Dive performance and aquatic thermoregulation of the world's smallest mammalian diver, the American water shrew (*Sorex palustris*)

by

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A Thesis Submitted to the Faculty of Graduate Studies
In partial Fulfillment of the Requirements
For the Degree of

MASTER of SCIENCE

Department of Biological Sciences
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Roman W. Gusztak

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirement of the degree

Of

MASTER OF SCIENCE

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Abstract

Weighing 12-17 g, the American water shrew, *Sorex palustris*, is the world's smallest mammalian diver. Allometry predicts the water shrew to have the smallest total body oxygen storage capacity (TBOSC), highest diving metabolic rate (DMR), lowest skeletal muscle buffering capacity and lowest concentration of glycolytic enzymes of any mammalian diver (Emmett and Hochachka 1981, Schreer and Kovacs 1997). Despite these constraints, *S. palustris* has a calculated aerobic dive limit (cADL; 14.2 s) similar to that predicted by allometry for larger-bodied divers. Shrews closely adhered to predicted aerobic dive limits while submerged, with only 3.1 % (10 °C water) and 2.3 % (30 °C water) of dives exceeding their cADL. However, skeletal muscle buffering capacity was similar to that of other larger semi-aquatic mammals (38.22±2.28 β), suggesting a heightened ability to adopt anaerobic pathways when oxygen stores are depleted. The cADL of adult water shrews diving in 30 °C water (14.2 s) was higher than in 10 °C water (10.7 s), due to an increased DMR at the lower water temperature (6.77 ml O_2 g^{-} h^{-1} verses 9.02 ml O_2 g^{-} h^{-1}, respectively). Adult *S. palustris* exhibited an elevated TBOSC (ca. 1.2x greater) compared to the similar-sized and strictly terrestrial short-tailed shrew, *Blarina brevicauda*. Oxygen bound to hemoglobin in adult water shrews (26.93±0.58 vol %) is the highest value recorded for any semi-aquatic mammal and is the largest contributor to TBOSC in this species (61%). The mean voluntary dive time of water shrews was 5.09 ± 0.08 s (N = 25, n = 1584), with a mean maximum dive time per trial of 10.32 ± 0.38 s. A maximum voluntary dive time of 23.65 s was recorded. Diving trials completed in 3, 10, 20 and 30 °C water showed that water temperature (T_w) significantly affected dive duration ($F_{3,68}$ = 5.033, P = 0.003), duration of longest dive ($F_{3,68}$ = 6.173, P

= 0.001), and total time in water ($F_{3,68} = 7.892$, $P < 0.001$), but not dive frequency ($F_{3,68} =$ 0.467, $P = 0.706$). Further, adult water shrews tested with ($N = 6$) and without ($N = 6$) an implanted abdominal body temperature (T_b) transmitter (6.7-9.7 % of body mass) showed no significant differences in any measured criteria of dive performance. This was attributed to the large buoyant force water shrews are required to overcome when diving, which is presumably much greater than the additional mass of the transmitter. Adult water shrews experienced a 1.33-fold increase in DMR when diving in 10 °C compared to 30 °C water, while maintaining a normothermic T_b (38.0 ± 0.3 °C, 39.2 ± 0.2 °C, respectively), suggesting diving shrews compensate for heat lost to cold water by increasing their heat production. In all voluntary dive trials, shrews regularly used behavioral thermoregulation to defend against immersion hypothermia, with only 5 % of dives occurring at T_bs < 37.3 °C.

Acknowledgements

I found my MSc to be the most challenging, and yet, most fulfilling task that I have completed. I am grateful for this opportunity and even more thankful for the people that I have had the pleasure to work with, learn from and become friends with. I am indebted to Allyson Hindle, Dean Jeske and Rob Senkiw for the many hours they assisted in trapping, feeding and tissue processing. Similarly, Chris Schneider assisted in these areas as my summer student, and without hesitation, also repaired leaky aquaria, ground up shrew food (a mixture of beef and chicken hearts, pork and chicken livers and a can of dog food), and made sure I always stayed awake while driving home after a sleepless night of trapping. I thank Shane Farrow and my brother, Stefan Gusztak, for also helping in the repetitive task of tissue processing. The friendly and helpful staff in Animal Holding were a pleasure to work with, and also contributed many mealworms and crayfish to the shrew's diet. I also appreciate all the advice and moral support from Ian McIntyre who was a great encouragement throughout the completion of this thesis.

There are many staff in the Zoology Department that made this experience an enjoyable one, and I will mention only a few. I thank Dr. Hare for always having an open door and a few minutes to talk about stats, ground squirrels or anything else that came to mind. I am grateful to Dr. Hahn, Tara Narayansingh, and Dr. Sealy for their continual interest in students and encouraging them to reach their full potential. Further, I appreciate the work that Madeline and Urmilla completed on my behalf, because being registered in two faculties simultaneously creates a lot of paperwork.

I would also like to recognize my committee members who assisted tremendously in this thesis. I thank Dr. Porter for her many insightful comments and participation on

my committee. It has been a great privilege to be Dr. MacArthur's last graduate student, and I am thankful for the opportunity to work along side this ingenious, kind-hearted and humble man. I was told, and now have experienced, that Dr. MacArthur always wants the best for his students and is willing to go the 'extra mile' to accommodate an individual's needs. I also appreciate the patience and understanding exhibited by both Dr. MacArthur and Dr. Campbell by allowing for other studies to interrupt the completion of this project. Having now worked with Dr. Campbell for over seven years, I do not know where to begin to show my gratitude. I think the highest compliment is that, seven years ago, I did not consider research as a part of my future career, but now am keenly interested in pursuing a clinical research role in medicine. Through Dr. Campbell's mentoring, I have come realize the enjoyment of problem solving (or pulling a MacGyver, as it was known in Dr. MacArthur's lab) and that when combined with a strong work ethic, leads to great scientific and personal accomplishments. I am eternally thankful for what I have learned under his mentoring.

I also thank my family for all their love and support throughout the completion of this thesis, especially my sister, Christina, who has helped me more than she will ever know. Lastly, I thank my parents for always believing in me and giving me every opportunity to excel.

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Abbreviations used in text

ADL	Aerobic dive limit
ANOVA	Analysis of variance
bADL	Behavioural aerobic dive limit
BTDL	Behavioural temperature diving limit
β	Buffering capacity measured in slykes
BMR	Basal metabolic rate
cADL	Calculated aerobic dive limit
DLT	Diving lactate threshold
DMR	Diving metabolic rate
diam.	Diameter
g	Gram
h	Hour
Hb	Hemoglobin
Hct	Hematocrit
Mb	Myoglobin
min	Minute
n	Number in sample size
N	Number of animals
O_2	Oxygen
MR	Metabolic rate
RMR	Resting metabolic rate
s	Second
STPD	Standard temperature pressure dry
TBOSC	Total body oxygen storage capacity
T_a	Ambient temperature
T_b	Body temperature
T_w	Water temperature
vol.	Volume
$\dot{V}O_2$	Rate of oxygen consumption

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Introduction

First coined by Kooyman *et al.* (1980), the term aerobic dive limit (ADL) is defined as the longest dive duration for which the post-dive blood plasma lactate concentration does not rise above pre-dive values. This variable provides an important estimate of the species' aerobic diving capability. Indeed, if a diver exceeds its ADL, then the animal's metabolism must shift from aerobic to anaerobic pathways and the cost of diving increases due to the production of lactate. However, owing to the inherent difficulties of deriving plasma samples, most studies calculate the ADL (cADL) by dividing total body oxygen stores (TBOSC) by its diving metabolic rate (DMR). In these cases, cADL is defined as the time at which time a diver has used up all available oxygen and is now relying completely on anaerobic metabolism. By comparison, the diving lactate threshold (DLT), which occurs sooner than the cADL, is defined as the time into the dive when there is a significant rise in blood-lactate levels above pre-dive levels (Butler 2006, Davies *et al.* 2007).

To date, the majority of empirical studies dealing with diving energetics and aquatic thermoregulation have been conducted on large-bodied marine mammals or birds (Lavigne *et al.* 1986, Butler and Jones 1997, Butler 2004), with relatively few comparative studies on smaller amphibious species (Dawson and Fanning 1981, MacArthur 1984a, Kruuk *et al.* 1997, McIntyre 2000). Given the significant theoretical challenges facing any small-bodied diver, there clearly exists a need for further studies examining the diving abilities and mechanisms of counteracting heat loss of these animals.

A suite of physiological changes classically referred to as the 'dive reflex', but more recently designated the 'dive response', has been described for diving mammals and birds while submerged (Scholander 1940, Davis *et al.* 2004). The dive response includes peripheral vasoconstriction with hypoperfusion, apnea, bradycardia, reduced O_2 consumption by working muscles and, for dives exceeding the diving lactate threshold (DLT), a post-dive lactate pulse in the blood plasma (Evans *et al.* 1994, Ponganis and Kooyman 2000, Butler 2006). The literature is replete with studies calculating the "theoretical" cADLs of large-bodied aquatic endotherms, and matching these estimates against the natural dive behaviour of these species, in order to determine the extent to which divers stay within these limits (Butler 2006). Examined on a cost:benefit basis, divers are most efficient when minimizing the surface time required to recoup onboard O_2 stores while maximizing their foraging time underwater (Kramer 1988). Consequently, if a diver exceeds its aerobic dive capacity, post-dive lactate levels will typically rise, leading to increased obligate time spent at the surface and, ultimately, decreased foraging efficiency. When lactate is produced, an additional cost is incurred since the diver must spend extra (wasted) time at the surface metabolizing the lactate, either during one long surfacing period or over many inter-dive periods following short aerobic dives. In theory, divers should adopt aerobic diving strategies that optimize the foraging time: surface time ratio. A diver can maximize its aerobic foraging time by increasing TBOSC and/or decreasing DMR. Constraints limiting the TBOSC of a diver are based primarily on physiological parameters such as lung volume and myoglobin (Mb) and hemoglobin (Hb) concentrations, which tend to be relatively stable. These variables do however, vary with age and for some species, season (MacArthur 2001).

Recently, Butler and Jones (1997) formulated the adaptive hypothermia hypothesis to explain why some species routinely exceed their cADL. By intentionally decreasing core T_b, divers can theoretically decrease DMR, due to the Q_{10} effect, and thereby extend dive duration while maintaining an aerobic metabolism (Geiser 1988). However, the benefit of increased aerobic dive endurance comes with the mandatory energetic cost of re-warming cooled tissues after immersion. Studies examining the "adaptive hypothermia" argument have yielded mixed results (Hall *et al.* 1987, Ponganis *et al.* 2003, Hindle *et al.* 2006). The water shrew offers an ideal model for testing this hypothesis. This is because it has the largest surface-area-to-volume ratio, smallest on-board O_2 stores and the highest mass-specific diving metabolic rate of any diver, all factors that predict rapid cooling and a limited scope for dive endurance.

The ideal method of determining an animal's aerobic dive capacity is to monitor post-dive plasma lactate concentrations. When sampling plasma lactate, Butler and Jones (1997) suggested using the term DLT to define the time into the dive when a diver's plasma lactate concentration significantly exceeds pre-dive baseline values. By differentiating between DLT and cADL (see below), a clear distinction is made of the method used in determining a diver's aerobic limit. Unfortunately, this method, which requires serial blood sampling, is technically difficult to implement because it requires central venous access. Consequently, post-dive blood lactate levels have only been measured for few species during voluntarily diving: adult Weddell seals (*Leptonychoties weddellii*; Kooyman *et al.* 1980), Weddell seal pups (Burns and Castellini 1996), Emperor penguin (*Aptenodytes forsteri*; Ponganis *et al.* 1997a), Baikal seals (*Phoca sibirica*; Ponganis *et al.* 1997b), white whale (*Delphinapterus leucas*; Shaffter *et al.*

1997) and bottlenose dolphins (*Tursiops truncates*; Williams *et al.* 1999). Based on these and other studies, it is generally accepted that divers perform ca. 90-95% of voluntary dives within their ADL.

Due to the difficulty in obtaining post-dive blood samples from small-bodied divers, only cADL estimates have been reported for these species. This entails calculating the diver's TBOSC then dividing this value by its DMR (Ponganis and Kooyman 2000, MacArthur 2001). One of the shortcomings of this technique is that of obtaining accurate estimates of DMR. The DMR can be influenced by many factors, including water temperature (T_w), level of activity underwater, dive depth and body temperature (T_b; Green *et al.* 2007). It is also difficult to obtain reliable estimates of DMR because traditional methods for measuring the $\dot{V}O_2$ of an exercising animal under steady-state conditions cannot be employed underwater (MacArthur and Krause 1989). One approach is to use open-circuit respirometry to measure DMR by assuming that the post-dive increase in $\dot{V}O_2$ above the pre-dive baseline reflects the cost of submergence (MacArthur and Krause 1989, McIntyre *et al.* 2002). Since $\dot{V}O_2$ is measured during the post-dive period, the disadvantage of this method is that the estimated costs of diving can be erroneously elevated if the animal is active after the dive or depressed if active before the dive. This is especially true for semi-aquatic species, which are typically small and often depend on vigorous post-dive grooming or preening to shed excess water from their pelage or feathers (MacArthur 1989).

Studies have also examined the costs of diving for a series of dives and recovery periods and recorded the average DMR over the entire dive cycle (MacArthur and Krause

1989, McIntyre *et al.* 2002). For many aquatic birds and mammals tested in 20-30 °C water, DMR has been experimentally estimated to be ca. 1.5–3.0 x RMR. For instance, the mean DMR of thick-billed murres, *Uria lomvia*, and common murres, *Uria aalge*, was measured at 2.6 and 1.6 x RMR, respectively (Butler and Jones 1997), while the DMR of star-nosed moles, *Condylura cristata*, and muskrats, *Ondatra zibethicus*, averaged 2.1 and 2.7 x RMR, respectively (MacArthur and Krause 1989, McIntyre *et al.* 2002).

It is worth noting that a few studies (MacArthur 1984a, MacArthur and Krause 1989, de Leeuw 1996) have also made the distinction between DMR and what has been termed by de Leeuw as 'excessive diving costs' associated with diving and grooming. These post-dive costs (e.g. grooming, re-warming) represent a significant energy expenditure to the animal which may be important from an ecological perspective. This is especially true for small amphibious species that are expected to incur faster rates of cooling and have higher mass-specific costs associated with post-dive re-warming than larger divers (MacArthur 1989). Post-dive grooming not only serves to shed water and restore the integrity of the pelage, but also provides a source of metabolic heat that helps to restore T_b following emergence of the animal from water.

The TBOSC of diving endotherms, representing the combined oxygen reserves of lungs, blood and muscle, is a key factor limiting underwater dive endurance, and it increases proportionally with body mass ($mass^{1.0}$). Concurrently, the mass-specific rate of O_2 consumption decreases according to the allometric relationship: $\dot{V}O_2 = mass^{0.75}$ (Kleiber 1975). Thus, allometry predicts that, compared to larger-bodied species, small divers have a higher mass-specific $\dot{V}O_2$, specified as diving metabolic rate (DMR),

coupled with a lower TBOSC, leading to an overall reduction in dive endurance (dive endurance = $mass^{0.25}$; Schreer and Kovacs 1997).

Behavioural dive profiles coupled with TBOSC estimates are crucial to determine if allometric predictions and dive trends developed for larger species hold true also for the smallest diving mammals. For instance, it is commonly accepted that most divers do not have exceptionally large lungs (Kooyman 1973, Snyder 1983) as this would likely increase buoyancy while diving and therefore also increase DMR. However, McIntyre *et al.* (2002) determined that star-nosed moles have remarkably large lungs (>1.74x mass-specific allometric predictions; Stahl 1967) suggesting these animals may strongly rely on lung oxygen reserves while diving.

The thermoregulatory ability of a diver in water is also important because it can greatly influence dive performance, especially in smaller semi-aquatic species that are more susceptible to immersion hypothermia (MacArthur 1989). As with dive endurance, thermoregulatory efficiency increases with body size, resulting in lower thermoregulatory costs for larger compared to smaller divers. Large-bodied diving mammals have a decreased surface area-to-volume ratio, thereby reducing sensible heat loss while submerged (MacArthur 1989). These divers are often endowed with additional blubber or fur that dramatically increase whole-body insulation. In contrast, small-bodied divers have a large surface area-to-volume ratio and limited capacity for enhancing pelage or tissue insulation. Both factors predispose most semi-aquatic species to higher rates of heat loss in the aquatic medium than is the case for their larger counterparts. On the basis of these arguments, MacArthur (1989) has suggested that a large body size is adaptive and should be selected for in aquatic and semi-aquatic endotherms. Supporting this view,

for example, is the observation that the semi-aquatic muskrat and the American water shrew, *Sorex palustris*, are both larger in size than closely related terrestrial species (MacArthur 1989, Gusztak and Campbell 2004).

Diving physiologists have given scant attention to small semi-aquatic divers, owing in part to the inherent difficulties in capturing, maintaining and working with these species in captivity. Consequently, the majority of diving studies have focused on marine mammals and birds and diving ducks (reviewed by Butler and Jones 1997, Ponganis and Kooyman 2000, Butler 2004). To date, only a handful of studies have examined diving endurance of mammalian divers <15 kg including beaver, *C. canadensis* (Butler 1991), muskrat, *O. zibethicus* (MacArthur and Krause 1989), platypus, *Orinthorynchus anatinus* (Evans *et al.* 1994) and mink, *Mustela vison* (Dunstone and O'Conner 1979, West and Van Vliet 1986). Further, dive data for the smallest mammalian divers (<100 g) are available only for star-nosed moles, *Condylura cristata* (McIntyre *et al.* 2002), European water shrews, *Neomys fodiens* (Köhler 1991, Vogel 1998), and, to a very limited extent, American water shrews, *S. palustris* (Calder 1969, McIntyre 2000).

As the smallest eutherian diver (12-17 g), *S. palustris* is an intriguing species for which to examine dive endurance and aerobic diving capability. Previous research (Gusztak *et al.* 2005) has determined that *S. palustris* has the highest mass-specific BMR of any eutherian diver examined to date (3x mass-predicted value for a similar-sized mammal). This suggests that the water shrew may also have an extremely high DMR and should be limited to very short dives if it routinely stays within its cADL.

Due to its small thermal inertia, the water shrew should also gain or lose heat rapidly in response to changes in environmental temperature. It is of interest, then, that *S.*

palustris encounters a broad range of environmental temperatures in nature, as they have been captured near both continental coasts in Canada and as far north as southeastern Alaska (Thomas *et al.* 1980, Cook *et al.* 1997). In these regions, water shrews inhabit many aquatic areas, but generally prefer foraging near fast-flowing streams and rivers under the cover of overhanging banks (Conaway 1952). As with other soricine (red-toothed) shrews, *S. palustris* is active year-round and presumably experiences appreciable energetic costs associated with winter foraging. Also, because of their elevated BMR, water shrews must consume relatively large amounts of food or face starvation in a matter of hours (Gusztak *et al.* 2005). Remarkably, these voracious predators are able to stay in energy balance throughout the winter while foraging under the snow and ice, usually in riparian environments where convective heat loss is likely to be high. Consequently, this diminutive insectivore is of interest not only in terms of its dive endurance, but also with respect to its thermoregulatory competence since, in theory, it should be highly susceptible to immersion hypothermia after even a brief period of aquatic activity.

Unfortunately, remarkably little is known of this species' dive performance or aquatic thermal biology. On the basis of limited forced-dive trials, Calder (1969) concluded that water shrews can stay submerged for an average of 37.9 s (maximum = 47.7 s) before drowning. He also obtained, using a rectal thermocouple, the first T_b estimates of water shrews during aquatic activity. These experiments demonstrated that *S. palustris* cooled extremely fast (2.8 °C/min) while submerged in 10–12 °C. Only one study has examined voluntary dive performance of *S. palustris* in captivity, and on the basis of dive data collected from a single shrew, McIntyre (2000) reported a mean dive

time of 5.7 s (n = 88). In another study, Vogel (1990) examined the aquatic thermal biology of the larger (14–18 g), independently derived European water shrew, *Neomys fodiens*, using an implanted abdominal T_b transmitter. He found that this species could maintain a stable T_b averaging 37 °C, while almost completely submerged in 1.8 °C water for 6 min. Vogel suggested that the disparity in cooling rates of the two water shrew species could be explained by inadequate holding conditions in Calder's study. He pointed out that in the latter study, animals were not required to routinely dive for prey nor were they provided with adequate substrate to burrow in. Both of those departures from natural conditions may have caused the pelage to lose its hydrophobic qualities that are essential for repelling water (Vogel 1990).

For semi-aquatic species, the greatest energy expense during aquatic foraging appears to be that of rewarming body tissues after exiting the water (MacArthur 1989, MacArthur and Krause 1989). By limiting the drop in T_b during aquatic foraging, a diver can minimize these energetic costs. A diver can reduce its body cooling during foraging by relying upon anatomical/physiological and/or behavioural mechanisms. The rate of cooling can be reduced by increasing the insulation and/or increasing the rate of heat production. A diver may also utilize behavioural thermoregulation to limit the foraging time in cold water (MacArthur 1979). Thus, most semi-aquatic species have physiological adaptations that they use in combination with behavioural mechanisms to limit cooling during aquatic foraging. For instance, it has been documented in nature that some semi-aquatic species such as muskrat and beaver, *Castor canadensis,* limit cooling by exiting the water at a consistent T_b that is remarkably close to their daily mean T_b (MacArthur 1979; Dyck and MacArthur 1992).

Like all divers, *S. palustris* has anatomical and physiological adaptations to forage in water, but since it is an amphibious predator, the water shrew is somewhat constrained in the extent to which it can specialize for aquatic activity. Anatomical specializations include thickened hairs around the toes of the front and hindlimbs, known as fibrillae, which serve to increase the surface area of the paddling appendages. The water shrew has been observed running along the surface of the water supported by the trapped air in the fibrillae, and for such a feat, is known as 'the water walker' (Jackson 1928, Findley *et al.* 1975). The long tail, which continually rotates in water like a corkscrew, is important to provide stability for the shrew during diving (Conaway 1952, Köhler 1991). The most important morphological feature allowing for the water shrew's amphibious habits, however, is its hydrophobic pelage (Hutterer 1985, Köhler 1991). The fur of water shrews is composed of specialized awn hairs that trap a layer of air as the shrew submerges. This insulative barrier inhibits water from contacting the skin (Hutterer 1985) and greatly aids in retaining body heat. However, it also dramatically increases its buoyancy, requiring diving shrews to continually move their appendages to stay submerged (Conaway 1952). To surface, water shrews cease all locomotion and effortlessly ascend in the water column.

Study Objectives

The primary goal of this study was to investigate the dive performance and aquatic thermoregulatory ability of wild-caught *S. palustris* in a laboratory setting. Trials were first completed to assess the diving behaviour of this species, as well as test for the occurrence of behavioural thermoregulation in water shrews voluntarily diving over a

range of water temperatures. In addition, several shrews implanted with T_b transmitters allowed for the collection of real-time telemetric T_b data during diving and re-warming periods over a range of water temperatures. Further, voluntary dives completed in 3 °C and 10 °C water with measured T_b recordings were utilized to test the "adaptive hypothermia" hypothesis. This hypothesis predicts that both dive duration and dive frequency should correlate inversely with Tb. A second major objective was to determine this species' cADL which required measuring DMR and determining its TBOSC. These data, combined with information on dive performance, provided insight into the extent to which diving water shrews depend on aerobic metabolism. Since diving species tend to have a higher O_2 storage capacity than similar sized non-diving species (Ponganis *et al.* 1999; McIntyre *et al.* 2002), the TBOSC of *S. palustris* was also compared to that of a strictly terrestrial shrew of similar mass, the short-tailed shrew, *Blarina brevicauda.*

Materials and Methods

Animal care

A total of 67 water shrews and 18 short-tailed shrews were captured between June 2001 and August 2006 in Whiteshell (49°47'N, 95°13'W) and Nopiming (50°28'N, 95°15'W) Provincial Parks, Manitoba, Canada, using Sherman live traps (256 x 76 x 76 cm). Trapping techniques and holding conditions in captivity are detailed elsewhere (Gusztak and Campbell 2004). Briefly, traps were baited with fish and set along the edges of fast-flowing streams or low-lying aquatic regions with abundant sedge (Conaway 1952). Traps set for short-tailed shrews were placed along trails covered by grass or along tunnels located a few meters inland from water shrew sets. Traps were inspected every 2 h. Immediately upon capture, shrews of each species were placed individually into covered 38-l plastic containers, supplied with soil, a layer of thick moss, rocks, logs, a nest box (100 x 100 x 125 mm), food and water trays. Provisions included meal worms, *Tenebrio molitor*, hulled sunflower seeds, ground Purina cat food™ and a prepared meat mixture (see below), along with any large invertebrates found while trapping. Shrews were brought to the University of Manitoba Animal Holding Facility within 12 h of capture.

Vogel (1990) suggested that captive water shrews lose the hydrophobic properties of their pelage when animals are not provided with holding conditions that permit diving and access to dry moss for burrowing following aquatic activity. Consequently, much care was taken in the design and continued maintenance of the holding tanks to ensure the fur of water shrews was always in optimal condition (Gusztak and Campbell 2004). Modified 264-l aquaria (88 x 50 x 60 cm) served as individual holding containers for

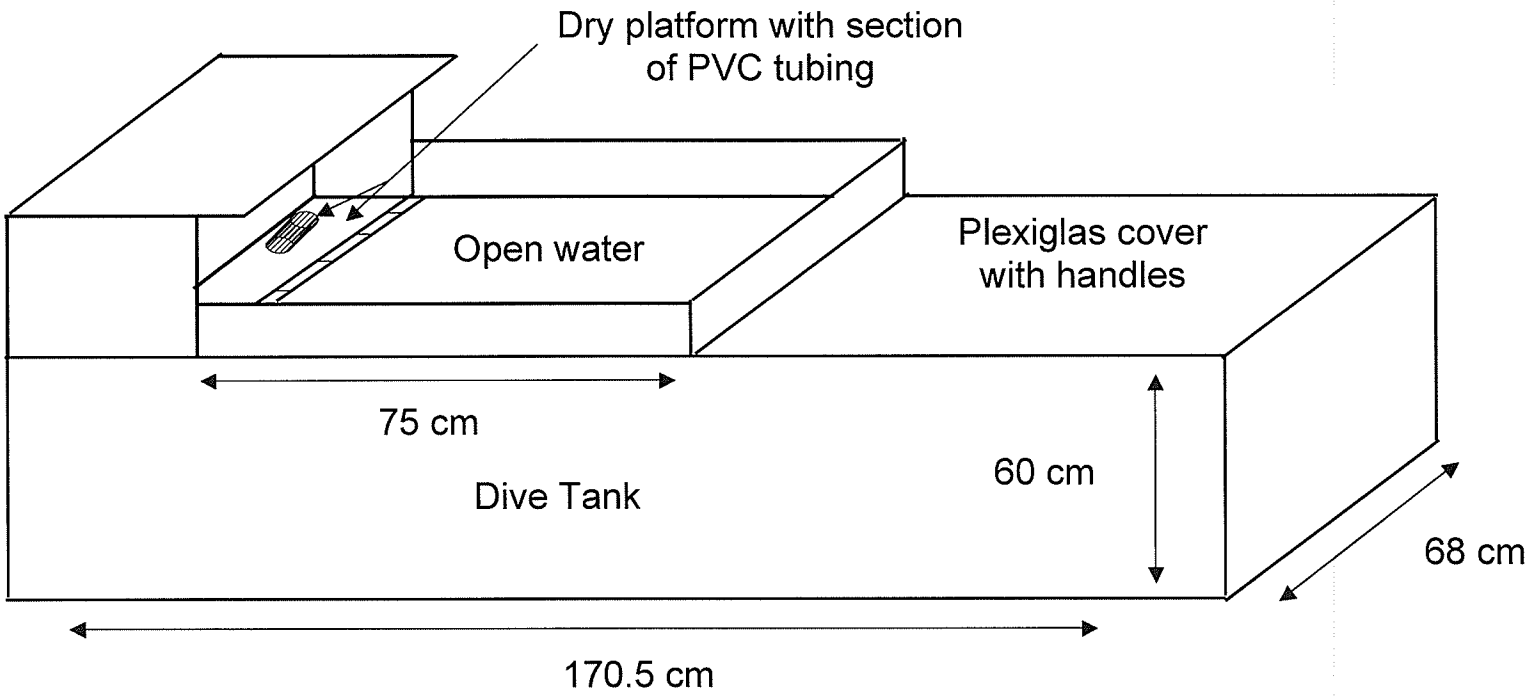
water shrews. Each holding tank had a discrete terrestrial (ca. 75%) and aquatic (ca. 25%) compartment separated by a 1-cm thick plexiglas partition (Gusztak and Campbell 2004). The terrestrial zone was furnished as described above for the transport containers. Short-tailed shrews were individually housed in 76-l terrestrial containers and were supplied with water dishes that were refilled every 12 h. Both shrew species were offered a prepared meat mixture (beef and chicken hearts, pig and beef liver, ground beef, fish fillets and canned dog food mixed with vitamin and calcium supplements) every 12 h. To feed, water shrews had to climb up a section of PVC tubing leading to the aquatic area, then swim across the tank and dive under a removable partition to access the feeding tray. This requirement ensured shrews were habitually diving in the setup. When available, mealworm larvae and aquatic prey (leeches, dragonfly nymphs and small crayfish) were also placed in the aquatic portion of the water shrew tanks to encourage natural foraging behaviour. When offered, aquatic prey was preferentially consumed over the meat ration. All wild shrews used in diving trials or for tissue processing were given three weeks to acclimate to holding conditions before trials were initiated or TBOSC calculated. Due to time constraints, TBOSC for some of the short-tailed shrews were completed after only one week of acclimation. Water shrews were allowed a recovery period of at least 48 h between successive experimental trials (see below). Water and short-tailed shrews were aged at post-mortem based on tooth wear examined under a microscope and subsequently divided into subadults (born the summer of capture) or adults (being reproductively active). All animals were cared for in accordance with the principles and guidelines of the Canadian Council of Animal Care and a university-approved research protocol.

Voluntary dive behaviour

As a first step in assessing the diving capabilities of American water shrews, I recorded the frequency and duration of voluntary dives by 25 captive animals (six of which were implanted with Tb transmitters, see below). No prey was placed in the dive tank, though shrews were offered a mealworm 10 min before each trial. The mass of each animal was recorded just before feeding commenced. All shrews completed a pre-trial training run in 30 °C water to familiarize themselves with the dive arena. Each behavioural dive trial was conducted in 3, 10, 20 or 30 °C water and was initiated when the shrew first entered the water. Trials lasted precisely 20 min.

The 170.5 x 68 cm fiberglass dive tank was filled with water to a depth of 60 cm and was divided into three sections (Figure 1). A transparent dive platform (17.5 x 68 cm) was situated at one end just above the waterline. On this platform, a 4-cm section of PVC tube (internal diam. = 3.5 cm) was fastened to provide a darkened refuge for the shrew. The center section of the tank contained open water (75 x 68 cm), while the remainder of the tank was covered with a sheet of 1-cm thick plexiglas (78 x 68 cm) to encourage exploratory diving behaviour. The plexiglas sheet was equipped with handles so it could be removed quickly if a shrew became disorientated beneath. Data were collected during the trial using a Sony Microcassete Recorder that facilitated post-trial analyses of dive durations and frequencies, inter-dive times, time in water, and grooming behaviour. Six adult water shrews that were surgically implanted with a customized 1.0-g abdominal temperature transmitter (see below) completed voluntary dive trials at all four T_w's. This allowed for a comparison of voluntary dive performance between implanted and non-implanted adult water shrews. In the pre- and post-trial periods, each

Figure 1. Schematic of dive tank used to study the voluntary dive behaviour of American water shrews, *Sorex palustris*. See text for details.


Dry platform with section
of PVC tubing
Open water
Plexiglas cover
with handles
75 cm
60 cm
Dive Tank
68 cm
170.5 cm

shrew was housed in a 38-l container with 6-8 cm of mixed soil, rocks and logs to simulate natural conditions and minimize stress.

Body temperature recordings

A total of 12 water shrews were equipped with a 1.0-g model X-M transmitter (Mini-mitter Inc., Bend, OR, USA) surgically implanted in their abdominal cavities. Each transmitter was modified from the original packaging to decrease its overall size and then calibrated following the method of Dyck and MacArthur (1992). Surgical procedures are described in detail by Gusztak *et al.* (2005). Briefly, shrews were anesthetized using the inhalent anesthetic Isoflurane, given at 3% during induction and then adjusted, as needed, to 2-3% to maintain a surgical plane of anesthesia. A midline incision through the skin and body wall was made along the *linea alba*. The sterilized transmitter was then placed into the abdominal cavity and incisions closed with sutures. Post-operative surgical care included placement of the shrews in a disinfected 38-l plastic container containing a nest box and shredded paper towel. Shrews were supplied with fresh food and water every 12 h and were transferred back to their holding tanks after 48 h. Aquatic trials started 7-10 days later. Surgical procedures were successful in 94% of cases (16 of 17 operations), with five shrews undergoing a second surgery 4-6 weeks later to implant new transmitters with a fresh battery.

Rates of body cooling and re-warming were determined for implanted shrews diving in 3-30 °C water. T_b data were recorded during the 5-min pre-trial, 20-min trial, and 10-min post-trial periods, respectively. T_b was recorded via a Sony Cassette-

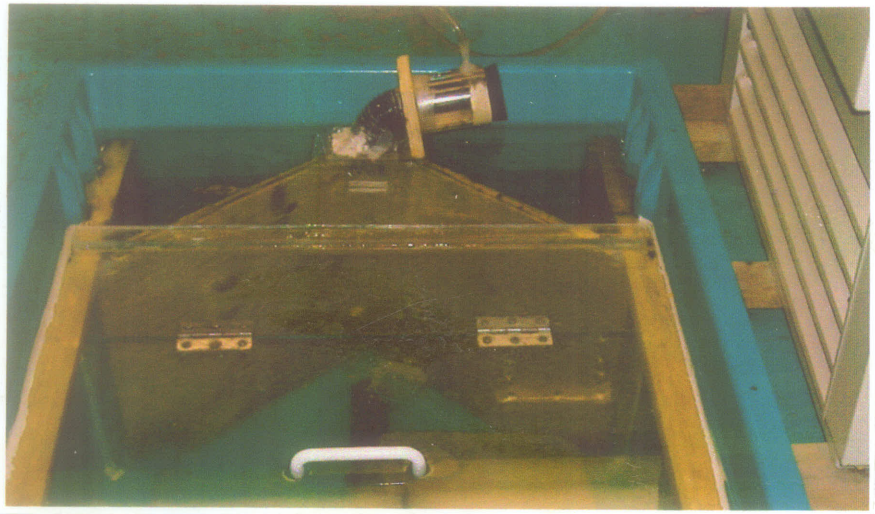
Recorder that was placed outside the terrestrial section of the tank and data subsequently analyzed at 1-min intervals throughout the 35-min experiments.

Costs of diving bouts and associated re-warming

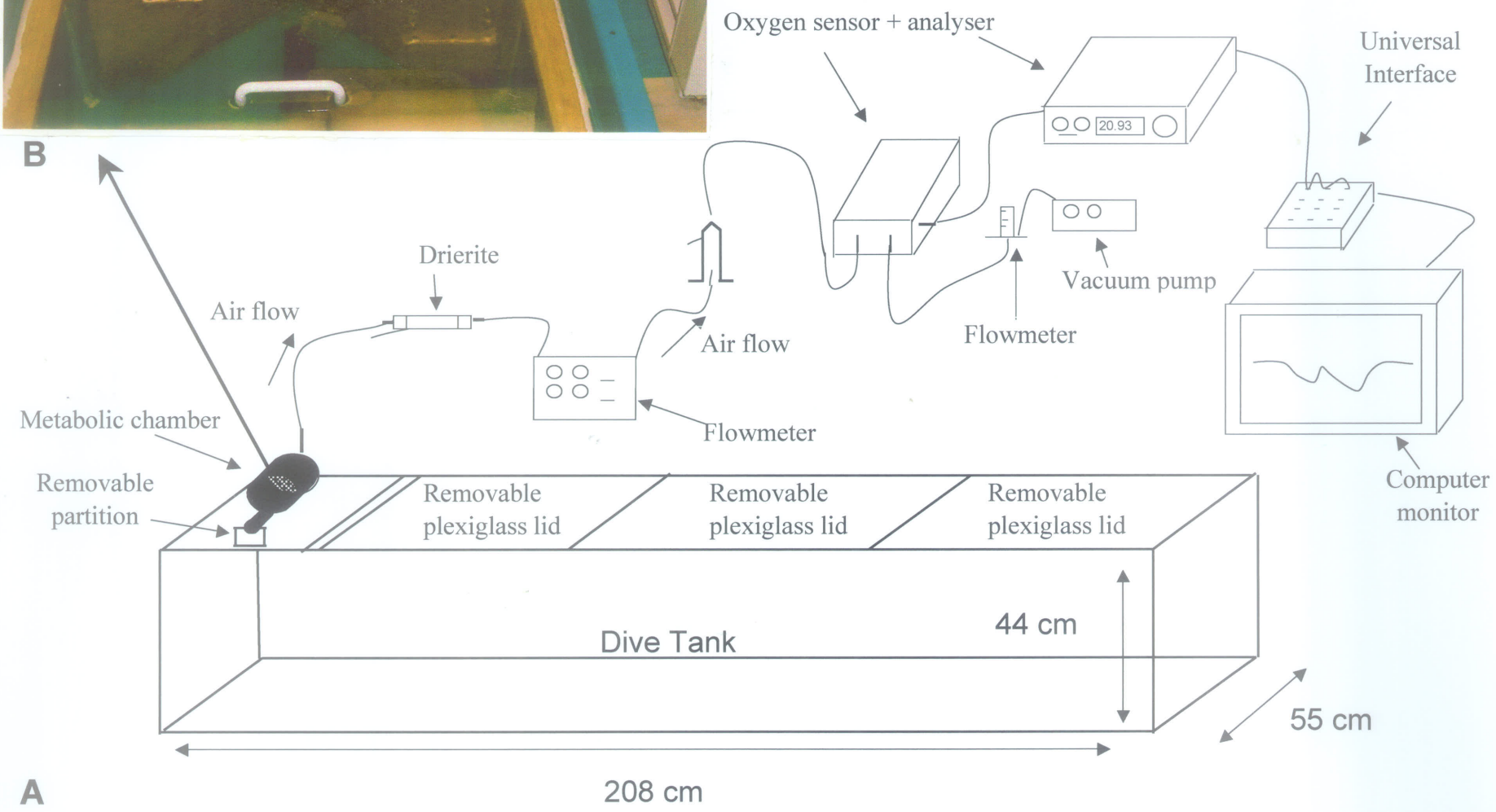
The costs of bouts of diving, grooming and re-warming were estimated for water shrews voluntarily diving in a 208 x 55 cm fiberglass tank filled with water to a depth of 44 cm (McIntryre *et al.* 2002). The tank was covered with three removable plexiglas panels and was filled with water so the panels were just submerged, making the metabolic chamber the only site in the tank where the animal could surface for air (Figure 2). A transparent 165-ml metabolic chamber was constructed from a 6-cm length of plexiglas tubing (internal diameter = 6 cm). At the rear of the chamber was a removable stopper allowing for easy transfer of shrews to and from the chamber. Attached to the opposite end of the chamber was a curved section of tubing 4 cm in diameter and 5 cm long that shrews had to traverse in order to enter the water. A removable partition was placed between the metabolic chamber and tank cover to prevent shrews from entering the water while pre-dive metabolic measurements were recorded. This partition was also employed to confine shrews to the metabolic chamber when recording post-dive recovery costs (see below).

An outlet port was installed in the top/rear portion of the metabolic chamber while six inlet holes, each 1 mm in diameter, were drilled into the base of the chamber at the opposite end to facilitate mixing of air. Air was drawn through the chamber at ca. 500 ml min^{-1} using a TR-SS2 gas analysis sub-sampler (Sable Systems Inc., Las Vegas, USA)

Figure 2. A) Dive tank and respiratory setup used to measure costs of diving in American water shrews. B) Close-up photo of diving metabolic chamber. Note: shrews could be prevented from entering the water by closing a moveable partition attached to base of metabolic chamber. See text for details.



B


Oxygen sensor + analyser
Universal Interface
20.93
Drierite
Air flow
Air flow
Vacuum pump
Flowmeter
Flowmeter
Metabolic chamber
Removable partition
Removable plexiglass lid
Removable plexiglass lid
Removable plexiglass lid
Computer monitor
Dive Tank
44 cm
55 cm
208 cm

A

calibrated against a bubble flowmeter (accurate to within ca. ± 2%; Levy 1964). Exhalent gas was dried and analyzed using an Applied Electrochemistry S-3A O_2 analyzer. Fractional O_2 content was recorded at 1-s intervals, while chamber T_a and T_w were recorded immediately prior to and following each trial. A respiratory quotient of 0.83 was assumed, based on an earlier study of fasted water shrews (Gusztak *et al.* 2005) and instantaneous $\dot{V}O_2$ derived, following the method of Bartholomew *et al.* (1981). Mean instantaneous $\dot{V}O_2$ measurements were calculated at 20-s intervals throughout the trial. Each shrew was implanted with an abdominal temperature transmitter (see above). The signal was recorded on a Sony Cassette-Recorder and subsequently analyzed at 1-min intervals throughout the pre-trial, diving and post-trial periods, respectively.

Concrete blocks and sections of PVC tubing were placed at the bottom of the tank to encourage longer exploratory dives, since preliminary trials suggested these objects increased time underwater. Each shrew completed a total of four trials presented in random order: two in 10 °C water and two in 30 °C water. Trials conducted at 30 °C provided an estimated DMR when thermoregulatory costs are presumably minimal (MacArthur 1989), while 10 °C water was chosen to assess the thermoregulatory costs associated with submersion in cold water.

The mass of each shrew was recorded immediately before placing the animal in the metabolic chamber and transmitter mass subtracted prior to calculating metabolic rates. Each trial consisted of a 10- to 15-min pre-trial period during which time the shrew was confined to the metabolic chamber and its lowest $\dot{V}O_2$ over 5 min taken as the baseline value. Estimates of RMR were also obtained, but occurred in only five (10 °C

water) and eight (30 °C water) trials, emphasizing the high levels of activity in these aquatic predators.

After the partition was removed, 10 min was allotted for voluntary diving, which commenced upon the animal's first entry into water. At the end of the 10-min dive session, the partition was gently slid back into place to prevent further dives. A trial duration of 10 min was chosen because in preliminary tests, water shrews exhibited the highest dive frequency within this initial time period (see results). The post-dive recovery $\dot{V}O_2$ associated with re-warming and grooming was recorded until the animal's $\dot{V}O_2$ or T_b returned to within 95% of the pre-trial baseline. If this had not occurred after 15 min in the chamber, the trial was ended. Dive durations, grooming behaviour and relative activity were recorded on a Sony Microcassete-Recorder and analyzed after each trial.

Metabolic costs of a single dive

The dive tank described above was also employed to estimate the metabolic cost of single dives in 30 °C water. Control trials with transmitter-implanted shrews were completed to ensure T_b did not decrease below resting values following a dive and, therefore, suggested that there were no, or only minimal thermoregulatory costs incurred during dives at this T_w. Only $\dot{V}O_2$ measurements of shrews without T_b transmitters were used to calculate individual dive costs. As shrews completed more diving trials or after being implanted with transmitters, they were less likely to rest during the pre-trial period of the metabolic chamber. This made for selecting shrews that were recently captured to

first complete these trials and then the other diving trials. Shrews were placed in, and confined to, the metabolic chamber via the removable partition until a stable pre-dive RMR of at least 60 s duration was obtained. Inactivity was verified visually and coincided with the minimum recorded $\dot{V}O_2$. After initial testing, certain shrews were preferentially chosen for these trials as some were more apt to rest in the metabolic chamber, while others remained continually active for the entire 45-min trial. Immediately after baseline measurements were completed, shrews were allowed access to the tank by sliding the partition out and allowing them to complete a single dive. Upon the shrew surfacing, the partition was closed to prevent further diving, and the shrew confined to the chamber for at least 7 minutes to allow any lactate produced from the previous dive to be cleared. However, the majority of dives recorded during these trials were extremely short (mean = 3.4 s, max = 10.0 s), with none surpassing the cADL, suggesting that minimal, if any, lactate accrued during diving. Diving $\dot{V}O_2$ measurements were calculated only for dives in which no post-dive activity occurred or when the shrew engaged in less than three episodes of grooming (each < 1 s), but otherwise rested throughout the post-dive recovery period. Post-dive recovery was monitored until $\dot{V}O_2$ returned to within 95% of the pre-trial resting value. If post-dive $\dot{V}O_2$ did not return to baseline due to increased animal activity, then the dive was discarded. Instantaneous $\dot{V}O_2$ measurements were analyzed at 3-s intervals and the mean of all measurements used to calculate the unit cost of an individual dive. Dive duration, grooming behaviour and level of activity were recorded concurrently on a Sony Microcassete Recorder and a computer for post-trial analyses.

Body oxygen stores

The TBOSC of water shrews was determined after completing all diving/behavioural trials on each animal. Short-tailed shrews were allowed a 1- to 3-week acclimation period in the lab prior to determining TBOSC. The mass of each shrew was recorded, after which the animal was deeply anesthetized with 3% Isoflurane inhalant anesthetic. A cardiac puncture was then performed to extract a blood sample for Hb and Hct determinations (MacArthur 1984b; McIntyre *et al.* 2002). All animals were administered an overdose of Isoflurane following the blood sample. The heart, forelimb and hindlimb muscles were then quickly removed and freeze-clamped in liquid nitrogen. Excised muscles were stored at -70 °C and Mb content and buffering capacity determined later following the methods of Reynafarje (1963) and Castellini *et al.* (1981), respectively. The lungs were carefully removed after the majority of the heart muscle had been cut away, and lung volume determined gravimetrically following the procedures described by Weibel (1970/71). Briefly, this included inserting a 3-cm section of P20 cannula 5-8 mm into the trachea. The cannula was secured in place with a 5-0 silk ligature. Vetbond™ was applied to the knot at the juncture of the cannula/trachea to ensure the preparation would not slip. The trachea/lung prep was submerged in saline (0.9 M NaCl) and then inflated at a constant pressure of 20 mmHg with humidified air for ca. 10–15 min before measurement. All recordings were corrected to STPD.

The total percentage of muscle mass, expressed as a fraction of digesta-free body mass, was also calculated for 12 water shrews and 2 short-tailed shrews. Skinned, eviscerated carcasses were submerged for ca. 24–48 h in a detergent solution at 32 °C to detach any skeletal muscle adhering to the bones. The total skeletal muscle mass was

then calculated by subtracting the dry bone mass from the initial carcass mass (MacArthur *et al.* 2001, McIntyre *et al.* 2002).

Total blood volume of water shrews was estimated from the allometric equation (Prothero 1980): blood volume (ml) = 76 $M^{1.0}$, where M = body mass in kg. Total body O_2 stores of *S. palustris* were determined following the procedures of Lenfant *et al.* (1970). This method assumes that water shrews dive with lungs fully inflated and with an initial lung oxygen concentration of 15%. The oxygen storage capacity of blood was calculated by assuming that 1/3 and 2/3 of the blood volume constituted the arterial and venous fractions, respectively, with the former having an oxygen saturation of 95% and the latter a 5% vol. decrease in O_2 content compared to arterial blood. Skeletal muscle Mb concentrations were determined as the mean from samples of the forelimb and hindlimb for each individual, multiplied by the mass of skeletal muscle in the body. Blood and Mb oxygen capacities were presumed to equal 1.34 ml O_2 g $pigment^{-1}$ (Lenfant *et al.* 1970, Kooyman *et al.* 1983).

TBOSC (ml O_2 in muscle, blood and lungs, corrected to STPD) were calculated and divided by the mean DMR (ml O_2 s^{-1}) measured in 10 °C and 30 °C water, in order to derive a cADL for water shrews diving at each T_w. This estimate hinges on the assumption that all O_2 stores are utilized during diving, before the animal switches to anaerobic respiration (Kooyman *et al.* 1980). I also calculated the bADL at 10 °C and 30 °C water, defined as the dive duration exceeded by only 5% of all voluntary dives (Kooyman *et al.* 1983).

Skeletal muscle buffering capacity

The skeletal muscle buffering capacities of short-tailed and water shrews were tested against non-bicarbonate buffers following the procedure of Castellini *et al.* (1981). Buffering capacity (β) was standardized to represent the μmol of base needed to increase the pH of 1 g of wet muscle mass from a pH of 6 to 7. A 0.3- to 0.5-g sample of frozen skeletal muscle comprised of both forearm and hindlimb tissue was ground up in 0.9 M NaCl, following which the solution was titrated with 0.2 M NaOH using an Accumet® AB 15/15+ pH meter and an AccuTupH sensing electrode.

Statistical analyses of data

All statistical analyses were performed using SPSS 9.0 for Windows. Means of dive variables were compared across water temperatures using one-way ANOVA, while two-way ANOVA was employed to test for possible interaction effects between T_w and the presence or absence of an implanted transmitter in the subject animal. When appropriate, differences between means were compared using Tukey's multiple range test. Dive profiles were compared between implanted and non-implanted adult water shrews using log likelihood ratio test (G-test, Zar 1974). Means of variables were compared using a 1-tailed Students *t*-test. Regression lines were fitted by the method of least squares. Significance was set at 5% and means presented as ± 1 S.E.M.

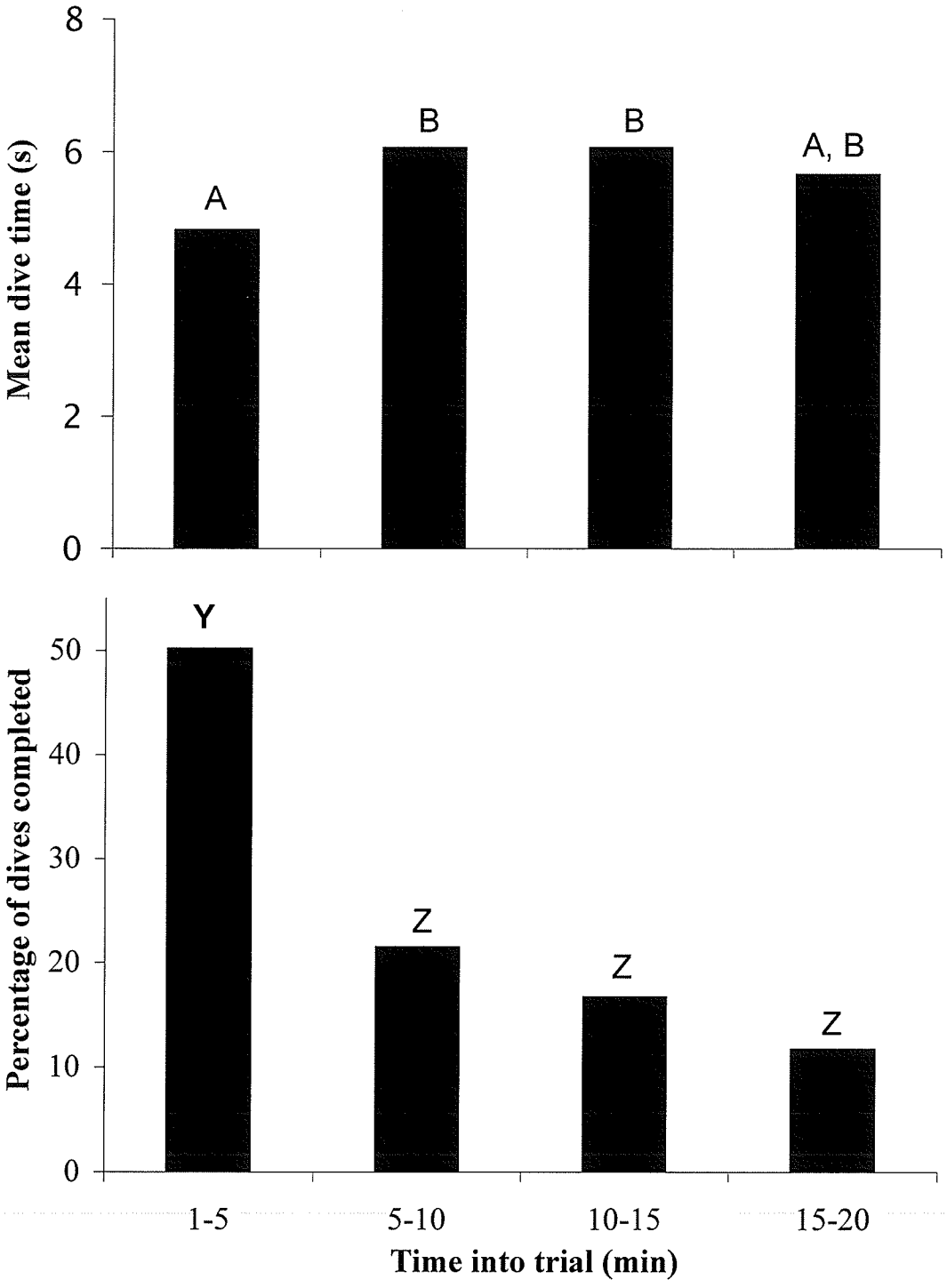
Results

Voluntary dive behaviour

In most trials, water shrews were hesitant to dive until they had fully explored the surfaces of both the terrestrial and aquatic sections of the tank. Shrews routinely initiated the first aquatic bout of each trial with short bursts of surface swimming that usually included exploration of the perimeter of the tank. Subsequently, predictable pre-dive behaviour was routinely observed. Water shrews would approach and briefly pause at the edge of the dive platform for 1–10 s, during which time repetitive head nodding occurred, causing the shrew's vibrissae to repeatedly touch the water. This behaviour was typically followed by the shrew diving from the platform. It was common for air released from the pelage of the shrew to become trapped beneath the plexiglas cover. This gas would accumulate throughout the trial, but usually was not large enough to be inspired via the nostrils of the shrew as the plexiglas cover was removed between each trial. However, during the first 30 °C water training session, gas would sometimes coalesce into a 5-10 mm bubbles as multiple trials would be completed without removing the plexiglas cover and shrews would be seen inspiring the trapped gas.

Water shrews in this study engaged in two distinct categories of dives. Dives were classified as either shallow (< 10 cm) or deep (reaching the tank bottom at 60 cm). Very few dives were completed between these depths, but if they occurred, were specified as shallow. During each 20-min dive trial, the mean number of dives completed during the first 5-min was significantly greater than for any of the three remaining 5-min periods ($F_{3,92} = 27.466$, $P < 0.0001$). Greater than 50% of the total number of voluntary dives occurred during the first 5 min of the trial (Figure 3). Data is shown only for

Figure 3. Percentage of total voluntary dives completed and corresponding mean dive times of six transmitter-implanted American water shrews during successive 5-min periods of each dive trial ($n = 317$). Percentages with different letters differ significantly from each other ($P < 0.05$).

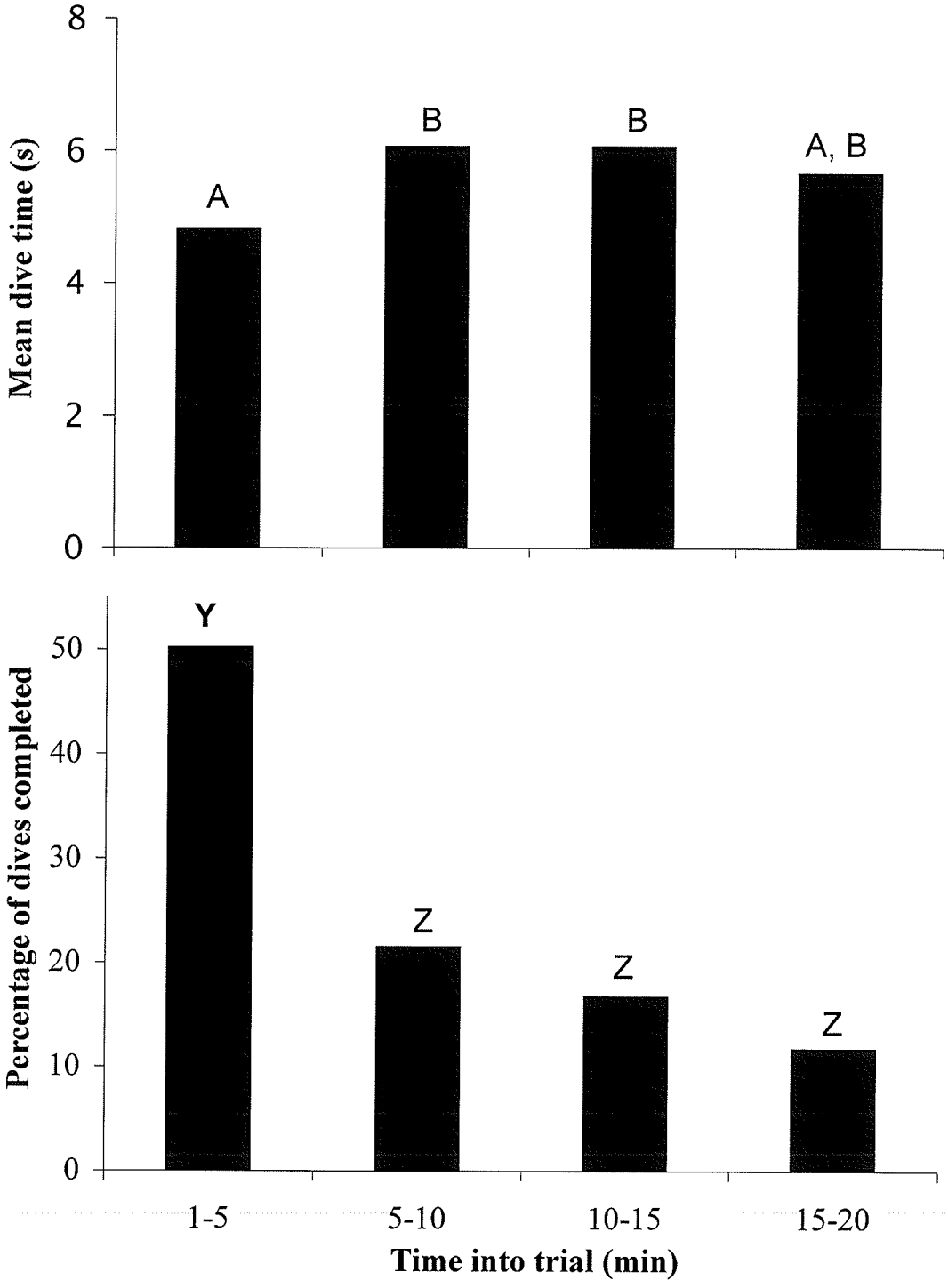

Mean dive time (s)
8
6
4
2
0
A
B
B
A, B
Percentage of dives completed
50
40
30
20
10
0
Y
Z
Z
Z
1-5
5-10
10-15
15-20
Time into trial (min)

shrews implanted with a transmitter, but non-implanted shrews showed a similar trend. Only data for implanted animals are included here, since dive times and the time (min) into the trial were recorded together only in this group, in order that the effect of T_b on dive time could also be examined.

Four subadult water shrews had a marginally shorter mean dive duration (4.91 ± 2.96 s) and a higher mean dive frequency (0.71 ± 0.21 dives min^{-1} $trial^{-1}$) than adult water shrews (5.17 ± 3.49 s and 0.66 ± 0.26 dives min^{-1} $trial^{-1}$; N = 12) but neither variable differed significantly between the two groups (t = 1.064, d.f. = 705, P = 0.14; t = -0.638, d.f. = 38, P = 0.26). Further, the dive performance of 6 adult water shrews with an implanted abdominal temperature transmitter did not differ significantly from 6 other non-implanted adults. Consequently, data for subadults and adults with and without transmitters were pooled in all subsequent analyses.

A total of 25 shrews participated in 111 individual dive trials with an overall recorded mean dive time of 5.09 ± 0.08 s (Figure 4). Of the 1584 voluntary dives recorded for these shrews, 311 (19.6%) were deep dives (60 cm) with an average duration of 8.06 ± 0.17 s (Figure 4). The five longest dives of each trial had a mean duration of 7.76 ± 0.28 s, and the mean longest dive per trial was 10.32 ± 0.38 s. The longest voluntary dive recorded was 23.65 s, with only three dives exceeding 20 s. One of these 20-s dives occurred at the end of the trial, so no inter-dive time was recorded (see below). During each 20-minute trial, shrews completed, on average, 14.70 dives (range = 0 to 53 dives).

Figure 4. Frequency distribution of voluntary dive times of 25 American water shrews diving in 3-30 °C water. See text for details.


Mean dive time (s)
8
6
4
2
0
A
B
B
A, B
Percentage of dives completed
50
40
30
20
10
0
Y
Z
Z
Z
1-5
5-10
10-15
15-20
Time into trial (min)

The average dive:surface ratio was 0.21 ± 0.01 s (Figure 5). There was a significant increase in inter-dive surface time with longer dives, suggesting longer dives require a longer recovery period than shorter dives ($F_{1,952}$= 20.28, $P < 0.0001$, $r^2 = 0.021$; Figure 5). To ensure that the two longest dives (20.07 and 23.65 s) were not significantly influencing the trend, the regression was also computed without these values. Even so, there was still a significant positive relationship between inter-dive surface interval and dive time ($F_{1,950} = 14.594$, $P < 0.0001$, $r^2 = 0.015$).

Influence of water temperature on dive behaviour

Water temperature significantly influenced the total time water shrews spent swimming and diving ($F_{3,68} = 7.892$, $P < 0.001$), with shrews voluntarily spending less than half as long in 3 °C, compared to 30 °C water (Table 1, Figure 6). Overall, mean dive duration increased with water temperature ($F_{3,68} = 5.033$, $P = 0.003$) as did the dive:surface ratio ($F_{3,68} = 7.146$, $P < 0.001$). On average, shrews surfaced for 101 s and 66 s before diving again in 3 °C and 30 °C water, respectively. For all dives combined, dive frequency was independent of T_w ($F_{3,68} = 0.467$, $P = 0.706$). However, the frequency of deep dives was significantly different between 3 and 30 °C water ($P = 0.037$). Conversely, the mean duration of deep dives did not vary with T_w (Table 1). Water temperature did appear to affect the longest dive of each trial ($F_{3,68} = 6.173$, $P = 0.001$), with water shrews diving an average of 35% longer in 30 °C, compared to 3 °C water. The bADL of water shrews diving in 10 °C and 30 °C water was calculated to be 10 and 12 s, respectively (Figure 6).

Figure 5. The relationship of inter-dive surface time to duration of preceding dive in water shrews completing consecutive dives during voluntary dive trials. Regression line was fitted by the method of least squares. D:S ratio = dive duration divided by time spent at the surface.

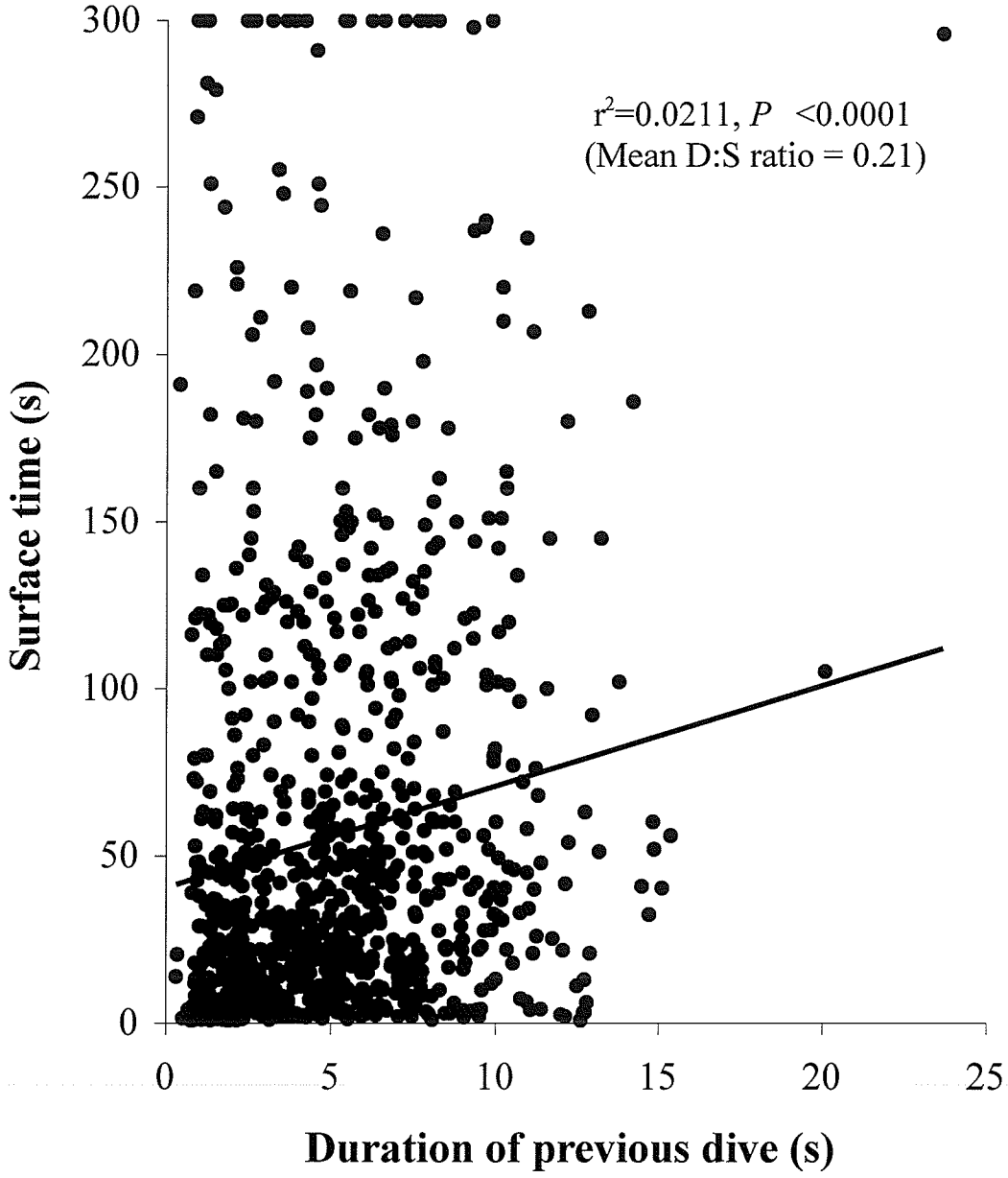

300
250
200
150
100
50
0
Surface time (s)
r²=0.0211, P <0.0001
(Mean D:S ratio = 0.21)
0
5
10
15
20
25
Duration of previous dive (s)

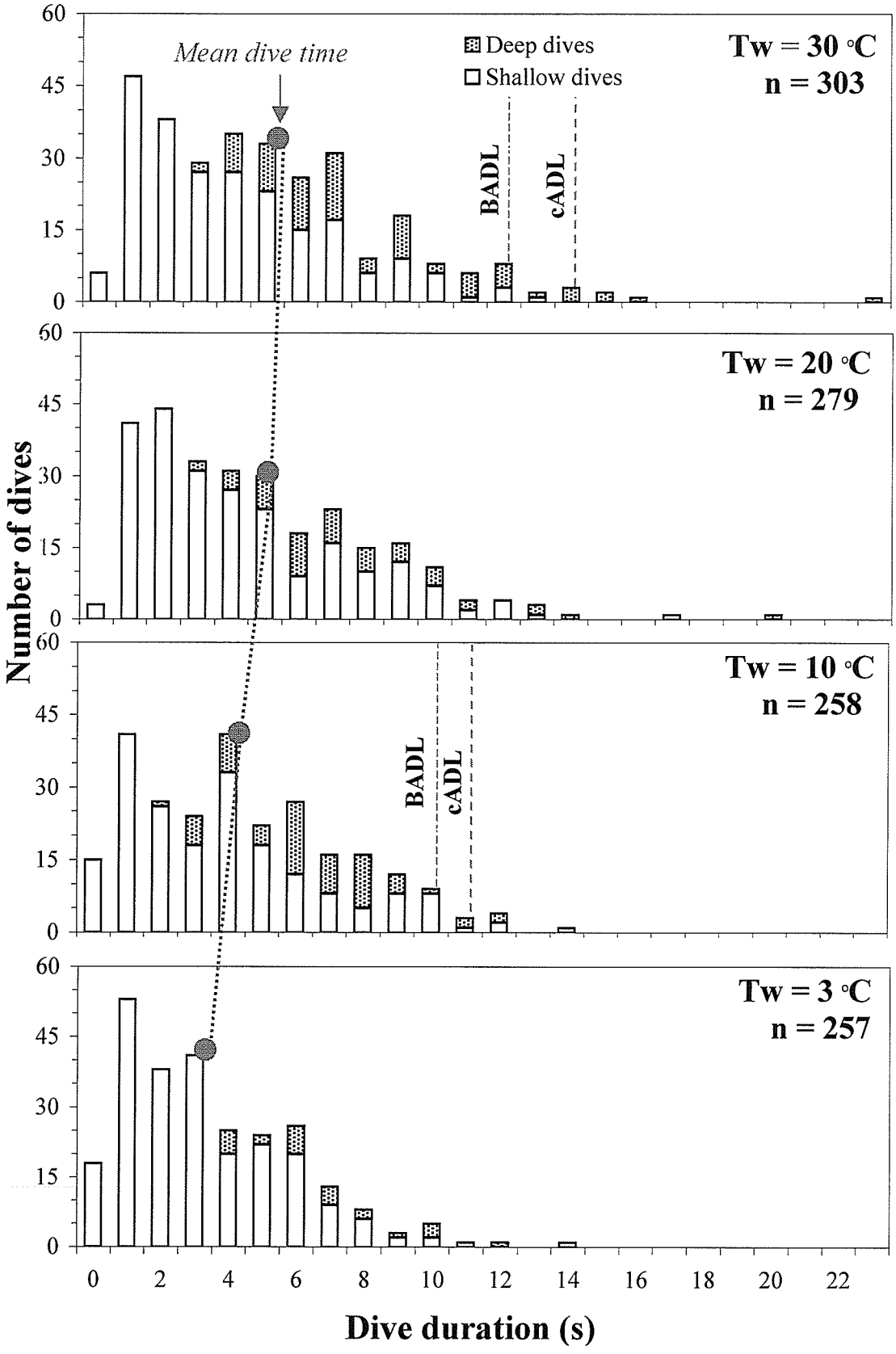
Table 1: The effect of water temperature on the voluntary dive behavior of 18 captive American water shrews (*Sorex palustris*).

	Water temperature (°C)			
	3	10	20	30
Total time in water (s)	111±14[a]	134±15[a]	184±21[ab]	235±25[b]
All dives				
Dive duration (s)	3.8±0.2[a]	4.8±0.5[ab]	5.3±0.3[b]	5.7±0.4[b]
Dive frequency (dives • min^{-1})	0.71±0.08[a]	0.71±0.09[a]	0.77±0.07[a]	0.84±0.11[a]
Dive:surface ratio	0.12±0.02[a]	0.20±0.02[ab]	0.25±0.09[b]	0.27±0.03[b]
Duration of longest dive (s)	8.0±0.8[a]	9.0±0.8[ab]	11.0±0.8[bc]	12.4±0.9[c]
Deep dives (> 50 cm)				
Dive duration (s)	6.6±0.6[a]	7.5±0.6[a]	7.7±0.6[a]	9.3±0.7[a]
Dive frequency (dives • min^{-1})	0.08±0.01[a]	0.17±0.04[ab]	0.14±0.03[ab]	0.23±0.05[b]
Dive:surface ratio	0.11±0.05[a]	0.15±0.02[a]	0.27±0.05[a]	0.25±0.05[a]

Values presented are means ± S.E.M.

Within each row, means sharing the same letter are not statistically different (P>0.05).

Figure 6. Influence of water temperature (T_w) on the diving behaviour of 18 American water shrews. Behavioural and calculated aerobic dive limits (bADL; cADL) in 10 °C and 30 °C water are denoted by dashed lines. Solid circles indicate overall mean dive time at each T_w.


Mean dive time
Deep dives
Shallow dives
Tw = 30 °C
n = 303
BADL
cADL
Tw = 20 °C
n = 279
Tw = 10 °C
n = 258
Tw = 3 °C
n = 257
Number of dives
Dive duration (s)
0
15
30
45
60
0
2
4
6
8
10
12
14
16
18
20
22

Body temperature measurements during voluntary diving

A total of 23 T_b data sets were obtained from six water shrews that completed a total of 24 dive trials. Owing to a mechanical error with the recording device, no T_b data were obtained for one water shrew diving in 10 °C water, but behavioural dive data were still obtained for this animal via audiotape. Pre-trial T_b measurements were elevated across the four T_ws, but were not significantly different between test groups ($F_{3,19}$ = 0.390, P = 0.76, Table 2). In the diving portion of the trial, water shrews usually incurred the largest drop in T_b during the first 5-7 min, but the decrease varied with T_w (Figure 7, Appendices 1 to 6). Following this initial curvilinear decline, T_b tended to plateau for the remainder of the dive trial, with re-warming evident only in the post-dive period, after shrews were removed from the dive tank (Figure 7). The lowest recorded T_b during the diving session did not differ significantly between T_ws ($F_{3,19}$ = 2.341, P = 0.11). During the post-trial period, water shrews rewarmed and attained a T_b similar to the pre-trial value, regardless of T_w (Table 2). Also, T_w did not significantly influence the highest post-trial T_b ($F_{3,19}$ = 0.767, P = 0.52). For all T_ws tested, the post-dive T_b was significantly higher than the lowest T_b over 1-min during the trial (Table 2).

Influence of transmitter implants on dive performance

Dive performance was compared in adult water shrews with (N = 6) and without (N = 6) an implanted 1.0-g abdominal temperature transmitter by first pooling the dive data for each group across all T_ws. There were no significant differences in dive performance between the two groups in any of the examined variables, including mean dive duration and frequency, longest dive time and total time in water (Table 3). Though

Table 2: Telemetered body temperatures (T_b) recorded from six American water shrews during voluntary dive trials in 3-30 °C water.

	Water temperature (°C)			
	3	10	20	30
Time in water (s)	106.9±22.4^{b}	152.5±34.0^{b}	180.4±29.4^{b}	162.1±18.7^{b}
Rate of cooling during first 5 min into trial (°C min^{-1})	1.61±0.63^{c}	0.92±0.24^{c}	0.99±0.31^{c}	0.43±0.15^{c}
T_b measurements (°C)				
Pre-trial T_b	40.0±0.2^{d,1}	40.1±0.1^{d,1}	39.9±0.1^{d,1}	40.1±0.2^{d,1}
Mean T_b during diving	38.9±0.3^{f,2,3}	39.1±0.2^{f,2}	38.7±0.4^{f,2,3}	39.4±0.2^{f,2}
Lowest T_b in trial	38.0±0.3^{e,3}	38.4±0.2^{e,3}	37.7±0.5^{e,3}	38.8±0.2^{e,3}
Highest post-trial T_b	39.5±0.2^{g,1,2}	39.8±0.2^{g,1}	39.8±0.2^{g,1,2}	39.5±0.1^{g,1,2}

Values presented are means ± S.E.M.
Values within a row sharing the same letter $^{(b\text{-}g)}$ do not differ significantly (P>0.05).
Values within a column sharing the same number $^{(1\text{-}3)}$ do not differ significantly (P>0.05).

Figure 7. Telemetered body temperatures of six water shrews voluntarily diving in 3-30 ^{o}C water. Prior to and after each trial, shrews were placed inside temporary holding containers that were furnished in a manner similar to their permanent holding tank (see text for details).

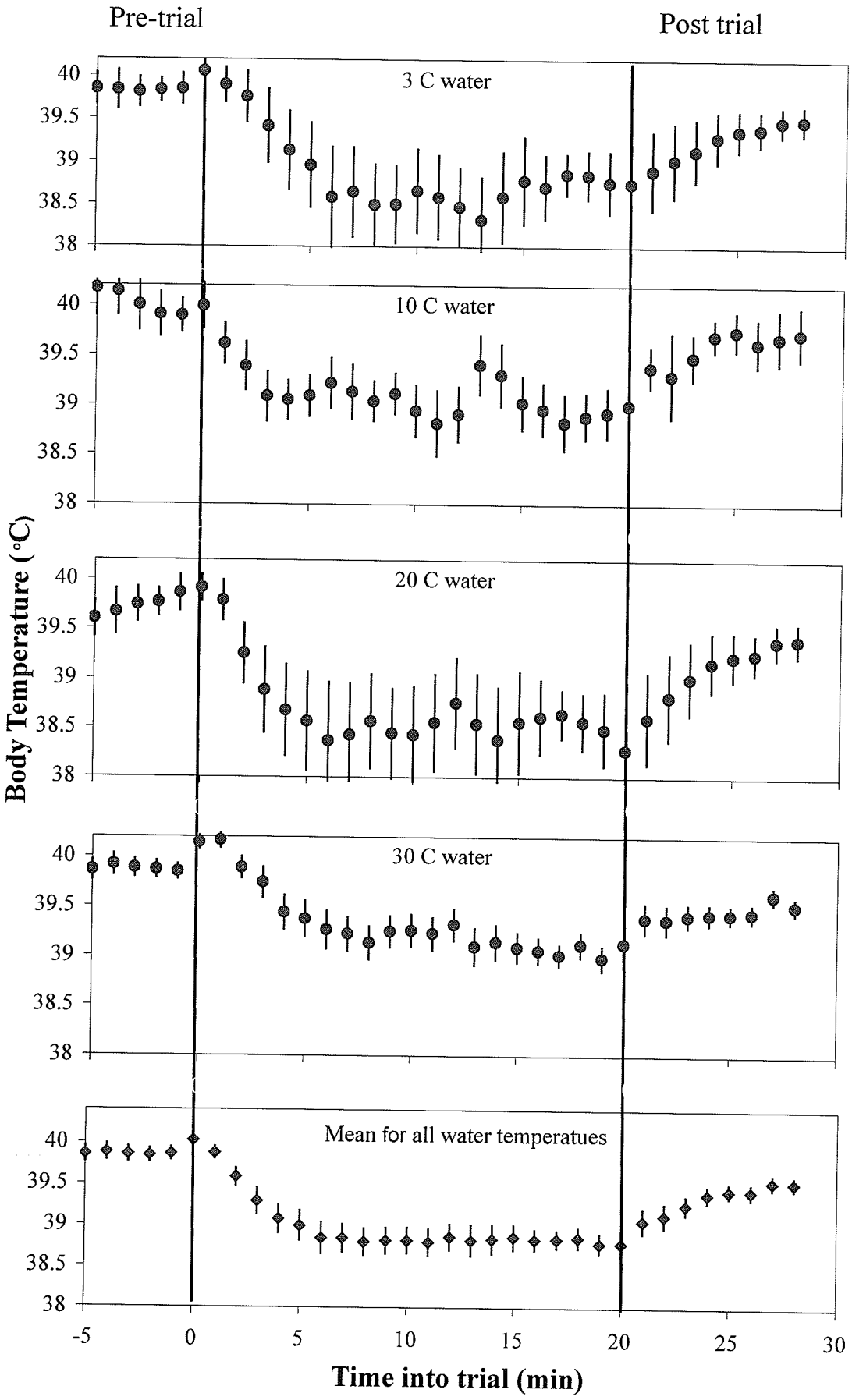

Pre-trial
Post trial
3 C water
10 C water
20 C water
30 C water
Mean for all water temperatues
Body Temperature (°C)
40
39.5
39
38.5
38
-5
0
5
10
15
20
25
30
Time into trial (min)

Table 3: A comparison of the dive performance of adult American water shrews with and without a surgically implanted abdominal temperature transmitter (1.0 g). Dive data for each animal are pooled values from trials completed in 3, 10, 20 and 30 °C water.

	Mass (g) (without transmitter)	Mean dive duration (s)	Mean dive frequency (dives min^{-1})[a]	Mean deep dive duration (s)	Mean deep dive frequency (dives min^{-1})[a]	Longest dive (s)	Dive:surface ratio	Total time in water per trial (s)
Without transmitter								
Shrew 1	16.3	5.1	0.39	8.8	0.05	12.2	0.21	132.0
Shrew 2	13.6	5.8	0.48	13.9	0.04	23.7	0.21	160.0
Shrew 5	14.0	6.4	0.85	8.5	0.41	13.2	0.26	170.5
Shrew 8	17.8	4.2	0.76	7.8	0.08	13.9	0.13	193.0
Shrew 9	13.3	5.3	0.94	8.5	0.20	11.7	N/A[b]	163.9
Shrew 10	15.6	4.1	0.53	7.4	0.10	12.6	0.31	83.9
MEAN	**15.1±0.8**	**5.2±0.4**	**0.66±0.10**	**9.2±1.1**	**0.15±0.06**	**14.6±2.0**	**0.22±0.03**	**150.5±17.0**
With transmitter								
Shrew 12	15.1	4.3	0.85	7.8	0.13	14.8	0.11	198.0
Shrew 13	14.1	5.9	0.40	8.1	0.14	16.7	0.10	108.2
Shrew 14	15.2	4.3	0.70	5.9	0.14	9.8	0.21	151.0
Shrew 15	14.0	6.3	1.00	8.8	0.29	20.1	0.33	185.8
Shrew 16	14.0	4.1	0.44	6.1	0.05	10.2	0.19	80.7
Shrew 17	14.2	7.0	0.58	9.7	0.14	14.5	0.22	167.8
MEAN	**14.4±0.3**	**5.3±0.6**	**0.66±0.11**	**7.7±0.7**	**0.15±0.03**	**14.4±1.8**	**0.19±0.04**	**148.6±20.4**
P-value[c]	0.199	0.397	0.490	0.124	0.487	0.468	0.263	0.468

[a]Trial length was 20 min.
[b]Dive data for shrew 9 was from McIntyre 2000; dive:surface ratio was not reported for this animal.
[c]Calculated using 1-tailed Student's t-test.

non-implanted shrews tended to make longer deep dives than implanted animals (Table 3), the difference was not significant ($P = 0.124$). The frequency distribution of dives by implanted and non-implanted shrews was also compared for dives times of 0 to 15 s, using log likelihood ratios to ensure the calculated mean value was not influenced significantly by outliers. Again, no significant difference was found between implanted and non-implanted adult water shrews (G value = 2.78, d.f. = 5, $P > 0.50$; Appendix 7).

I also determined if the dive behaviour of water shrews was significantly influenced by the interaction of transmitter effect and T_w, using a 2-way ANOVA. There was no significant interaction effect on dive duration ($F_{3,40} = 1.013$, $P = 0.397$), dive frequency ($F_{3,40} = 0.304$, $P = 0.822$), dive:surface ratio ($F_{3,35} = 0.378$, $P = 0.770$), deep dive duration ($F_{3,30} = 1.182$, $P = 0.333$), deep dive frequency ($F_{3,40} = 0.032$, $P = 0.992$), dive:surface ratio of deep dives ($F_{3,20} = 0.409$, $P = 0.748$), the longest dive for each trial ($F_{3,40} = 0.659$, $P = 0.582$) or the total time in water ($F_{3,40} = 2.321$, $P = 0.0898$). Shrews implanted with a transmitter did spend, on average, 78 s less time diving than did the non-implanted animals in 30 °C water. However, water shrews implanted with a transmitter spent more time in water at the other three T_ws than non-implanted animals.

Diving metabolic rate

The costs of repetitive diving and re-warming were determined for 12 implanted water shrews (3 subadults, 9 adults) that completed 579 dives over 40 trials. Since there were not enough data points to statistically analyze each cohort separately, all calculated values are based on pooled $\dot{V}O_2$ data from both cohorts. T_b measurements were not obtained for 6 of the 40 trials, due to weak/absent signals from some transmitters. During

the 10-min period available for voluntary diving, shrews spent an average of 51 ± 7 s (8.5% of total time) and 78 ± 10 s (13% of total time) diving in 10 °C and 30 °C water, respectively (t = 2.24, d.f. = 38, P = 0.01). Mean dive times in 10 °C and 30 °C water were 3.8 ± 0.2 s (n = 256) and 5.4 ± 0.3 s (n = 269), respectively, (t = 2.54, d.f. = 32, P = 0.007) with an associated mean trial T_b of 38.9 ± 0.3 °C and 39.1 ± 0.2 °C (t = 0.47, d.f. = 32, P = 0.319). The mean dive duration of water shrews completing behavioural and metabolic diving trials were not statistically different at 10 °C (t = 1.36, d.f. = 33, P = 0.18) and 30 °C water (t = 0.15, d.f. = 33, P = 0.88).

There were a number of confounding factors that dramatically increased the shrew's $\dot{V}O_2$ while in the diving metabolic chamber, including level of activity, integrity of the air boundary in the fur and T_b. Shrews that became wet during the trial also tended to experience a greater drop in T_b than dry shrews (Table 4). Wet individuals were more likely to exhibit increased post-dive activity in the metabolic chamber than dry shrews. It was also observed that a third category of shrews were active in the metabolic chamber even though they completed few dives during the trial. These shrews displayed a heightened MR, exhibited little diving and had a minimal drop in T_b. To account for these factors, shrews completing a dive trial were categorized as dry, wet, or active (Table 4).

Pre-dive RMR did not differ statistically between 10 °C (4.11 ± 0.15 ml O_2 g^{-1} h^{-1}; n = 5) and 30 °C water (4.02 ± 0.14 ml O_2 g^{-1} h^{-1}; n = 8; t = 0.43, d.f. = 11, P = 0.337), although the associated T_a inside the metabolic chamber was significantly different at 19.2 ± 0.3 °C and 21.8 ± 0.3 °C, respectively (t = 5.93, d.f. = 11, P < 0.001). RMR data for both trials were pooled to derive an average RMR (4.06 ± 0.09 ml O_2 g^{-1} h^{-1}) for

Table 4: Diving metabolic rates (MR) and abdominal temperatures (T_b) of American water shrews voluntarily diving in 10 °C and 30 °C water.

	Water temperature (°C)					
	10			30		
	Dry pelage (n = 5)	Wet pelage (n = 7)	Active (n = 7)	Dry pelage (n = 9)	Wet pelage (n = 7)	Active (n = 3)
Total dive time (s)	68.4 ± 1.6[a]	54.1 ± 9.4[a]	28.5 ±7.4	93.1 ± 17.6[a]	85.3 ± 11.9[a]	38.4 ± 14.1
Resting metabolic rate (VO_2 ml O_2 g^{-1} h^{-1})	4.11 ± 0.15	4.11 ± 0.15	----	4.06 ± 0.09	4.06 ± 0.09	----
Pre-trial MR (VO_2 ml O_2 g^{-1} h^{-1})	5.61 ± 0.37[a]	8.69 ± 0.86[b]	9.17 ± 1.10	5.80 ± 0.31[a]	8.26 ± 0.94[b]	9.19 ± 0.93
Diving MR (VO_2 ml O_2 g^{-1} h^{-1})	8.77 ± 0.39[a]	12.76 ± 1.13[b]	11.05 ± 1.21	6.57 ± 0.27[a]	11.12 ± 1.06[b]	10.34 ±1.49
Post-trial MR (VO_2 ml O_2 g^{-1} h^{-1})	8.05 ± 0.31[a]	12.25 ± 1.11[b]	10.83 ± 1.20	6.50 ± 0.33[a]	11.48 ± 1.38[b]	11.59 ± 2.02
Pre-dive T_b (°C)	39.73 ± 0.26[a]	39.47 ± 0.19[a]	40.02 ± 0.41	40.06 ±0.18[a]	39.43 ± 0.20[b]	40.29 ± 1.16
Lowest T_b in trial (°C)	37.79 ± 0.29[a]	37.03 ± 0.16[b]	39.41 ± 0.37	39.00 ± 0.21[a]	37.14 ±0.27[b]	38.97 ± 0.80
T_b at end of diving (°C)	38.0 ± 0.33[a]	37.33 ± 0.22[a]	39.65 ±0.36	39.16 ± 0.23[a]	37.33 ± 0.35[b]	38.99 ± 0.77
T_b after re-warming (°C)	39.17 ± 0.14[a]	38.38 ± 0.21[b]	39.16 ± 0.53	39.57 ± 0.20[a]	37.70 ± 0.36[b]	39.02 ± 0.04

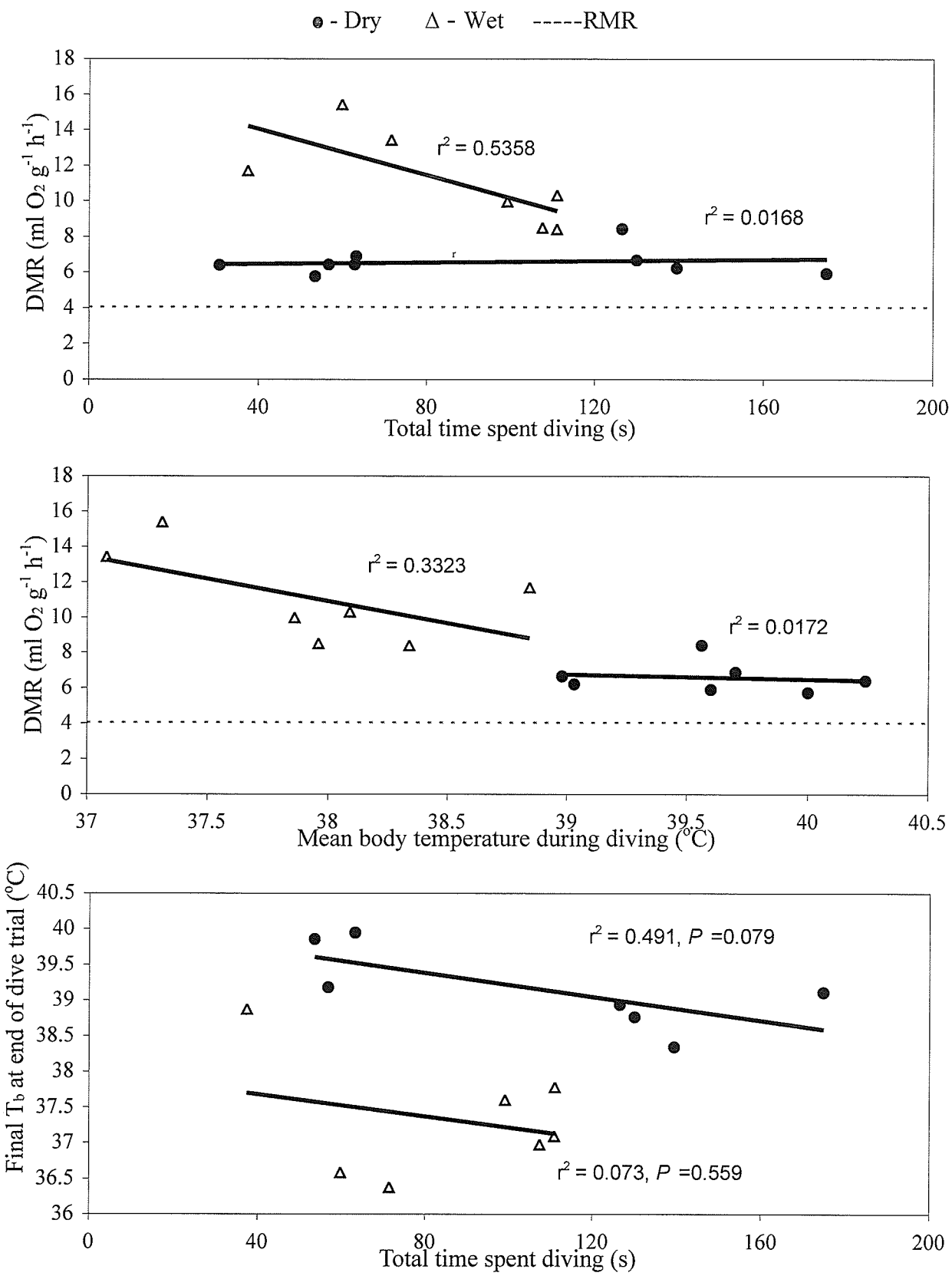
Values presented are means ± S.E.M., n = number of shrews sampled.
For each water temperature, means sharing the same letter within a row are not statistically different (P>0.05).

comparison with DMR values. These resting periods had an average duration of 110 ± 10 s ($t = 0.58$, d.f. = 11, $P = 0.283$) and with a mean T_b of 39.9 ± 0.2 °C ($t = 0.042$, d.f. = 11, $P = 0.483$).

At T_w = 30 °C, the DMR of dry shrews was 6.57 ± 0.27 ml O_2 g^{-1} h^{-1}, while shrews that became wet had a significantly higher DMR of 11.12 ± 1.06 ml O_2 g^{-1} h^{-1} (t = 5.031, d.f. = 14, $P < 0.0001$; Table 4). This 1.69-fold increase in DMR for wet animals occurred even though dry and wet shrews spent a similar time diving ($t = 0.366$, d.f. = 14, $P = 0.72$). Shrews that became wet also had a lower T_b at the end of the diving session (t = 4.733, d.f. = 12, $P < 0.0001$) and re-warmed to a lower T_b during the post-trial recovery period, compared to dry shrews ($t = 4.897$, d.f. = 12, $P < 0.0001$). For both wet and dry shrews, there was no statistical difference between the DMR and post-dive MR within each group, but wet shrews had a post-dive MR that was 1.77-fold greater than for dry shrews (Table 4). DMR did not correlate with the total submergence time of water shrews ($r^2 = 0.058$, $P = 0.367$), but did vary inversely with the mean T_b of the shrew during the diving portion of the trial ($r^2 = 0.668$, $P < 0.0001$; Figure 8).

Dry water shrews diving in 10 °C water had a mean DMR of 8.77 ± 0.39 ml O_2 g^{-1} h^{-1} while shrews that became wet had a DMR that was 1.45-fold greater (12.76 ± 1.13 ml O_2 g^{-1} h^{-1}, Table 4). Shrews that remained dry throughout the trial did spend more time diving than wet shrews, but the difference was not significant ($t = 0.844$, d.f. = 10, P = 0.419). Wet and dry shrews had a similar T_b at the start of the trial ($t = 0.932$, d.f. = 10, P = 0.373), but wet shrews experienced a significantly greater drop in T_b during diving (t = 2.749, d.f. = 10, $P = 0.021$) and also re-warmed to a significantly lower final T_b than dry animals ($t = 3.132$, d.f. = 10, $P = 0.011$). Wet shrews tended to be more active than dry

Figure 8. The relationship of diving metabolic rate (DMR) to total submergence time and mean body temperature (T_b) of American water shrews voluntarily diving in 30 °C. The relationship between the final T_b recorded during each diving session and total submergence time is also presented for water shrews having a dry (●) and wet (Δ) pelage.


● - Dry
Δ - Wet
-----RMR
DMR (ml O2 g-1 h-1)
r2 = 0.5358
r2 = 0.0168
Total time spent diving (s)
r2 = 0.3323
r2 = 0.0172
Mean body temperature during diving (°C)
Final Tb at end of dive trial (°C)
r2 = 0.491, P =0.079
r2 = 0.073, P =0.559
Total time spent diving (s)

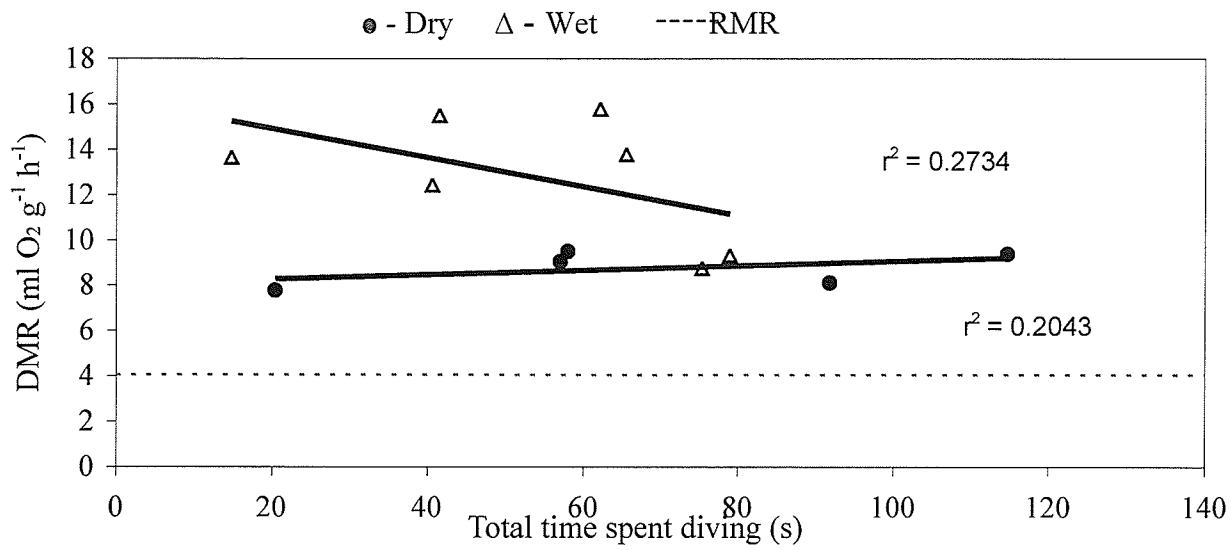
shrews in the metabolic chamber, which was likely reflected in their higher estimate of DMR. As with 30 °C water, dry and wet shrews did not exhibit a significant increase in post-dive MR (Table 4). In fact, for both wet and dry animals, post-dive MR was lower than the DMR, while wet shrews had a post-dive MR of 1.52x that of dry shrews (Table 4). Post dive analysis determined that DMR of dry shrews did not increases with total time diving ($r^2 = 0.203$) and mean T_b during diving ($r^2 = 0.227$; Figure 9). Though not statistically significant, T_b at the end of the dive trial varied inversely with total time diving in both wet and dry shrews (Figure 9).

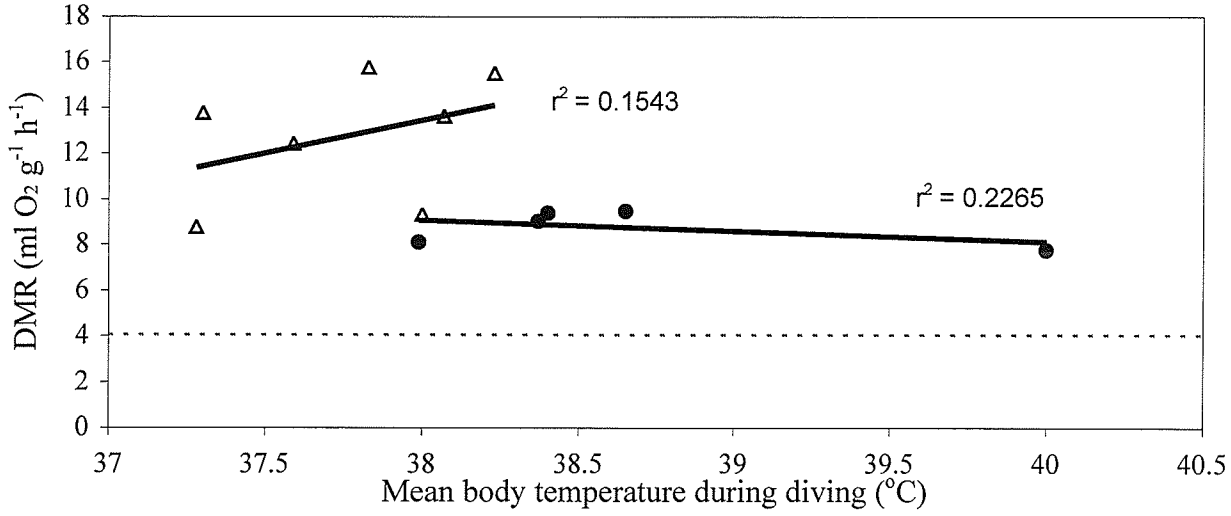
Measured DMR values of 9.02 ± 0.37 ($n = 4$) and 6.77 ± 0.40 ml O_2 g^{-1} h^{-1} ($n = 6$) were used to estimate the cADL at 10 °C and 30 °C water, respectively. These are mean values derived only for measurements from water shrews that remained dry throughout the trial and dove for at least 10% of the 10 min trial. The DMR of water shrews diving in 10 °C and 30 °C water was 2.21x and 1.67x greater than their respective pre-dive RMR while the mean DMR for shrews diving in 10 °C water was 1.33x greater than in 30 °C water.

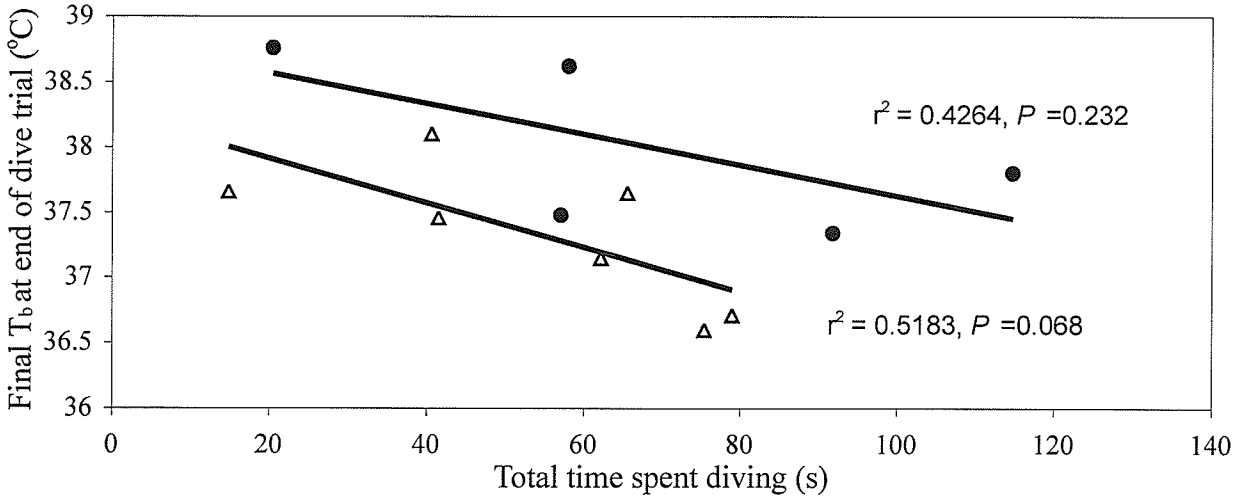
Cost of an individual dive

Costs of single dives were measured from 12 shrews that completed 39 trials and dove 119 times. For 24 of these dives, there was little or no activity during the post-dive recovery period. The remaining 95 dives were discarded due to the occurrence of significant post-dive motor activity. The pre-dive RMR averaged 3.86 ± 0.12 ml O_2 g^{-1} h^{-1} and did not differ significantly from the RMR of 4.06 ± 0.09 ml O_2 g^{-1} h^{-1} obtained in earlier diving trials ($t = 1.363$, d.f. = 23, $P = 0.185$). The majority of dives with no post-

Figure 9. The relationship of diving metabolic rate (DMR) to total submergence time and mean body temperature (T_b) of American water shrews voluntarily diving in 10 °C water. Comparisons are made for animals diving with dry (●) and wet (Δ) fur. Dashed line denotes resting metabolic rate (RMR) of dry shrews at thermoneutrality.


● - Dry Δ - Wet ----RMR
DMR (ml O2 g-1 h-1)
Total time spent diving (s)
r2 = 0.2734
r2 = 0.2043


DMR (ml O2 g-1 h-1)
Mean body temperature during diving (°C)
r2 = 0.1543
r2 = 0.2265


Final Tb at end of dive trial (°C)
Total time spent diving (s)
r2 = 0.4264, P =0.232
r2 = 0.5183, P =0.068

dive activity were of short duration (< 5 s), with only two dives > 5 s. All dives were performed near the surface, just under the plexiglas cover, with a mean submergence time of 3.41 ± 0.47 s (n = 24; N = 5). The mean cost of an individual dive (4.53 ± 0.07 ml O_2 g^{-1} h^{-1}) was 1.17 x RMR and was 1.49-fold lower than the estimated DMR at 30 °C (6.77 ml O_2 g^{-1} h^{-1}) used to determine the cADL. The cost of a single dive increased significantly with dive duration (r^2 = 0.317, P = 0.004; Figure 10).

Testing for adaptive hypothermia

Dive duration was plotted against T_b to determine if shrews with lower T_bs dove longer in cold water. To maximize the data set, dive data were combined for shrews completing voluntary dive trials in 3 °C and 10 °C water and shrews completing DMR trials in 10 C water (n = 309). A statistically significant negative relationship was found between dive duration and T_b (r^2 = 0.0795, P < 0.0001; n = 309; Figure 11). The upper T_b limit was then changed to 39.6 °C, based on the mean maximum value measured during 24-h terrestrial activity trials (Gusztak *et al.* 2005). This was completed to account for the "artificial" elevation in T_b at the onset of the trial due to handling stress, while also minimizing the influence of short exploratory dives which coincide with the start of the trial. Again, a statistically significant negative relationship was determined (r^2 = 0.0278, P = 0.0158; n = 209). In contrast, dive frequency significantly decreased with decreasing T_b, except at the highest T_b which also had a lower dive frequency ($F_{4,301}$ = 37.461, P < 0.001; Figure 11). Similar to the bADL calculation, a lower T_b threshold, or behavioural temperature dive limit (BTDL), was calculated. The BTDL corresponds to the T_b threshold below which only 5% of all voluntary dives were observed. The BTDL

Figure 10. Relationship between the metabolic cost of a single dive and dive duration in five American water shrews voluntarily diving in 30 °C water (n = 21 dives; see text for details). RMR = resting metabolic rate at thermoneutrality.

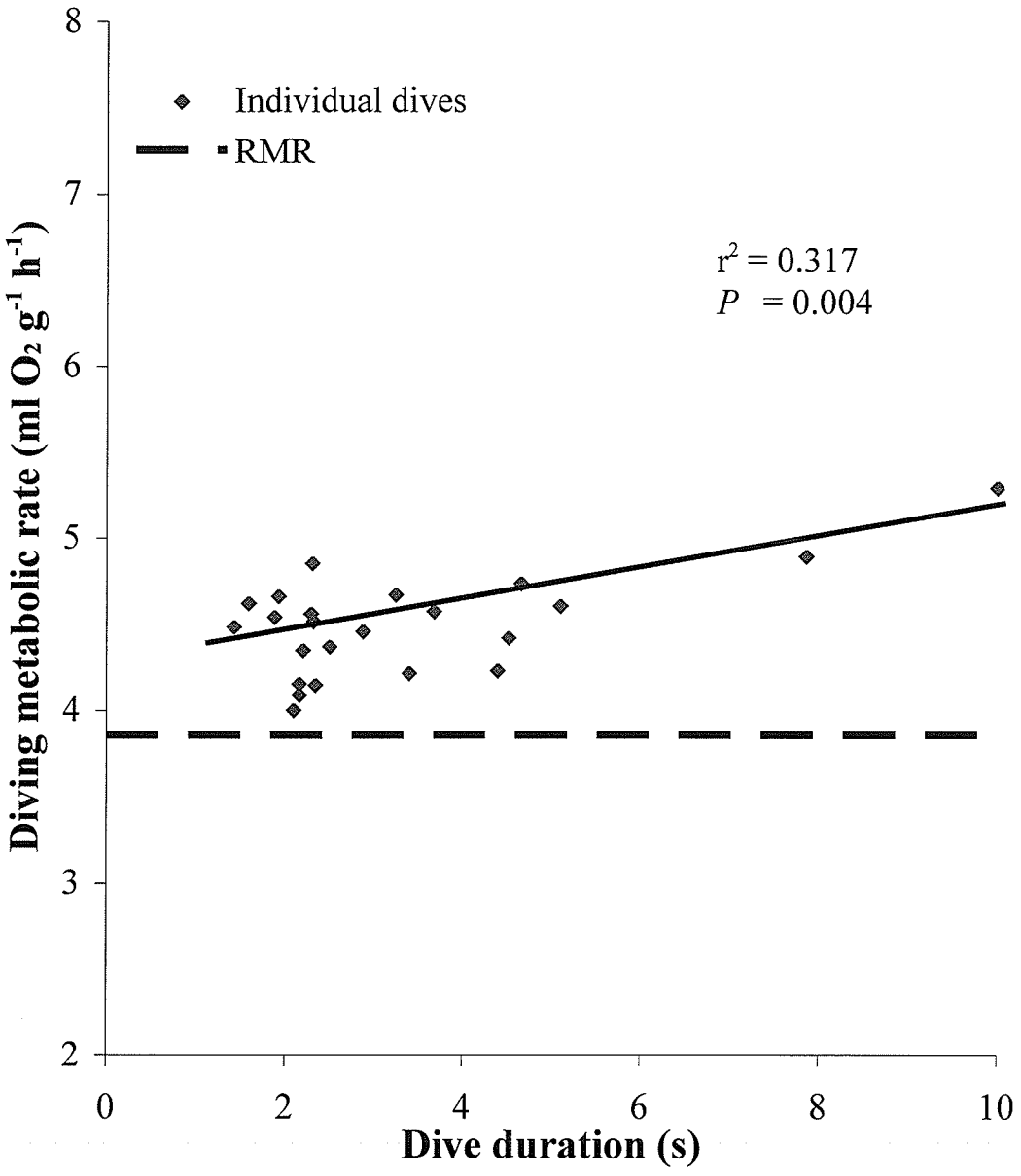
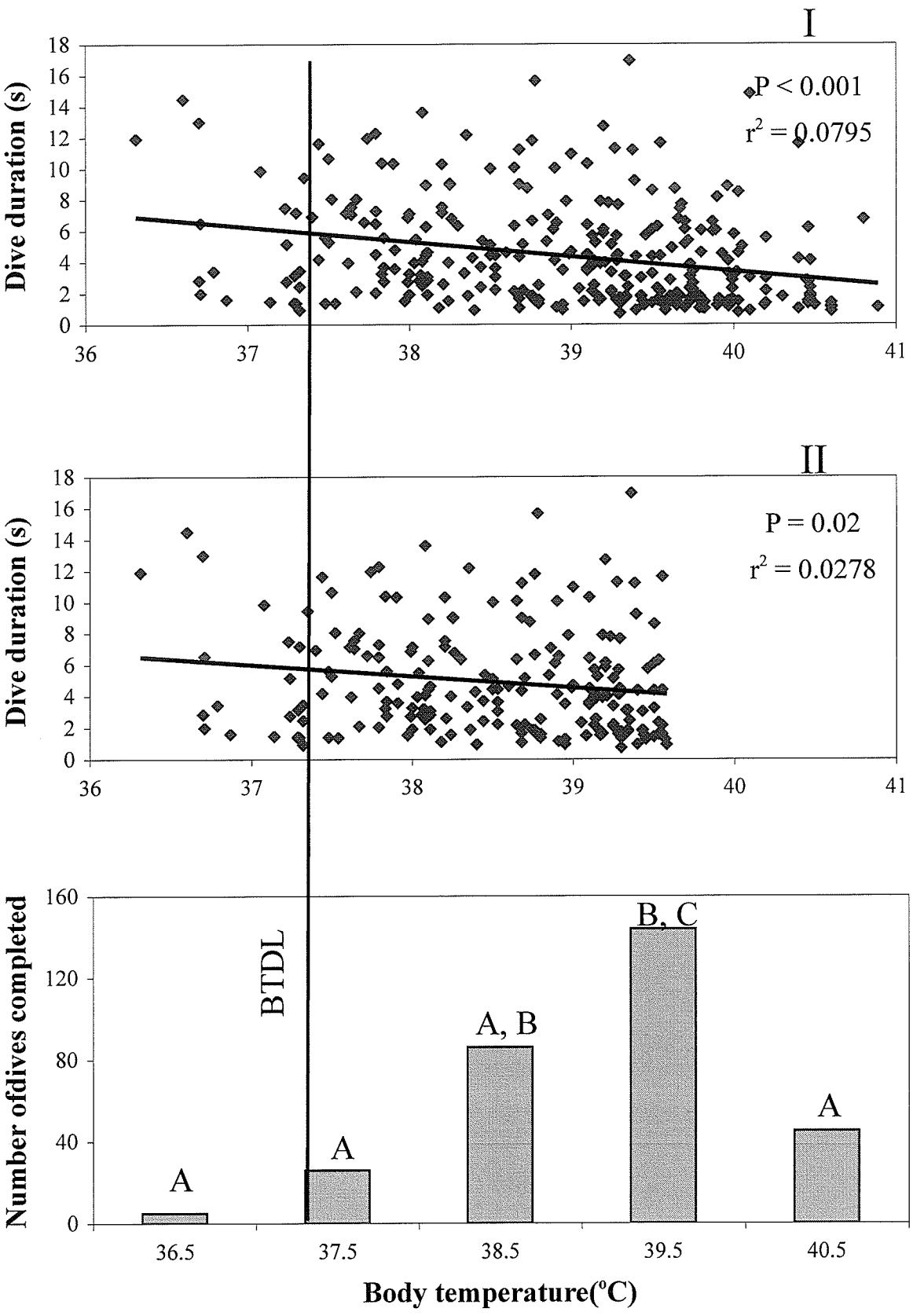

Individual dives
RMR
$r^2 = 0.317$
$P = 0.004$
Diving metabolic rate (ml O_2 g^{-1} h^{-1})
Dive duration (s)
8
7
6
5
4
3
2
0
2
4
6
8
10

Figure 11. Pooled behavioural and body temperature (T_b) data for American water shrews implanted with a 1.0-g intraabdominal transmitter and voluntarily diving in 3 °C and 10 °C water. The relationship between dive duration and core T_b is shown for all data combined (I) and only those cases when $T_b \leq 39.6$ °C (II). The bottom figure shows the frequency distribution of T_b recordings. Frequency distributions with different letters differ significantly from each other ($P < 0.05$). The behavioural temperature diving limit (BTDL) is shown by the solid line at 37.3 °C. See text for details.

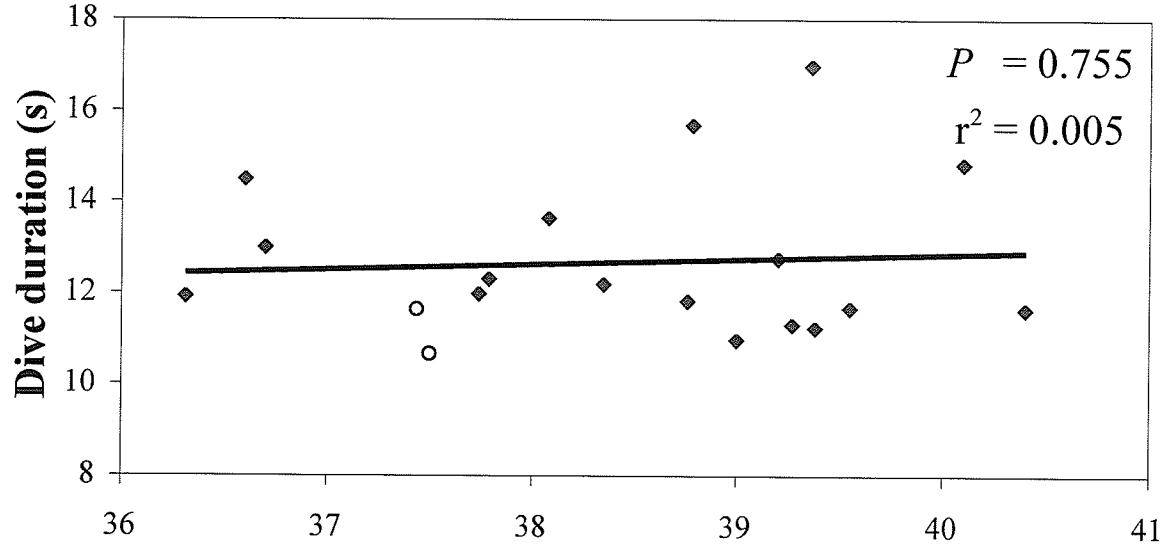

I
Dive duration (s)
P < 0.001
r² = 0.0795
II
Dive duration (s)
P = 0.02
r² = 0.0278
Number ofdives completed
BTDL
A
A
A, B
B, C
A
36.5
37.5
38.5
39.5
40.5
Body temperature(°C)

for *S. palustris* was 37.29 °C for all dives combined (n = 309) or 37.23 °C (n = 209) if only T_b values ≤ 39.6 °C were included (Figure 11). The adaptive hypothermia hypothesis attempts to explain why some divers routinely exceed their cADL. The cADL of water shrews diving in 10 °C is 10.7 s. Thus, a regression analysis between dive duration and T_b was completed for all dives ≥ 10.7 s to examine if shrews exceeded the cADL to a greater extent with a lower T_b (Figure 12). The majority of these longer dives were completed in 10 °C water (n = 17) compared to 3 °C water (n = 17), so the cADL estimate represents most of these data. Regardless of T_b, shrews displayed no significant increase in dive duration for dives ≥ 10.7 s (r^2 = 0.006, P = 0.755; n = 19), and had no significant increase in dive frequency with lower T_bs ($F_{4,14}$ = 2.105, P = 0.448; Figure 12).

Body oxygen stores and muscle buffering capacity

Mb concentration did not differ significantly between forelimb and hindlimb samples for either adults or subadults in the two shrew species sampled (Table 5). The mean (forelimb and hindlimb) Mb concentration (mg g of wet tissue^{-1}) of adult water shrews (6.04 ± 0.25; N = 9) was significantly higher than in subadults (3.76 ± 0.12; N = 10; t = 7.0743, d.f. = 18, P < 0.0001) and nearly 2x greater than for adult short-tailed shrews (3.03 ± 0.12; N = 9; t = 7.624, d.f. = 17, P < 0.0001). Skeletal muscle buffering capacity exhibited a similar trend, with adult water shrews having a higher value (38.22 ± 2.28 β; N = 13) than either subadult water shrews (30.67 ± 2.34 β; n = 11; t = 2.395, d.f. = 22, P = 0.012) or adult short-tailed shrews (24.88 ± 1.40 β; n = 10; t = 4.808, d.f. = 21, P < 0.0001).

Figure 12. Pooled behavioural and body temperature (T_b) data for American water shrews implanted with a 1.0-g intraabdominal transmitter and voluntarily diving in 3 °C (o) and 10 °C (◆) water. The relationship between dive duration and core T_b is shown only for dives greater than the calculated aerobic dive limit at 10 °C water (10.7 s) for *S. palustris*. Frequency distributions with different letters differ significantly from each other ($P < 0.05$). See text for details.


Dive duration (s)
18
16
14
12
10
8
36
37
38
39
40
41
P = 0.755
r2 = 0.005

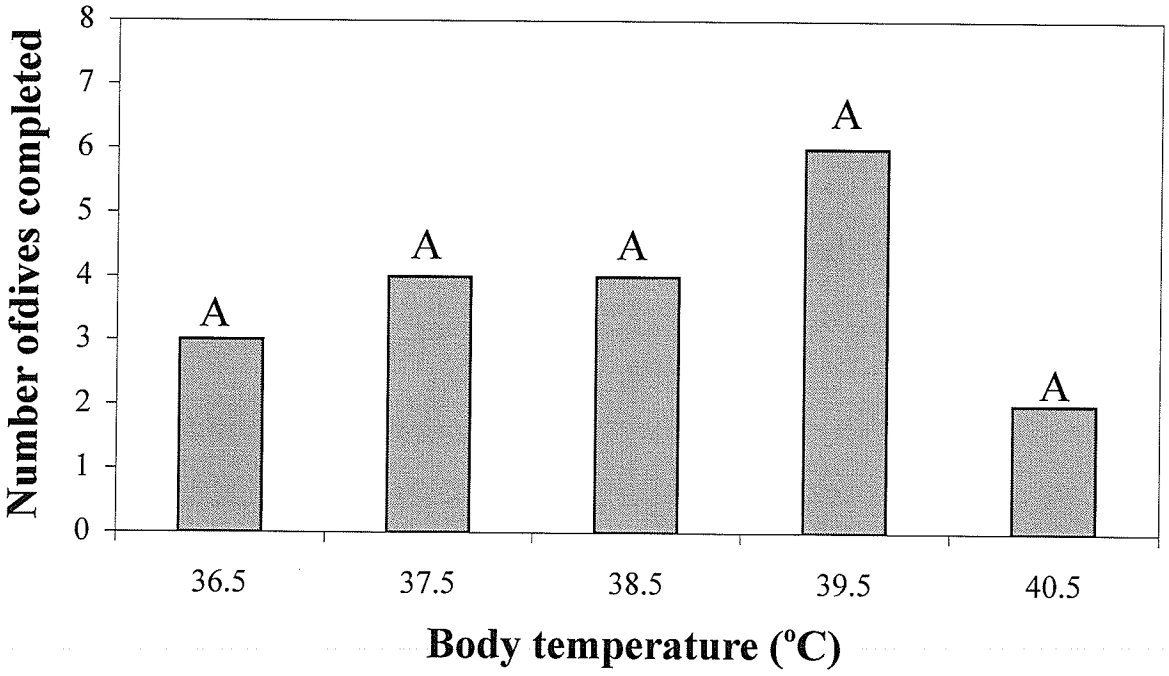

Number ofdives completed
8
7
6
5
4
3
2
1
0
A
A
A
A
A
36.5
37.5
38.5
39.5
40.5
Body temperature (°C)

Table 5: Respiratory characteristics of Short-tailed and American water shrews. Values are presented as means ± S.E.M., with number of animals sampled indicated in parentheses.

Variable	American water shrew		Short-tailed shrew	
	Subadult	Adult	Adult	Subadult
Body mass (g)	13.00±0.45 (20)	14.57±0.36 (21)[a]	24.26±1.41 (10)[b]	21.78±0.13 (7)
% total body muscle mass	31.74±4.51 (4)	41.45±3.45 (8)	28.09±2.56 (2)[b]	---------
(mg g wet tissue $^{-1}$)				
Forelimb	3.75±0.22 (10)	6.18±0.31 (10)[a]	2.98±0.32 (7)[b]	---------
Hindlimb	3.76±0.16 (10)	5.89±0.45 (9)[a]	3.01±0.40 (6)[b]	---------
Skeletal muscle (mg g wet tissue^{-1})[χ]	3.76±0.12 (10)	6.04±0.25 (9)[a]	3.03±0.26 (9)[b]	2.70±0.49 (7)
Heart	10.97±1.22 (5)	9.47±0.63 (6)	8.77±0.55 (5)	8.77±0.79 (5)
Buffering capacity, β[γ] (slykes)	30.67±2.34 (11)	38.22±2.28 (13)[a]	24.88±1.40 (10)[b]	25.05±1.92 (6)
Total lung volume (ml STPD 100 g^{-1})	4.55±0.18 (9)	4.57±0.28 (5)	3.34±0.07 (6)[b]	3.43±0.11 (5)
Hematocrit (%)	50.57±1.33 (10)	50.23±1.33 (15)	48.78±2.50 (8)	50.17±2.50 (7)
Hemoglobin content (g 100 ml^{-1})	19.69±1.02 (8)	20.09±0.44 (13)	17.29±0.62 (7)[b]	16.87±1.54 (6)
Blood O_2 capacity (vol %)	26.38±1.37 (8)	26.93±0.58 (13)	23.18±0.84 (7)[b]	22.61±0.14 (6)

[a] Values for adult and subadult water shrews are significantly different ($P<0.05$).
[b] Values for adult water shrews and adult short-tailed shrews are significantly different ($P<0.05$).
[χ] Mean Mb of forelimb and hindlimb muscles.
[γ] Slyke = μmoles of base required to titrate the pH of 1 g of wet muscle by 1 pH unit.

Obtaining lung volume measurements of water shrews proved challenging due to difficulty cannulating the trachea and excising the preparation from the carcass while not perforating the lungs. Recorded lung volumes of adult (4.57 ± 0.28 ml STPD 100 g^{-1}; N = 5) and subadult (4.55 ± 0.18 ml STPD 100 g^{-1}; N = 9) water shrews were similar (Table 5). These values are ca. 1.1x greater than predicted by allometry (Stahl 1967) for a mammal of 15.20 g (adult) and 14.13 g (subadult). Lung volumes of adult short-tailed shrews (3.34 ± 0.07 ml STPD 100 g^{-1}; N = 6) were significantly less than those recorded for adult water shrews (t = 7.401, d.f. = 9, $P < 0.0001$) and 6% less than that predicted by allometry for a 22.8 g mammal.

Total blood oxygen capacity of adult and subadult water shrews was high, averaging 26.57 ± 0.82 and 25.57 ± 0.43 vol %, respectively (Table 5). Adult water shrews also had a significantly higher blood oxygen capacity than adult short-tailed shrews (23.18 ± 0.84 vol %; t = 0.896, d.f. = 9, $P < 0.0001$). Hct levels were high in both adult water shrews and short-tailed shrews, and did not differ statistically between the two species (t = 0.735, d.f. = 21, $P < 0.235$). The calculated total O_2 storage capacity of summer-caught adult and subadult water shrews was 27.13 and 24.48 ml O_2 STPD kg^{-1}, respectively (Table 6). The mass-specific O_2 storage capacity of adult water shrews was 1.19x greater than that of summer-caught adult short-tailed shrews (Table 6). The largest single contributor to total O_2 stores in adults of both shrew species was O_2 bound to Hb in the blood, accounting for 61 and 67 % in water shrews and short-tailed shrews, respectively.

Table 6: Oxygen storage capacities of the lungs, blood and skeletal muscle of adult and subadult American water shrews (*Sorex palustris*) and adult short-tailed shrews (*Blarina brevicauda*).

		Oxygen stores (ml O_2 STPD kg^{-1})					Calculated aerobic dive limit (s)[a]	
							Water temperature (°C)	
Species	N	Lung	Arterial Blood	Venous Blood	Muscle	Total	10	30
S. palustris								
Adult	6	6.6	6.3	10.2	4.0	27.1	10.7	14.2
Subadult	3	6.3	6.1	9.9	2.1	24.5	9.6	12.8
B. brevicauda	2	4.8	5.9	9.3	2.8	22.7		

[a]Calculated for a 14.0 g water shrew assuming a diving metabolic rate of 9.02 ml O_2 g^{-1} h^{-1} (10 °C water) and 6.77 ml O_2 g^{-1} h^{-1} (30 °C water; see text for details).

Calculated aerobic dive limit

Assuming that diving animals fully deplete their O_2 reserves before initiating anaerobic respiration, and using the DMR estimate of 6.77 ml O_2 g^{-1} h^{-1} in 30 °C water, adult and subadult American water shrews have cADLs of 14.2 and 12.8 s, respectively (Table 6). This calculation is consistent with the estimated bADL at 30 °C (12 s), determined from voluntary dive profiles (n = 303 dives; Figure 6). Further, the cADL of water shrews was reduced by ca. 33 % when diving in 10 °C compared to 30 °C water, due to the increased DMR (9.02 ml O_2 g^{-1} h^{-1}). The cADL for adult and subadult water shrews in 10 °C water is 10.7 and 9.6 s, respectively (Table 6). Again, these calculated limits closely match the bADL (10 s) determined for water shrews diving in 10 °C water (n = 258 dives). Only 3.1 % and 2.3 % of voluntary dives in 10 °C and 30 °C water, repectively, exceeded the cADLs for adult *S. palustris*.

Discussion

Dive performance

Captive *S. palustris* exhibited a mean voluntary dive duration of 5.1 s, which is virtually identical to the value (5.2 s) reported by McIntyre (2000) for a single captive shrew, and is consistent with the range (3-6 s) reported for *N. fodiens* diving in captivity (Churchfield 1985, Ruthardt and Schröopfer 1985). Vogel (1998) suggested that dive times of captive water shrews are probably shorter than those of shrews in the wild due to a small tank size and absence of aquatic prey. The familiarity of the dive tank to the water shrew may also have affected dive behaviour. Köhler (1991) noted that *N. fodiens* would usually dive in a novel setting only after exploring the surface of the aquatic environment. Similar, cautious pre-dive behaviour was observed in *S. palustris* during voluntary dive trials. After becoming familiar with the terrestrial section followed by the dive tank's water surface, the shrew would usually complete a series of short, exploratory dives (1-2 s) before initiating longer dives. Short exploratory dives at the onset of the trial in captivity have also been recorded for double-crested cormorants, *Phalacrocorax auritus* (Enstipp *et al.* 2006).

In this study, tank size should have been adequate to determine the overall dive performance of the water shrew, since the dive tank used was one originally designed for studying beaver. This study thus likely demonstrates an accurate representation of the water shrew's dive ability, since voluntary dive times ranged from 1 to 23 s, but the dive frequency in this study is likely skewed towards more shorter dives than expected under natural conditions. It is interesting to note that the mean dive duration of water shrews significantly increased when placed in an artificial riverbank environment with 3 °C

water for a 24-h period (Appendix 8). The same dive tank was used as in the voluntary dive trials, but was modified with an artificial riverbank attached to one side and river-washed rocks were placed at the bottom of the tank. Adjacent to the aquatic area was a terrestrial tank furnished in a similar fashion as the regular holding tanks. Connecting the two areas was a piece of plexiglas tubing. Dive time was recorded via video and analyzed at a later date. In this setup, all prey items were placed in the water, ensuring that the shrews would dive. The mean voluntary dive duration in this setup was 6.88 ± 0.13 s (N = 3; n = 227; Appendix 8). This value is significantly greater than the mean dive endurance of 3.8 s for water shrews voluntary diving in 3 °C water (t = 12.25, d.f .= 482, P < 0.0001; Table 1) and also greater than the overall mean dive duration of all dives combined (5.1 s; t = 7.92, d.f. = 809, P <0.0001). The dive data in Appendix 8 also has a bell curve distribution with a rather abrupt drop in dive duration between 10-11 s. These dive data yielded a bADL of 10.7 s, which is identical to the cADL determined for adult water shrews diving in 10 °C water and further suggests strict adherence to an aerobic diving schedule in this species (see below).

Even under laboratory conditions, *S. palustris* is an impressive diver compared to other small-bodied divers. Another semi-aquatic insectivore, the star-nosed mole, is 4-5x larger (50-60 g) than the water shrew, yet exhibits a mean dive duration that is only 1.8 times greater (9.2 s; McIntyre *et al.* 2002). The dipper (*Cinclus mexicanus*) is the smallest avian diver (ca. 50 g) and its mean recorded voluntary dive time in a natural riparian environment is comparable to that of star-nosed moles and water shrews (5-15 s; Murrish 1970). Likewise, the much larger 325-450 g Bufflehead (*Bucephala albeola*) has a mean dive time of only 12.5 s (Gauthier 1993), while mink (850 g) have a mean

dive duration of just 9.9 s (Dunstone and O'Connor 1979). Juvenile (300-500 g) and adult muskrats (650-900 g) recorded similar average dive times ranging from 19.2-22.0 s while a mean of 31.3 s was recorded for 1.5-2.0 kg free-ranging platypus, *Ornithorhynchus anatinus*, diving in a lake (Bethge *et al.* 2003).

As previously mentioned, captive American water shrews have the capacity to dive much longer than the recorded mean, as 14 % of all dives exceeded 9 s. Deep dives comprised 19.6 % of all dives, and on average, were 1.6x longer than the overall mean dive duration. The longest dive per trial (10.3 s) was 2.0x greater than the mean dive time (Table 1). Similar individual maximums of 8.3 to 15.6 s were recorded for *N. fodiens* diving in captivity (Köhler 1991). It is interesting to note that the mean maximum dive time for the star-nosed mole was only 7 s greater than the water shrew (17.7 s; McIntyre *et al.* 2002), but that of juvenile and adult muskrats were much longer at 51-86 s and 121-224 s, respectively, presumably due to a heightened ability to decrease DMR in the latter species (MacArthur *et al.* 2001).

Prior to this study, very little was known about the voluntary breath-hold capacity of *S. palustris* (Calder 1969, McIntyre 2000). Calder (1969) evaluated "dive duration" using forced-dive methods that timed the duration of submergence until the shrew stopped moving. He determined a mean maximum dive time of 37.9 s (max value of 47.7 s) for *S. palustris*, a value twice the maximum voluntary dive time observed in this study. Calder's conflicting values may be explained in part by the different pathophysiology associated with voluntary versus forced dives (Davis *et al.* 1999, Davis *et al.* 2004). Foremost, an exaggerated dive response is elicited by forced dives due to the body's urgent response to conserve O_2. Accordingly, forced dives lead to extreme

bradycardia followed by strong peripheral vasoconstriction as the body tries to shunt blood to the organs essential for survival, namely the brain and heart (Zapol *et al.* 1979). These are all basic defenses against asphyxia in which, in the case of a forced dive, prolong the underwater survival time of the diver by conserving O_2 for the most oxygen-sensitive organs. It follows that other tissues must substantially decrease their O_2 consumption or use anaerobic means to obtain energy. The dive response associated with voluntary dives also includes bradycardia and peripheral vasoconstriction, but the response is less intense than forced dives, since the peripheral tissues are thought to rely primarily on aerobic metabolism (Davis *et al.* 2004). Thus, the maximum dive endurance of *S. palustris* recorded by Calder would not be experienced routinely in nature, but may occur if a shrew became disoriented under ice. Further, the maximum dive time of 23.7 s recorded in this study for the American water shrew is virtually identical to that (24 s) recorded for the slightly larger European water shrew that was observed diving in a 2-m deep stream (Scholetch 1980).

Influence of transmitter implants on dive behaviour

In this study, transmitter mass (1.00-1.16 g) did not surpass the recommended upper limit (10%; Brander and Cochran 1967), but came close in some of the lighter shrews, as transmitter mass ranged from 6.7-9.7% of total body mass. However, adult water shrews equipped with a transmitter did not show any significant changes in dive performance compared to transmitter free animals (Table 3). The mean deep dive duration was the variable with the greatest (though not statistically significant) difference between implanted (7.7 s) and non-implanted (9.2 s) animals. If the additional mass of

the transmitter did have an adverse effect on deep dives, then the deep dive frequency and duration of the longest dive should also have been negatively influenced since the longest dive was usually a deep dive. Instead, these variables were nearly identical between the two groups. The longer mean deep dive duration for non-implanted shrews can be attributed, in part, to the impressive dive performance of shrew #2, which also exhibited the longest voluntary breath-hold capacity of any shrew in the study. This may have been due to an elevated O_2 storage capacity corresponding to an increased blood volume associated with pregnancy and lactation (Mattlin *et al.* 1998), since three weeks prior to completing voluntary dive trials, shrew #2 had weaned three shrews born in captivity (Gusztak and Campbell 2004). Crocker *et al.* (2001) noted that lactating New Zealand sea lions (*Phocarctos hookeri*) exhibited the longest dive duration for any otariid (eared seals), with 44% of dives surpassing the cADL. Although shrew #2 was no longer lactating, she still may have been in a heightened postpartum physiological state that could account for the increased mean deep dive duration.

There was also no significant interaction effect of T_w and transmitter status on any of the dive performance variables. Shrews implanted with a transmitter did spend an average of 33% (79 s) less time in 30 °C water than non-implanted shrews. But implanted shrews had a greater overall time in water (diving and swimming) for the other T_ws.

The large amount of air trapped in the pelage of the water shrew was probably the most important factor accounting for the similarity in dive performance of the two groups. McIntyre (2000) measured the volume of air bound to the pelage in the water shrew and recorded a mass-specific air capacity of 0.35 ml g^{-1}, which is greater than in

the star-nosed mole (0.19 ml g^{-1}; McIntyre 2000). This coincides with American water shrews also having a lower specific gravity (0.761), or stronger buoyant force in water, compared to star-nosed moles (0.826) and muskrats (0.952; MacArthur 1992, McIntyre 2000), but less than European water shrews (0.726 Köhler 1991). The relatively large amount of air trapped in the pelage of *S. palustris* would lead to increased energetic costs of submerging to depth, but also allow quicker ascents. It would also decrease the energetic cost of surfacing with large prey and shorten the travel time while ascending the water column, thereby lessening the probability of prey escaping. For instance, *S. palustris* in captivity was observed to routinely captured 2-3 g crayfish from the bottom of a 30 cm tank and effortlessly return them to the surface. Likewise, Köhler (1991) recorded that *N. fodiens* could pick up a 10 g (max 12.8 g) snail shell filled with lead from the bottom of a tank and carry it to land. It is reasonable to suggest then, that the large buoyancy created by the insulative air layer can easily compensate for a water shrew diving with an implanted 'onboard mass' of 1 g, even though it is almost 1/10th its mass.

Influence of water temperature on dive behaviour and body temperature

Many small amphibious divers decrease or limit core T_b cooling behaviourally, by exiting the water to re-warm. As expected, water shrews demonstrated behavioural thermoregulation, since it is energetically more efficient to prevent large drops in T_b than incur the costs of re-warming (MacArthur 1989). Based on voluntary dive trials in 3, 10, 20 and 30 °C water, shrews did not consistently show a statistically significant difference

in any measured variable across adjacent T_ws. Instead, most variables examined showed a gradual positive relationship between behavioural indices of dive performance and increasing T_w. However, there was a large difference in dive performance between the two extreme T_ws. Comparing 3 and 30 °C water, *S. palustris* experienced a 2-fold increase in the total time spent in water, a 1.5-fold increase in dive duration, a 1.6-fold increase in the longest dive duration and a 1.4-fold increase in deep dive duration. Further, deep dive frequency was almost 3x greater in 30 °C than in 3 °C water while the dive frequency for all dives was 1.2x greater (the only non-significant variable between the two T_ws). Many other small-bodied semi-aquatic mammals and birds have shown a similar positive correlation of dive duration and frequency with T_w (MacArthur 1984a, de Leeuw *et al.* 1999, McIntyre *et al.* 2002). As mentioned, the air boundary surrounding the water shrew adds buoyancy, but more importantly, acts as a protective insulator to limit transfer of heat to the water (Vogel 1990, Köhler 1991). Dive depth and hydrostatic pressure vary linearly, so that birds and mammals with a compressible air layer experience a reduction in insulation as hydrostatic pressure rises with depth. The end result is increased peripheral cooling (Wilson *et al.* 1992). Further loss of the air layer is cause by rapid movements of the appendages while diving, and since deep dives are significantly longer than surface dives, more air should be jettisoned.

Rates of cooling calculated for water shrews completing voluntary swimming and diving was completed over the first 5 min of each trial (Table 2) and averaged 0.92 °C/min in 10 °C water. The first 5 min of each trial was chosen because shrews completed a significantly higher number of dives (Figure 3) and had the quickest and largest drop in T_b (Figure 7) during this portion of the diving trial. Using rectal

thermocouples, Calder (1969) calculated cooling rates of *S. palustris* in 10 °C water to be 2.06 °C/min (swimming) and 2.46 °C/min (diving). Shrews in this study likely had lower rates of cooling because much effort was taken to keep the pelage of the shrews in optimal condition, and so, maintain a larger air layer while submerged.

Regardless of T_w, water shrews showed a similar trend in T_b throughout all voluntary dive trials, including initial cooling, a plateau and then increasing T_b with re-warming after transfer to a holding container. Likewise, a single star-nosed mole diving in the same apparatus showed a comparable T_b profile (McIntyre 2000).

Water shrews in this study likely had an artificially elevated T_b (40.0 ± 0.7 °C) at the start of each dive trial, since wild-caught *S. palustris* housed in a terrestrial environment for 24-h trials had a mean maximum T_b of 39.6 ± 0.2 °C that correlated with peak activity (Gusztak *et al.* 2005). Thus, despite my precautions, the pre-trial increase in T_b observed in this study is likely a stress response associated with transfer of the shrew from the holding container to dive tank. Regardless of T_w, shrews experienced the greatest drop in T_b during the first 5 minutes of a diving trial. They also completed >50% of all voluntary dives during this interval (Figure 3). These telemetered T_b data suggest that water shrews experience cooling no matter the T_w, and have likely maximized heat production and total peripheral resistance in order to limit a drop in core T_b, similar to other amphibious small-bodied divers (MacArthur 1989). Body temperature then plateaued for the remaining 15 min, demonstrating some variability between T_ws that was not statistically significant (Table 2). In most trials, water shrews maintained a T_b >37.5-38.0 °C by decreasing aquatic activity. This suggests that after water shrews have fully engaged vasopressor responses to limit heat loss, they rely principally on behavioural

thermoregulation to limit a further decline in T_b and avoid immersion hypothermia. Both free-ranging muskrats and beavers were observed to maintain core T_b within ± 1 °C of normothermic T_b throughout most semi-aquatic activity, and similarly used behavioural thermoregulation to limit body cooling (MacArthur 1979, Dyck and MacArthur 1992).

Diving metabolic rate

Consistent with allometric predictions, the American water shrew has the highest recorded DMR of any diver (6.57 ml O_2 g^{-1} h^{-1} in 30 °C water). The high DMR of *S. palustris* reflects its inherently high mass-specific BMR (Gusztak *et al.* 2005), possibly combined with a high buoyancy when submerged and a disproportionately high drag while diving, due to its high mass-specific surface area (de Leeuw 1996). However, the DMR of *S. palustris* is 1.6 x RMR, which is less than star-nosed moles (2.1 x RMR; McIntyre *et al.* 2002) and muskrats (2.7 x BMR; MacArthur and Krause 1989) measured under similar protocols, but similar to sea otters diving and capturing prey (1.6 x RMR on water; Yeates *et al.* 2007).

It is of interest to note that using the DMR calculated for a single dive, the ratio of DMR to RMR was similar for water shrews (1.2 x RMR) and sea otters (1.3 x RMR on water), and in both mammals, a positive relationship between DMR and dive duration was recorded (Figure 10; Yeates *et al.* 2007). For *S. palustris*, this DMR estimate for individual dives should be taken as an absolute minimum cost of submergence. This is because all such dives were completed along the surface of a plexiglas cover in 30 °C water, with many being shorter than the recorded mean dive duration. Further, Enstripp *et al.* (2006) reported for double-crested cormorants (*Phalacrocorax auritus*) that

increasing dive depth from 1 to 10 m resulted in a large increase in DMR than either a decrease in T_a or T_w. Water shrews would have also likely shown this relationship due to the effects of hydrostatic pressure on air insulation, and also suggests the DMR estimate for individual dives is not representative of normal foraging. Instead, this DMR is likely representative of a short exploratory dive, but also may suggest that the cost of swimming directly under "ice" is surprising low.

There are many factors that affect DMR estimates, including T_w, T_b, dive depth and duration, swim velocity, drag and post-dive activity (Sparling and Fedak 2004, Green *et al.* 2007). Indeed, accurate estimates of DMR have proved challenging due to the many variables influencing it (Ponganis *et al.* 1993). As it is not technically feasible to obtain instantaneous DMR estimates, post-dive MR is assumed to reflect the costs of the previous duration submerged. Estimations of DMR for any semi-aquatic species will also be affected by the metabolic cost associated with any post-dive grooming or activity within the metabolic chamber. The DMR estimate of 6.57 ± 0.27 ml O_2 g^{-1} h^{-1} for *S. palustris* was determined for dry animals diving in 30 °C water with a having a mean diving T_b of 39.0-40.2 °C, a mean dive duration of 5.4 s and with 79 % of dives occurring 1-2 cm below the plexiglas cover (the other 21 % diving to 30 cm depth). In 10 °C water, the estimated DMR for dry water shrews with a mean T_b of 37.9-40.0 °C, an average dive time of 3.75 s and completing 14% of all dives to a depth of 30 cm was 8.77 ± 0.39 ml O_2 g^{-1} h^{-1}. Thus, a 1.3-fold increase in DMR was associated for dry shrews diving in the colder water. This value is slightly lower than for muskrats that exhibited a 1.7- to 2.0-fold increase in DMR when diving in 3-10 °C water compared to 20-30 °C water (MacArthur 1984a).

The greatest influence on DMR for *S. palustris* was the integrity of the pelage while diving. In 10 °C and 30 °C water, wet shrews experienced 1.5-1.7 fold increase in DMR, compared to dry shrews, respectively, even though wet and dry shrews dove for similar time in both T_ws. As expected, wet shrews experienced a greater drop in T_b than dry shrews, but still maintained a mean minimum $T_b > 37.0$ °C, and re-warmed to a lower mean T_b (37.3 °C) by the end of the trial, albeit at a slower rate than dry shrews (Table 4). Vogel (1990) noted that European water shrews only became wet after being in captivity and attributed it to holding conditions that did not afford tunneling in moss to ensure optimal maintenance of the pelage. Even though much effort was put into care and maintenance of *S. palustris* in captivity, about half of shrews become wet during the diving trials suggesting the pelage was not in optimal condition. However, the few American water shrews seen diving in the wild have not appeared wet (Conaway 1952). Thus, it is unlikely shrews would become water logged in nature, and these data stress the importance of a water shrew maintaining the insulative qualities of its pelage in order to prevent these increased thermoregulatory costs.

No significant positive relationship was observed between total time diving and DMR for dry shrews in either 10 °C or 30 °C water, which is in contrast to the significant positive relationship between individual dive time and DMR. Further, DMR for dry shrews paralleled the RMR regardless of time in water for both T_ws. The shrews diving in 10 °C and 30 °C water spent the majority of the time (ca. 80-90%) in the metabolic chamber which will confound the DMR estimate with that of terrestrial activity. The DMR estimate for 10 °C and 30 °C water was 1.56x and 1.13x that of the pre-trial metabolic rate at a T_a of 22 °C (Table 4). Since the water shrew has an inherently

elevated mass-specific rate of heat production, the extra cost associated with submergence in 30 °C water appears to be only slightly greater than its MR associated with activity while in the thermal neutral zone (Gusztak *et al.* 2005).

There was also no significant increase in post-dive MR relative to DMR for wet or dry water shrews diving in either 10 °C or 30 °C water. In fact, there was a slight decrease in some cases. This suggests that some costs associated with re-warming were likely met during the inter-dive periods, and involved grooming and heat generated from motor activity. Further, during the post-dive recovery portion of the trial, shrews maintained a similar, or slightly decreased MR compared to the DMR, even though they had not fully re-warmed to pre-trail T_bs. This suggests that if shrews experience a mild to moderate decline in T_b (1.5-2.5 °C below pre-trails values) that they will utilize heat produced by terrestrial motor activity to gradually re-warm, instead of greatly elevating their MR to re-warm more quickly.

Although no trials were specifically designed to test the adaptive hypothermia hypothesis in the American water shrew, voluntary dive data in 3 °C and 10 °C water were combined with core T_b and analyzed to determined if shrews utilize hypothermia to increase dive endurance. Consistent with this hypothesis, water shrews did show a statistically significant relationship between increased dive duration and a decrease in core T_b (Figure 11). However, statistically significant fewer dives were completed at lower T_bs. Notably, shrews freely diving in 3 °C water in a semi-natural, semi-aquatic environment over 24 hrs, also revealed a similar trend ($r^2 = 0.0461$, $P = 0.0103$; $F_{3,138} = 224.895$, $P < 0.001$; Appendix 9). For water shrews to demonstrate "adaptive hypothermia", a drop in core T_b must be accompanied by an increase in dive capability.

Thus, shrews should show an increased dive duration and dive frequency with decreasing T_b. Dive duration increased, but dive frequency dropped significantly in both laboratory and semi-natural environments. Butler and Jones (1997) proposed the adaptive hypothermia hypothesis to explain why some divers consistently dive longer than their cADL or can greatly exceed it. A regression analysis completed for dives only exceeding the cADL at 10 °C water (10.7 s) and T_b did not show a significant correlation ($r^2 = 0.05$, $P = 0.755$, $n = 19$; Figure 12). The same analysis was completed for shrews in the semi-natural environment diving. A lower cADL was assumed (9.7 s) since shrews were diving in 3 °C water, and a similar statistically insignificant regression was calculated ($r^2 = 0.001$, $P = 0.871$, $n = 28$; Appendix 10). Further, dive frequency did not significantly correlate to T_b in either the laboratory (Figure 12) or seminatural environment ($F_{2,26} = 48.414$, $P = 0.057$; Appendix 10). Thus, these last two analyses suggest that *S. palustris* does not utilize hypothermia to extend its dive endurance past its cADL or increase dive frequency while hypothermic.

Further, all T_b measurements were recorded after the dive had been completed, so if shrews had completed a long deep dive and incurred increased cooling, they would be more likely to exit the water for an extended period of time to re-warm, hence the observed drop in dive frequency. It was observed that during a foraging bout, shrews would complete multiple diving sorties before returning on land to re-warm. Once on land, shrews would incur a dramatic drop in core T_b, reflecting the peripheral cooling incurred throughout the foraging bout (Gusztak, unpub. obs.)

The decrease in dive frequency at lower T_b occurred in both the semi-natural and laboratory setting (Appendix 9; Figure 11), and exemplifies the strict thermoregulatory

control *S. palustris* exhibits during aquatic activity. I propose a new term, the behavioural temperature dive limit (BTDL), which is analogous to the bADL, and indicates the threshold differentiating the upper 95% and lower 5% of core T_b measurements associated with voluntary diving. It is noteworthy that the calculated BTDL in the semi-natural setting (37.29 °C; n = 142) is the same as in the laboratory trials (37.23 °C; n = 209) and that these values are similar to the mean lowest T_b recorded during 24-h terrestrial activity (37.5 °C; Gusztak *et al.* 2005). Further, the highest recorded T_b for a shrew diving in the semi-natural setting (39.6 °C) was identical to the mean maximum T_b obtained in semi-natural terrestrial settings. This strongly suggests, that like the semi-aquatic muskrat and beaver, the water shrew regularly maintains T_b within a ± 1 °C range for terrestrial and aquatic activity, while also using behavioural thermoregulation to avoid immersion hypothermia (MacArthur 1979, Dyck and MacArthur 1992).

Body oxygen stores and muscle buffering capacity

Divers are known for their physiological adaptations to increase breath-hold capacity, especially those relating to their disproportionately large body oxygen stores (Butler and Jones 1997, McIntyre *et al.* 2002). Not surprisingly, the mass-specific TBOSC of the water shrew was almost 1.2x greater than that of the strictly terrestrial short-tailed shrew (Table 6).

Many researchers in this field consider the primary indicator of a diver's breath-hold capacity to be its muscle oxymyoglobin concentration (Kooyman and Ponganis 1998, Ponganis *et al.* 1999), but *S. palustris* appears not to follow this trend. The skeletal

muscle Mb concentration of adult water shrews (0.604 g 100 g^{-1}; Table 5) is half that recorded for star-nosed moles (1.36 g 100 g^{-1}; McIntyre *et al.* 2002), muskrats (1.21-1.38 g 100 g^{-1}; MacArthur *et al.* 2001) and beaver (1.2 g 100 g^{-1}; McKean and Carlton 1977). Conversely, skeletal Mb concentrations of adult water shrews are 2-4x that of strictly terrestrial short-tailed shrews (0.303 g 100 g^{-1}; Table 5; 0.16 g 100 g^{-1} Stewart *et al.* 2005). Similarly low Mb concentrations have been recorded for the 1.8 g Etruscan shrew, *Suncus etruscus* (0.15 g 100 g^{-1}; Jürgens 2002).

Mb concentration of skeletal muscle is correlated to muscle fiber type (Ordway and Garry 2004), and although the latter has not been studied in *S. palustris*, it has been examined for other shrews. Jürgens (2002) studied the muscle fibers in *S. etruscus* and the musk shrew, *Crocidura russula*, and concluded that they had exclusively Type II fast-twitch fibers in their extensor digitorum longus and soleus muscles and no Type I slow-twitch fibers. Functionally, Type I fibers are important for maintaining posture, and become more important as size increases, since gravitational forces are proportional to body mass (Jürgens 2002). Notably, Mb concentration correlates with fiber type, with Mb levels following the pattern: Type IIB < Type IIA < Type I muscle fibers (Ordway and Garry 2004). Thus, the low Mb levels recorded in shrews likely arises from their paucity of Type I muscle fibers. This also suggests, however, that the elevated Mb concentration of the water shrew muscle compared to other shrews is probably an adaptation to diving. Overall, Mb stores in *S. palustris* account for only 14.7% of total body O_2 stores, which is similar to that of humans (15%), but substantially lower than the average reported for pinnipeds (33%; Kooyman 1989).

The water shrew is able to compensate for its low skeletal muscle Mb concentration, yet still exhibit an elevated TBOSC, owing to the potential gain in O_2 stores in the lungs and, especially, the blood. The mean lung volume of adult water shrews (4.57 ml STPD 100 g^{-1}; Table 5) was ca. 1.1x greater than predicted by allometry for a 15.2 g mammal (Stahl 1967). Further, this species has a mass-specific pulmonary O_2 storage capacity that is 1.37x greater than for adult short-tailed shrews. The mass-specific lung volume of the American water shrew is also larger than for other shrews including, *N. fodiens*, *Sorex minutus*, and *Suncus etruscus* with respective volumes of 3.56, 3.81 and 4.02 ml STPD 100 g^{-1} (Gehr *et al.* 1980). These moderately increased lung O_2 stores of *S. palustris* could serve to increase buoyancy, as well as provide an important source of O_2 while diving. However, lung volume of *S. palustris* is almost half than that of the adult star-nosed moles (8.1 ml STPD 100 g^{-1}; McIntyre *et al.* 2002).

By far, the most important source of O_2 for a diving water shrew is its large reserve in the blood, which comprises 61 % of its O_2 storage capacity. This is reflected in the high Hb (20.0 g 100 ml^{-1}) and Hct (50.2 %) values, with the former resulting in a mean blood O_2 capacity of 26.9 vol % for an adult water shrew. Many species of shrews examined to date have recorded high Hb (range: 15-18 g 100 ml^{-1}) and Hct (range: 45-50%) values, some of which are near the upper limits recorded for any mammal (Wolk 1974, Gehr *et al.* 1980). Even so, the blood O_2 capacity recorded for the American water shrew (26.9 vol %) is the highest value recorded of any shrew, including the European water shrew (23.9 vol %; Wolk 1974), the Eutruscan shrew (23.3 vol %; Bartels *et al.* 1979) and adult short-tailed shrew (23.1 vol %; Table 5). Adult water shrews also exhibited a higher blood O_2 capacity than other semi-aquatic divers studied to date,

including adult star-nosed moles (23.0 vol %; McIntyre *et al.* 2002) and muskrats (24.1 vol %; MacArthur *et al.* 2001). But these values were substantially lower than for more highly specialist divers such as white whales (28.0 vol %; Shaffer 1997) and adult harbour seals (30.0-32.2 vol %; Burns *et al.* 2005).

Buffering capacity of skeletal muscle has been shown to increase with increasing body mass and is also important for prolonging burst activity in species utilizing sprinting (Castellini *et al.* 1981, Hochachka and Mommsen 1983). Specifically, elevated buffering capacity is correlated with Type IIA and IIB fast-twitch muscle fibers, which as expected, are found in higher concentrations in mammals using burst activity (Abe 2000, Nakagawa and Hattori 2002). In anaerobic or severely hypoxic conditions, as may be encountered during prolonged diving or for shorter periods of time during sprinting, glycolysis is the only means of ATP production, albeit an inefficient one. This process is inhibited if intracellular pH drops too low, therefore intracellular buffers are critical to ensure an optimum pH for glycolysis to occur while exercising in low O_2 environments.

Adult water shrews have a skeletal muscle buffering capacity (38.2 β) that is 1.2x greater than for subadult water shrews and significantly greater (1.5-fold) than for adult short-tailed shrews. Other semi-aquatic species have a similar buffering capacity, including adult platypus (38.2 β; Evans *et al.* 1994), semi-aquatic adult star-nosed moles (44.1 β; McIntyre *et al.* 2002) and adult summer-caught muskrats (51.5 β; MacArthur *et al.* 2001). Since shrews have primarily Type IIA and IIB muscle fibers, it is expected that they would have an increased buffering capacity compared to animals with a greater proportion of Type I fibers (Abe 2004). The ecological benefit of an increased buffering capacity in *S. palustris* could be important in terrestrial and, especially, aquatic

environments for both capturing prey and escaping predation. An increased buffering capacity also gives support to the tenet that shrews use a flush-pursuit method of foraging as proposed by Catania *et al.* (2008). Allometric scaling of glycolytic enzymes to mass predicts that water shrews should have the poorest ability of any diver to utilize anaerobic glycogenolysis (Emmett and Hochachka 1981, Hochachka *et al.* 1987). Although glycolytic enzymes were not specifically examined in this study, it seems counterproductive to invest in increasing buffering capacity while not having a modest glycolytic benefit. Glycolytic pathways probably do not play a large role in the majority of dives completed by water shrews, since few voluntary dives exceeded the cADL, but may still be critically relied upon at times. For instance, shrews likely relied on glycolytic pathways to complete forced dives of up to a maximum of 47.7 s in 10-12 °C water (mean = 37.9 s, Calder 1969). This duration is ca. 4-5-fold greater than the cADL of shrews diving in 10 °C water and suggests that water shrews can dramatically increase dive duration, if required.

Aerobic dive limits and diving behaviour

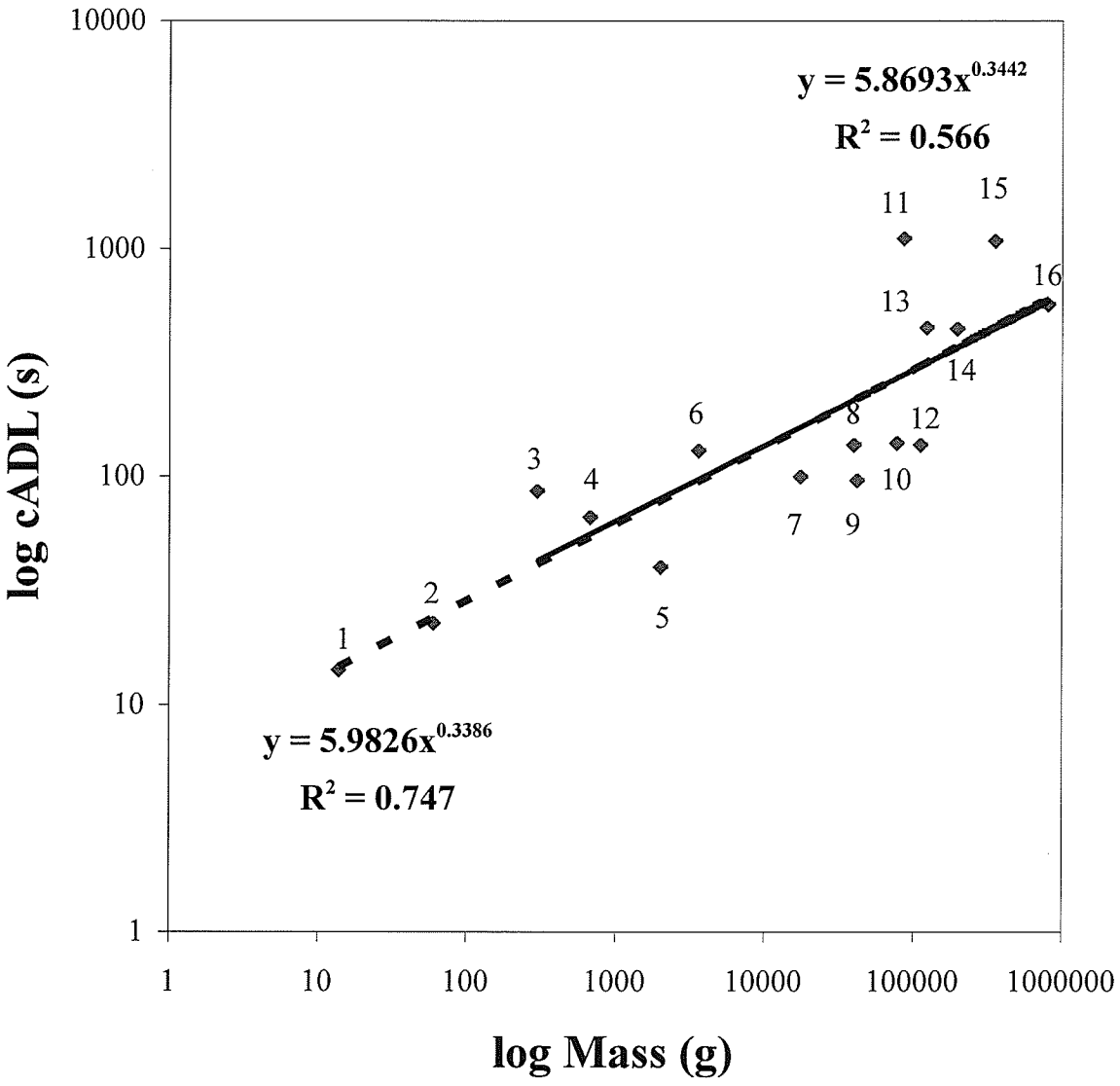
As predicted by allometry, the diminutive size (12-17 g) of *S. palustris* severely limits its aerobic dive endurance. Indeed, these shrews possess the smallest total oxygen storage capacity, highest mass-specific DMR, and lowest cADL of any diver studied to date (Tables 4 and 5). Moreover, water shrews have the highest surface-area-to-volume ratio of any endothermic diver, a fact that should make it extremely susceptible to immersion hypothermia (MacArthur 1989). It is truly remarkable then, that this small insectivore can efficiently utilize aquatic foraging as a means to sustain its inherently

elevated rate of heat production, since aquatic foraging is known to be one of the most energetically costly methods of foraging (Fish 2000). This energetic burden is demonstrated by the fact that the metabolism of the American water shrew requires it to consume at least its body weight in prey on a daily basis (Gusztak *et al.* 2005).

The water shrew rarely dove longer than its cADL, an observation consistent with studies of many other diving mammals (MacArthur *et al.* 2001, McIntyre *et al.* 2002, Butler 2006). Only 3.1 % and 2.3 % of dives by adult water shrews in 10 °C and 30 °C water exceeded their cADLs of 10.7 s and 14.2 s, respectively. The water shrew has a dive endurance that is relatively brief, but in an ecological context, may enable appreciable underwater swim distances in search of prey. For instance, using a cADL of 14 s and assuming that *S. palustris* has an underwater swimming speed similar to that of *N. fodiens* (52 cm s^{-1}; Köhler 1991) and is diving for benthic prey in 1-m deep water, an estimate of underwater foraging range can be calculated. A conservative estimate of transit time to the bottom and back to the surface is 6 s, leaving 8 s during which the shrew could travel up to 4.0 m in search of prey, while maintaining an oxygen-based metabolism. This is a substantial foraging distance for the water shrew, as it is equivalent to ca. 28 body-lengths. Approached from another perspective, if the cADL of *S. palustris* is scaled up to the mass of a 375 kg Weddell Seal, the resulting cADL is close to 106 hrs!

To determine if the aerobic breath-hold capacity of *S. palustris* follows allometric predictions established for other divers (Schreer and Kovacs 1997), log cADL was plotted against log mass (range 14 g – 800 kg) for a number of diving species for which cADL has been determined (Figure 13). A regression line fitted by the method of least squares predicts cADL = mass*$5.9826^{0.3386}$ (mass in g). Allometry predicts aerobic dive

Figure 13. Relationship between the log calculated aerobic dive limit and log body mass of diving mammals ranging from 14 g to 800 kg. Regression analysis was completed with data points 1 and 2 (dotted line) and without (continuous line). 1 American water shrew (this study), 2 star-nosed mole (McIntyre *et al.* 2002), 3 juvenile muskrat (MacArthur *et al.* 2001), 4 adult muskrat (MacArthur *et al.* 2001), 5 platypus (Bethge *et al.* 2003), 6 macaroni penguin (Green *et al.* 2003), 7 harbour seal pups (Jorgensen *et al.* 2001), 8 California sea lion (Ponganis *et al.* 1997c), 9 Antarctic fur seal (Costa *et al.* 2001), 10 Australian sea lion (Costa *et al.* 2001), 11 juvenile elephant seals (Irvine *et al.* 2000), 12 New Zealand sea lion (Costa *et al.* 2001), 13 Weddell seal pup (Burns and Castillini 1996), 14 juvenile leopard seal (Kuhn *et al.* 2006) 15, adult Weddell seal (Ponganis *et al.* 1993), 16 white whale (Shaffer *et al.* 1997). See text for details.


10000
1000
100
10
1
log cADL (s)
y = 5.8693x^0.3442
R² = 0.566
y = 5.9826x^0.3386
R² = 0.747
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
1
10
100
1000
10000
100000
1000000
log Mass (g)

endurance to increase with body mass according to the 0.25 power, but this value seems to underestimate breath-hold capacity of diving mammals since an exponent of 0.35 was calculated. The greater than predicted scaling exponent likely arises from the elevated body oxygen stores of divers compared to non-diving species (McIntyre *et al.* 2002). The cADL of the water shrew falls on the best-fit regression line, suggesting that the aerobic dive capacity of *S. palustris* is consistent with that of other divers, when corrected for body mass differences (Figure 13). This conclusion is supported by the observation that the best-fit line is not affected by the removal of the water shrew and star-nosed mole from the calculation ($cADL = mass*5.8693^{0.3442}$).

The relevance of determining cADL for certain diving species has been called into question since some large-bodied, benthic divers tend to have a significant number of dives exceeding their cADL. For instance, deep diving Australian and New Zealand sea lions routinely exceeded their cADLs by 1.4 and 1.5-fold, respectively (Costa *et al.* 2001). Meanwhile the shallow-diving Antarctic fur seal routinely dove to only 80% of its cADL, suggesting that calculating the ADL for this species yields a good representation of its natural diving behaviour, unlike Australian and New Zealand sea lions (Costa *et al.* 2001). Small amphibious divers < 2 kg submerge to relatively shallow depths and have less interspecific variability in dive depth than larger species, but have also been noted to rarely dive beyond their cADL. For instance, the star-nosed mole (50 g) and muskrat (680 g) only exceeded their cADLs during 2.9 % and 6 % of all voluntary dives, respectively (MacArthur *et al.* 2001, McIntyre *et al.* 2002). Thus, determining the cADL of small-bodied divers seems to be a useful estimate of their breath-hold capacity, even though this is not always the case for larger divers. Further, the different diving patterns

of large-bodied divers may also explain the increased variability around the predicted best-fit line in Figure 12, compared to the lower variability for the small-bodied divers, which have relatively shallow dive depths.

The longest voluntary dive completed by *S. palustris* was 23.7 s, a duration 112 % greater than the maximum dive time predicted by allometry by Schreer and Kovacs (1997) for a 16.3 g diver (21.2 s; $1.62M^{0.37}$) or 269 % greater than predicted by Halsey *et al.* (9.3; $35.5M^{0.326}$; 2006). Halsey *et al.* (2006) examined the allometry of diving using phylogenetic analysis, which they suggested is a better way to examine these variables since it accounts for relatedness between species. Unfortunately, Halsey *et al.* (2006) did not include dive data from the small semi-aquatic insectivores, rodents, platypuses and otters in their allometric analyses. Thus the derived estimate of maximum dive time for the water shrew (269 %) should be interpreted cautiously since these data are being extrapolated to a much smaller diver. This deficiency highlights the need for additional studies to examine the dive performance of small-bodied divers, so as to allow for a thorough statistical comparison to their larger-bodied counterparts.

Recent studies by Catania (2006) and Catania *et al.* (2008) have examined the impressive sensory abilities of American water shrews to detect aquatic prey. This includes underwater "sniffing", in which animals by quickly exhale and re-inhale air bubbles, thereby enabling them to use olfactory cues to find prey. Sensitive vibrissae along the snout of the water shrew can also detect the fine, rapid movement of water caused by fleeing prey or used to quickly discriminate between prey and non-prey while foraging. It is these superb sensory modalities that allows water shrews to forage with equal success in day or night (Catania *et al.* 2008).

Water shrews appear also to re-breath air bubbles trapped under the ice during winter foraging, and so are able to increase aerobic dive endurance. MacArthur (1992) observed that muskrats re-breathing air bubbles had a significantly increased total immersion time and duration of individual excursions over a 15-min trial. Anatomically, the long, slender nose of *S. palustris* is ideal for puncturing air bubbles. Since water shrews commonly co-habit waterways with beavers and muskrats (Conaway 1952), it is expected that many air bubbles of significant size could be exploited by *S. palustris* diving under the ice.

Conclusions

Water shrews face the greatest challenge during aquatic foraging in the winter, as they are required not only to dive, but also must detect and capture prey in ice-cold water, often in total darkness (Catania *et al.* 2008). The water shrew must balance the continual threat of immersion hypothermia while submerged, with the pressure to increase its aerobic dive endurance and anaerobic capacity to ensure adequate foraging time. The world's smallest mammalian diver is able to achieve this feat by diving, at what is probably the physiological limit for a mammal, while depending critically on behavioral thermoregulation to avoid immersion hypothermia. It also has an impressive set of sensory modalities to efficiently capture aquatic prey (Catania *et al.* 2008).

As often reported for other diving mammals, water shrews rarely completed dives beyond the cADL, but demonstrated the capability to, on occasion, voluntarily dive ca. 1.7x this limit. *S. palustris* also had a greater TBOSC (ca. 1.2-fold) compared to the similar sized, but strictly terrestrial, short-tailed shrew. Nearly two-thirds of the TBOSC of the water shrew is accounted for by O_2 bound to Hb. The elevated O_2 stores of the water shrew are able to offset its increased DMR, resulting in a cADL that is similar to that predicted by allometry. Further, water shrews rely on an insulative air layer in their pelage to initially limit cooling, but then use behavioural means to avoid immersion hypothermia. Indeed, only 5% of recorded dives had a T_b less that 37.3 $^{\circ}C$, suggesting shrews rarely become hypothermic while diving. A positive relationship between increased dive duration and decreased T_b was noted, but dive frequency was reduced at lower T_bs, suggesting shrews do not utilize a decreased T_b to increase underwater endurance. The elevated muscle buffering capacity of the water shrew may be associated

with diving, especially if they adopt a flush-pursuit method of aquatic foraging (Catania *et al.* 2008) that involves periods of brief, intense motor activity. It would be especially advantageous for *S. palustris* to use this method of foraging in cold water, since the Q_{10} effect is expected to slow the reaction time of the prey, while the normothermic water shrew would be unaffected during foraging.

Further studies should be completed to examine the glycolytic capacity of the water shrew, since mass-specific allometry predicts this species to have the lowest values of any diver, while this study has shown it to have a higher than expected buffering capacity. Quantifying the skeletal muscle fiber types of *S. palustris* might also help explain the observed Mb concentrations in this mammal.

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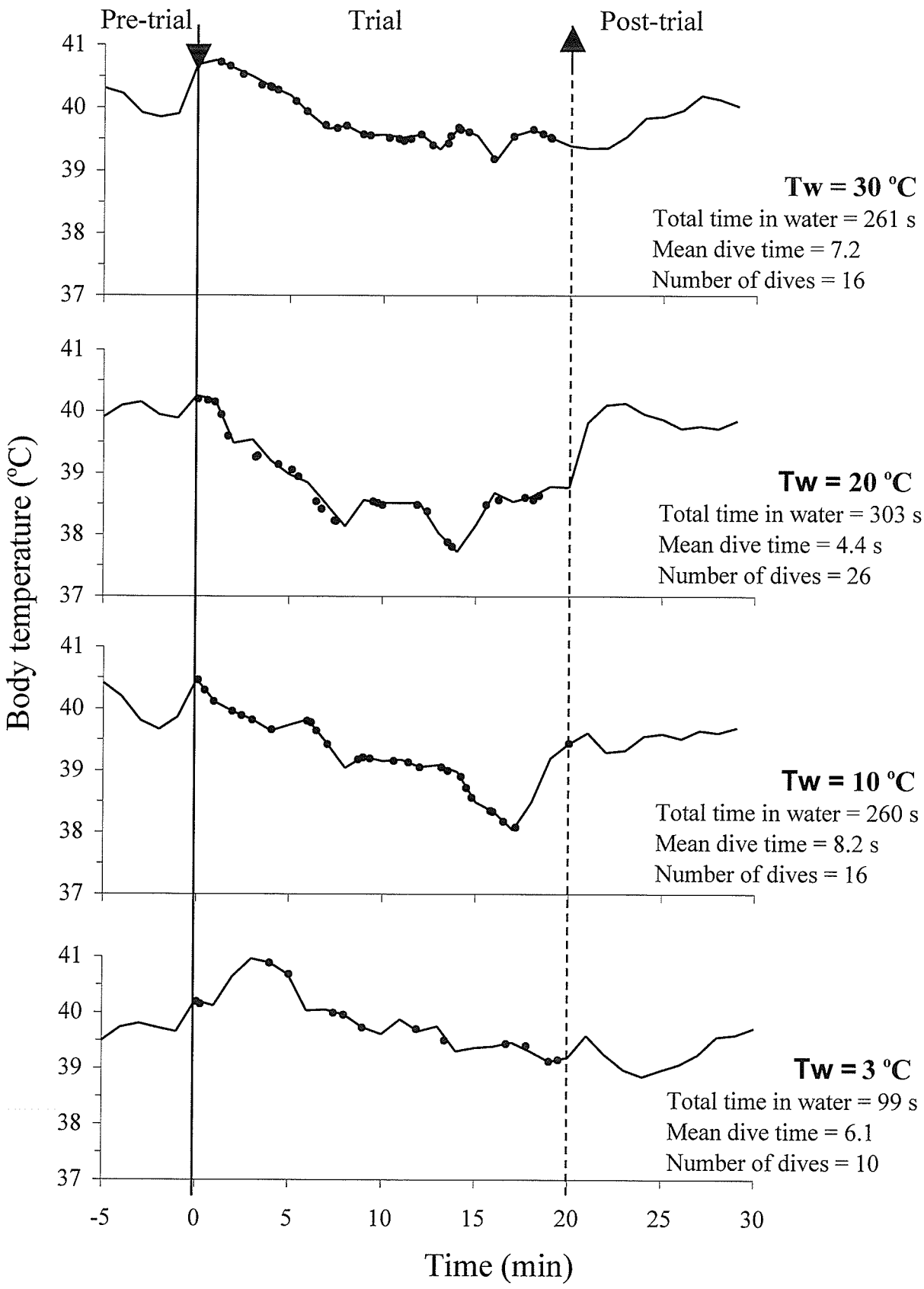
A1. Tb dynamics of water shrew # 12



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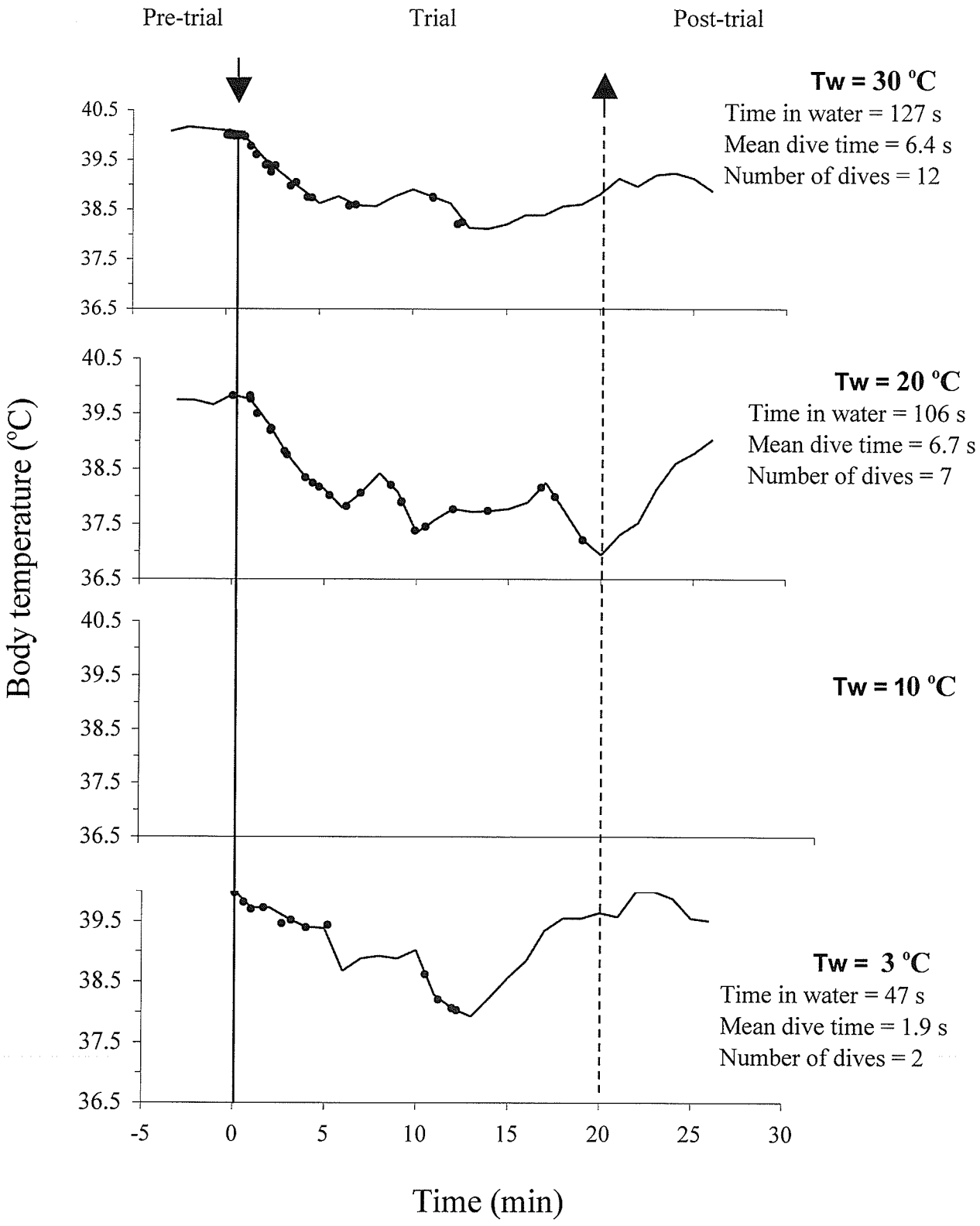

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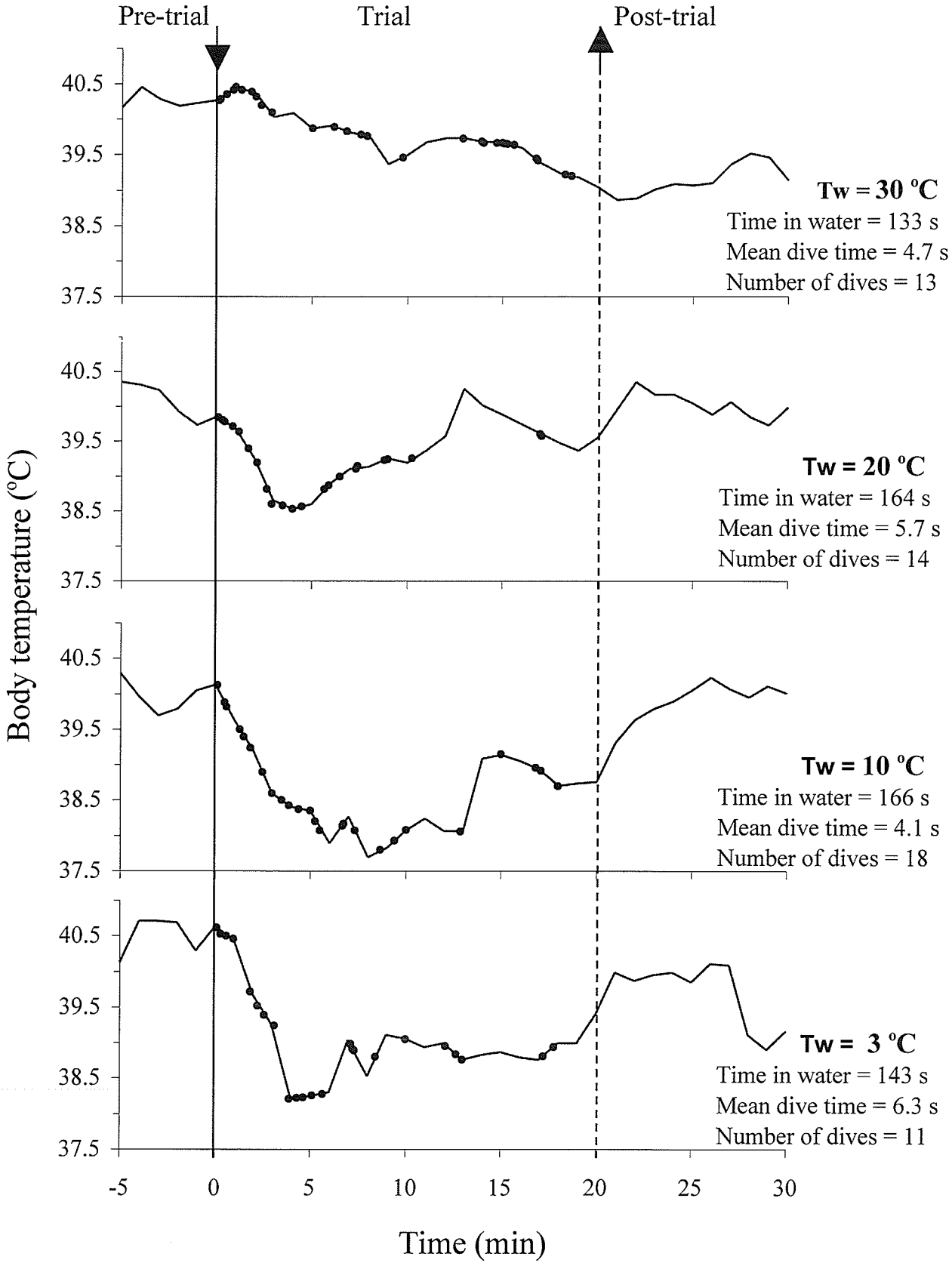

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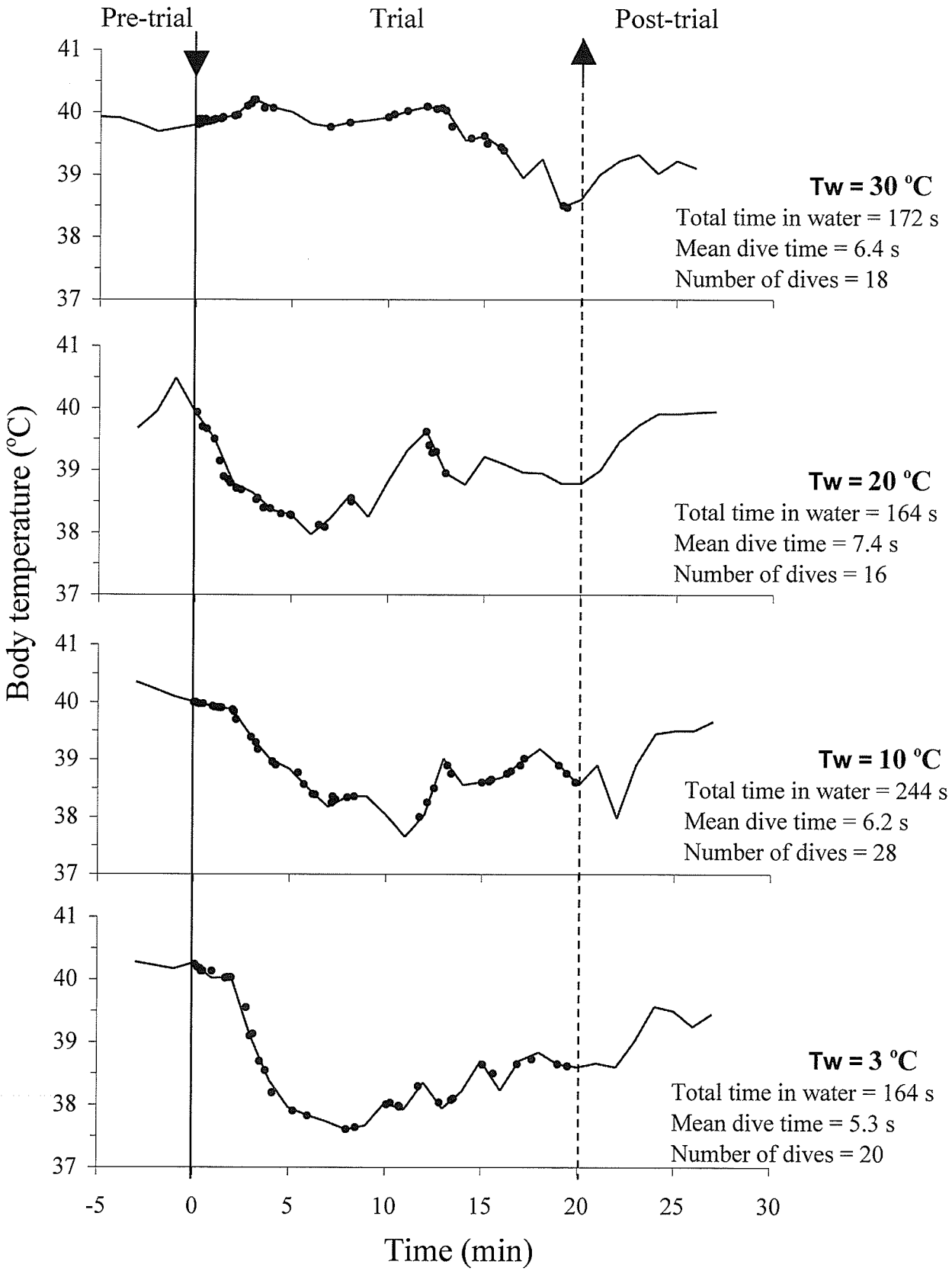

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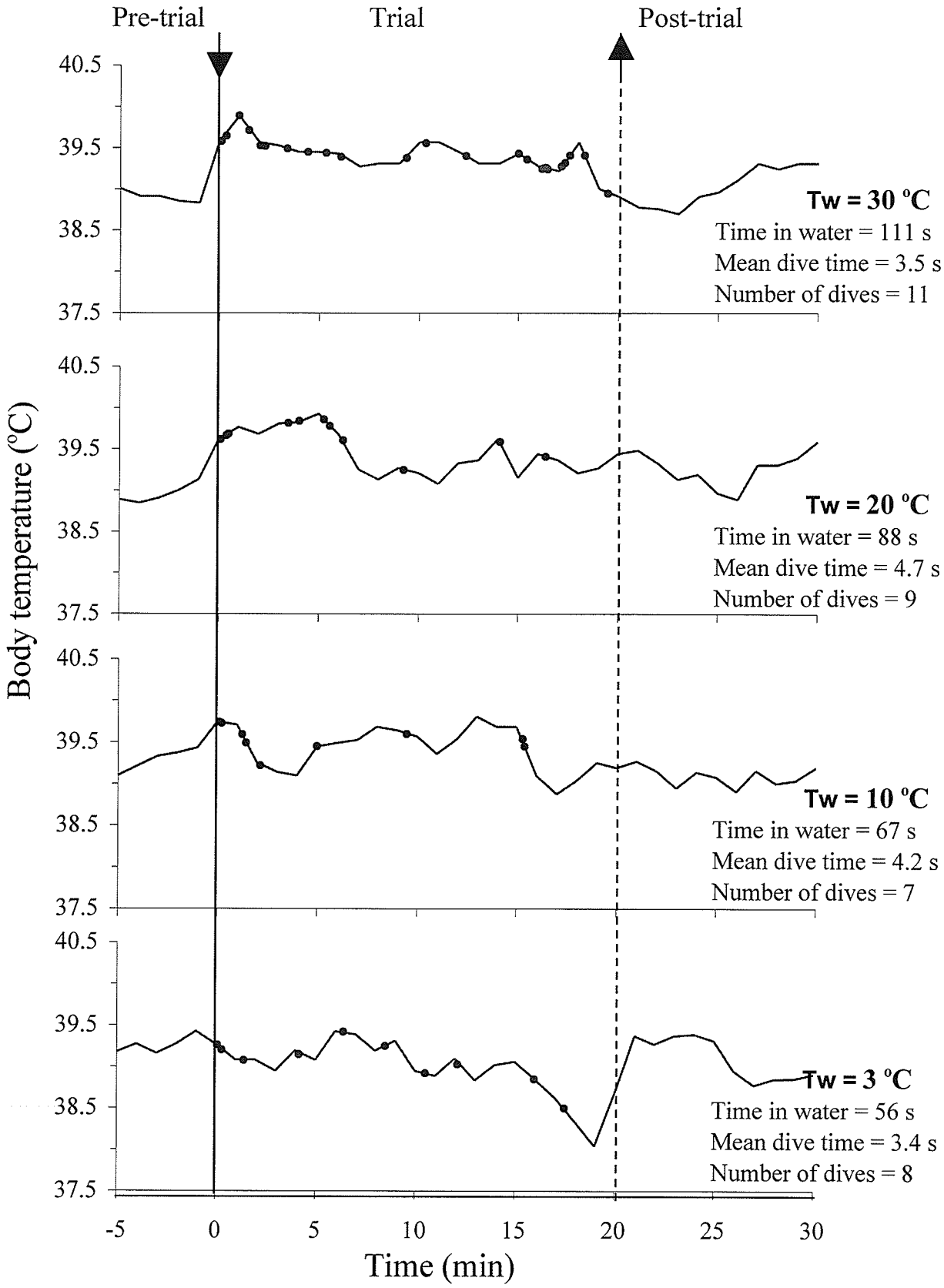

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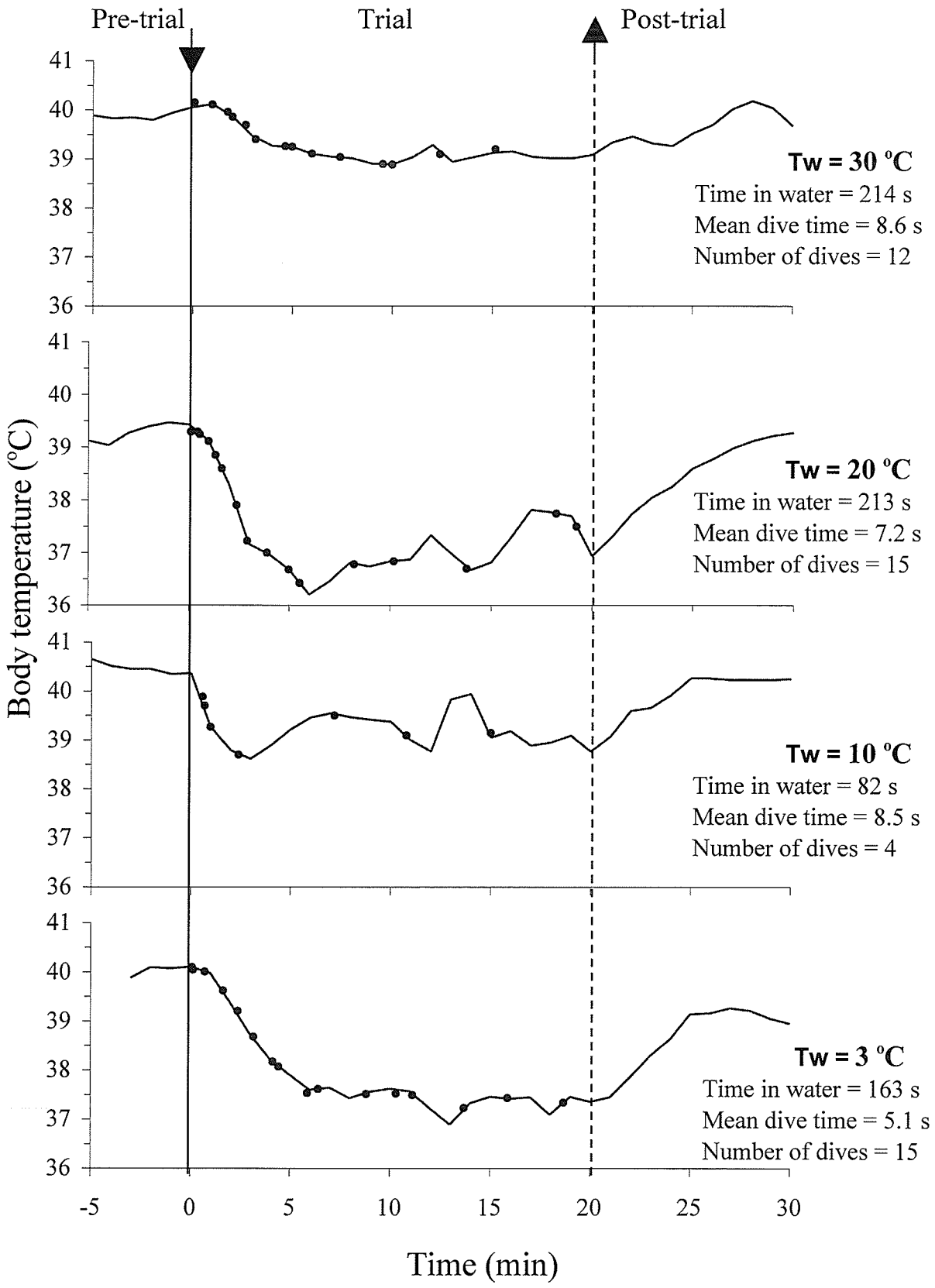
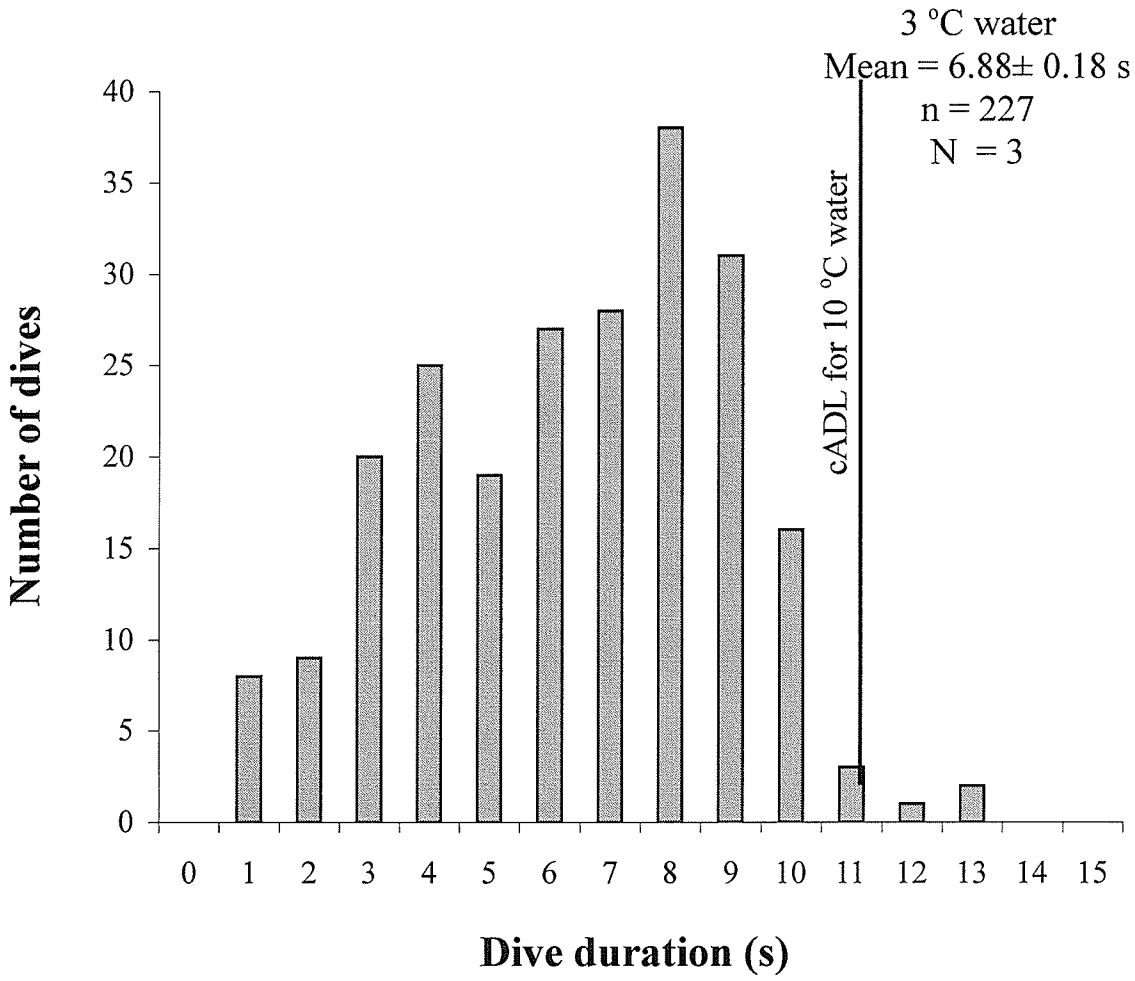


A7: A contingency table using the method of log-likelihood ratio to compare the frequency distribution of voluntary dives by adult American water shrews with (N=6) and without (N=6) an implanted abdominal temperature transmitter (1.0 g).

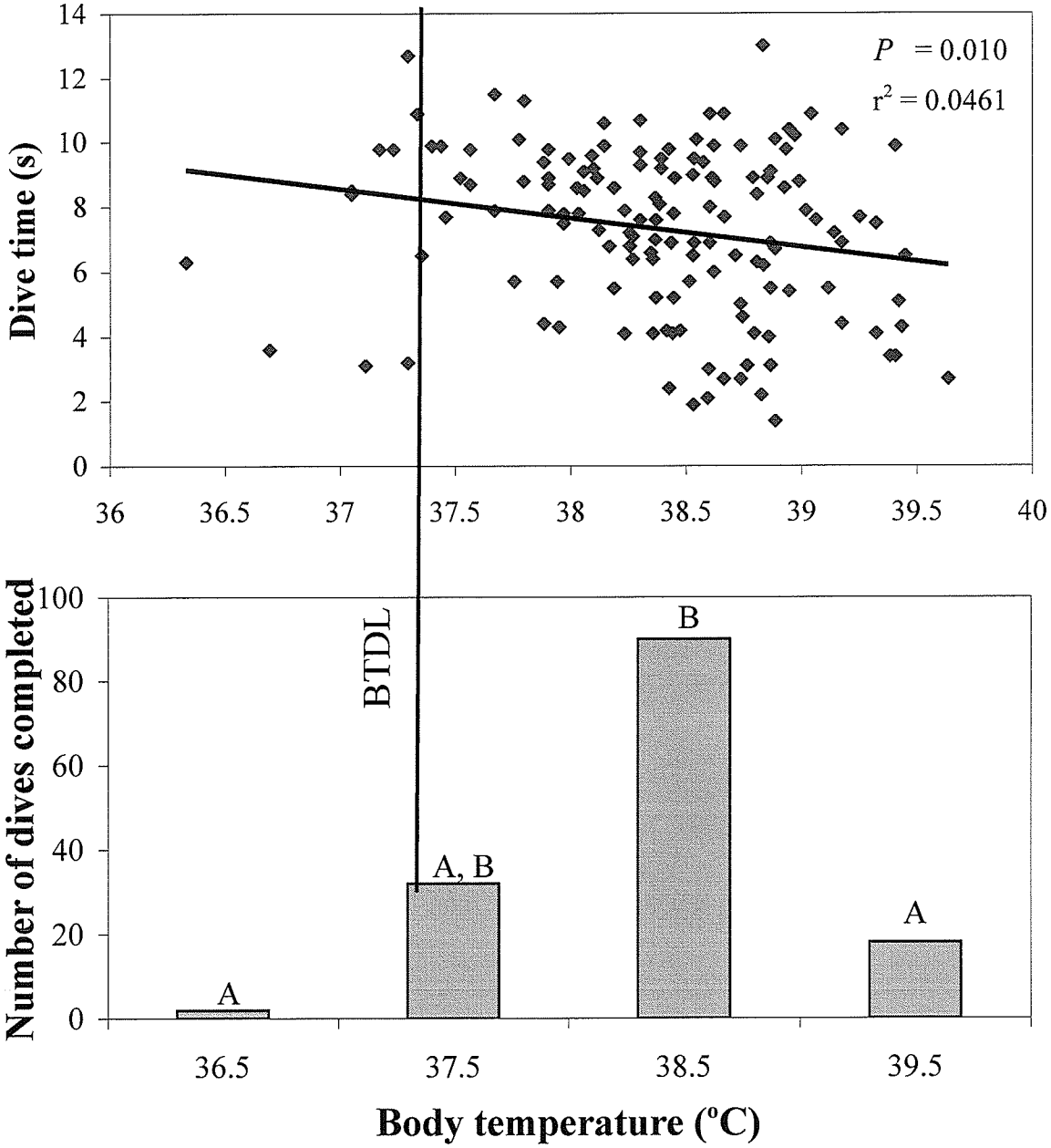
	Dive duration (s)						Total
	0-2	3-5	6-8	9-11	12-14	>15	
Number of dives completed by water shrews without transmitter	109	96	69	29	13	3	319
Number of dives completed by water shrews with transmitter	92	104	75	36	10	3	320
Total	201	200	144	65	23	6	639

d.f.=5, critical value = 11.07.
Calculated G-value=2.78, P>0.50.

A8: The distribution of dive times for three American water shrews diving in a semi-natural riparian environment in 3 °C water over a 24-h period. The calculated aerobic dive limit (cADL) of 10.7 s for adult water shrews diving in 10 °C water is marked with a black line. See text for details.



A9: Relationship between voluntary dive time and core body temperature (Tb) of American water shrews implanted with a 1.0-g intraabdominal transmitter during aquatic foraging in 3 °C water in a semi-natural environment. The bottom figure shows the distribution of dive frequency with Tb. Frequency distributions with different letters differ significantly from each other ($P < 0.05$). The behavioural temperature diving limit (BTDL) was calculated to be 37.3 °C and is represented by the solid black line intersecting both figures. See text for details.



A10: Body temperature (T_b) data for American water shrews implanted with a 1.0-g intraabdominal transmitter and voluntarily diving in 3 °C water in a semi-natural environment. The relationship between dive duration and core T_b is shown only for dives greater than 9.7 s (Assumed less than the calculated aerobic dive limit of 10.7 s for S. palustris diving in 10 °C water). Frequency distributions with different letters differ significantly from each other ($P < 0.05$).