences that these moles display in terms of habitat preference, behaviour and phylogenetic history, as the star-nosed mole diverged from the main talpid line early in the evolutionary history of this group (Yates and Greenbaum, 1982). The star-nosed mole is an accomplished diver that relies on aquatic insects and annelids for a substantial proportion of its diet (Hamilton, 1931). Conversely, the coast mole is exclusively fossorial and preys mainly on terrestrial oligochates (Sheehan and Galindos-Leal, 1997).

As a comparative study, my research focused on the star-nosed mole and the comparable-sized coast mole as a terrestrial reference species when appropriate. I investigated the extent and partitioning of total body O_2 stores in both mole species in order to assess the potential contribution that habitat preference makes in augmenting O_2 stores. For the star-nosed mole, O_2 storage data were then used, in combination with estimates of the cost of diving (VO_2) measured by open-flow respirometry, to determine the theoretical aerobic dive limit (ADL) of this species. To place these estimates in an ecological context, I then compared the correspondence of the ADL to the pattern of voluntary dive behaviour determined for star-nosed moles. An

additional objective of this study was to examine the thermoregulatory competence of the star-nosed mole in air and water. To achieve this goal, I needed to establish the limits of thermoneutrality and estimate the BMR of this species. I further determined the Tb dynamics and conductance of star-nosed moles in air and water under varied temperature conditions. Subsequent studies focused on the strategies by which star-nosed moles avoid immersion hypothermia, including behavioural tactics that minimize exposure to cold water, surface area and pelage characteristics, the potential benefit of an elevated BMR to reduce the costs of aquatic thermoregulation and the possible exploitation of the Heat Increment of Feeding (HIF) provided by a protein-rich diet. Limited data were also obtained for the water shrew, especially as they pertain to the diving ability and BMR of this diminutive diver.

CHAPTER 1

BODY OXYGEN STORES, AEROBIC DIVE LIMITS AND DIVING BEHAVIOR OF THE STAR-NOSED MOLE (*CONDYLURA CRISTATA*) WITH COMPARISONS TO NON-AQUATIC TALPIDS

Introduction

There is now a considerable body of literature supporting the conclusion that most endothermic divers sustain an oxygen-based metabolism underwater (see Kooyman, 1989; Butler and Jones, 1997). In theory, aerobic diving results in enhanced foraging efficiency since disturbances in blood chemistry and obligate recovery times at the surface are minimized. However, most of the existing literature is based on studies of pinnipeds, seabirds and diving ducks. Relatively few studies have been conducted on small-bodied mammalian divers, including muskrat, *Ondatra zibethicus* (MacArthur and Krause, 1989; MacArthur, 1989), mink, *Mustela vison* (Dunstone and O'Conner, 1979; West and Van Vliet, 1986) and beaver, *Castor canadensis* (Butler,1991). With the exception of the European water shrew, *Neomys fodiens*, no published data exist on the voluntary dive performance of mammalian divers with adult body masses < 500 g. Moreover, virtually nothing is known about the oxygen storage capacity of these diminutive divers, nor of the relationship of their oxygen stores to dive behaviour. Such studies are critical to confirm whether allometric predictions of vertebrate diving endurance (Schreer and Kovacs, 1997) hold for the smallest endothermic divers.

One such animal is the star-nosed mole, *Condylura cristata*. Of the seven species of North-American moles (F. Talpidae), only the star-nosed mole is semi-aquatic. This accomplished diver frequents tunnel systems excavated along the edges of streambeds and lakes. Though star-nosed

moles prey mainly on terrestrial oligochaetes, they also forage underwater for benthic and pelagic invertebrates (Hamilton, 1931; Rust, 1966). Despite its small size and presumed susceptibility to immersion hypothermia (MacArthur, 1989), this insectivore is reported to actively forage in near-freezing water during the frigid winter months (Merriam, 1884; Hamilton, 1931). In a preliminary study of the diving behaviour of six star-nosed moles, I observed dive durations that greatly exceeded predictions based on allometric theory. Average dive times of these moles rivalled those of the mink (Dunstone and O'Connor, 1979*a*), a semi-aquatic mustelid that is approximately 20 times larger than *Condylura*. Given its small mass, inherently high basal metabolic rate, BMR (2.25 ml g^{-1} h^{-1}, Campbell *et al.*, 1999), and hence potentially high rate of O_2 utilization underwater, the star-nosed mole thus presents an intriguing model for investigating mammalian dive endurance. The paucity of existing data likely reflects the difficulty in obtaining live animals, as this species is notoriously difficult to capture and maintain in captivity.

The purpose of this study was three-fold. First, I wished to determine the extent of total body O_2 stores and measure the diving metabolic rate (DMR) of star-nosed moles in order to estimate the "theoretical" ADL of this species. A second goal was to assess the correspondence, if any, between the calculated ADL and behavioural indices of dive performance of star-nosed moles. My final objective was to compare oxygen storage capacity and the potential for anaerobic metabolism in 2 talpids of similar mass and phylogenetic history: the semi-aquatic star-nosed mole and the exclusively

terrestrial coast mole, *Scapanus orarius*. This comparison is important because all moles are specialized for burrowing and it is useful to know to what extent diving has modified respiratory functions in the star-nosed mole. Since respiratory specializations for diving are potentially convergent with patterns seen in fossorial species, this study hopefully will shed light on the mechanisms underlying hypoxia tolerance in talpids generally. For example, previous studies have consistently demonstrated high Mb concentrations in divers (Kooyman, 1989; MacArthur, 1990) and in non-diving, actively burrowing mammals such as the echidna, *Tachyglossus aculeatus* (Hochachka *et al.*, 1984) and the mole rat, *Spalax ehrenbergi* (Widmer *et al.*, 1997). Given the low O_2 content of mole tunnels (Schaefer and Sadleir, 1979), it is conceivable that fossorial moles also exhibit enhanced oxygen stores, possibly to compensate for prevailing hypoxic conditions in the closed burrow microenvironments of these animals. If this were found to be the case, the question would then arise: are the tissue oxygen stores of star-nosed moles even further enhanced by a dependence on diving? In addition to comparing the partitioning of body oxygen reserves, the potential for anaerobic metabolism was assessed from measurements of buffering capacity and glycogen concentration in the fore- and hindlimb muscles of each mole species.

Materials and Methods

Animals

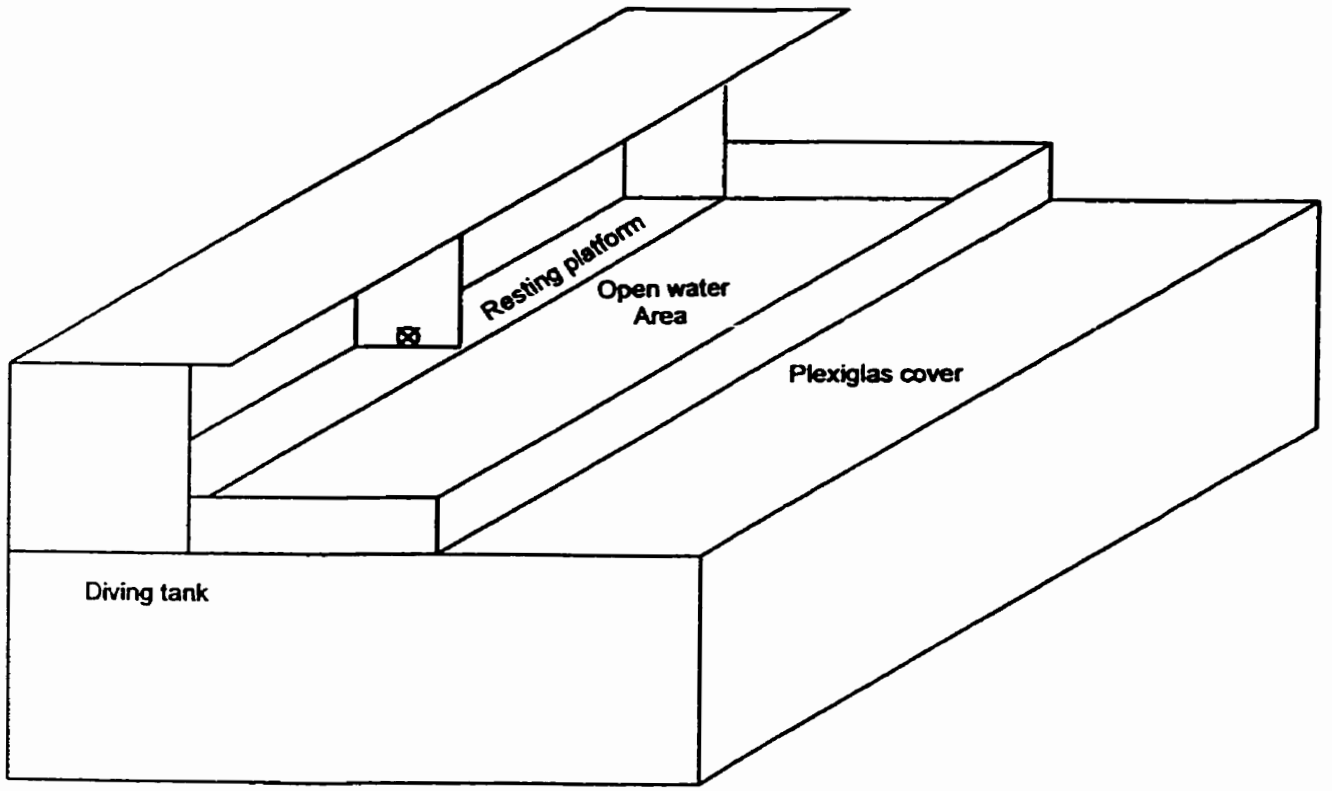
A total of 31 star-nosed moles were live-trapped at Piney, Moose River, Rennie and Caddy Lake, Manitoba, between June 1997 and September 1999. Within 24 h of capture, moles were transported to the University of Manitoba Animal Holding Facility and held individually in a controlled environment chamber maintained at 20 ± 1°C with a 12L:12D photoperiod. Animal care procedures are described in detail in Campbell *et al.* (1999). Briefly, star-nosed moles were housed individually in large glass aquaria (88 cm x 50 cm x 60 cm) fitted with vertical Plexiglas partitions that divided each tank into aquatic and terrestrial compartments. The terrestrial section comprised ca. three-quarters of the tank and was filled to a depth of 45 cm with sterilized soil in which the moles readily constructed tunnel systems. On the aquatic side, a 24-cm-deep pool of 20°C water was provided for moles to swim or dive. Animals were maintained on a diet of earthworms, *Lumbricus spp.*, leeches, *Nephelopsis obscura*, mealworms *Tenebrio molitor*, and a meat ration containing vitamins and a calcium supplement (Campbell *et al.*, 1999). Of the 11 star-nosed moles from which morphometric and body O_2 stores data were collected, 4 individuals were identified as juveniles on the basis of total body length (Simpson, 1923; Hamilton, 1931) and tooth wear characteristics (Hartman, 1995).

In May 1999, 10 coast moles were live-trapped near Abbottsford, British Columbia by the direct capture method (Sheehan and Galindo-Leal, 1995) and immediately transported to the Department of Zoology, University of British Columbia. Holding conditions were identical to those adopted for the star-nosed mole, with the exception that coast moles were housed in large soil-filled plastic containers (46 cm x 33 cm x 38 cm) with no provision for swimming. Carcasses of an additional 15 coast moles were provided by a local mole trapper and immediately frozen at –70°C for later analysis at the University of Manitoba. This study complied with University of Manitoba and University of British Columbia regulations governing animal research and at all times, animals were cared for in strict accordance with Canadian Council on Animal Care guidelines.

Dive Behaviour

Following 3 weeks acclimation to animal holding facilities, a study of voluntary dive behaviour was initiated on 18 star-nosed moles. Dive trials were performed in a large fibreglass-lined plywood tank (143.5 cm x 68 cm) fitted with removable wooden and Plexiglas panels. The tank was provided with a dry resting platform (17.5 x 68 cm) and the swimming/diving section was covered by a Plexiglas sheet except for an open swimming area (75 x 68 cm) immediately adjacent to the platform (Fig. 1-1). The tank was filled to a depth of 60 cm with 3, 10, 20 or 30°C water. Each mole was exposed to all four water temperatures presented in random order and on different days.

Figure 1-1. Voluntary dive apparatus for determining the behaviour of star-nosed moles. Note the presence of the partition in the center of the resting platform, which contains a passageway constructed of PVC pipe.


Resting platform
Open water
Area
Plexiglas cover
Diving tank

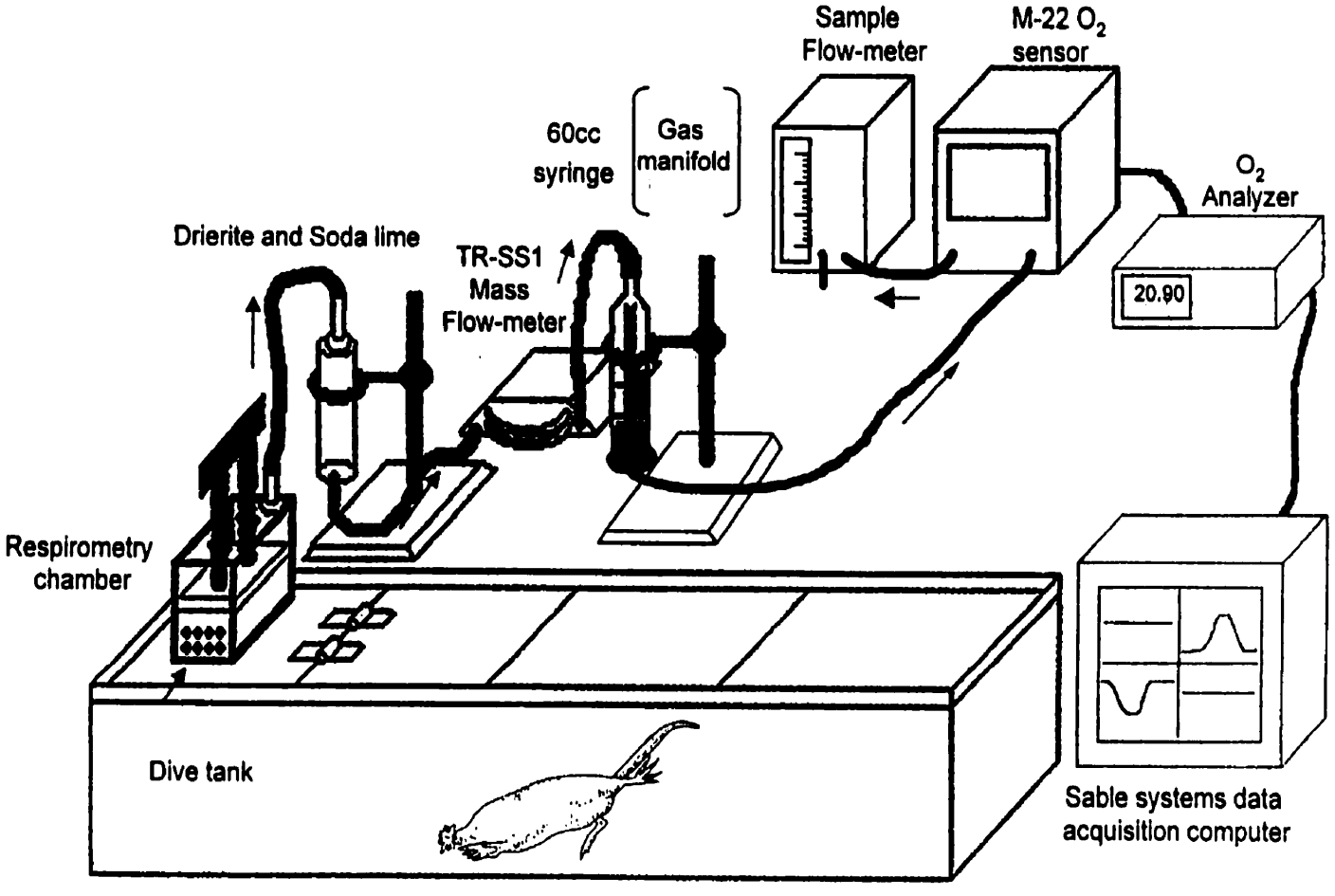
Further, animals were not fasted prior to testing. To maximize sample size dive data were pooled for all water temperatures, however the absolute thermal effects on indices of dive behaviour are presented in chapter 2.

At the start of each trial, the mole was placed on the dry resting platform. For a 20-min observation period the animal was then free to move throughout the tank. The durations and frequencies of all diving, swimming, resting and grooming episodes were recorded on audiotape for subsequent analyses.

Diving Respirometry

To estimate the cost of diving, a series of diving trials was conducted in a fibreglass tank (208 cm x 55 cm x 52 cm) filled with water to a depth of 44 cm. Four removable Plexiglas panels covered the tank. Consequently, the animal was prevented from surfacing anywhere in the tank except in a 2.55-L Plexiglas metabolic chamber (30 cm x 22 cm x 15 cm) mounted on one of the cover panels (Fig. 1-2). The chamber was similar in design to a larger version constructed for muskrats (MacArthur and Krause, 1989). Air entered the chamber via a series of small holes bored in one of the walls near water level, and was drawn by vacuum through the chamber via a ceiling exhaust port located at the opposite side of the structure. Gas mixing was facilitated by an electric fan installed in the chamber ceiling (MacArthur and Krause, 1989). Flow rate was maintained at 940 ml min^{-1} using a combination pump/mass flow meter (TR-SS1 gas analysis subsampler, Sable Systems Inc.,

Figure 1-2. Open-flow respirometry system for the estimation of the cost of diving in star-nosed moles, showing the respirometry chamber, gas-analyzing equipment and the data acquisition computer. The direction of gas flow is indicated by the arrows. The plunger assembly described on p. 13 is shown within the respirometry chamber (Modified after MacArthur and Krause, 1989).


Sample
Flow-meter
M-22 O_2
sensor
60cc
syringe
Gas
manifold
O_2
Analyzer
Drierite and Soda lime
TR-SS1
Mass
Flow-meter
20.90
Respirometry
chamber
Dive tank
Sable systems data
acquisition computer

Henderson, NV, USA). Excurrent air was drawn sequentially through a column of soda lime and a column of Drierite to eliminate CO_2 and H_2O vapour, respectively. A 250-ml subsample of dry, CO_2-free exhaust gas was drawn through the M-22 sensor of an Applied Electrochemistry S3-A oxygen analyzer for determination of the fractional oxygen content of expired gas (FeO_2). Air flow rate through the metabolic chamber (ml min^{-1}), FeO_2 and water temperature (°C) were recorded every 5 s using a Sable Systems Universal Interface and Datacan V data acquisition software (Sable Systems Inc.).

Pretrial training sessions were conducted to familiarize animals with the diving tank and metabolic chamber. During training, the length of the tank available for diving was varied using removable Plexiglas partitions. Training runs were performed at an initial tank length of 90 cm, which was subsequently extended to 144 cm and finally, to 191 cm. Prior to each trial, the water level was adjusted to the prescribed depth to ensure a constant gas volume in the metabolic chamber, and water temperature (Tw) was maintained at 30 ± 0.5 °C. At this temperature, star-nosed moles exhibited maximal diving activity in the 20- min behavioural trials described above and were presumably under minimal thermal stress. Moles were weighed to the nearest 0.01 g approximately 10 min before the start of each trial.

In 1997, the duration of diving metabolic trials was maintained at exactly 8 min. However, as my preliminary findings indicated that the proportion of time spent diving increased with trial duration, trials were

extended to 10 min in 1999. Animals gained access to the tank and metabolic chamber via a hinged door mounted on one of the Plexiglas panels. Dive behaviour as well as activity in the metabolic chamber, were closely monitored and recorded on a Sony tape recorder. Upon completion of the trial, a plunger mounted in the ceiling of the metabolic chamber was gently depressed, prompting eviction of the animal without interrupting gas analysis (MacArthur and Krause, 1989). The animal was then permitted to leave the water and enter a dry nest-box, at which point it was immediately transferred to a large container of soil. All animals were fed prior to metabolic testing. To facilitate analysis, the mean rate of oxygen consumption (VO_2) was calculated for the entire run following the method of Withers (1977, equation 4a). This value represents the combined costs of diving and surface swimming by the mole during either an 8- or 10-min test period in water

To obtain baseline metabolic measurements for intra- and interspecific comparisons, I also measured the resting metabolic rate (RMR) of star-nosed moles and coast moles at thermoneutrality. In this case, the metabolic chamber consisted of a modified 0.95-litre paint can with a flat black interior that was fitted with inlet and outlet air ports and furnished with 3-4 mm of dry, sterilized soil. Otherwise, the procedure for determining VO_2 of resting animals in air was identical to that described for diving/swimming star-nosed moles. For trials in air, the chamber was housed in a controlled environment cabinet set at 30 ± 0.5°C for ca. 1 h. The lowest VO_2 maintained over at least a 3-min period was taken as RMR and absence of motor activity was verified

independently using a motion activity detector (MAD-1, Sable Systems Inc.) mounted directly beneath the metabolic chamber.

Body Oxygen Stores

After completion of aquatic trials, star-nosed moles were killed with an overdose of inhalant anesthetic (Halothane, MTC Pharmaceuticals) in order to assess O_2 storage capacities of the blood, lungs and skeletal muscles. While moles were deeply anesthetized, a blood sample was drawn by cardiac puncture for hemoglobin (Hb) and hematocrit (Hct) determinations (MacArthur, 1984*b*).

In freshly euthanized individuals, samples of forelimb and hindlimb muscles were harvested and immediately frozen at – 70°C. These tissues subsequently were analyzed for Mb concentration following the procedure of Reynafarje (1963). Muscle samples from coast moles were treated in an identical manner, with the exception that muscle was freeze-clamped in liquid N_2 prior to freezing at –70°C. In all animals, the lungs were carefully excised and lung volume was determined gravimetrically (Weibel, 1970/71; MacArthur, 1990). For this purpose, lungs were immersed in saline (0.9 M NaCl) and inflated with humidified air at a constant pressure of 20 mmHg. Final lung volume was corrected to standard temperature and pressure. I also calculated muscle mass as a percentage of the digesta-free body mass. Following removal of the internal organs, skin, eyes and brain, the eviscerated carcass was weighed and immersed in a detergent solution and

boiled for approximately 48 h to remove the remaining skeletal muscle. The weight of the dry skeleton was subsequently determined and subtracted from eviscerated carcass wet weight to derive total skeletal muscle mass.

Blood volumes of star-nosed moles and coast moles were estimated from the allometric equation: blood volume (ml) = 76 $M^{1.0}$, where M = body mass in kg (Prothero, 1980). Otherwise, the calculation of lung, blood and muscle O_2 stores followed conventional protocol (Lenfant *et al.*, 1970; Kooyman, 1989; MacArthur, 1990; Lyderson *et al.*, 1992). The calculated ("theoretical") ADL (s) was determined by dividing the total body oxygen stores (ml O_2, STPD) by the mean swimming / diving VO_2 (ml O_2 s^{-1}) obtained for each mole. Implicit in these calculations is the assumption that all oxygen reserves are fully exploited underwater (Kooyman, 1989). I also determined the behavioral ADL (BADL), previously defined by Kooyman *et al.* (1983) and Burns and Castellini (1996) as the dive time exceeded by only 5% of all voluntary dives.

Muscle Buffering Capacity

Buffering capacities of forelimb and hindlimb muscles were determined following the procedure of Castellini and Somero (1981). Briefly, a 0.5 g muscle sample was homogenized in 0.9 M NaCl and then titrated at 37°C with 0.2 M NaOH. The pH of the homogenate was determined using a Corning model 360 pH meter equipped with an ISFET electrode. Buffering capacity, measured in Slykes (β), is defined as the amount of base required

to raise the pH of 1 g of wet weight of muscle from pH 6 to 7 (Van Slyke, 1922).

Glycogen Determination

The glycogen contents of forelimb and hindlimb muscles were determined according to the methods of Kemp and Van Heijmingin (1954). Accordingly, a thawed muscle sample was initially homogenized in 80% methanol to eliminate free glucose. Following 6 min centrifugation in an IEC clinical centrifuge, the supernatant was discarded and 5 ml of deproteinizing solution (5g TCA, 0.1g $AgSO_4$ in 100 ml H_2O) was added to the remaining sample in a tube that was submerged in boiling water for 15 min. After boiling and readjusting the level of deproteinizing solution, the homogenate was recentrifuged for 5 min. The resulting supernatant was pipetted into a clean test tube with 3 ml H_2SO_4 and boiled for exactly 6.5 minutes. The absorbance of the H_2SO_4-treated solution was then measured spectrophotometrically at 520 nm and compared to a glucose standard curve to determine glycogen concentration in mg g wet tissue^{-1} (Kemp and Van Heijmingin, 1954).

Statistical Treatment of Data

Two-sample comparisons of mean values were made using either Student's or Welch's t-test. For interspecific comparisons of muscle biochemistry, a split-plot design was employed (Steel, 1980). Regression

lines were fitted by the method of least squares. Significance was set at the 5% level and means are presented as ± 1 SE.

Results

Diving Behaviour of Star-Nosed Moles

There was no statistical difference between the dive times of juvenile and adult star-nosed moles; hence data for these cohorts were pooled. Exploratory dives tended to fall into one of two groups: those that were limited to depths < 10 cm ("shallow dives") and those that involved exploration of the bottom of the tank at swimming depths of 50-60 cm ("deep dives"). Deep dives accounted for 11.5% of all voluntary dives and averaged 11.6 ± 0.56 s in duration. Based on a total of 722 voluntary dives involving 18 star-nosed moles, the overall mean dive time was 9.2 ± 0.24 s (Fig. 1-3). The mean duration of the 5 longest exploratory dives in each trial was 13.2 ± 0.79 s, while the mean maximum dive time per trial was 17.7 ± 1.40 s. The longest recorded exploratory dive by a freely diving star-nosed mole in this study was 47.0 s. However, one exceptional individual that briefly became disoriented in the tank dove for 58.8 s. For all animals combined, the mean diving frequency was 0.46 ± 0.14 dives min^{-1} (range 0 - 0.95 dives min^{-1}).

The inter-dive surface interval of star-nosed moles averaged 34 s and the mean dive:surface ratio was 0.21 ± 0.10. No relationship was observed between the inter-dive surface interval and the duration of the preceding dive (Fig. 1-4), suggesting that, in general, longer dives did not require extended recovery periods at the surface. The dive:surface ratios of 6 individuals for

Figure 1-3. Frequency distribution of all voluntary dive times recorded for 18 star-nosed moles, tested in 3, 10, 20 and 30 °C water. Data are presented from moles captured between 1997 and 1999. Vertical dashed lines denote behavioural and calculated aerobic dive limit (see methods for details).

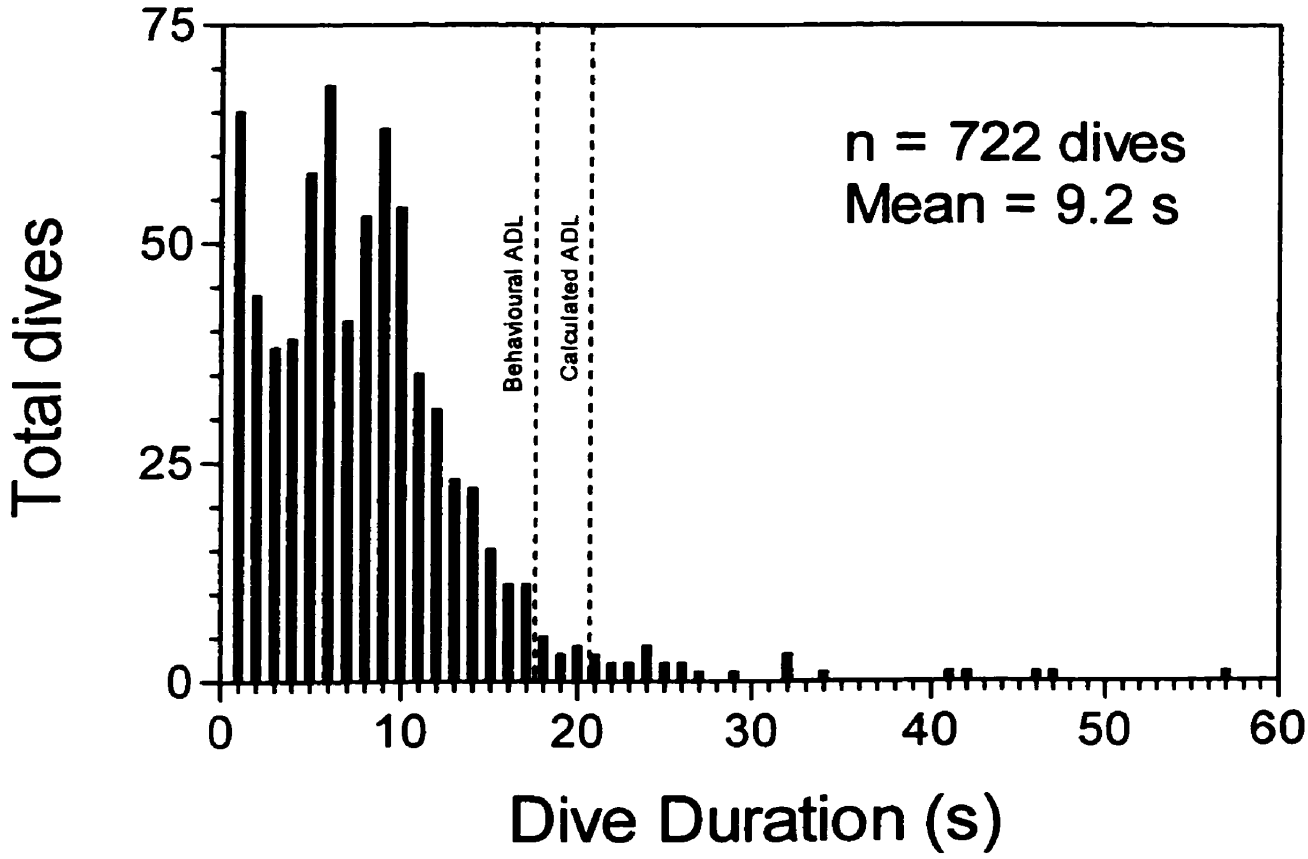
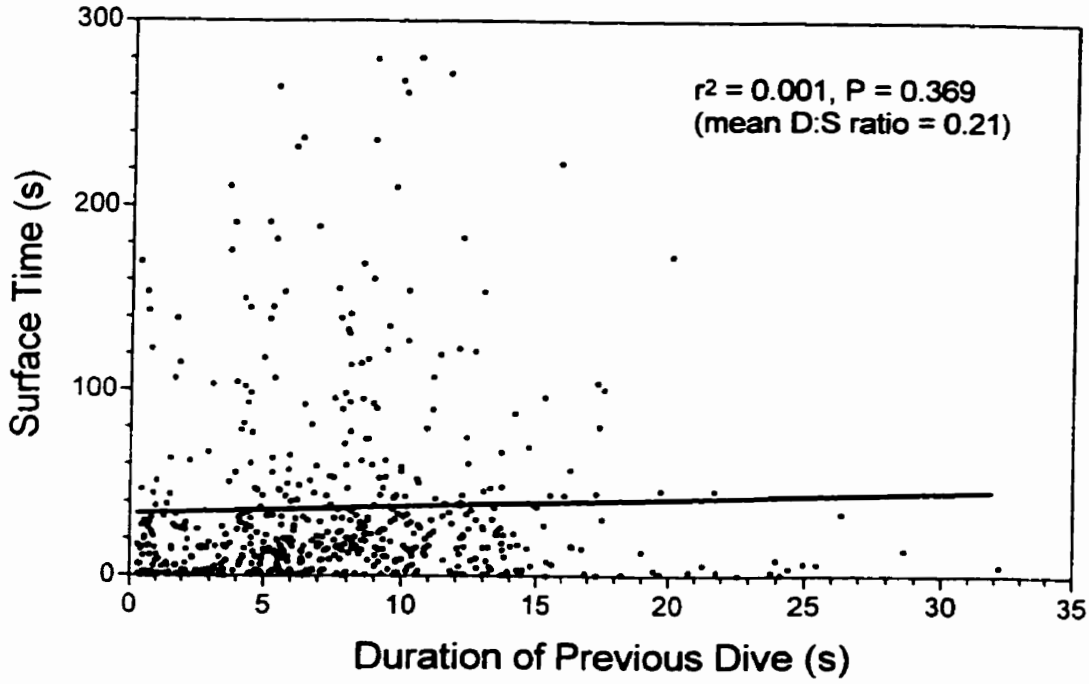

75
50
25
0
Total dives
0
10
20
30
40
50
60
Dive Duration (s)
Behavioural ADL
Calculated ADL
n = 722 dives
Mean = 9.2 s

Figure 1-4. The relationship between inter-dive surface period and length of preceding dive in 15 freely-diving star-nosed moles. Regression line is fitted by the method of least-squares. D:S ratio = ratio of dive duration to length of subsequent recovery time at surface.


300
200
100
0
Surface Time (s)
0
5
10
15
20
25
30
35
Duration of Previous Dive (s)
r2 = 0.001, P = 0.369
(mean D:S ratio = 0.21)

which body O_2 stores data were also available ranged from 0.09 to 0.25 (Table 1-1).

Metabolic Rates and Aerobic Dive Limits of Star-Nosed Moles

Metabolic costs of surface swimming/diving were obtained for 11 star-nosed moles (2 juveniles, 9 adults) and all of these animals adapted well to the aquatic respirometry set-up. The lack of metabolic data for juveniles precluded statistical comparisons with adults and data for both cohorts were pooled. In 22 trials, these moles made a total of 519 dives and spent 0 - 51% (mean = 20.28 ± 3.80%) of each test session diving. I observed no statistical relationship between mean VO_2 in water and either % time diving (Fig. 1-5A) or diving frequency (Fig. 1-5B) in the 11 moles tested. The mean VO_2 of star-nosed moles in water was 5.38 ± 0.35 ml O_2 g^{-1} h^{-1} (n = 11) and was 2.1 times greater than the mean RMR recorded for the same individuals in air (2.56 ± 0.01 ml O_2 g^{-1} h^{-1}, n = 18). By comparison, the RMR of coast moles averaged only 1.33 ± 0.03 ml O_2 g^{-1} h^{-1} (n = 9), a rate nearly half that of the star-nosed mole.

Assuming that the total body O_2 stores of star-nosed moles = 34.0 ml O_2 STPD kg^{-1} (see below), that all of these stores are exploited during diving, and that their diving metabolic rate = 5.38 ml g^{-1} h^{-1}, then the calculated aerobic dive limit of adult moles is 22.8 s. This value exceeds that derived for juvenile star-nosed moles by only 2.3 s, or 10.2% (P > 0.05, Table 1-2). For all animals combined, the proportion of voluntary dives exceeding the

Table 1-1. Intraspecific variability in voluntary dive times and calculated aerobic dive limits for 8 star-nosed moles.

Mole	Cohort	Mass (g)	Duration of longest dive (s)[a]	Mean duration of 5 longest dives [a]	Dive frequency (dives min^{-1}) [a]	Dive : surface ratio[b]	CADL[c] (s)
M1/97	A	57.30	24.41	22.24	0.95	0.223	24.57
M3/97	A	52.20	16.94	14.39	0.42	0.184	25.83
M7/97	A	43.28	21.60	13.23	0.15	0.177	26.03
M2/98	J	53.12	17.50	13.27	0.20	0.162	21.04
M3/98	J	54.65	—	—	—	—	20.27
M5/99	A	53.44	3.09	—	0.15	—	19.41
M7/99	A	47.76	17.49	16.70	0.95	0.252	19.00
M8/99	A	50.22	15.56	11.91	0.85	0.094	21.70

[a] For each animal, values are means of trials in 3, 10, 20 and 30 °C water (n = 4).
[b] Dive : surface ratio = ratio of dive duration to length of subsequent recovery time at surface.
[c] CADL = Calculated aerobic dive limit: total body O_2 stores (ml, STPD) ÷ mean metabolic rate in water (ml O_2 g^{-1} h^{-1}).
A = Adult, J = Juvenile.

Figure 1-5. Relationship between mean VO_2 of star-nosed moles in water (*n* = 11 animals) and (A) percent time diving and (B) dive frequency, during 10-min immersion trials in 30°C water. Solid lines indicate least-squares regressions; dashed lines denote the mean resting VO_2 at thermoneutrality in air (2.56 ml $O_2\,g^{-1}\,h^{-1}$).

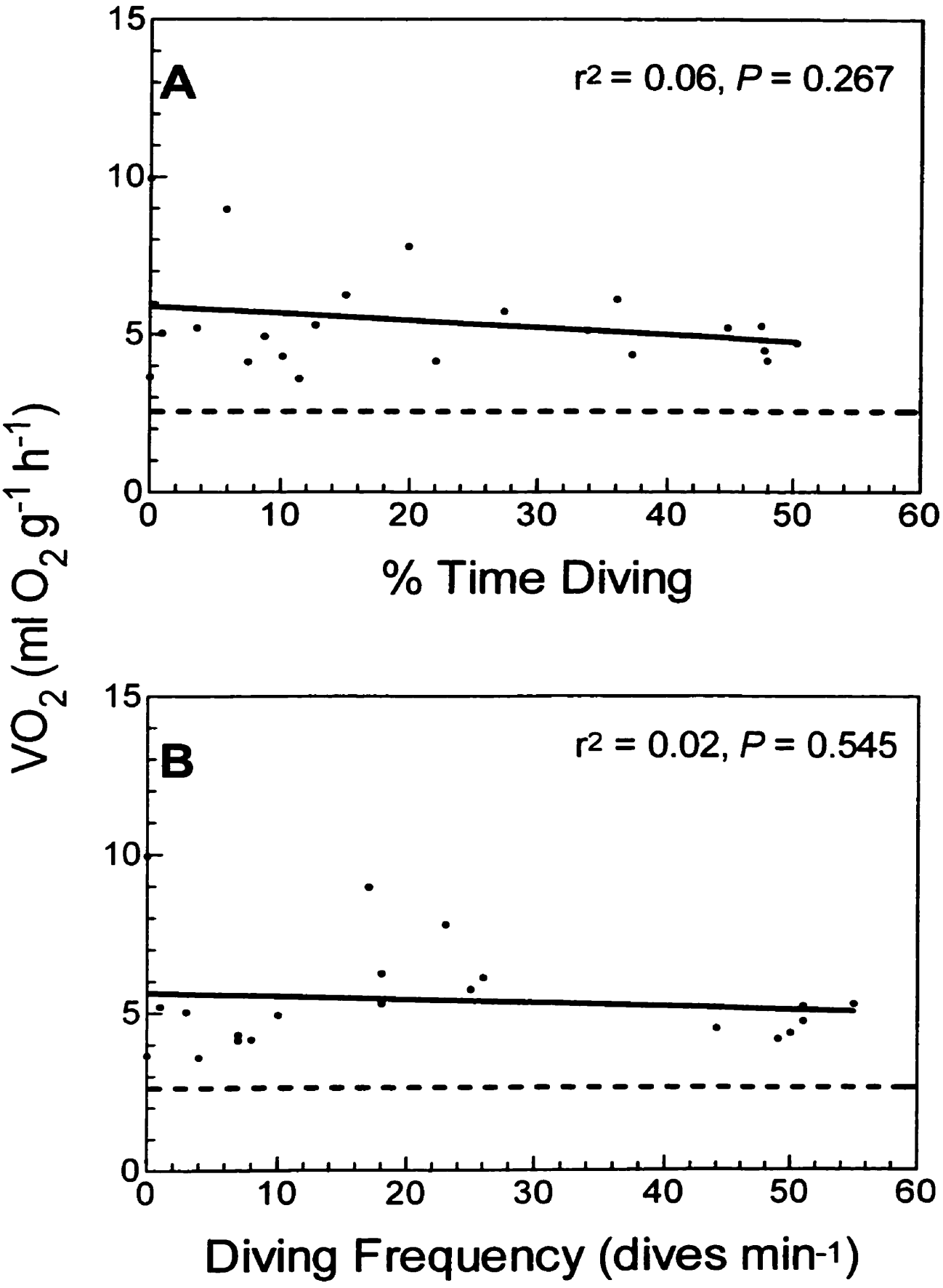

A
r2 = 0.06, P = 0.267
15
10
5
0
0
10
20
30
40
50
60
% Time Diving
B
r2 = 0.02, P = 0.545
15
10
5
0
0
10
20
30
40
50
60
Diving Frequency (dives min-1)
VO2 (ml O2 g-1 h-1)

Table 1-2. Oxygen storage capacities of lungs, blood and skeletal muscles of star-nosed moles (*Condylura cristata*) and coast moles (*Scapanus orarius*).

	Oxygen stores (ml O_2 STPD kg^{-1})					Calculated aerobic dive limit[a]
Species	Lung	Arterial blood	Venous blood	Muscle	Total	(s)
C. cristata						
Juveniles	14.1	5.0	7.5	4.3	30.9	20.7
Adults	12.2	5.6	8.6	7.6	34.0	22.8
S. orarius	7.2	6.0	9.4	6.8	29.4	———

[a] Calculated for a 50-gram animal assuming a diving metabolic rate of 5.38 ml O_2 g^{-1} h^{-1}.

calculated ADL was only 2.9% (Fig. 1-3). In adults, 2.8% of all voluntary dives exceeded the calculated ADL while for juveniles, the corresponding figure was 3.6%. The mean behavioral aerobic dive limit determined for adults and juveniles combined (n = 18) was 17.5 s.

Partitioning of Body O_2 stores in Star-Nosed Moles and Coast Moles

Hct, blood Hb content and blood O_2 capacity were similar in the three groups tested ($P > 0.05$, Table 1-3). However, significant intra- and interspecific differences were observed in both lung and muscle O_2 stores. The total lung capacity of adult star-nosed moles was 16% lower than in juveniles of this species ($P = 0.015$), but was 40% greater than for adult coast moles ($P = 0.0001$, Table 1-3). The lungs accounted for 45.6 and 35.9% of the total body O_2 stores of adult and juvenile star-nosed moles, respectively, compared to only 24.5% for adult coast moles (Table 1-2). While total body O_2 stores were statistically similar in all groups ($P > 0.05$), adult star-nosed moles exhibited the highest overall total O_2 storage capacity, with the lung and muscle reserves contributing most to the observed intra- and interspecific differences (Table 1-2). Differences in mean skeletal muscle Mb concentration between adult (13.57 $\pm$ 0.40 mg g wet tissue^{-1}) and juvenile (7.10 $\pm$ 0.42 mg g wet tissue^{-1}) star-nosed moles were highly significant ($P < 0.0001$). Consequently, intraspecific differences in muscle O_2 storage capacity also were strongly significant ($P = 0.0004$). Mean muscle Mb content also differed between the two mole species ($P = 0.0008$, Table 1-3). Despite

Table 1-3. Comparisons of lung, blood and muscle characteristics of star-nosed moles (*Condylura cristata*) and coast moles (*Scapanus orarius*). Values are presented as means ± SE.

Variable	Star-nosed mole		Coast mole	Difference (adult *C. cristata* – adult *S. orarius*)
	Juvenile	Adult	Adult	
Body mass (g)	48.58 ± 3.26 (4)	50.93 ± 1.69 (7)	64.18 ± 2.59 (13)	-13.3**
Total lung capacity (ml STPD $100g^{-1}$)	9.67 ± 0.385 (3)	8.09 ± 0.289 (6)*	4.89 ± 0.437 (7)	+3.2**
Hct (%)	49.92 ± 4.58 (2)	50.51 ± 2.57 (8)	46.75 ± 1.98 (11)	+3.8
Hb (g 100 ml^{-1})	15.50 ± 0.347 (2)	17.17 ± 1.24 (7)	17.42 ± 0.838 (11)	-0.25
Blood O_2 Capacity (vol. %)	20.77 ± 0.469 (2)	23.01 ± 1.66 (7)	23.35 ± 1.12 (11)	-0.34
Skeletal muscle Mb [a] (mg g wet $tissue^{-1}$)	7.10 ± 0.419 (2)	13.57 ± 0.399 (7)*	11.36 ± 0.343 (11)	+2.2**

[a] Mean for forelimb and hindlimb muscles.
* Adult-juvenile differences significant ($P<0.05$).
** Interspecific differences significant ($P<0.05$).
Values in parentheses denote number of animals tested.

being nearly 21% smaller ($P = 0.0005$) and possessing a slightly lower percentage of skeletal muscle, adult star-nosed moles exhibited a total muscle O_2 storage capacity that was 13.5% higher than for the more fossorial coast mole. However, mean skeletal muscle Mb content of both star-nosed moles and coast moles exceeded values measured for the non-fossorial shrew mole, *Neurotrichus gibbsii* (mean = 8.8 mg g wet tissue^{-1}, $n = 2$). In all moles examined, the Mb content of the forelimb swimming and digging muscles exceeded levels in the hindlimb muscles and ventricles. However, only in adults of the two species were these differences statistically significant (Table 1-4). A split-plot model revealed a significant effect of both age ($F_{1,9} = 109.15$, $P < 0.0001$) and muscle site ($F_{1,9} = 19.35$, $P = 0.0017$) on Mb concentration, but there was no evidence of interaction effects between these variables in either adult or juvenile star-nosed moles ($F_{1,9} = 0.79$, $P = 0.398$). Though the differences were only marginally significant ($P = 0.032$), the Mb concentration of the forelimb muscles also exceeded that of the hindlimbs in the coast mole. Interspecific comparisons of mean skeletal muscle Mb content yielded statistically significant differences between forelimb ($P = 0.0001$) and hindlimb ($P < 0.0001$) for adult star-nosed moles and coast moles (Table 1-4). A split-plot analysis revealed significant differences in mean Mb content between species ($F_{1,14} = 6.76$, $P = 0.021$) and limb site ($F_{1,14} = 15.99$, $P = 0.0013$), with no evidence of interaction between these variables ($F_{1,14} = 0.2$, $P = 0.66$).

Table 1-4. Buffering capacities and myoglobin and glycogen concentrations of skeletal muscles of star-nosed moles (*Condylura cristata*) and coast moles (*Scapanus orarius*). Values are presented as means ± SE.

Variable		Star-nosed mole		Coast mole	Difference (adult *C.Cristata* - adult *S. orarius*)
		Juveniles	Adults	Adults	
Myoglobin (mg g wet tissue^{-1})					
	Heart	7.74 ± 0.457 (4)	8.91 ± 0.379 (6)	9.24 ± 0.276 (7)	-0.3
	Forelimb	8.33 ± 0.825 (4)	14.39 ± 0.517 (7)	12.10 ± 0.252 (10)	+2.3
	Hindlimb	5.86 ± 0.161 (4)	12.75 ± 0.432 (7)	10.61 ± 0.560 (10)	+2.1
Buffering Capacity (β)[a]					
	Forelimb	43.65 ± 2.54 (4)	44.12 ± 2.96 (6)	37.33 ± 2.10 (10)	+6.8
	Hindlimb	43.18 ± 1.25 (4)	48.01 ± 4.26 (6)	38.94 ± 1.77 (10)	+9.1
Glycogen (mg g wet tissue^{-1})					
	Forelimb	1.09 ± 0.544 (4)	1.47 ± 0.273 (6)	3.43 ± 0.808 (6)	-2.0
	Hindlimb	2.23 ± 1.12 (4)	1.68 ± 0.460 (6)	5.24 ± 1.42 (6)	-3.6

[a] β = Slyke (μmoles of base required to titrate the pH of one gram wet weight of muscle by one pH unit). Values in parentheses denote number of animals tested

Muscle Biochemistry of Star-Nosed Moles and Coast Moles

The buffering capacity and glycogen concentration of locomotor muscles were analyzed to assess the tolerance of these animals to anaerobic metabolism. Muscle buffering capacities tended to be highest in adult star-nosed moles but only the hindlimb value for this species was statistically different from that of adult coast moles ($P = 0.038$, Table 1-4). Mean glycogen concentrations of forelimb and hindlimb muscles were similar ($P > 0.05$) in both juvenile and adult moles. Though muscle glycogen concentration was similar in juvenile and adult star-nosed moles, split-plot analysis revealed significant effects due to limb site ($F_{1,8} = 11.17$, $P = 0.01$) and age-limb interaction ($F_{1,8} = 6.17$, $P = 0.034$) in this species. With respect to muscle glycogen content, mean values for coast moles exceeded those of star-nosed moles and hindlimb concentration exceeded forelimb. Furthermore, a split-plot analysis revealed significant effects due to species ($F_{1,10} = 6.17$, $P = 0.032$) and limb sampling site ($F_{1,10} = 5.88$, $P = 0.036$), but no evidence of interaction effects. A significant difference also existed between species in hindlimb glycogen concentration ($P = 0.036$).

Discussion

It is widely acknowledged that the breath-hold endurance of vertebrate divers depends critically on the parsimonious use of limited "on-board" oxygen stores while diving. Allometry predicts that while total body oxygen stores scale close to unity, the rate of O_2 consumption scales to ca. 0.75

power of mass (Schmidt-Nielsen, 1985; Hudson and Jones, 1986; de Leeuw, 1996). Therefore, relative to their O_2 stores, larger divers should have lower mass-specific metabolic rates than their smaller counterparts. Since O_2 stores increase faster than their rate of depletion underwater, with an increase in body size the ADL should, in theory, scale to the 0.25 power of mass (Hudson and Jones, 1986). It is not surprising, then, that the diving capacity of endothermic divers tends to increase with body mass (Scheer and Kovacs', 1997). The cost of diving in small semi-aquatic endotherms is further exacerbated because drag increases with mass-specific surface area (Carbone, 1995) and air trapped in the pelage substantially increases buoyancy of small-bodied divers.

Despite these theoretical limitations, the diving ability of star-nosed moles exceeded Schreer and Kovacs (1997) predicted maximum dive duration by 31.9%. The average dive time of star-nosed moles (9.2 s) was comparable to that of the considerably larger mink (9.9 s, mass = 850 g, Dunstone and O'Connor, 1979) but was lower than the mean dive durations recorded for weaned, juvenile muskrats (19 s, mass = 254-360 g, MacArthur *et al.*, submitted). Few other comparative empirical studies of dive behaviour exist for small-bodied, endothermic divers. Mean voluntary dive times are available for adult platypus, *Ornithorynchus anatinus*, (28 s, mass = 900-1500 g, Evans *et al.*, 1994), the European water shrews (4-10 s, mass = 10-20 g, Kohler, 1984; Churchfield, 1985) and the North American water shrew, *Sorex palustris* (5.9 s, 14 g, McIntyre, see Appendix 1). Calder (1969)

reported a maximum dive time of 37.9 s in a single water shrew subjected to a forced dive. Surprisingly, the average dive time of star-nosed moles was only slightly lower than that reported for the tufted duck, *Anthya fuligula*, an accomplished avian diver (mean dive time = 10.9 s, mass = 638 g, Stephenson *et al.*, 1989) that possesses significantly higher total oxygen stores than *Condylura*.

The question that arises, then, is how does one account for the exceptional dive performance of the star-nosed mole? Potential factors contributing to the enhanced dive endurance of this species could include: a relatively low rate of oxygen depletion underwater, higher-than-expected O_2 reserves, or a strong dependence on anaerobic pathways during diving.

Conventional estimates of diving metabolic rates (DMR) are frequently assumed to be twice the BMR or RMR of the species in question (Burns and Castellini, 1996). Consistent with this assumption, the estimated DMR of star-nosed moles was 2.1 X RMR. However, this ratio is slightly less than that reported for muskrats (MacArthur and Krause, 1989) or tufted ducks (Woakes and Butler, 1983; Butler, 1988), in which DMR averaged 2.73 and 3.5 times BMR, respectively. Moreover, the mean metabolic cost of surface swimming/diving of star-nosed moles was low when compared to the mean value reported for mink (6.54 ml O_2 g^{-1} h^{-1}, Stephenson *et al.*, 1988), suggesting that this insectivore displays a relatively low mass-specific cost of submergence. Star-nosed moles, like muskrats and tufted ducks, are strongly positively buoyant (mean specific gravity of moles = 0.826 $\pm$ 0.008, n = 8,

McIntyre, unpub. obs.), a factor that may contribute to the 2- to 3.5-fold increase in the estimated cost of diving by these species. Marine birds, such as the Humboldt penguin, *Spheniscus humboldti*, that are almost neutrally buoyant, exhibit little change in VO_2 during voluntary diving (Butler and Woakes, 1984).

That body oxygen stores are often elevated in vertebrate divers appears well established (Weber *et al.*, 1974; Stephenson *et al.*, 1989; Evans *et al.*, 1994; Butler and Jones, 1997). Consistent with this trend, I found that skeletal muscle Mb levels of star-nosed moles (Table 1-4) were comparable to those reported for other semi-aquatic mammals including platypus (1.43 g $100g^{-1}$, Evans *et al.*, 1994), beaver (1.2 g $100g^{-1}$, McKean and Carlton, 1977), and winter-acclimatized muskrats (1.3 g $100g^{-1}$, MacArthur, 1990). The mean Hct of adult star-nosed moles (50.5%) was similar to that reported for platypus (52%, Parer and Metcalfe, 1967), but exceeded values reported for muskrat (46.7%, MacArthur, 1990) and beaver (42.1%, Kitts *et al.*, 1958). Combining lung, blood and muscle estimates, the total mass-specific body O_2 stores of adult star-nosed moles (34 ml kg^{-1}) exceeded those of platypus (25 mlO_2 kg^{-1}, Evans *et al.*, 1994), but were similar to values reported for winter-acclimatized muskrats (35.7 mlO_2 kg^{-1}, MacArthur, 1990). A causal link between oxygen storage capacity and dive endurance is often assumed in interspecific comparisons (Kooyman, 1989; Butler and Jones, 1997) and my findings suggest that the exceptional diving ability of *Condylura* may be attributed, at least in part, to elevated body O_2 stores.

The absence of correlation between muscle indices of anaerobic potential (buffering capacity and glycogen levels) and muscle Mb content suggests that variations in Mb, and hence muscle aerobic capacity, are not matched by compensatory adjustments in buffering capacity or glycogen content of working muscles. Without adequate buffering capacity, even muscles possessing large glycogen deposits cannot function anaerobically for extended periods, because falling pH inhibits enzyme function and impedes further glycolytic activity (Castellini and Somero, 1981). The low buffering capacity and glycogen content of the skeletal muscles of star-nosed moles suggest little dependence on anaerobic metabolism while diving. This conclusion is supported by behavioural observations in which only 2.9% of all voluntary dives exceeded the calculated ADL, a finding that presumably reflects adoption of an aerobic diving schedule to maximize underwater search time and hence optimize foraging efficiency (Butler and Jones, 1997).

A major objective of this study was to compare the O_2 storage capacity of star-nosed moles and coast moles. The rationale for this comparison is the premise that several hypoxia-driven respiratory adaptations are potentially convergent in burrowers and divers, and it is informative to know to what extent diving has modified the O_2 storage capacity of star-nosed moles. My findings indicate that the large mass-specific O_2 stores of the star-nosed mole are primarily accounted for by the lungs and muscle, rather than blood, as is typically the case for diving rodents and pinnipeds (MacArthur, 1990; Burns and Castellini, 1996). As in other highly fossorial species, including the

fossorial mole rat and valley pocket gopher, *Thomomys bottae*, the O_2-carrying capacity of coast mole blood is high relative to non-burrowing, terrestrial mammals. Though lower than in star-nosed moles (Table 4), the skeletal muscle Mb concentration of the exclusively fossorial coast mole (1.14 g $100g^{-1}$) was comparable to that of other burrowers, including the echidna (1.26g $100g^{-1}$, Hochachka *et al.*, 1984). These findings underscore the potential significance of Mb in species that routinely encounter hypoxia associated with diving or burrowing. Skeletal muscle Mb levels varied with both age and sampling site in star-nosed moles. Consistent with several recent studies (e.g. Ponganis *et al.*, 1999, MacArthur *et al.*, submitted), my results suggest ontogenetic changes in muscle Mb levels. Ponganis *et al.* (1999) reported myoglobin contents of post-molt juvenile and pre-molt chicks of the emperor penquin, *Aptenodytes forsteri*, that were only 24% - 31% those of adult values. Similarily, our results indicate that mean adult muscle myoglobin concentration of star-nosed moles exceeded juvenile values by 48%. Interestingly, the tendency for Mb levels of the forelimbs to greatly exceed hindlimb values in both mole species is reversed from the trend observed in muskrats (MacArthur, 1990). Whereas the forelimb muscles of moles are the primary locomotor digging muscle, the hindlimbs of muskrat are the primary propulsive organs underwater (Fish, 1982).

Clearly, the high total body O_2 stores of star-nosed moles cannot be attributed solely to the selective pressures of diving, since the similar-sized coast mole also has comparable total O_2 stores. However, differential

partitioning of O_2 stores was evident in the two species. Of particular interest was my finding that the lung volume of star-nosed moles was 1.74 times greater than the predicted allometric value. By comparison, lung volume of the coast mole conformed to standard allometric predictions for eutherian mammals (Stahl, 1967).

The observed differences in RMR between star-nosed and coast moles are also of interest. The inherently high RMR of the star-nosed mole follows the conventional pattern for small semi-aquatic endotherms that are subject to chronic cold exposure (Iverson, 1972; MacArthur and Campbell, 1994; Campbell *et al.*, 1999), while the RMR of the coast mole is consistent with a fossorial lifestyle (McNab, 1979). It is important to note that these two mole species exhibit dramatic differences in habitat, and that star-nosed moles presumably diverged from the main talpid line early in the evolutionary history of the group (30-49 mya, Yates and Greenbaum, 1982). Recent studies have also suggested that star-nosed moles may have secondarily acquired a semi-aquatic lifestyle (Grand *et al.*, 1998; Campbell *et al.*, 1999). It is conceivable, then, that the thermal environment of star-nosed moles may have selected for an elevated BMR to augment heat production. Moreover the large lung volume of this species not only augments total O_2 stores during diving, but may also provide positive buoyancy during surface swimming, thus reducing trunk surface area exposed to water and potentially minimizing the metabolic cost of aquatic thermoregulation. As in the star-nosed mole, an exceptionally large lung volume has been reported for the sea otter, *Enhydra*

lutris (Lenfant *et al.*, 1970). When foraging, sea otters mainly undertake short and shallow dives, and their large lung volume has been suggested to facilitate floating at sea between periodic thermogenic bouts of activity (Costa and Kooyman, 1984).

In summary, the results of this study reveal that like the coast mole, the star-nosed mole exhibits a large mass-specific total O_2 reserve. This finding likely reflects convergence arising from hypoxia-inducing conditions associated with subterranean burrowing or diving. In star-nosed moles, the principal O_2 reservoir is provided by the substantive lung and muscle compartments. I suggest that the need to attenuate thermoregulatory costs associated with a semi-aquatic existence, perhaps coupled with a need to augment O_2 reserves during diving, may account for the exceptionally large lung volume in this species. These factors, coupled with a high blood O_2 storage capacity and a relatively low metabolic cost of aquatic activity, likely contribute most to extending the dive duration of this peculiar mammal. Clearly, additional studies are needed to explain the exceptional dive performance of semi-aquatic insectivores.

CHAPTER 2

AQUATIC THERMOREGULATION IN THE STAR-NOSED MOLE (*CONDYLURA CRISTATA*) WITH COMPARISONS TO NON-AQUATIC TALPIDS AND THE WATER SHREW (*SOREX PALUSTRIS*)

Introduction

The energetic demands of foraging in the aquatic medium often impose severe thermoregulatory penalties on endothermic vertebrate divers. Water has a thermal conductivity that is approximately 25 X that of air, resulting in almost zero resistance to heat flow (Rapp, 1971). While large-bodied marine species tend to benefit from high thermal inertia, small semi-aquatic mammals with large surface area-to-volume ratios are often susceptible to heat loss in water (Costa and Kooyman, 1982). Furthermore, the considerable air boundary provided by pelage renders small mammals more buoyant (de Leeuw, 1996) than larger marine divers whose primary source of insulation is usually substantial deposits of subcutaneous fat rather than air (Scholander *et al.*, 1950). Metabolic rate and thermal conductance are both inversely related to body mass in homeotherms (Calder, 1969). Consequently, as mass decreases, rate of O_2 consumption (VO_2) per gram of tissue increases, resulting in accelerated heat loss. Attempts by animals to mitigate conductance in water are frequently hampered by mechanical disruption of the air boundary contained within the pelage (Stephenson, 1995). Thus diving poses 2 principle thermoregulatory problems for an endotherm. First, compression of the pelt reduces whole-body insulation underwater. Further, the rate of heat loss during diving often exceeds the capacity for metabolic heat production, resulting in a state of negative energy balance and, consequently, a fall in Tb with attendant post-immersion recovery costs.

These energetic constraints should, in theory, be greatest in the world's smallest mammalian divers, which include 2 species of water shrews (*Neomys fodiens* and *Sorex palustris*) and the star-nosed mole, *Condylura cristata*. As the sole semi-aquatic member of the family Talpidae, the star-nosed mole frequently encounters frigid water temperatures during winter foraging excursions (Merriam 1884; Seton, 1909; Hamilton, 1931). While terrestrial oligochaetes compose much of the star-nosed mole's diet, several authors have suggested that aquatic prey items may predominate during winter, as tunnel excavation and consequently earthworm predation are impossible in frozen ground. Yet, it appears that virtually nothing is known regarding the abilities of this insectivore to thermoregulate in air or water. Such investigations are essential to enhance our understanding of how semi-aquatic mammals in general, and this species in particular, thermoregulate in water.

The small body size of insectivores should dictate a high mass-specific metabolic rate, yet severely limit their ability to enhance insulation and expand body fuel reserves. Moreover, the presence of large, sparsely-haired appendages specialized for digging suggests that thermal conductance in *Condylura* may be relatively high (Grant and Dawson, 1978*b*; Reynolds, 1993). Consequently, the potentially high thermoregulatory costs of underwater activity should select for maximal foraging efficiency in these small mammals (MacArthur, 1984*a*). Combined with the elevated VO_2 required to sustain aerobic metabolism during diving, the cooling effects of

cold water may impose strict limitations on the underwater endurance of star-nosed moles. Further, attempts by these animals to attenuate heat loss through insulatory adjustments involving increased air retention in the pelt may, by default, incur higher hydrodynamic costs owing to increased buoyancy (de Leeuw, 1996).

The purpose of this study was to first investigate the thermoregulatory ability of star-nosed moles in air and water. The metabolic costs of aquatic thermoregulation were assessed from measurements of VO_2 and whole-body conductance at varied water temperatures. To estimate the contribution of air contained within the pelt to total insulation, the volume of this gas and its role in retarding heat loss also were investigated. A second objective was to test the hypothesis that the star-nosed mole derives a thermoregulatory benefit from the obligatory increase in metabolism that ensues following feeding. This heat increment of feeding (HIF) is associated with the cost of mastication, peristalsis, excretion and metabolic conversion of ingested food (Costa and Kooyman, 1984; MacArthur and Campbell, 1994, Campbell *et al.*, 2000). HIF has been suggested to defray thermoregulatory costs, particularly in species that predominantly consume protein-rich foods that produce a strong HIF response. The star-nosed mole offers an excellent model for testing this hypothesis, since it eats mainly earthworms which, on a dry-matter, ash-free basis are approximately 80% protein (French, 1957; Campbell *et al.*, 2000). Consequently, I investigated the potential role of the HIF response in attenuating the metabolic costs of aquatic thermoregulation in this species.

Where relevant, comparisons were made between the star-nosed mole and the exclusively fossorial coast mole, *Scapanus orarius*, to determine the relationship between indices of thermoregulatory competence and habitat selection among these talpids. The coast mole affords an ideal model for comparison with the star-nosed mole, since the two species have similar body masses and are geographically disjunct. The coast mole is found only in extreme southwestern British Columbia and northern Washington (Sheehan and Galindos-Leal, 1997), while the star-nosed mole is the most northern talpid, occurring throughout southeastern Canada and the northern USA, extending as far west as Manitoba. In addition, limited comparative data are presented for the smallest mammalian diver, the North-American water shrew. The challenges of aquatic thermoregulation should be greatly exacerbated in this diminutive species which is frequently sympatric with the star-nosed mole.

Materials and Methods

Animals

Laboratory studies were performed on 29 star-nosed moles collected from Piney, Rennie and Caddy Lake, Manitoba, between June 1997 and Sept. 1999. A local mole trapper in Abbottsford, British Columbia, provided a total of 20 coast mole carcasses in 1999. An additional 2 coast moles were live-trapped in June of 1998. Animal care and collection procedures are

described in detail in Campbell *et al.*, (1999) Sheehan and Galindos-Leal (1997) and chapter 1, respectively.

North American water shrews (n = 8) were caught near Moose River, Nopoming Provincial Park, Manitoba, during late August and early September of 1998 and 1999. Water-shrews were captured along streambanks, close to water, using pitfall traps (Anderson, 1934). For this purpose, the streambed was excavated and a 2-L tin can was submerged at ground level, thus acting as a pitfall. A barrier constructed of heavy polyethylene sheeting was drawn across the center of the can and extended for 0.2 m on land and 1 m into the water, and was secured into the substrate with thin copper rods placed at regular intervals (Lisa Hartman, pers. comm.). The latter provision acted as a corral to direct shrews into the trap. I also used ca. 180-200 Sherman and Longworth traps per session that were baited with pork fat and peanut butter. Despite the additional provision of dry nesting material and frequent trap checks at 2-3 h intervals, placement of traps in close proximity to water resulted in 75% mortality of trapped animals. However, 2 water shrews were successfully live-trapped and transported to the University of Manitoba Animal Holding Facility within 24 h of capture where they were held individually in a controlled environment chamber maintained at 20 ± 1°C with a 12L:12D photoperiod. Shrews were housed in two large plastic containers (46 cm x 31 cm x 38 cm) connected in series via PVC pipe, thus dividing the tank into terrestrial and aquatic compartments. The terrestrial section was furnished with peat moss, while on the aquatic side, a 24-cm-deep pool of 20°C water

was provided for shrews to swim and dive. Animals were maintained on a diet of mealworms, *Tenebrio molitor*, leeches, *Nepholopsis obscura*, and a meat ration containing vitamins and a calcium supplement. In addition, live minnows were provided whenever possible. In captivity, these agile divers readily captured minnows; they emerged from the water to consume only the head and viscera and rejected the remainder of the carcass. This foraging behaviour is analogous to observations of water shrews in nature (Buckner, 1970), suggesting that these animals adapted well to holding conditions. Animals were cared for in accordance with Canadian Council on Animal Care guidelines.

Dive behaviour

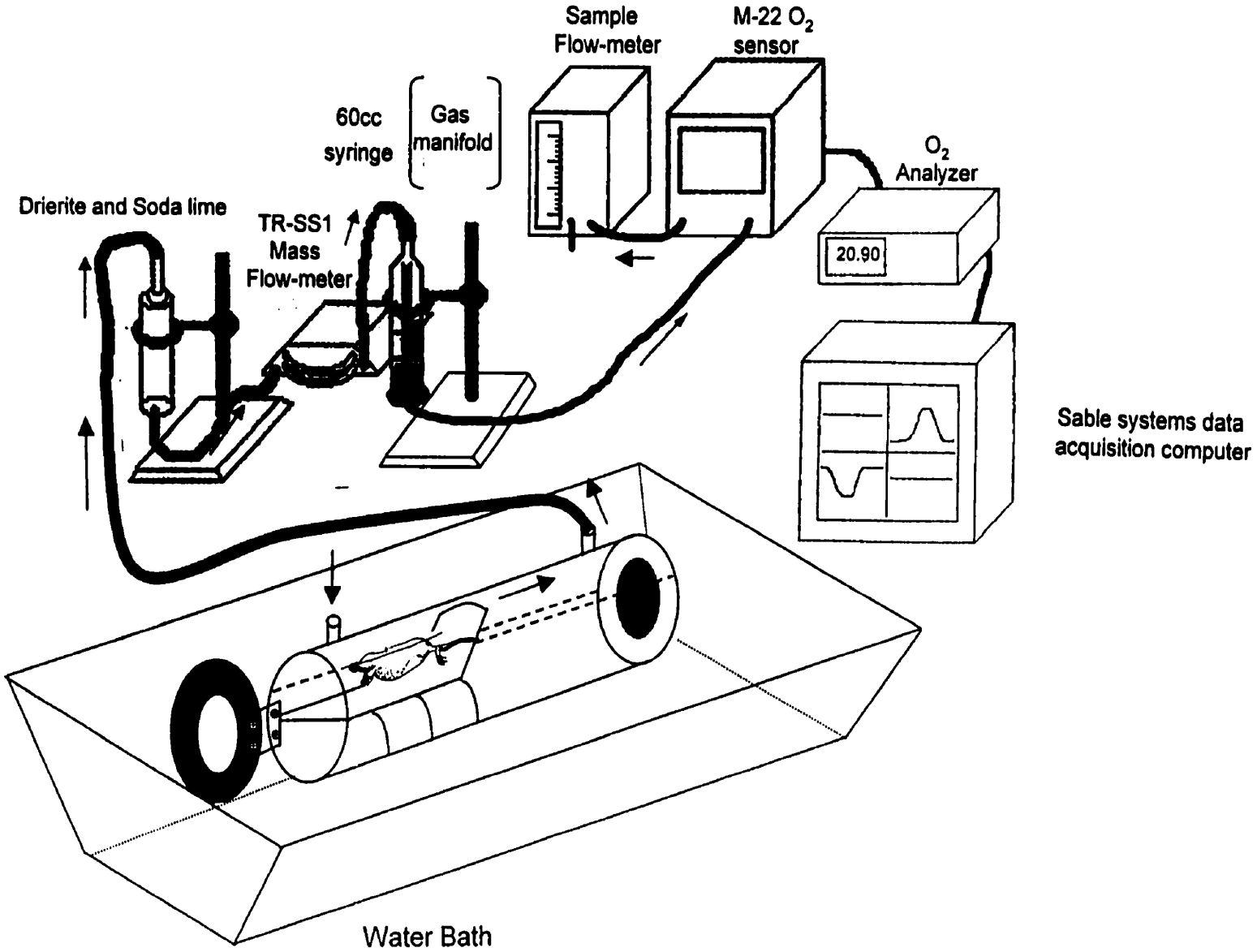
To assess the influence of water temperature on voluntary dive times, a study of voluntary dive behaviour of star-nosed moles was initiated soon after the animals arrived at the University of Manitoba Animal Holding Facility. A series of 20-min trials were performed in a large wooden plywood tank that included an open swimming area and a dry resting platform (See Fig 1-1). Prior to each trial, the tank was filled with 3, 10, 20 or 30°C water, randomly presented to prevent thermal acclimation. All bouts of swimming, diving, resting and grooming activity were recorded for subsequent analyses.

Metabolic rate measurements

Measurements of resting rates of O_2 consumption (VO_2) in air were measured over a range of ambient temperatures (0-33°C) using negative-pressure, open flow respirometry. Following weighing, animals were introduced into a metabolic chamber consisting of a 0.95-L metal can painted flat black inside and installed in a controlled temperature cabinet. Exhaust gas was treated in an identical manner to that previously described (in Chapter 1). Animals were held in the chamber for ca. 1 h and the lowest VO_2 occurring over at least a 3-min period was taken as the resting metabolic rate (RMR) in air. The absence of motor activity during resting metabolic rate determinations was verified independently using a motion activity detector (MAD-1, Sable Systems Inc.) mounted directly beneath the metabolic chamber.

To estimate RMR at different ambient water temperatures, a series of short-term immersion trials was conducted. For this purpose, a 7-L Plexiglas cylinder (98.5 cm long, 10 cm dia.) that was immersed in a constant temperature water bath containing either 3, 10, 20 or 30°C water, served as the respirometry chamber (Fig. 2-1). At one end of the cylinder, a wire mesh floor connected to a spring-loaded ramp provided a resting platform. The ramp could be raised to prevent access of the animal to the remaining water-filled section of the tube. The cylinder was fitted with inlet and outlet air ports and excurrent air was drawn by vacuum from the cylinder at a rate of 940 ml min^{-1}. Two copper-constantan thermocouple probes were used to continually monitor cylinder and bath temperature throughout the experiment.

Figure 2-1. Apparatus for determining RMR in water of live star-nosed moles. Immersion cylinder is shown within a constant temperature water bath maintained at either 3, 10, 20 or 30°C. Note presence of resting platform and the inlet and outlet gas ports positioned at opposite ends of the respirometry chamber. Arrows denote direction of gas flow. Dashed lines within the cylinder indicate approximate water level within the respirometer.


Sample
Flow-meter
M-22 O_2
sensor
60cc
syringe
Gas
manifold
O_2
Analyzer
Drierite and Soda lime
TR-SS1
Mass
Flow-meter
20.90
Sable systems data
acquisition computer
Water Bath

To assess RMR during immersion, the resting platform was flooded to a sufficient depth that the animal was mostly submerged, yet able to maintain continuous contact with the floor. Exhaust air was routed through Drierite, and then to a second column containing soda lime / Drierite. The dry, CO_2-free air sample was subsequently directed to the M-22 sensor of an Applied Electrochemistry S3-A O_2 analyzer for determination of FeO_2 (%). The rate of O_2 consumption was calculated using the method of Withers (1977, equation 4a).

Following weighing, the animal was introduced into the chamber and the entrance sealed with a rubber stopper. After 20 min of baseline recording, the resting platform was flooded by gently tipping the cylinder. An immersion period of 20 min followed. However, at lower temperatures it was necessary to reduce immersion time in order to avoid hypothermia. At the end of the trial, the cylinder was tilted in the reverse direction, draining water from the resting platform and permitting metabolic measurement during a 20-min post-immersion recovery period.

Body Temperature

A limited number of moles were instrumented with intra-abdominal transmitters (Mini-mitter Inc., Sunriver, OR, USA) that permitted monitoring of deep body temperature (Tb). Prior to implantation, transmitters were individually calibrated against an NBS-certified mercury thermometer in a circulating water bath. Transmitter calibration and implantation procedures

are described elsewhere (MacArthur, 1979). Briefly, the animal was weighed to the nearest 0.01g and anesthetized with an intraperitoneal injection of ketamine hydrochloride (100mg kg^{-1}) presented in combination with rompun (10mg kg^{-1}). The sterilized transmitter was then implanted into the abdominal cavity and the midline abdominal incision promptly closed. Animals were allowed a 2-week post-operative recovery period prior to initiating aquatic trials. I encountered significant obstacles when trying to anesthetize these animals, as star-nosed moles are highly susceptible to anesthesia-induced mortality (Allison, 1970; this study). In a total of 12 surgical procedures, only 2 animals survived. However, I discovered subsequently that rectal temperatures are easily measured and that star-nosed moles can be adequately restrained when provided access to a blind ending, 3-cm-diameter tunnel cut in a foam block. Animals readily entered the tunnel within I min following each trial. Tb was measured using a 30-Ga. copper-constantan probe lubricated with mineral oil and inserted 2 cm into the rectum. The temperature probe was coupled to a Physitemp BAT-12 digital thermometer (Sensortek Inc., Clifton, NJ, USA).

Determination of thermal conductance

Surface area (SA) is a critical variable in heat flux calculations, but is often difficult to measure (Reynolds, 1993). Techniques for determining SA include measuring the excised pelt or applying geometric approximations to calculate regional SAs that are then summed (Innes *et al.*, 1990). To estimate

the whole-body thermal conductance (C) in water, surface areas of star-nosed moles were evaluated following a procedure modified from Costa and Kooyman (1982) and MacArthur (1984*a*). The identical procedure was applied to coast moles and water shrews to obtain the meeh factor for each species (see below). Briefly, the flattened pelt and appendages were traced onto a sheet of paper and their respective areas determined with a SigmaScan digitizing tablet and microprocessor (Jandel Scientific, Corte Madera, CA, USA). To assess the contribution of the naked appendages to overall SA, the feet and tail were painted with a rubber latex (Amaco, American Art Clay Co., Inc., Indianapolis, IN, USA) which, when dry, was dissected free from the underlying tissue, photocopied and traced with the digitizing tablet. The surface area of the 22-tentacle nose was determined using a Nikon model SMZ-U dissecting microscope coupled to a Sony 3-CCD video camera, which captured images of the tentacles for later analysis using Northern Eclipse image processing software. All images were calibrated using a micrometer. The magnified image represented the SA for 11 tentacles, which was then traced with the digitizing tablet and the resulting area multiplied by 4, assuming equal area for both right and left and dorsal and ventral portions of the nose. For each animal, the Meeh factor (K) was derived from the equation:

$$SA = K\,W^{0.67} \quad (1)$$

where SA = surface area (m^2) and W = body weight in kg (Stitt *et al.*, 1971). The Meeh factor is a proportionality constant that measures the extent of

streamlining in animals (Innes, 1990; Reynolds, 1993). Those species possessing a more fusiform body shape, should also expect to have a lower Meeh factor.

Thermal conductance was estimated using the equation:

$$(2).\quad C = \frac{\text{Heat production} - \text{EHL} \pm \text{Heat storage}}{(T_b - T_a)\,SA}$$

where EHL= evaporative heat loss, assumed to be negligible and therefore excluded from calculations of C (MacArthur, 1984*a*), Ta = ambient temperature. Heat production was calculated, assuming that 1 ml O_2 min^{-1} kg^{-1} = 0.335 W kg^{-1} (Costa and Kooyman, 1982; MacArthur, 1984*a*). Heat storage was determined using a conversion factor of 3474 J kg^{-1} for each 1°C change in Tb.

Following established procedures (Morrison and Tietz, 1957; Bozinovic and Merritt, 1992; de Vries and van Eerden, 1995), carcass cooling curves were used to estimate minimum resting C in water. To facilitate comparisons with literature values, thermal conductance (W m^{-2} $°C^{-1}$) in still water with and without an intact air boundary in the pelage was calculated using the equation:

$$(3).\quad \text{Thermal conductance} = b \times Hm\ (J\ g^{-1})$$

where b = slope of ln(Tb-Ta) vs. Time (min) and

Hm = specific heat of mammalian tissue (3.47 J g^{-1} $°C^{-1}$; Morrison and Tietz, 1957).

Carcasses were heated in a convection oven with rectal probes inserted to a depth of 2 cm to continuously monitor core Tb. When core Tb reached 40°C, the carcass was removed from the oven and suspended vertically in still, 20 °C water until Tb equaled water temperature. Following shampooing of the carcass to remove the insulative air boundary and oils present on the pelt (Harlow, 1983), the animal was reheated until Tb = 40 °C and the immersion trial repeated.

Specific gravity determinations

In order to assess the contribution of air trapped within the pelage to overall buoyancy, specific gravity estimates were obtained for all 3 insectivore species. To eliminate residual gas contained within the lungs, a pneumothorax was performed on each cadaver with a 21-gauge syringe. The carcass was then weighed in air and the volume of each animal in water was determined gravimetrically (Scherle, 1970; Weibel, 1970/71). Shampooing the pelt in a light detergent solution enabled the calculation of the volume of air trapped in the pelage (MacArthur, 1992).

Heat Increment of Feeding

Campbell *et al.* (2000) reported that the "biochemical HIF", or rise in VO_2 associated with the metabolic transformation of food, was maximal at 2 h following feeding. Consequently, the potential for thermal substitution should in theory, also be maximized at this time. A series of 20-min immersion trials were conducted on 7 star-nosed moles in the same cylinder used for determining VO_2 and C at different water temperatures. Animals were initially

fasted for 8 h. They were then placed in an empty container and either fed a pre-weighed sample of earthworms that had been washed and patted dry, or were sham-fed to provide an experimental control. Following the 15-min feeding session, all orts were collected and weighed, and the animal was immediately transferred to a container of clean, earthworm-free soil. The water in the cylinder was then adjusted to the appropriate depth and temperature (10 °C). At 120-130 min post-feeding, Tb was recorded and the animal was forced to enter the metabolic chamber. Throughout the immersion period, VO_2 and water temperature were continuously monitored. Upon completion of the trial, a final Tb was recorded and the mole was then returned to soil.

Statistical treatment of data

Mean values were compared with Student's t-test, Welch's t-test, analysis of variance (ANOVA) and, where appropriate, the Tukey-Kramer multiple comparisons test. Regression lines were fitted by the method of least squares. Significance was set at the 5% level and means are presented ± 1 SE.

Results

Thermal influences on Diving behaviour

Water temperature affected both the duration ($F_{3,328}$ = 5.21, P = 0.0016; Table 2-1) and the frequency ($F_{3,24}$ = 3.82, P = 0.02; Table 2-1) of voluntary dives by star-nosed moles. In fact, mean dive duration of moles swimming in 30°C water exceeded that of moles diving in 3°C water by 3.6 s, or 35.3%. Least-squares regression analysis revealed a significant, albeit modest positive correlation between dive frequency and water temperature (Fig. 2-2A). However, mean dive frequency was only statistically different between trials in 3 and 30 °C water (Table 2-1, P = 0.006).

Body temperature

Body temperature of star-nosed moles was independent of air temperature ($F_{3,42}$ = 0.536, P = 0.66) over the entire range of ambient temperatures tested (0-33 °C). A regression of Tb on air temperature revealed no statistical relationship (r^2 = 0.01, P = 0.41) between these variables (Fig. 2-3A). The mean Tb of fasted, resting star-nosed moles in air was 37.7 ± 0.05 °C (n = 50).

In the 20-min immersion trials, Tb varied positively with water temperature (r^2 = 0.55, P < 0.0001; Fig. 2-3A) and analysis of variance revealed a significant effect of water temperature on mean Tb ($F_{3,30}$ = 12.74, P < 0.0001, Table 2-1). In all immersion trials, moles displayed a net decline in Tb below initial resting values in air. The greatest decline in Tb during 20-

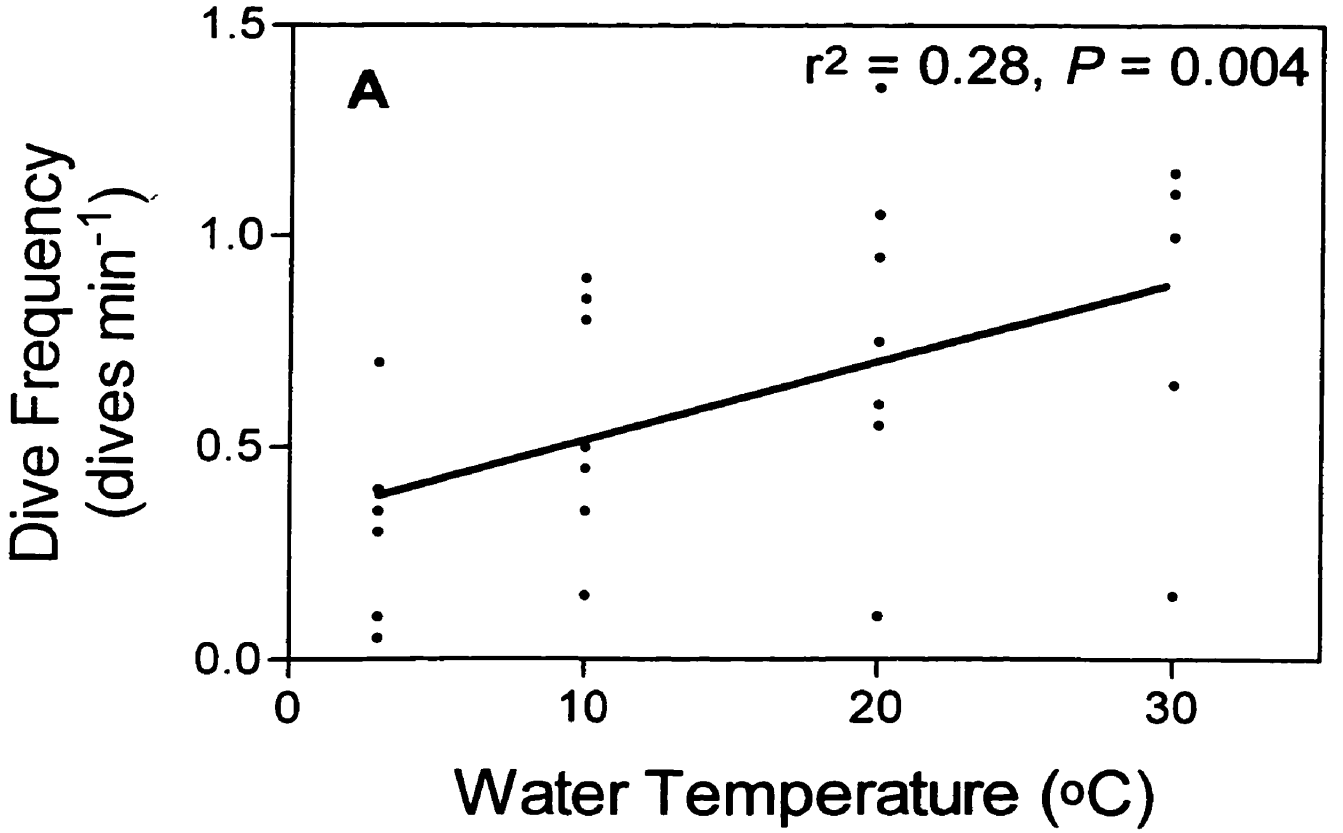
Table 2-1. The influence of water temperature on thermal conductance, body temperature and voluntary dive behaviour of star-nosed moles. Values presented are means ± SE.

Variable		Temperature (°C)			
		3	10	20	30
Conductance ($W\ m^{-2}\ °C^{-1}$)					
	Air	2.82 ± 0.14 (9)^{a}	3.22 ± 0.14 (11)^{a}	4.13 ± 0.19 (11)^{b}	6.14 ± 0.23 (10)^{c}
	Water	7.83 ± 0.67 (6)^{a}	11.65 ± 1.30 (6)^{ab}	11.92 ± 0.66 (11)^{b}	17.84 ± 1.34 (6)^{c}
Mean Tb (°C)*					
	Air	37.96 ± 0.50 (10)^{a}	37.48 ± 0.53 (13)^{a}	38.01 ± 0.086 (11)^{a}	38.05 ± 0.12 (12)^{a}
	Water	34.06 ± 0.91 (6)^{ab}	33.47 ± 0.60 (7)^{a}	36.05 ± 0.45 (12)^{bc}	37.70 ± 0.21 (9)^{c}
Cooling rate ($°C\ min^{-1}$)*		0.364 ± 0.11 (4)^{ab}	0.63 ± 0.17 (5)^{a}	0.208 ± 0.027 (11)^{b}	0.059 ± 0.011 (8)^{b}
Dive duration (s)		6.6 ± 0.56 (7)^{a}	7.2 ± 0.42 (7)^{a}	8.4 ± 0.44 (7)^{a}	10.2 ± 0.90 (7)^{b}

Dive frequency (dives min^{-1})	0.29 ± 0.084 (7)[a]	0.57 ± 0.11 (7)[ab]	0.76 ± 0.15 (7)[ab]	0.84 ± 0.14 (7)[b]

Note: Within each row, means sharing the same letter are not statistically different.
Values in parentheses denote number of animals tested.
* Based on rectal measurements at start and end of trial (see text for details).

Figure 2-2. The relationship of (A) dive frequency and (B) dive duration to water temperature in 7 star-nosed moles. Values are presented for moles during 20-min voluntary dive trials in 3, 10, 20 and 30 °C water.


A
r² = 0.28, P = 0.004
Dive Frequency (dives min⁻¹)
1.5
1.0
0.5
0.0
0
10
20
30
Water Temperature (°C)

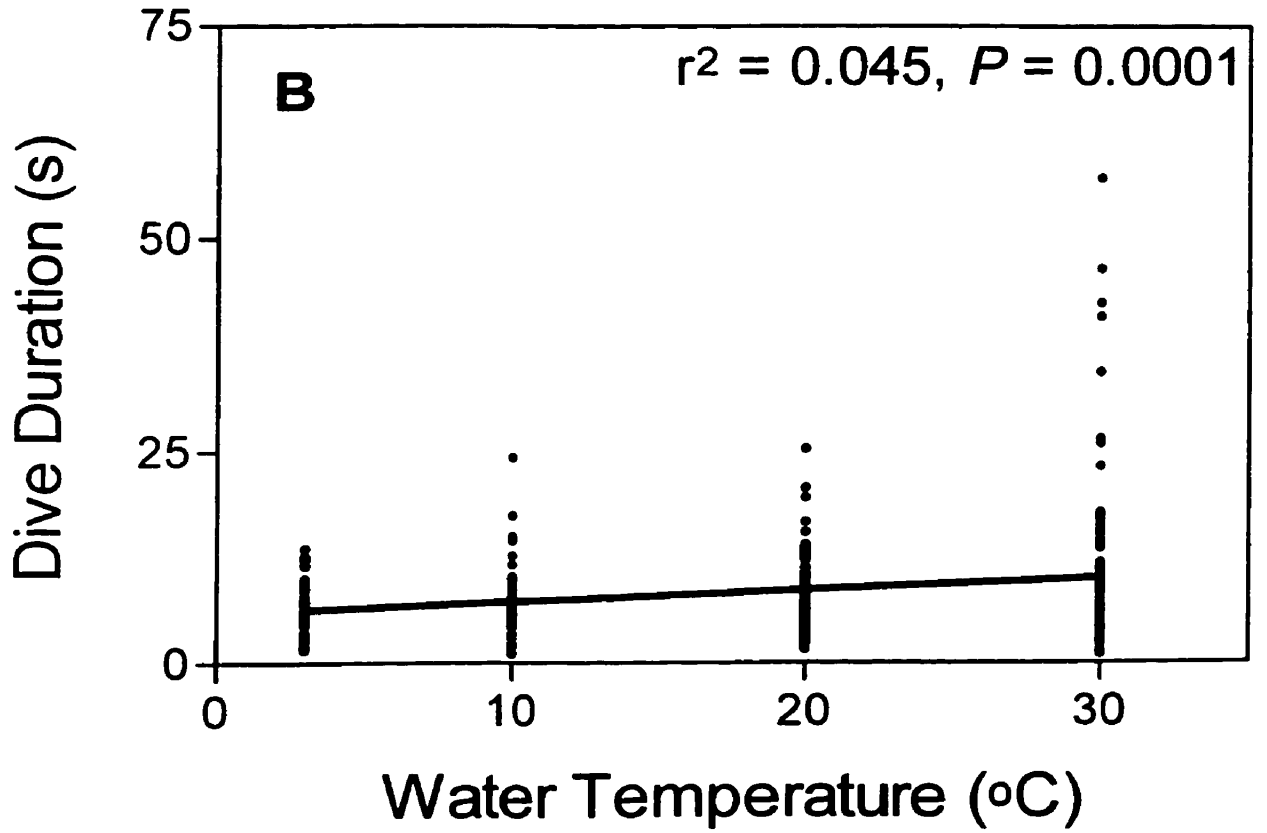
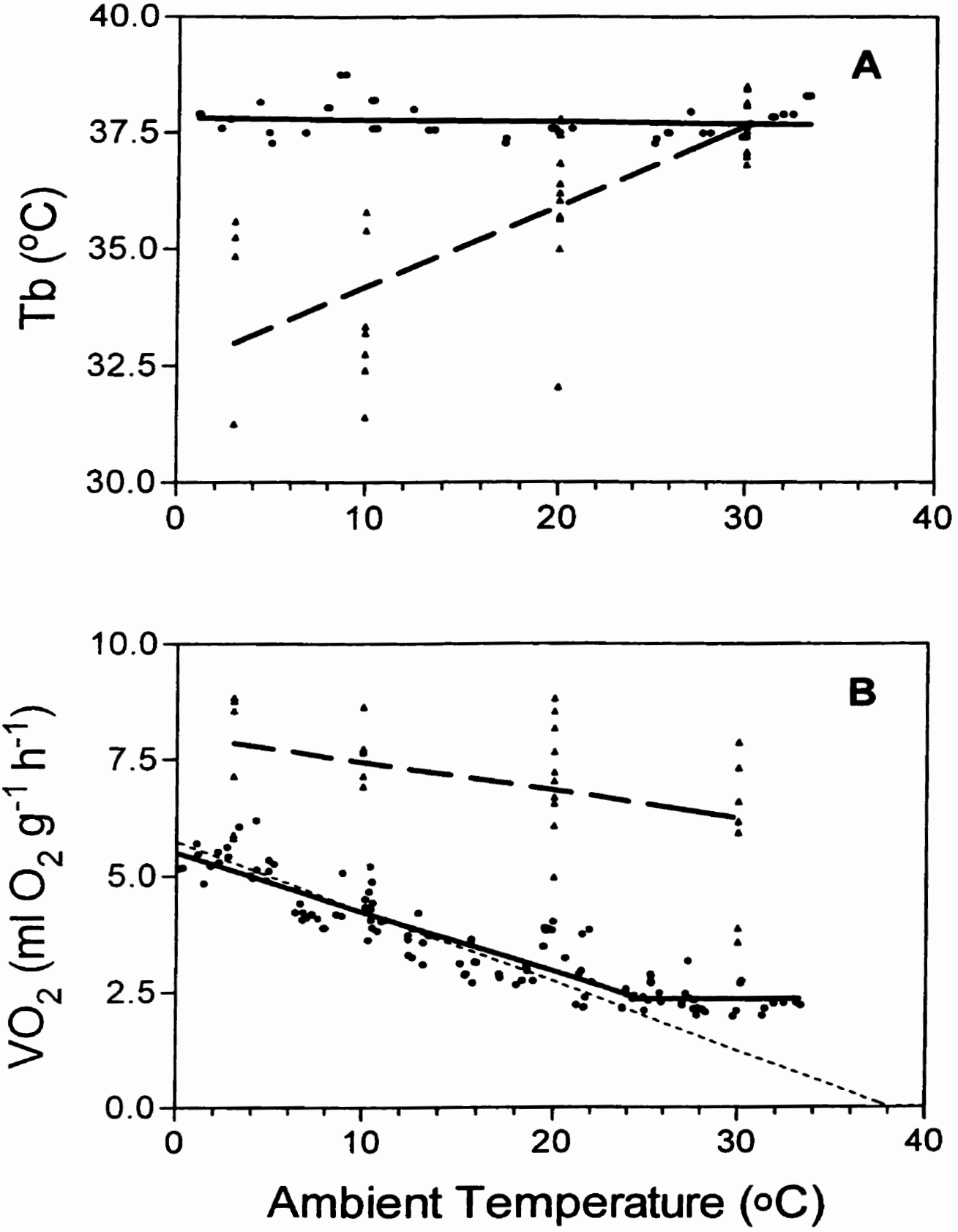

B
r² = 0.045, P = 0.0001
Dive Duration (s)
75
50
25
0
0
10
20
30
Water Temperature (°C)

Figure 2-3. The relationship of (A) body temperature (Tb) and (B) resting rate of O_2 consumption (VO_2) to air temperature (closed circles) in 12 fasted star-nosed moles and to water temperature (closed triangles) in 11 fed moles. Solid lines represent the least-squares regressions of Tb and VO_2 on air temperature; dashed lines denote the regressions of Tb and VO_2 on water temperature. The dotted regression line was derived by forcing the regression of VO_2 on air temperature to intersect the abscissa at Tb = 37.7 °C.


A
40.0
37.5
35.0
32.5
30.0
Tb (°C)
0
10
20
30
40
B
10.0
7.5
5.0
2.5
0.0
VO2 (ml O2 g-1 h-1)
0
10
20
30
40
Ambient Temperature (°C)

min immersion sessions was observed in trials conducted in 10 °C water. As expected, cooling rates (°C min^{-1}) generally increased with declining water temperature ($F_{3,24}$ = 10.31, P = 0.0002), though moles appeared to cool faster in 10 °C, than in 3 °C water (Table 2-1).

A single radio-implanted star-nosed mole defended Tb within narrow limits during a 20-min period of immersion in 20 °C water (Fig. 2-4). During this immersion period, the maximum drop in Tb of this mole was 0.95 °C. In sharp contrast, a radio-implanted coast mole exposed to the same period of immersion displayed a dramatic 13.0 °C decline in Tb (Fig. 2-4), and exhibited symptoms of severe hypothermia following the trial, including disorientation, lethargy and intense shivering. Limited data for the same radio-implanted star-nosed mole were also obtained for 20-min immersion trials in 3, 10, 20 and 30 °C water (Fig. 2-5A-D). The net Tb decline in this animal was greatest in 3 °C (mean Tb drop = 3.8 °C), where the mean cooling rate was 0.19 °C min^{-1}.

Metabolic rates

In air, the lower critical temperature (LCT) of star-nosed moles was estimated to be 24.0 °C (Fig. 2-3B). Below this temperature, VO_2 increased steadily with declining air temperature (VO_2 = 5.297 – 0.129 Ta; r^2 = 0.76, n =127, P < 0.0001). While the upper critical temperature could not be clearly delineated, metabolic rate was independent of Ta from 24.0 to approximately

Figure 2-4. Telemetered abdominal temperatures of a radio-implanted star-nosed mole (closed circles) and a radio-implanted coast mole (open circles) during a 20-min immersion period in 20°C water. Arrows denote times of animal entry to (↓) and exit from (↑) the Immersion chamber.

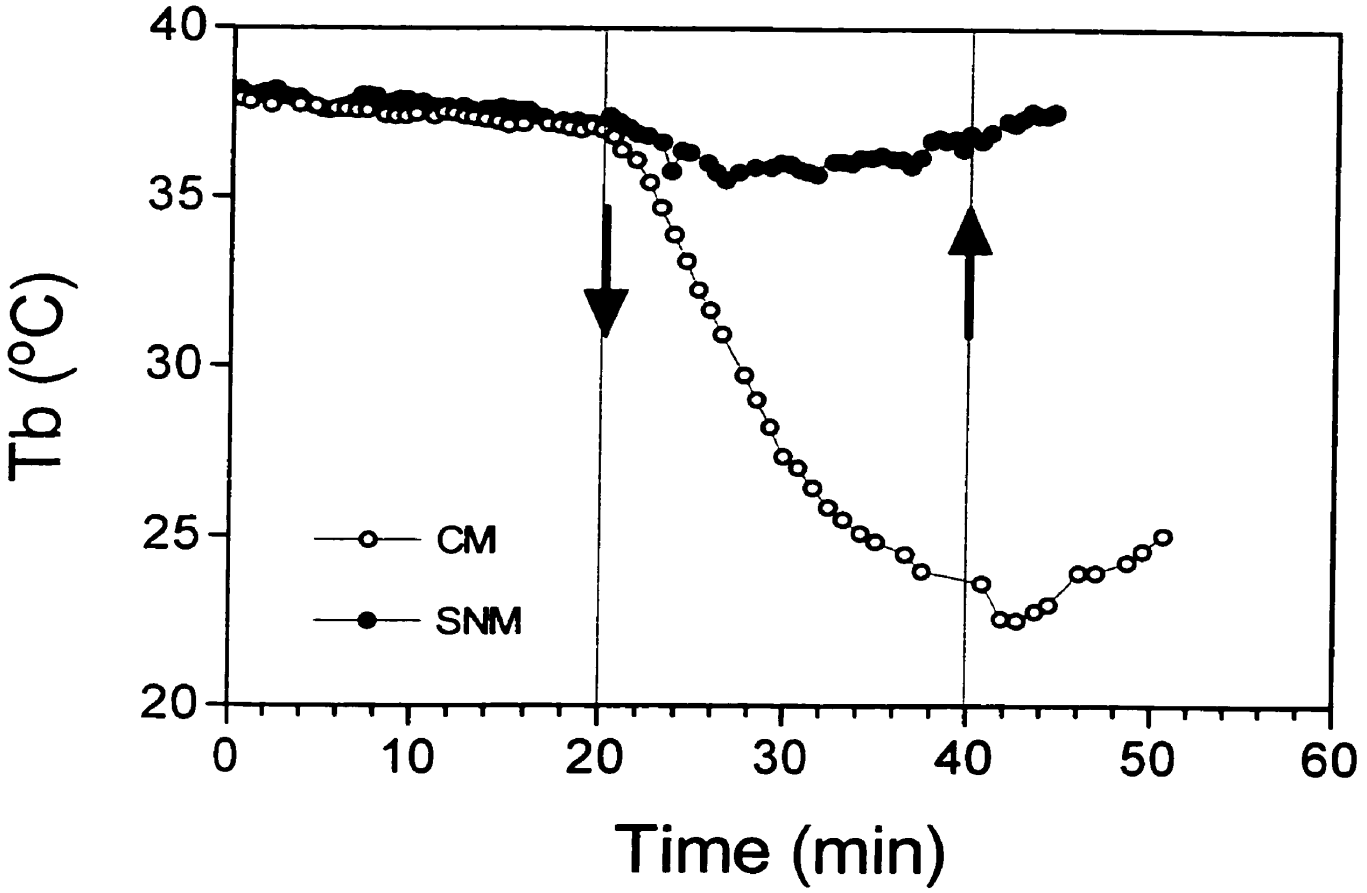
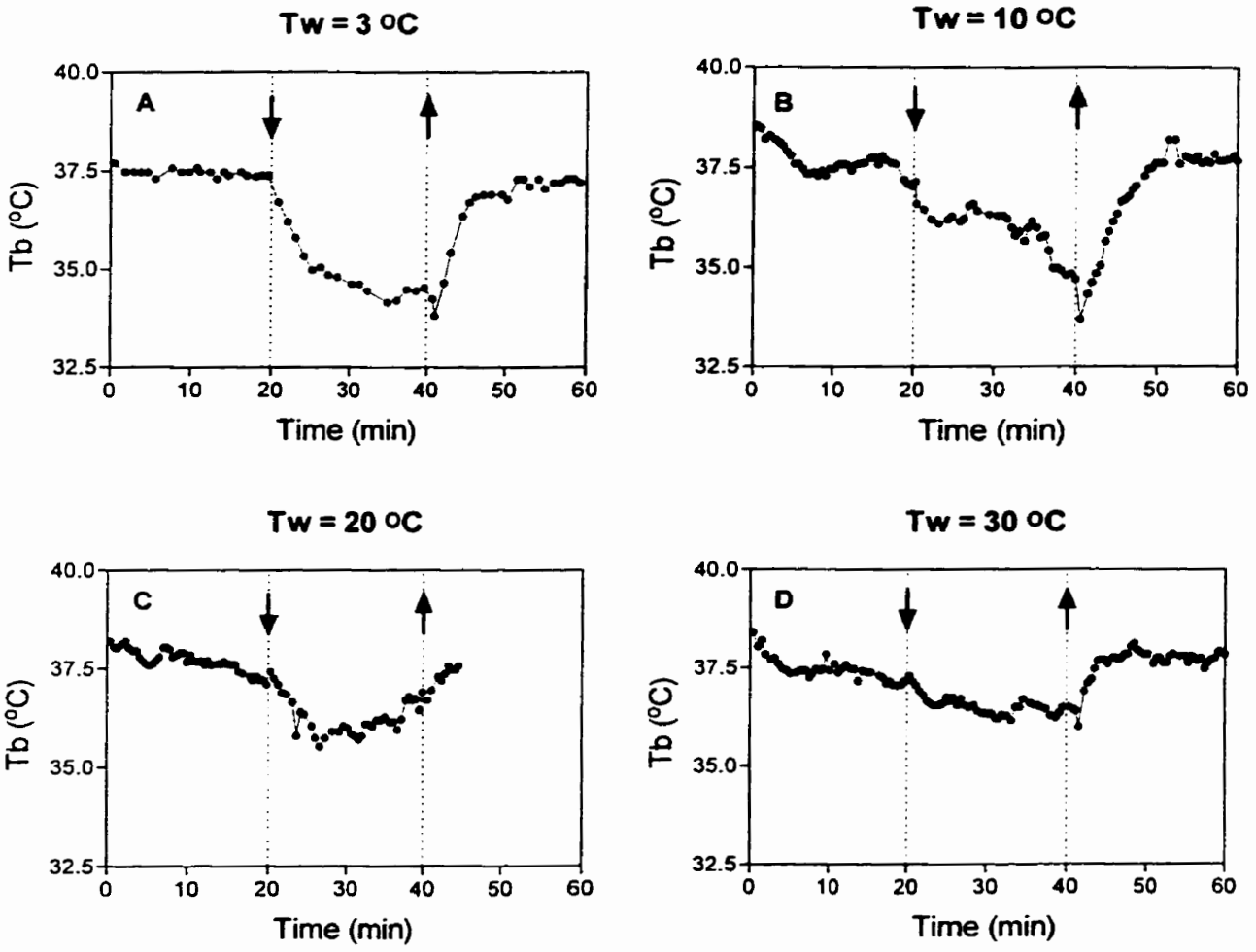

40
35
30
25
20
Tb (°C)
CM
SNM
0
10
20
30
40
50
60
Time (min)

Figure 2-5. Temporal variation in telemetered abdominal temperature of a single star-nosed mole immersed for 20 min in 3, 10, 20 and 30°C water. Arrows denote mole entry to (↓) and exit from (↑) the immersion apparatus.


Tw = 3 °C
A
Tb (°C)
40.0
37.5
35.0
32.5
0
10
20
30
40
50
60
Time (min)
Tw = 10 °C
B
Tb (°C)
40.0
37.5
35.0
32.5
0
10
20
30
40
50
60
Time (min)
Tw = 20 °C
C
Tb (°C)
40.0
37.5
35.0
32.5
0
10
20
30
40
50
60
Time (min)
Tw = 30 °C
D
Tb (°C)
40.0
37.5
35.0
32.5
0
10
20
30
40
50
60
Time (min)

33 °C, resulting in a thermal neutral zone (TNZ) spanning 9 °C (Fig. 2-3B). The mean BMR of 12 star-nosed moles was 2.35 ± 0.05 ml O_2 g^{-1} h^{-1}, or, 47.2 J g^{-1} h^{-1} (n = 33). Assuming an average body mass of 50 g, this value is 2.1 times that predicted from allometry for eutherian mammals (VO_2 = 3.45 $Mass^{-0.287}$; McNab, 1988).

During 20-min immersion trials, VO_2 increased directly with declining water temperature (r^2 = 0.19, P = 0.02; Fig. 2-3B). Average VO_2 in 10 °C water exceeded the mean value for fasted animals resting in air at the same temperature by 1.7 times, or 3.2 ml O_2 g^{-1} h^{-1}. The maximum VO_2 of a star-nosed mole in water was 8.53 ml O_2 g^{-1} h^{-1}, which exceeded the maximum predicted value by 14.6% (Maximum VO_2 (ml O_2 g^{-1} h^{-1}) = 0.499 $W^{0.678}$; Sherer and Wunder, 1979; MacArthur, 1984).

Surface areas and Thermal Conductance estimates

Surface area measurements indicated that the naked extremities accounted for 22.9 ± 0.008 and 17.7 ± 0.004% of total surface area in star-nosed moles and coast moles, respectively, while the corresponding value for water shrews was 18.7 ± 0.01%. Significant differences in total surface area were observed between the three species ($F_{2,31}$ = 111.74, P < 0.0001). Total forelimb area of coast moles was nearly twice that of the similar sized star-nosed mole (P < 0.0001, Table 2-2). The surface area of the robust tail of star-nosed moles (8.18 ± 0.59 cm^2) greatly exceeded that of both coast moles (3.62 ± 0.14 cm^2, P <0.0001) and water shrews (3.82 ± 0.204 cm^2, P <

Table 2-2. Surface areas, specific gravities and pelt gas volumes of 3 insectivores: the star-nosed mole, *Condylura cristata*, the coast mole, *Scapanus orarius* and the water shrew, *Sorex palustris*. Values presented are means ± SE.

Variable	Species		
	C. cristata	*S. orarius*	*S. palustris*
Mass (g)	50.7 ± 2.73 (8)[a]	61.7 ± 4.68 (7)[a]	10.8 ± 0.80 (8)[b]
Surface area (cm^2)			
Trunk	87.4 ± 2.84 (13)[a]	108.18 ± 4.15 (13)[b]	41.23 ± 2.36 (8)[c]
Forelimbs	7.84 ± 0.19 (13)[a]	12.81 ± 0.34 (13)[b]	1.88 ± 0.066 (8)[c]
Hindlimbs	7.45 ± 0.21 (13)[a]	6.57 ± 0.17 (13)[b]	3.58 ± 0.11 (8)[c]
Tail	8.18 ± 0.59 (13)[a]	3.62 ± 0.14 (13)[b]	3.82 ± 0.204 (8)[b]
Nose	2.30 ± 0.05 (13)	———	———
Total Body	113.16 ± 2.45 (13)[a]	131.18 ± 4.49 (13)[b]	50.50 ± 2.31 (8)[c]
Meeh factor	0.081 ± 0.002 (9)[a]	0.088 ± 0.002 (8)[a]	0.105 ± 0.003 (8)[b]
Mass-specific gas vol. in fur (ml STPD g^{-1})	0.19 ± 0.009 (8)[a]	0.177 ± 0.026 (7)[a]	0.35 ± 0.027 (8)[b]
Fur contribution as a % of total gas volume	15.68 ± 0.645 (7)[a]	14.93 ± 1.99 (7)[a]	26.75 ± 1.70 (8)[b]
Specific gravity	0.826 ± 0.008 (8)[a]	0.853 ± 0.016 (7)[a]	0.761 ± 0.013 (8)[b]

Note: Within each row, means sharing the same letter are not statistically different ($P > 0.05$).
Values in parentheses denote sample sizes.

0.0001). Thermal conductance (C) of star-nosed moles in air and water varied over a range of 2.4 - 7.0 and 4.9 - 22.0 W m^{-2} $°C^{-1}$, respectively (Table 2-1). Whole-body C of star-nosed moles correlated strongly with ambient temperature both in air (r^2 = 0.74, P < 0.0001; Fig. 2-6A) and in water (r^2 = 0.62, P < 0.0001; Fig. 2-6B). Mean C in water was, on average, 2.8 - 3.6 X higher than the corresponding values for star-nosed moles tested in air (Table 2-1).

Thermal conductances of carcasses with an intact air boundary varied between species ($F_{2,17}$ = 2107954, P < 0.0001; Table 2-3). Cooling constants derived from the regression of ln (Tb – Ta) vs. time were highly significant (r^2 = 0.85 to 0.999, P < 0.0001). In star-nosed moles, removal of air entrapped in the pelage increased C by 40.5% (P < 0.0001). The corresponding figure for water shrews and coast moles was 38.4% (P < 0.0001) and 0.01% (P < 0.0001), respectively.

Specific gravity

The specific gravities of star-nosed moles, coast moles and water shrews with undisturbed pelage and deflated lungs varied from only 0.76 to 0.85, suggesting that these animals were strongly positively buoyant in water (Table 2-2). Though specific gravity varied among the three species ($F_{2,20}$ = 14.34, P = 0.0001), mean values for the star-nosed mole and the coast mole were similar (P > 0.05, Table 2-2). The volume of air entrapped in the pelage and the percentage of total body volume accounted for by this air boundary

Figure 2-6. The relationship of whole-body thermal conductance (C) to Ambient temperature (Ta) in live star-nosed moles. Data are presented for (A) fed animals tested in 3-30°C air and for (B) fed animals immersed for 20 min in 3 - 30°C water.

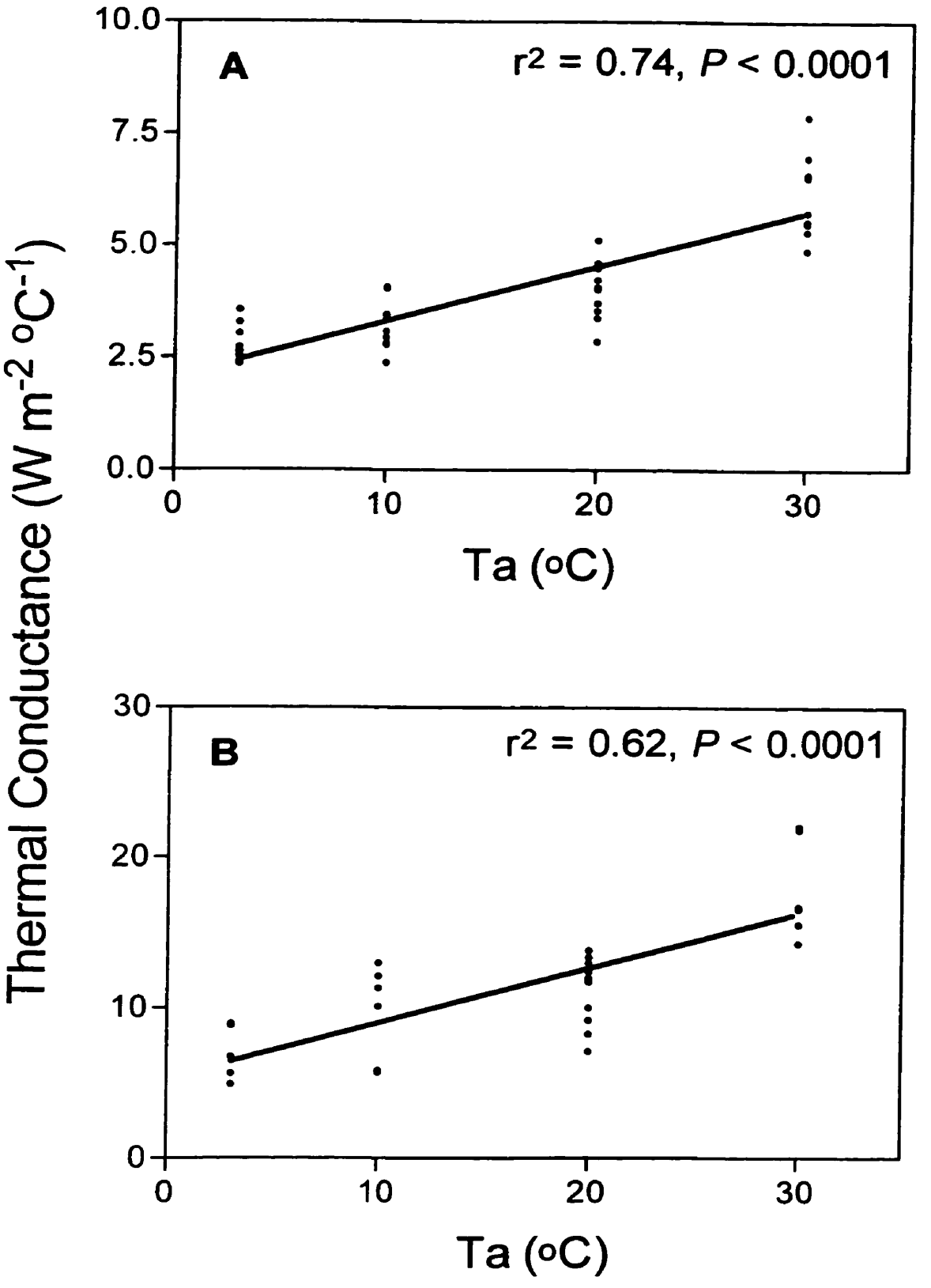

A
r2 = 0.74, P < 0.0001
10.0
7.5
5.0
2.5
0.0
0
10
20
30
Ta (oC)
B
r2 = 0.62, P < 0.0001
30
20
10
0
0
10
20
30
Ta (oC)
Thermal Conductance (W m-2 oC-1)

Table 2-3. Carcass thermal conductances of 3 insectivores, the non-aquatic coast mole, *S. orarius*, the semi-aquatic star-nosed mole, *C. cristata*, and the water shrew, *S. palustris*. Data are presented for animals with and without an insulative air boundary. Values are means ± SE.

Species	Conductance ($W\ m^{-2}\ °C^{-1}$)	
	Air boundary intact**	Wet to skin**
S. orarius	29.90 ± 0.0005 (7)	30.10 ± 0.005 (5)
C. cristata	21.84 ± 0.0003 (9)	36.71 ± 0.004 (9)
S. palustris	21.40 ± 0.018 (4)	34.46 ± 0.05 (6)

* Intraspecific differences significant ($P < 0.0001$).
** Interspecific differences significant in all cases ($P < 0.0001$).
Values in parentheses denote number of animals tested.

are presented for each species in table 2-2. On a mass-specific basis, water shrew pelts held the greatest air volume (0.35 ml g^{-1}; Table 2-2). For a 50-g star-nosed mole, the combined gas volume contributed by the pelt (0.19 ml g^{-1}, Table 2-2) and lungs (0.081 ml g^{-1}; Table 1-3) accounted for 26.1% of total body volume. By comparison, the equivalent analysis for coast moles, assuming a mass of 60 g, suggested that lung (0.049 ml g^{-1}, Table 1-3) and pelt gas volumes (0.18 ml g^{-1}, Table 2-2) accounted for 22.0% of total body volume.

Heat Increment of Feeding

The average metabolic rate of fasted moles in water was not statistically different from that of fed subjects ($P > 0.05$; Table 2-4). The magnitude of the net Tb drop during the 20-min immersion period did not vary between fed and fasted moles ($P > 0.05$). Further, I observed no effect of meal size on post-prandial VO_2 in water for the 7 moles tested ($P > 0.05$).

Discussion

Cold water poses formidable thermoregulatory challenges for endothermic divers. Maintenance of a stable Tb in water dictates accelerated metabolic heat production to compensate for heat that is rapidly absorbed by the surrounding aquatic medium. This demand for metabolic heat is consistent with the growing body of empirical evidence indicating that many

Table 2-4. Average metabolic rate and net body temperature decline of fed and fasted star-nosed moles ($n = 7$) during a 10-min immersion period in 10°C water. Values presented are means ± SE.

Variable	Trial	
	Fed	Fasted
Mass (g)	55.30 ± 2.30	51.52 ± 2.21
VO_2 (ml O_2 g^{-1} h^{-1})	7.98 ± 0.27	8.28 ± 0.30
Net Tb drop (°C)*	2.99 ± 0.28	3.23 ± 0.40

* Pre-immersion Tb – final Tb recorded at end of 10-min immersion trial.

semi-aquatic endotherms are endowed with elevated BMRs. For example, the platypus, *Ornithorynchus anatinus*, sea otter, *Enhydra lutris*, and coypu, *Myocaster coypus*, all exhibit BMRs that greatly exceed those of other closely related terrestrial species (Grant and Dawson, 1978*b*; Costa and Kooyman, 1982; Doncaster *et al.*, 1990). In the present study, the BMR of star-nosed moles (2.35 ml O_2 g^{-1} h^{-1}) was 2.1 X the predicted value for a comparable-sized eutherian mammal. This value is nearly twice that of the coast mole (1.33 ml O_2 g^{-1} h^{-1}, Chapter 1), and substantially exceeds the BMR reported for other talpids, including the broadfooted mole, *Scapanus latimanus* (1.25 ml O_2 g^{-1} h^{-1}, Contreras and McNab, 1979), the eastern mole, *Scalopus aquaticus* (1.41 ml O_2 g^{-1} h^{-1}, McNab, 1979), and the larger Townsend's mole, *Scapanus townsendii* (0.82 ml O_2 g^{-1} h^{-1}, Kenagy et al., 1982). Interestingly, the semi-aquatic water shrew also exhibits an inherently high BMR (4.75 ml O_2 g^{-1} h^{-1}, See Appendix 2) compared to other insectivores of similar mass, including: the short-tailed shrew, *Blarina brevicauda* (3.22 ml O_2 g^{-1} h^{-1}, Dawson and Olsen, 1987), and the shrew-mole, *Neurotrichus gibbsii* (3.85 ml O_2 g^{-1} h^{-1}, Campbell and Hochachka, 2000).

While Tb of star-nosed moles was independent of air temperature, these moles displayed an average Tb (37.7°C) that exceeds values previously reported for other talpids (36.0 – 37.1°C; McNab, 1979; Contreras and McNab, 1989), a finding consistent with the elevated BMR of this species. It is noteworthy that the thermal neutral zone of star-nosed moles (24-33°C) exceeds the thermoneutral range reported for other talpids

examined to date by 1.5°C. However, I was unable to establish the limits of thermoneutrality for moles in water, since activity precluded measurement of resting VO_2 in this medium.

Clearly, thermoregulatory strategies favouring a semi-aquatic lifestyle are at variance with physiological specializations associated with subterranean habitation. The relatively low BMRs and Tbs of non-aquatic talpids, including the coast moles investigated in this study, likely reflects the need to dissipate heat within a confined burrow environment (McNab, 1979; Campbell *et al.*, 1999). Conversely, burrowing species that are endowed with a high BMR are faced with an increased heat load that is difficult to dissipate within the constraints of a burrow microenvironment, thus resulting in an increased risk of hyperthermia.

Despite an elevated basal rate of heat production, moles readily cooled in 8- to 20-min forced immersion trials at water temperatures that are routinely encountered by this species in nature. This propensity for cooling may explain, at least in part, my observation that star-nosed moles exhibited behavioural avoidance of cold water in 20-min voluntary dive trials conducted in the laboratory. Moles showed a decline in dive duration and diving frequency, particularly in 3 and 10°C water (Table 2-1). These findings are consistent with those reported for muskrats, *Ondatra zibethicus* (MacArthur, 1984*a*), in which mean dive duration and diving frequency were reduced in cold (3-10°C) water. This trend among semi-aquatic mammals that alternate between foraging in water and resting or feeding on land appears to result

from an inability to sustain stable Tb during cold-water immersion, favouring instead behavioural strategies that minimize exposure to cold water. To date, the platypus and, surprisingly, the European water shrew, *Neomys fodiens*, are the smallest mammals known to maintain a stable Tb at low water temperatures (Grant and Dawson, 1978*a*; Vogel, 1990). However, like the star-nosed mole, the Australian water-rat, *Hydromys chrysogaster* (Dawson and Fanning, 1981), the muskrat (MacArthur, 1984*a*) and the mink, *Mustela vison* (Williams, 1986) all exhibit pronounced abdominal cooling at water temperatures below 20°C. The sea otter also demonstrates a decline in abdominal Tb when post-absorptive and at rest in 20 °C water (Costa and Kooyman, 1984).

Cooling was dramatic in star-nosed moles, as several animals became severely hypothermic in 3 and 10 °C water. However, limited radio-telemetry measurements for a single star-nosed mole revealed that this animal was capable of defending Tb in 20 °C water, while the telemetered Tb of a single coast mole plummeted over the same period, showing little sign of recovery for 10 min following immersion (Fig. 2-4). Similarly, the same radio-implanted star-nosed mole displayed a continued decline in Tb during immersion in 3 and 10 °C water, and the Tb of this animal continued to fall after the immersion period ended (Fig. 2-5 A and B). The continued decline in Tb following immersion may have resulted from vasodilation of cooled peripheral tissues, resulting in a transient drop in Tb as cooled venous blood returned to the core (MacArthur, 1984*a*).

Whole-body thermal conductance is an additional index of thermoregulatory ability in cold water, reflecting the combined thermal resistances of body tissues and pelage insulation (MacArthur, 1989). Conductance estimates may be derived from body temperature and metabolic measurements of resting or floating animals. Cooling constants obtained from carcass cooling curves (Morrison and Tietz, 1957) also provide measures of the combined thermal resistances of body tissues and pelage insulation to heat loss. Despite my observation that Tb declined at all water temperatures < 30°C, the C of live star-nosed moles averaged only 2.8-3.6 X higher in water than in air. By comparison, the C of immersed, resting water rats in 20 °C water was nearly 5 X the value recorded for these same animals resting in air (Dawson and Fanning, 1981). As in muskrats (C = 8.32-10.87 W m^{-2} $°C^{-1}$; MacArthur, 1984*a*), little variation in C was observed in water rats resting on a submerged platform in 15-30 °C water (10.6-8.2 W m^{-2} $°C^{-1}$; Dawson and Fanning, 1981). Not surprisingly, the thermoregulatory ability of star-nosed moles in water appears modest compared with that of much larger muskrats or Australian water rats. For both rodent species, thermal conductance in 20°C water was 16 and 25.4% less than was the case for star-nosed moles resting at the same water temperature. Similarly, C estimates of platypus resting quietly in 5-20°C water (Grant and Dawson, 1978*b*) were approximately 64-58% lower than my values for the star-nosed mole. While much of the interspecific variation in C may be attributed to the fact that the mass of star-nosed moles is only 3.1 – 7.8% that of the platypus (0.8 – 1.6

kg), muskrat (0.64 – 1.16 kg) and water rat (0.68 – 1.12 kg), the observed differences may also arise from variation in pelage characteristics. The superior quality of platypus fur may account for its comparatively low C in water. With the exception of the sea otter, the platypus has the highest hair density yet reported for a mammal (600-900 hairs / mm^2; Sokolov, 1962; Grant and Dawson, 1978*b*). While comparable data for the star-nosed mole are lacking, Ivanter (1994) found that among Insectivores, the European mole, *Talpa europa*, has the highest hair density (130 hairs / mm^2), which also marginally exceeds values reported for the muskrat (110-120 / hairs mm^2; Sokolov, 1962). These comparisons may account, at least partially, for the dramatic difference in C between the star-nosed mole and platypus, relative to differences reported between the muskrat and the star-nosed mole.

The value of insulative pelage in water is dependent on the integrity of the attendant air layer adjacent to the skin (Williams, 1986). The results of this study suggest that the insulative air boundary entrapped within the pelage of the star-nosed mole reduces the minimal thermal conductance in water 40.5% beyond that of mole fur without air (Table 2-3). The same comparison for water shrews yielded a 38.4% difference in thermal conductance between shrews having an intact pelt and those with fur that was wet to the skin (Table 2-3). These findings are consistent with previous observations of water shrews (Calder, 1969) demonstrating that carcass C is increased by a factor of 2 when the air boundary is eliminated. In another, parallel study, Harlow (1983) compared Tb changes of live muskrats exposed to immersion

following either removal of the hardarian gland, an organ that produces lipid used to waterproof the fur, or shampooing. Shampooed muskrats showed a significant increase in cooling compared to control subjects in which the pelage air boundary was intact. Hardarian-ectomized individuals exhibited intermediate cooling responses, suggesting that lipids on the fur may be vital in retaining the air layer that muskrats depend upon for insulation (Harlow, 1983). It is conceivable that lipids may also aid in retention of the air boundary in star-nosed moles. Eadie (1954) reported an increase in glandular activity in the dorsal head, chin and wrist regions of star-nosed moles that is apparently timed to coincide with breeding periods.

Surprisingly, the thermal conductance of carcasses exceeded values obtained for live animals (Table 2-3). Williams (1986) previously observed this phenomenon in mink and attributed the increase in C in carcasses to the lack of circulation in dead animals. Presumably, corpses possess uniform temperature from the core to the periphery, while in live animals, vasoconstriction of the extremities results in cooling of peripheral tissues relative to that of the core. Consequently, this regional heterothermy may reduce the gradient for heat loss and therefore result in reduced total C.

While providing an obvious thermoregulatory benefit, the air boundary in the pelage rendered star-nosed moles and especially water shrews positively buoyant. The specific gravity of water shrews was lower than that of any other insectivores tested. This finding was consistent with the greater % gas volume and higher mass-specific gas volume trapped in the fur of this

species (Table 2-2). The specific gravities of all 3 insectivores were lower than the mean value reported for muskrats (0.952, MacArthur, 1992). Though not tested in this study, the buoyant force provided by the air boundary is likely to increase the energetic cost of diving in both water shrews and star-nosed moles.

Since the pelage air boundary provides such an obvious reduction in C, the most important aspect of surface area for any aquatic mammal should be the proportion of the body that is not covered by hair. The naked appendages of star-nosed moles, coast moles and water shrews accounted for 22.9%, 17.7% and 18.7%, respectively, of the total body surface area. These values are comparable to those reported for beaver, *Castor canadensis* (16.1-20.7%; MacArthur and Dyck, 1990), but are less than those calculated for platypus (30.9 – 33.6%; Grant and Dawson, 1978*b*) and muskrat (21- 26.9%; MacArthur, 1984*a*). As well as being good swimmers and divers, star-nosed moles are adept burrowers (Catania and Kaas, 1996). It might therefore be argued that star-nosed moles and, in particular, coast moles should exhibit a less fusiform shape when compared to species more specialized for swimming and diving. However, the meeh factors of these moles were similar to those reported for beaver (0.073; Reynolds, 1993; 0.091; MacArthur and Dyck, 1990) and muskrat (0.086; MacArthur, 1984*a*). Surprisingly, the meeh factor of water shrews exceeded that of both star-nosed moles and coast moles, despite the fact that the surface area devoted to the appendages of shrews was intermediate with respect to the two mole

species. The finding that the tail surface area of star-nosed moles exceeded that of both coast moles and water shrews, may be attributed to my observation that larger tails in star-nosed moles contained substantial deposits of subcutaneous fat. Hamilton (1931) suggested that the enlarged tail of star-nosed moles may serve as a fat reserve that may be exploited when these animals are nutritionally compromised.

Finally, the highly specialized nose of the star-nosed mole is likely to provide a major avenue for heat loss both in air and water, owing to its large exposed surface area. In this study, moles clearly used their tentacles to probe the surface of the walls and bottom of the diving tank, even in 3 °C water. The highly innervated nose receives a rich blood supply and is the primary sensory organ of this species (Catania, 1996). In this context, a substantial decrease in skin temperature would be unfavourable with respect to tactile sensitivity (Dehnhardt *et al.*, 1998). A previous study conducted on the highly innervated facial vibrissae of pinnipeds showed that diving harbour seals, *Phoca vitulina*, maintained vibrissae temperature (18.1 °C) well above water temperature (1 °C). It is therefore tempting to speculate that the star-nosed mole may tolerate high thermoregulatory costs imposed by the potentially high C of the well-circulated nose, in order to maintain tactile functions at all water temperatures. If so, this attribute might also contribute to the high whole-body C of this talpid, relative to other semi-aquatic forms (see above).

The benefit of the heat increment of feeding should, in theory, be maximized in species that consume protein-rich diets and are subject to chronic cold exposure (Costa and Kooyman, 1984; MacArthur and Campbell, 1994; Janes and Chappell, 1995; Hawkins *et al.*, 1997). I found no evidence however that the biochemical HIF, which reaches a maximum at 120 min post-feeding in star-nosed moles (Campbell *et al.*, 2000), substituted for active thermogenesis during immersion trials in water. The concept of substitution is based on the premise that exploitation of heat generated incidental to feeding will reduce the need for active thermogenesis in water. Consequently, the proposed benefit of HIF assumes that nutrient assimilation continues unabated during cold-water immersion (MacArthur and Campbell, 1994). In this context, vasoconstriction of the gut to enhance heat conservation and O_2 economy during diving may override the need for adequate splanchnic blood flow necessary for digestion. Furthermore, the functional anatomy of the forelimbs and the diving behaviour of star-nosed moles dictate that prey consumption occurs solely on land. Campbell et al. (2000) observed a transient mechanical HIF (0.5 – 1 h) in star-nosed moles that coincided with passage of digesta through the alimentary canal and was accompanied by a 0.8 °C elevation in Tb that persisted for 90 min. In winter, the cold temperature of ingested food may impose additional costs associated with consuming a meal, including a transient decline in abdominal Tb (Wilson and Culik, 1991; Handrich *et al.*, 1997).

Summary

In summary, the results of this study demonstrate that star-nosed moles are capable endotherms in air, but exhibit limited tolerance to cold-water immersion. The star-nosed mole and water shrew appear to be endowed with intrinsically elevated BMRs, a trait possibly linked to the high metabolic demands of aquatic activity.

The highly specialized nose of *C. cristata* is likely a critical avenue for heat loss, which may partially explain the high C of this species (Table 2-1). Whole-body conductance of star-nosed moles in water exceeded values previously reported for semi-aquatic species. However, the relatively large surface area of the appendages, including the nose, did not predispose moles to excessive cooling in water (Table 2-2). In fact, meeh factors for moles were similar to previous estimates derived for semi-aquatic mammals. The observation that the meeh factor for water shrews exceeded that of both mole species was potentially compensated for by the greater mass-specific air volume entrapped in the pelage of water shrews. The possibility that HIF substitutes for active thermogenesis in water could not be substantiated in star-nosed moles.

General Conclusions

Diving Physiology of the Star-nosed mole

The results of this study suggest that star-nosed moles, *Condylura cristata*, are exceptional divers, despite physiological constraints that should theoretically impose limits on dive duration. Of particular importance is the potential conflict between underwater thermoregulatory requirements and the O_2-conserving tactics that accompany diving. With few exceptions, vertebrate divers sustain an O_2-based metabolism while diving to support the increased O_2 demand of exercising muscles (Butler and Jones, 1997).

The underwater rate of O_2 depletion is a critical parameter when examining questions of diving endurance. Despite the substantial buoyancy derived from air entrapped within the fur, the cost of aquatic activity for star-nosed moles, relative to resting rates of O_2 consumption in air, was lower than that reported for other semi-aquatic mammals that are specialized for swimming and diving. (Stephenson *et al.*, 1988; MacArthur and Krause, 1989). The large O_2 stores were contributed mainly by the dramatic lung reservoir and elevated muscle Mb concentration of this species. Together, the substantial body O_2 reserves and relatively low cost of aquatic activity in star-nosed moles were manifested in dive durations that greatly exceeded allometric predictions. Aerobic pathways appeared to predominate during diving, based on the low buffering capacity and glycogen content of skeletal muscles, as well as behavioural observations suggesting that the percentage

of voluntary dives that exceeded the ADL in this species was low (2.9 % of total).

Thermal Biology of the Star-nosed mole

The remarkable diving endurance of star-nosed moles was not matched by a commensurate ability to maintain a stable Tb in water. Moles demonstrated dramatic core cooling in water cooler than 20 °C, and exhibited limited ability to defend Tb during immersion, except at the highest water temperatures (20 and 30 °C). Moreover, thermal conductance estimates of *Condylura* exceeded values previously reported for semi-aquatic mammals (Dawson and Fanning, 1981; MacArthur, 1984*a*; Williams, 1986). I suggest that the highly perfused nose substantially contributes to the observed increase in thermal conductance of the star-nosed mole. Star-nosed moles appeared to tolerate passive cooling in water, a finding that is consistent with results obtained for muskrats, *Ondatra zibethicus* (MacArthur, 1979; 1984*a*). I suggest that avoidance of prolonged immersion, as demonstrated by a reduction in both dive duration and dive frequency in cold water (Table 2-1), together with a tolerance to passive cooling and the parsimonious use of O_2 stores may augment foraging efficiency of star-nosed moles by minimizing surface recovery costs.

Additional mechanisms for conserving body heat may retard, but do not entirely eliminate core body cooling in water. The combined gas volume contributed by the pelt and lungs of a 50-g star-nosed mole accounted for 26.1% of total body volume, and rendered these animals positively buoyant.

By comparison, the sea otter, *Enhydra lutris*, also possesses a high mass-specific lung volume (Lenfant *et al.*, 1970). It has been suggested that modulation of this gas volume has substantial effects on buoyancy of sea otters resting at the surface and, therefore, their tendency to lose body heat to water (Costa and Kooyman, 1982). It appears reasonable then, that the large lung volume of star-nosed moles, while primarily acting as an O_2 reservoir, may, by enhancing floatation, also minimize trunk surface area exposed to water, and thus contribute to heat conservation.

The elevated BMR and Tb of star-nosed moles is also of interest, as this surfeit of metabolic heat may attenuate the effects of immersion hypothermia. As MacArthur (1979) observed in muskrats, initiating aquatic activity at an elevated Tb may endow semi-aquatic mammals with the ability to withstand a greater degree of cooling in water. The hypothesized exploitation of the heat increment of feeding during aquatic activity was not substantiated in star-nosed moles. However, previous reports (Campbell *et al.*, 2000) of a transient elevation in Tb arising from the mechanical processing of ingested food is suggested to potentially augment Tb recovery in moles that haul-up on land to feed following bouts of aquatic foraging.

Interestingly, preservation of the insulative air boundary entrapped in the pelage of star-nosed moles is achieved by vigorous and systematic grooming, which is potentially expensive for this species. The resulting intact air boundary, however, reduces cooling significantly.

Evolution of Hypoxia Tolerance in the Talpidae

One of the objectives of this study was to compare respiratory properties of the blood, muscle and lungs of two phylogenetically and geographically isolated talpids. The coast mole, *Scapanus orarius* and star-nosed mole provide useful models for studies of comparative hypoxia tolerance, since both species are chronically exposed to low ambient O_2 concentrations in burrow microenvironments and, in the case of the star-nosed mole, asphyxia associated with diving. Numerous recent theories have been advanced to account for the origin and diversity of the Talpidae (Grand *et al.*, 1998; Campbell *et al.*, 1999; Whidden, 2000). It is widely believed that talpids arose from a primitive ancestor of the extant desman, *Galemys pyrenaicus* (Richard, 1985). The desman is a non-burrowing semi-aquatic mammal possessing water-proof pelage, excellent diving abilities, and similar sensory adaptations to the star-nosed mole, including the highly specialized Eimer's organs of the snout. It is tempting, therefore, to speculate that the elevation of body O_2 stores occurred first in the Desmaninae, and was subsequently retained in the star-nosed mole and coast mole, but lost in the least fossorial talpid, the shrew-mole. In fact, my finding that the myoglobin concentration and Hematocrit of the hypoxia tolerant star-nosed mole and coast mole exceeded that of the shrew mole appears to support the latter hypothesis. However, anatomical evidence clearly suggests that *Condylura* passed through an ancestral fossorial phase and secondarily acquired a semi-aquatic lifestyle (Grand *et al.*, 1998). It is therefore possible that the

thermal environment of the star-nosed mole selected for an expanded lung volume and elevated BMR and Tb in order to accommodate the newly acquired costs of aquatic thermoregulation in this species. In contrast, a low BMR and high conductance were retained in the coast mole, providing a metabolism compatible with the thermoregulatory limitations posed by a confined burrow environment. Furthermore, the elevation of myoglobin, a hallmark of diving mammals, may have occurred subsequent to a fossorial evolutionary stage in the star-nosed mole. Finally, the possibility that the elevation in body O_2 stores occurred independently in both mole species cannot be ruled out, suggesting that the underlying evolutionary mechanism still awaits elucidation.

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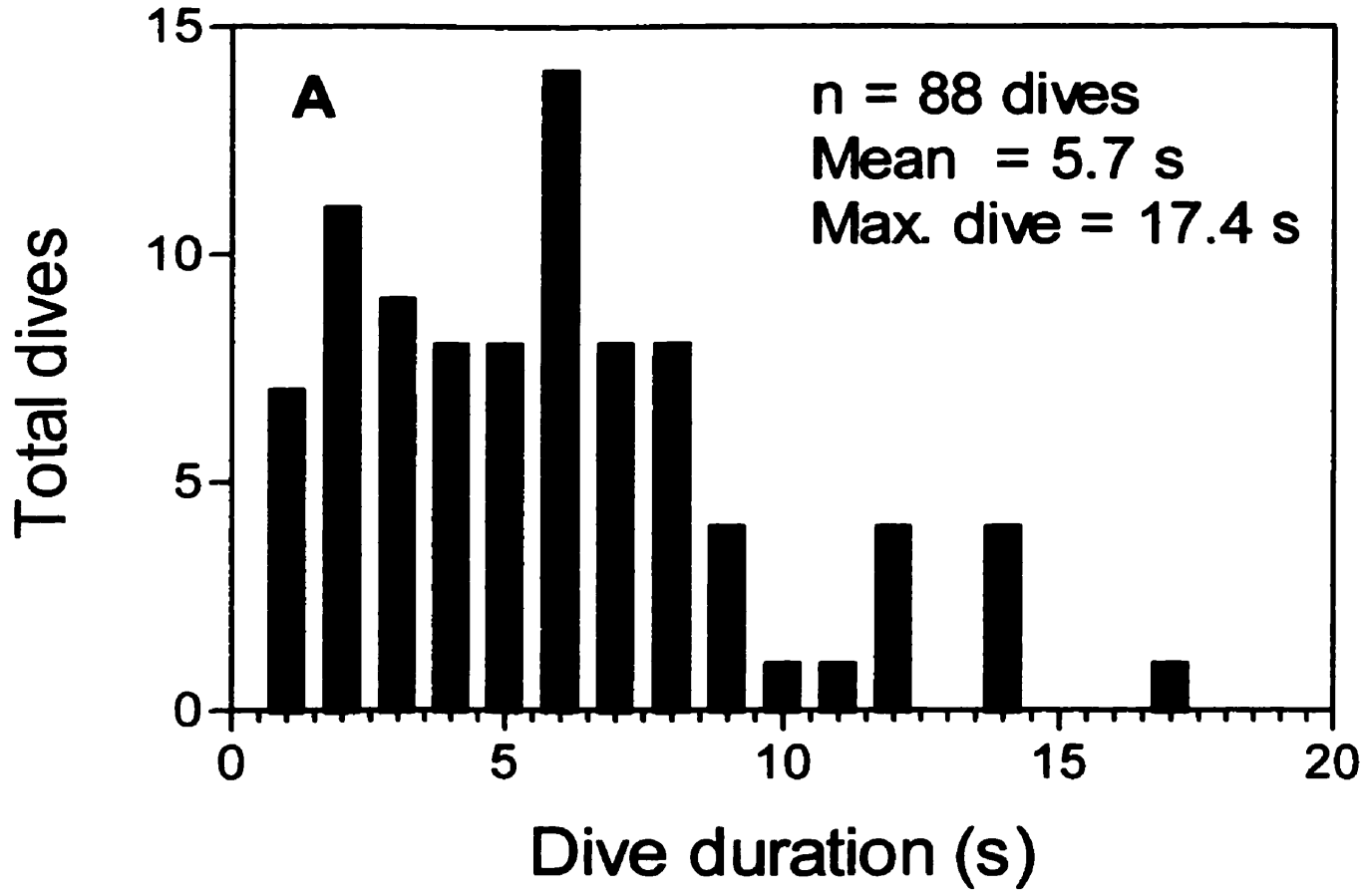
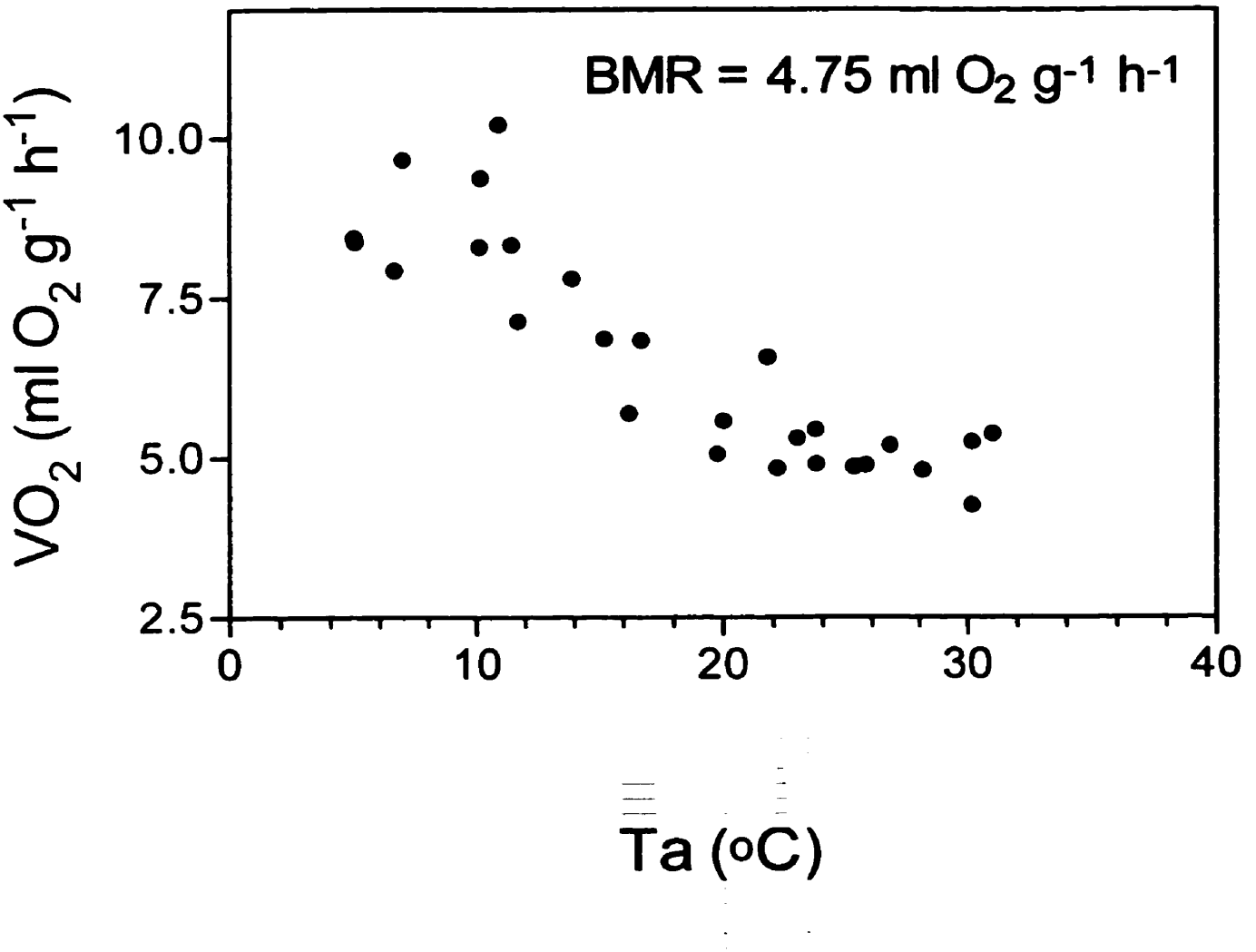
Appendix 1. Frequency distribution of voluntary dive times for a single North-American water shrew (*Sorex palustris*).



Appendix 2. The relationship of resting rate of O_2 consumption (VO_2) to air temperature (Ta) in 2 water shrews.



Appendix 3: Organ masses and % muscle mass in star-nosed moles

Mole	Mass (g)									
	Heart	Kidneys	Liver	Spleen	Brain	Pelt /skin	Gut	Digesta free gut	skeleton	% muscle mass
M1-97	0.327	0.429	2.0336	0.0385			7.10			
M3-97	0.254	0.370	1.9169	0.9420			3.06			
M7-97	0.256	0.401	2.0309	0.0944			6.19			
M2-98	0.244	0.416	2.0070	0.1051			7.84			
M3-98	0.285	0.469	2.3898	0.2476	0.303	11.785	8.12	2.394	3.4128	43.58
M6-98	0.267	0.505	2.6035	0.2079			6.72			
M14-98	0.289	0.391	1.9505	0.1296			7.44			
M5-99	0.260	0.429	2.0522	0.1730			4.46			
M7-99	0.242	0.369	2.1094	0.2640			4.70			
M8-99	0.231	0.377	1.7638	0.1412			4.66			
M1-99	0.312	0.435	2.1208	0.1604	0.551	8.806	8.82		4.0226	
M2-99	0.299	0.336	1.3502	0.1123	0.671	6.278	5.38	2.115	4.4865	42.78
M6-99	0.465	0.485	2.2301	0.0750	0.639	13.231	9.72	1.952	4.3728	34.77
M6-99a	0.245	0.402	0.9172		0.616	7.768	5.28	1.678	3.4602	42.67
M9-99	0.323	0.455	1.8505	0.2313	0.701	7.833	5.03	1.510	4.4210	42.19
M11-99	0.242	0.304	1.2341		0.498	6.646	5.04	1.580	3.4332	41.29
M13-99	0.382	0.508	2.6203		0.742	10.033	10.3	1.998	3.2379	40.69
UM1-99	0.349	0.353	1.4523	0.2006	0.602	6.824	6.79	2.301	2.9199	50,14
UM2-99	0.347	0.453	1.6904		0.577	9.902	6.16	2.065	3.3085	39.83
UM3-99	0.230	0.359	1.1483		0.664	7.663	7.95	2.884	3.1506	41.23
UM-99	0.345	0.686	2.8826	0.2216	0.351	11.046	8.55	3.624	2.454	40.04

Appendix 4: Organ masses and % muscle mass in coast moles

Mole	Mass (g)									
	Heart	Kidneys	Liver	Spleen	Brain	Pelt /skin	Gut	Digesta free gut	skeleton	% muscle mass
CM1-99	0.374	0.757	3.3160	0.1945			6.39			
CM3-99	0.363	0.687	2.2900	0.1117			8.39			
CM4-99	0.360	0.722	3.5779	0.2105			5.72			
CM5-99	0.371	0.653	4.2510	0.1404			9.29			
CM6-99	0.346	0.676	3.5451	0.1096			4.91			
CM7-99	0.353	0.641	3.2716	0.0979			5.50			
CM8-99	0.218	0.402	1.3977	0.1104			5.31			
CM9-99	0.377	0.854	3.8056	0.1775			11.4			
CM10-99	0.398	0.893	2.9534	0.2182			7.49			
CM3-00	0.359	0.625	2.5753	0.1686			11.2			
CM4-00	0.648	0.780	3.9493	0.1054			7.73			
CM6-00	0.617	0.635	2.5247		0.932	19.58	6.51	2.019	5.824	38.77
CM8-00	0.443	0.460	1.8520	0.6038	0.913	8.92	5.63	1.907	4.335	44.47
CM9-00	0.653	0.585	2.6395		0.943	14.75	6.26	2.015	5.069	42.91
CM10-00	0.436	0.597	2.7494		0.412	14.04	5.47	2.110	5.322	45.79
CM11-00	0.450	0.676	2.1866	0.1225	0.737	11.40	7.40	2.879	5.782	36.18
CM12-00	0.466	1.324	2.3399	0.3063	0.742	13.34	6.07	2.163	5.531	41.39
CM13-00	1.027	0.673	3.9075		0.979	12.27	8.69	1.854	6.344	43.61
CM14-00	0.627	0.522	1.2771		0.892	5.53	7.66	1.744	5.563	49.22
CM15-00	0.876	0.343	2.4039		0.934	9.85	3.17		5.481	43.25
CM16-00	0.468	0.449	2.0182		0.925	8.78	5.00	1.698	4.590	50.00
CM17-00	0.639	0.569	1.9545		0.838	10.09	5.66	1.521	5.047	42.35

Appendix 5: Identity of star-nosed moles used in each experiment.
Last 2 digits of each code number denote sampling year.

Experiment								
Body O_2 stores	Voluntary dive behaviour	Diving VO_2	Live Conductance in air	Live Conductance in water	Metabolism temperature curve	HIF	Carcass Conductance	Dive behaviour vs. Water temperature
M1/97	M9/99	M1/97	M13/99	M13/99	M7/98	M5/99	Ren1	M4/98
M3/97	M8/99	M4/97	M12/99	M3/99	M7/97	M4/99	Ren2	M4/97
M7/97	M4/99	M5/97	M7/99	M4/99	M8/98	M7/99	Ja21	M5/97
M2/98	M5/99	M4/99	M3/99	M5/99	M4/98	M8/99	M7/98	M3/97
M3/98	M7/99	M5/99	M9/99	M6/99	M3/97	M9/99	M8/98	M8/98
M6/98	M13/99	M7/99	M8/98	M7/99	M4/97	M12/99	JD	M7/97
M14/98	M6/99	M8/99	M7/98	M8/99	M5/97	M6/99	M4/98	M7/98
M5/99	M3/99	M9/99	M6/99	M9/99	M6/97		M6/97	
M7/99	M11/99	M10/99	M4/98	M7/98	M1/97		M13/98	
M8/99	M10/99	M12/99	M5/98	M8/98	M2/97			
	M1/98	M6/99		M10/98	M3/97			
	M2/98			M12/99	M5/98			
	M4/98			M11/99				
	M7/98			M4/98				
	M8/98							
	M1/97							
	M3/97							
	M4/97							
	M5/97							
	M7/97							