Implications of Aquatic Hypothermia for Dive Performance in the Semi-Aquatic Muskrat, *Ondatra zibethicus*

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 Zoology
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THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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IMPLICATIONS OF AQUATIC HYPOTHERMIA FOR DIVE PERFORMANCE IN THE SEMI-AQUATIC MUSKRAT, *ONDATRA ZIBETHICUS*

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ALLYSON G. HINDLE

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

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ABSTRACT

The "adaptive hypothermia" hypothesis predicts that hypothermia, via the Q_{10} effect, reduces whole-body oxygen consumption by depressing metabolism of cooled tissues. This hypothesis has gained prevalence in large part due to published reports of dramatic body cooling in free-ranging animals foraging in cold water. Because the evidence to date implicating hypothermia as an oxygen sparing strategy is largely circumstantial, I undertook an investigation of the influences of mild hypothermia on the semi-aquatic muskrat diving voluntarily in a laboratory setting.

Pre-chilling elicited no overt behavioural changes to diving in adult muskrats. In fact, the only impact of hypothermia on the aquatic behaviour of adults was a tendency for hypothermic individuals to spend more total time immersed in cold water (P = 0.006). Both diving and average rates of oxygen consumption (VO_2) of adults were unaffected by hypothermia when animals were tested in 30°C water. However, significant interactions between water and body temperatures (T_b) were observed for diving (P = 0.045) and average (P = 0.040) VO_2, resulting in significantly higher (P = 0.045-0.017) VO_2 values for hypothermic adults diving from the water surface in 10°C water. Hypothermia reduced diving heart rate only in dives < 25 s (P = 0.007) and did not appear to affect the onset or temporal pattern of diving bradycardia. An interesting aspect of this study was the demonstration that heart rate was significantly correlated with VO_2 during both terrestrial (grooming) and aquatic (diving) activity. However, neither calibration was influenced by T_b. Also, heart rate and diving

$\dot{V}O_2$ were each inversely related to dive duration, demonstrating that muskrats may modulate diving metabolic rate according to demands.

Post-dive excess $\dot{V}O_2$ was higher for pre-chilled than for normothermic muskrats in short dives only. This discrepancy was eliminated for longer (2 min) dives, suggesting that a comparable or maximal dive response was achieved in both groups during longer dives, regardless of T_b. I also observed that following hypothermic diving, T_b of adults continued to recover (27 ± 1 min) well beyond the post-dive period (6 min) during which $\dot{V}O_2$ was significantly elevated.

In the youngest cohort tested (200 - 400 g), hypothermia was associated with elevated $\dot{V}O_2$ and decreased diving during continuous immersion. This suggests intolerance to chilling in juvenile muskrats, but a development of tolerance to hypothermic aquatic activity with increasing body size. Following hypothermic diving, $\dot{V}O_2$ of juvenile muskrats was elevated for only 2 min post-immersion. It appears that juveniles incur high thermoregulatory costs associated with hypothermia and engage thermogenic pathways during the immersion period, whereas adults tend to incur lower costs, which they defer until they have emerged from the water.

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ABBREVIATIONS USED IN TEXT

Ad	adult (< 600 g)
ADL	aerobic dive limit
BAT	brown adipose tissue
DMR	diving metabolic rate
D:S	dive: surface ratio
ECG	electrocardiogram
F_{IO_2}	fractional oxygen content of inspired air
$F_{E_{eq}}$	equilibrium fractional oxygen content of expired air with no further changes in VO_2
F_{E_t}	fractional oxygen content of expired gas at an arbitrary time
Juv1	juvenile (200-400 g)
Juv2	juvenile (400-600 g)
KAR	ketamine-atropine-rompun anesthetic agent
NST	non-shivering thermogenesis
RMR	resting metabolic rate
T_b	body temperature
T_w	water temperature
V	chamber volume
$\dot{V}$	flow rate of dry, CO_2-free air
VO_2	rate of oxygen consumption

INTRODUCTION

Literature Review

Diving within aerobic limits

Animals that forage underwater should, in theory, exhibit traits that maximize their breath-hold capacity. It is generally acknowledged that an animal's dive time is limited by finite on-board oxygen stores (Kooyman 1989) and that its aerobic dive limit (ADL) defines the maximum dive duration that can be supported by an oxygen-based metabolism (Kooyman *et al.* 1980). Theoretically, ADL is equivalent to accessible body oxygen stores divided by the rate at which these stores are depleted underwater. In order to maximize time spent underwater, animals require strategies that enhance their ADL, their capacity for recovery from diving, or their ability to continue diving beyond the ADL (*i.e.* their capacity for anaerobic metabolism).

Behavioural studies suggest that, for the most part, divers do not exceed the calculated ADL in a given dive bout (Kooyman 1989; Butler and Jones 1997). This preference for aerobic diving is supported by the absence of any biochemical evidence of increased capacity for anaerobic metabolism in marine, compared to terrestrial mammals (Castellini *et al.* 1981). Given the tendency for anaerobic end-products (*e.g.* lactate) to accumulate in metabolizing tissues during prolonged periods of submergence, dives beyond the ADL typically are associated with long recovery periods at the surface during which times these end-products are processed. Therefore, maintenance of an aerobic diving schedule maximizes potential foraging time by reducing obligate surface time.

For example, in Weddell seals, *Leptonychotes weddellii*, blood lactate levels are not significantly elevated over the course of sequential feeding dives. However, their lactate production is closely correlated to submergence time in longer exploratory dives (Kooyman *et al.* 1980), indicating that anaerobic metabolism is possible, but not preferred in this species. Ecologically, these long anaerobic dives are most advantageous for active aquatic predators that exploit ephemeral food sources (*e.g.* schools of fish; Kooyman 1989; Butler and Jones 1997). For example, foraging dives beyond the calculated ADL are routinely documented in avian predators such as western grebes, *Aechmorphus occidentalis* (Ydenberg and Clark 1989) and thick-billed murres, *Uria lomvia* (Croll *et al.* 1992).

In the absence of obvious adaptations for either rapid post-dive recovery or enhanced anaerobic capability, potential mechanisms for enhancing ADL must be examined to explain the impressive breath-hold capacity of divers. In general, divers are characterized by elevated oxygen stores resulting from high blood volume, hematocrit, and hemoglobin and myoglobin concentrations (Kooyman 1989). However, of perhaps greater importance than the maximum potential oxygen stores is the extent to which these stores can be exploited during the course of a dive. For instance, in pinnipeds and cetaceans diving to depth, pressure results in lung collapse, forcing air into the non-compressible bronchii and trachea where gas exchange cannot occur (Snyder 1983). This essentially precludes the lung as a potential oxygen reservoir in deep-diving animals, leaving the diver to rely on oxygen stores in the blood and muscle for aerobic fuel.

The dive response

The "classic dive response" was originally described by Scholander (cited in Butler and Jones 1982) to consist of apnea, decreased cardiac output, intense bradycardia and peripheral vasoconstriction, with selective perfusion of hypoxia-sensitive tissues. These vascular responses combine to preserve pre-dive arterial blood pressure and therefore provide adequate perfusion to working muscle and hypoxia-intolerant regions. The validity of the "classic dive response" and attendant hypometabolism is supported for mammals and, to a lesser extent, birds, by several studies using techniques such as heart rate telemetry (Butler and Jones 1997; Kooyman and Ponganis 1998), radioactive tracers (Castellini *et al.* 1985; Castellini 1988), and direct measurement of cardiac output and arterial blood flow (Bevan and Butler 1992).

When surface swimming in a flume, marine mammals demonstrate a typical exercise response (*i.e.* tachycardia, increased oxygen consumption, peripheral vasodilation) and show a normal capacity for aerobic work (Castellini *et al.* 1985; Butler 1988; Davis *et al.* 1991). However, in some divers at least (*e.g.* harbour seals, fur seals and sea lions), hematocrit levels and the mitochondrial volume densities and myoglobin content of swimming muscles are comparable to those of elite athletes such as dogs and racehorses (Kanatous *et al.* 1999). At the very least, this finding provides evidence that the swimming muscles of marine mammals are adapted for aerobic metabolism during the hypoxic conditions of diving (Kanatous *et al.* 1999). Considering that the dive response should theoretically blunt aerobic scope, it is possible that this high

capacity for aerobic metabolism is simply a mechanism to ensure an adequate delivery of oxygen to hypoperfused tissues during diving (Kanatous *et al.* 1999).

It follows, therefore, that a depression in diving metabolic rate (DMR) may at least partially explain the impressive breath-hold capacity of some divers. In fact, Scholander's classic dive response is based on his observation that the post-dive oxygen debt is lower than predicted from the diving oxygen deficit. In many studies, calculated DMR based on known body oxygen stores as well as diving patterns and behaviour, has been predicted to be equivalent to, or lower than the resting metabolic rate, or RMR (see Butler and Jones 1997 for review). For example, these predictions have been made for several penguin species (gentoo, king, emperor; Butler 2000), and for southern elephant seals (Hindell and Lea 1998). However, calculations of DMR based on indirect calorimetry do not universally support the general predictions derived from calculated ADLs and diving behaviour. Studies such as that of Nagy *et al.* (2001) involving king penguins have used doubly labeled water to demonstrate that "at sea" metabolic rates are comparable to RMR (see Butler and Jones 1997 for review). In laboratory studies, measurements of the rate of oxygen consumption ($\dot{V}O_2$) indicate that DMR is lower than RMR in juvenile northern elephant seals (26% reduction; Webb *et al.* 1998), and California sea lions (47% reduction in long dives; Hurley and Costa 2001). However, laboratory measurements of $\dot{V}O_2$ reveal an elevated DMR in tufted ducks (DMR = 3.5X RMR; Butler 2000), humboldt penguins (DMR =1.26X RMR; Butler and Woakes 1984) and muskrats (DMR = 2.75X RMR; MacArthur and Krause 1989).

The varied estimates of DMR illustrate the apparent conflict between the oxygen demands of underwater exercise and the need to ration oxygen in order to extend submergence time. These opposing demands imply that a balance between exercise and diving must be achieved in order for a diver to optimize oxygen usage (Hochachka 1986; Butler 1988; Castellini 1988). Butler (1988) suggests that in shorter dives there is a bias toward the exercise response, whereas in longer dives a bias towards the classic diving response is important in maximizing aerobic metabolism.

Since DMR is not consistent with respect to steady-state RMR measured at the surface, it is not surprising that measurements of various metabolic indices have thus far failed to yield a universal response in divers. The most widely measured variable of the dive response is heart rate, as it is relatively easy to telemeter from a free-diving animal. Many researchers have made a case for the use of heart rate as an indirect measure of metabolic rate in free-ranging animals (*e.g.* Butler and Woakes 1982, Butler 1993, Boyd *et al.* 1999, Green *et al.* 2001) and the well-documented occurrence of bradycardia during voluntary diving provides some support for the existence of diving hypometabolism (Butler and Jones 1997; Kooyman and Ponganis 1998).

Hypothermia-induced hypometabolism

The nature of a potential hypometabolic state in the diving animal is as yet poorly understood, though many hypotheses have been advanced (Hochachka and Guppy 1987; Fedak and Thompson 1993). Based on lack of evidence for reliance on anaerobic pathways, it is generally acknowledged that an overall

metabolic depression must result from suppression of aerobic pathways without a switch to anaerobiosis (Butler and Jones 1997). A drop in body temperature (T_b) is often observed in association with diving (Butler and Jones 1997; Kooyman and Ponganis 1998), leading researchers to implicate hypothermia as a possible contributing factor to diving hypometabolism (Herbert and Jackson 1985; Hill *et al.* 1987; Bevan and Butler 1992). It is well established that a drop in temperature reduces rates of cellular reactions ("Q_{10} effect") and this phenomenon may account for the hypometabolic state of cooled tissues (Geiser 1988). That a hypothermia-induced depression in metabolism may play a vital role in diving is suggested also by the common occurrence of counter-current heat exchangers which, while preserving heat in the body core, also promote cooling of peripheral tissues. As well, Willford *et al.* (1990) have demonstrated that the oxygen-binding affinity of hemoglobin in phocid seals (specifically the harbour seal) is remarkably insensitive to temperature change by comparison to humans and dogs. Since oxygen affinity of mammalian hemoglobin is typically increased in cold, a reduced sensitivity to temperature may serve to maintain the unloading of oxygen to cooled tissues during diving. Considering the significant thermal challenges posed by aquatic habitats, it is perhaps not entirely surprising that small endotherms do not always defend T_b in cold water (see MacArthur 1989 for review). However, hypometabolism has been linked to T_b decrease in a variety of large-bodied vertebrate divers as well (Herbert and Jackson 1985; Hill *et al.* 1987).

Abdominal temperature drops significantly in many diving birds and mammals (Butler and Jones 1997; Kooyman and Ponganis 1998). This usually has been attributed to convective heat loss to cold water, though in some cases, it has been associated with stomach cooling upon ingestion of cold prey (Wilson and Culik 1991; Handrich *et al.* 1997). To date, the most accurate measure of core T_b during diving has been taken from the dorsal aorta of Weddell seals (Kooyman *et al.* 1980; Hill *et al.* 1987). These studies report a decrease in the temperature of aortic blood that occurs immediately upon diving and which persists throughout the entire dive. However, more recent studies should also be highlighted, which do not indicate whole-body cooling, but rather, adoption of heterothermy, leaving the body core and major abdominal organs unaffected despite substantial cooling of peripheral tissues (Ponganis *et al.* 1993; Ponganis *et al.* 2001). For example, though abdominal temperature drops dramatically in emperor penguins, *Aptenodytes forsteri*, foraging in cold water, a temperature probe placed in the inferior vena cava of these birds revealed that their core T_b remained constant or even increased during foraging bouts (Ponganis *et al.* 2001).

Even assuming that hypothermia occurs routinely in aquatic endotherms, the "adaptive hypothermia" hypothesis (Butler and Jones 1997) still raises questions amongst diving physiologists. First, adoption of hypothermia is not without costs. For example, at low T_b, locomotor and brain function may be impaired. Also, thermoregulatory costs following a bout of hypothermia would likely represent a significant component of a diver's energy budget, given that

metabolic rate in water increases as water temperature declines (MacArthur 1984; Bevan and Butler 1992; Kruuk *et al.* 1994). Second, there is disagreement as to whether a reduced metabolism during diving is a direct response to a T_b decrease via the Q_{10} effect. For example, research conducted on hibernators suggests that T_b is lowered as a consequence of metabolic suppression, rather than the reverse argument based on Q_{10} considerations (Snyder and Nestler 1990).

Diving capabilities of juveniles

While young birds do not enter the water until they have fledged, mammalian divers may experience dive-induced hypoxia even in the womb. From allometry, we know that mass-specific oxygen stores scale close to the first power of body mass (Calder 1984), whereas whole-body metabolic rate scales close to the 0.75 power of mass (Calder 1984; Schreer and Kovacs 1997). A smaller diver should therefore be expected to exhibit relatively higher underwater $\dot{V}O_2$ with respect to its body oxygen stores than would be the case for a larger animal. This would theoretically result in significantly lower ADLs for juveniles. However, Jorgensen *et al.* (2001) suggest that the primary limiting factors in the development of diving capability in harbour seal (*Phoca vitulina*) pups are experience and learning. Regardless, juveniles present an interesting case since their reduced body size (and higher surface area-to-mass ratios) imply faster cooling rates and decreased thermal inertia. Coupled with the possibility of incomplete thermoregulatory competence in early life stages (*e.g.* MacArthur and Humphries 1999), juveniles are particularly susceptible to immersion

hypothermia. Thus, given their inherent reduced diving capability, juveniles perhaps offer the best candidates for investigating the benefits of immersion hypothermia on dive performance in mammalian and avian divers.

Objectives of Study

The semi-aquatic muskrat (*Ondatra zibethicus*) is an excellent model for investigating the "adaptive hypothermia hypothesis", since it routinely cools during voluntary swimming and diving (MacArthur 1979b, 1984) and apparently does not engage thermogenic pathways underwater (MacArthur 1986). Both heart rate (MacArthur and Karpan 1989) and T_b (MacArthur 1979b, 1984) of muskrats decline during voluntary diving, and active rewarming via brown adipose tissue (BAT) does not appear to occur until surfacing (MacArthur 1986). Given these observations, I hypothesized that hypothermia in diving muskrats may enhance their dive performance by lowering DMR. However, it should be cautioned that the high expected costs of post-dive recovery from hypothermia and the overall higher cost of diving in cold water (MacArthur 1984) suggest that the total cost of a dive bout (including recovery) may, in fact, be elevated for hypothermic animals. From an ecological perspective, this could make hypothermic diving disadvantageous to muskrats.

Potential benefits of hypothermia for the diving muskrat were assessed from both behavioural and metabolic data. Metabolic rates of normothermic and hypothermic animals were recorded for animals diving freely in warm and cold water. Normothermic muskrats dive significantly less in cold water (MacArthur 1984), therefore, dive times were also analyzed to determine the effects, if any, of hypothermia on diving behaviour. Finally, post-immersion recovery costs of normothermic and hypothermic muskrats were assessed for dives of controlled durations (0.5 – 2 min). These measurements were related to recovery costs in

the absence of diving to assess the true costs associated with hypothermic diving.

T_b dynamics and diving were also investigated in juvenile animals. Surprisingly, these animals are adept divers at an early age (Errington 1963; MacArthur *et al.* 2001), yet they are highly susceptible to immersion hypothermia (see above). Moreover, MacArthur *et al.* (2001) showed that ADL scales similarly to body mass in both adult and juvenile muskrats. The youngest cohort tested by these researchers (4.5 – 6 wks) dove significantly more often and at lower cost than the adults. Since these young did not exhibit elevated anaerobic capacity based on their muscle buffering capacity (MacArthur *et al.* 2001), I hypothesized that a hypothermia-induced depression of DMR may extend aerobic dive times of juvenile muskrats.

Because heart rate is often viewed as a universal and instantaneous index of metabolic status in divers (Butler and Jones 1997), cardiac responses of normothermic and hypothermic muskrats were also examined. Based on the premise that DMR is depressed with hypothermia, I predicted a parallel reduction in diving heart rate. Beat-to-beat changes in heart rate during submergence were also analyzed to determine if the pattern of bradycardia normally observed during diving is affected by hypothermia. To validate metabolic inferences derived from telemetered heart rate, concurrent measurements of V_{O_2} and heart rate were gathered for muskrats engaged in varying intensities of diving and surface activities.

METHODS AND MATERIALS

Study Animals

Adult ('Ad' cohort, >600 g) and weaned juvenile ('Juv2' cohort, 400-600 g) muskrats were captured from May through October at Oak Hammock Marsh, Manitoba (50°06' N, 98°20' W), using National live traps in overnight sets. In spring, the larger natal lodges on which adults were captured were also examined for the presence of litters ('Juv1' cohort, 200-400 g). Captured animals were transported immediately to the Animal Holding Facilities, University of Manitoba, where they were held at 14 ± 1°C with a 12L:12D photoperiod. Animals were caged individually or in pairs (if caught on the same lodge) in wire cages (90 × 30 × 30 cm) with attached nest boxes. Females with litters were housed in larger (106 × 53 × 48 cm) wire cages. Cages were positioned in shallow flow-through tanks so as to provide muskrats access to a 5–10 cm depth of water (12 ± 1°C) while keeping nest boxes dry. Animals were maintained on a diet of commercial rodent chow (ProLab HMG 3000) supplemented daily with apples and carrots. Animals were acclimated to holding conditions for a minimum period of 3 weeks prior to surgery or initiation of experiments.

Surgical Procedures

Muskrats were surgically implanted with either temperature-sensitive radio transmitters, following the methods of MacArthur (1979b, 1984), or with electrocardiogram (ECG)/T_b transmitters, following the procedure recommended by the manufacturer (Data Life Sciences International, St. Paul, MN). Prior to

surgery, muskrats were weighed and initially anesthetized with the inhalant anesthetic, Halothane (MTC Pharmaceuticals, Cambridge, ON). To obtain a surgical plane of anesthesia, muskrats were then given an intraperitoneal injection of a drug mixture (KAR) prepared by combining 10 mL ketamine hydrochloride (100 mg mL^{-1}, Bimeda-MTC, Cambridge, ON), 3 mL atropine sulfate (0.5 mg mL^{-1}, MTC Pharmaceuticals), 1 mL rompun (xylazine, 20 mg mL^{-1}, Bayer Inc., Etobicoke, ON) and 2 mL sterile water. The dosage of KAR administered was 0.5 mL kg^{-1} for adults and 0.55 mL kg^{-1} for juveniles. Animals were immobilized within 1-5 min of injection and remained anesthetized for approximately 30 min. If required, KAR was supplemented with additional Halothane applied from a cone covering the nose and mouth.

For temperature transmitters, a 2 × 4 cm area on the lower abdomen was shaved and cleaned with Hibitane (4% chlorohexidine succinate) followed by 70% alcohol. A 3-cm midline incision was made through the skin followed by a 2-cm incision through the body wall, along the *linea alba*. Sterilized transmitters were inserted into the body cavity and the body wall and skin wounds were closed with 3-0 catgut and 3-0 silk sutures, respectively.

For ECG/T_b transmitters, a 2 × 5 cm area on the ventral side just caudal to the xyphoid space as well as 1 × 1 cm areas near the right shoulder and left lower rib were shaved and cleaned (see above). On the abdomen, a 4-cm incision was made through the skin, followed by a 3-cm incision in the body wall along the *linea alba*. After insertion of the sterilized transmitter into the abdominal cavity, the two ECG leads of the unit were exteriorized to either side

of the body wall incision by passing them through a 14 Ga. needle. With the aid of a hollow trochar, the leads were passed subcutaneously to the electrode attachment sites (right shoulder and left lower rib), where a 1-cm incision was made in the skin allowing each lead tip to be secured to the underlying muscle with a single stitch. As the abdominal incision was closed, suture ribs on the transmitter casing were secured by stitches to the ventral body wall. For this procedure, all incisions were closed with 3-0 silk sutures.

Upon completion of surgery, wounds were cleaned with alcohol and treated with 2% xylocaine gel (lydocaine hydrochloride, Astra Pharma Inc., Mississauga, ON). A long-lasting antibiotic (Derapen, Ayerst Veterinary Laboratories, Guelph, ON) was then administered intraperitoneally at a dosage of 0.22 mL kg^{-1}. Experimentation on instrumented animals did not begin until at least 1 week post-surgery.

Measurements of Body Temperature

Muskrat body temperatures were determined using calibrated model X-M temperature transmitters (Mini-Mitter Inc., Sunriver, OR). Prior to implantation (see above), each transmitter was calibrated at 1–2°C increments between 21 and 41°C, using a Haake A81 circulating water bath. Weighing approximately 1.5 g and free-floating in the abdominal cavity, these transmitters provided temperature data on an AM frequency. Data were subsequently gathered from unrestrained animals using a Sony AM receiver positioned above the dive tank. These measurements were assumed to represent core T_b of test animals.

Measurements of Heart Rate/Body Temperature

For experiments involving measurement of heart rate, ECG and abdominal T_b were monitored concurrently using pre-calibrated model ETA-F40 transmitters (4g; Data Life Sciences). ECG/T_b data were collected using two receivers (models RC1 and RCN3000) positioned above the dive tank. Receivers were mounted in a purpose-built acrylic box suspended just above the water surface. The box was suspended by a sliding carriage from a beam running the full length of the tank. This enabled the receivers to be moved manually along the beam, maintaining an ideal 30-cm distance from the swimming animal. Data were recorded and later analyzed on computer using DataQuest Advanced Research Technology software (Data Life Sciences).

Metabolic Measurements

Rates of O_2 consumption in the metabolic chamber (see below) were determined using a negative-pressure, open-circuit respirometry setup similar to that described by MacArthur and Krause (1989). Briefly, room air was drawn through the metabolic chamber at a rate of 10 L min^{-1}. Exhaust gas was dried and made CO_2-free by passing it sequentially through columns of Drierite and soda lime. A sub-sample of the treated gas was drawn through a second, smaller set of columns containing Drierite and soda lime to ensure all water and CO_2 were removed. This gas sample was drawn at a rate of 250 mL min^{-1} into the M-22 sensor of an Applied Electrochemistry S3-A oxygen analyzer and the fractional O_2 content of the sample measured. All data were recorded on

computer using the Datacan Data Acquisition System (Sable Systems Inc., Henderson, NV).

Though mean $\dot{V}O_2$ was sufficient to describe metabolic rate during post-immersion recovery on the dry platform (see below), instantaneous measurements of $\dot{V}O_2$ (Bartholomew *et al.* 1981) were also derived because they are more precise than mean estimates and thus should allow better separation of $\dot{V}O_2$ associated with brief episodes of diving and surface swimming. It should be noted that estimates of $\dot{V}O_2$ derived from mean and instantaneous calculations were comparable during steady-state activity (both aquatic and terrestrial).

Instantaneous $\dot{V}O_2$ was calculated using the equation (Bartholomew *et al.* 1981):

$$\dot{V}O_2 = \frac{\dot{V}(FIO_2 - FE_{eq})}{1 - FIO_2} \qquad [1]$$

where $\dot{V}$ is the flow rate of dry, CO_2-free air in $cm^3\ min^{-1}$, FIO_2 is the fractional concentration of O_2 in the incurrent air, and FE_{eq} represents the equilibrium fractional O_2 content in expired air if no further changes in $\dot{V}O_2$ were to occur. Bartholomew *et al.* (1981) define FE_{eq} by the expression:

$$FE_{eq} = \frac{FE_t - FE_{t-1} + FE_t\text{-}1}{1 - e^{-\dot{V}\Delta t/V}} \qquad [2]$$

where $\dot{V}$ is the flow rate of dry, CO_2-free air in $cm^3\ min^{-1}$ and V represents the chamber volume. FE_t and FE_{t-1} are the fractional O_2 concentrations of expired gas at two arbitrary times, separated by Δt min. These values are representative of the washout characteristics of the metabolic chamber at the defined flow rate.

Instantaneous estimates of $\dot{V}O_2$ were used specifically for calculation of diving $\dot{V}O_2$. Since hypothermia is proposed to enhance dive capability, some estimate of the cost of diving was necessary. Diving $\dot{V}O_2$ was estimated using the equation of MacArthur and Krause (1989) and converted into mass-specific units:

$$\text{Diving } \dot{V}O_2\ (\text{mL g}^{-1}\ \text{min}^{-1}) = \qquad [3]$$

$$\frac{\text{Total } \dot{V}O_2\ (\text{mL g}^{-1}) - [\text{Cumulative surface time (min)} \times \text{Resting } \dot{V}O_2\ (\text{mL g}^{-1}\ \text{min}^{-1})]}{\text{Cumulative dive time (min)}}$$

Resting $\dot{V}O_2$ was calculated for each treatment group from instantaneous $\dot{V}O_2$ data, when animals were observed to be calm and resting at the surface. Diving $\dot{V}O_2$ was also calculated for animals with access to a dry platform, in which case resting $\dot{V}O_2$ was estimated for each treatment group from instantaneous $\dot{V}O_2$ data for animals engaged mainly in post-immersion grooming activity.

Chilling Procedure

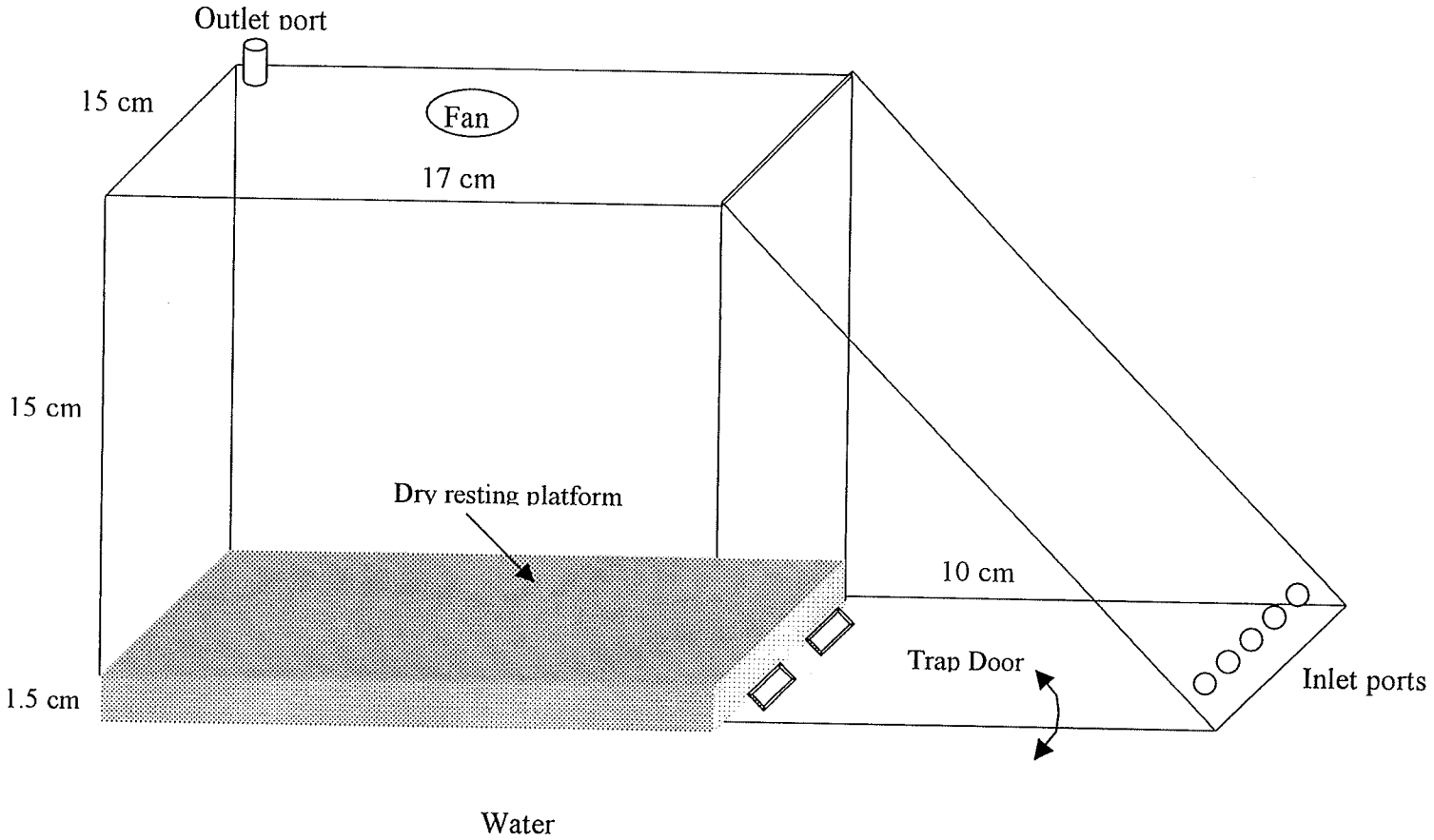
Prior to dive trials, muskrats were placed in a "chilling tank" where they were immersed to a depth of 15 cm in 6 ± 1°C water for up to 1 h, in order to

induce mild hypothermia. Preliminary laboratory observations showed that abdominal T_b of captive muskrats drops 0.5-3.5°C when animals are immersed in cold (6 ± 1°C) water for 1 h. This T_b decline encompasses the range reported for free-ranging muskrats foraging in winter (MacArthur 1979a). To minimize exercise during the immersion period and to facilitate transfer of subjects between tanks, animals were confined to a National live trap (60×16×16 cm) immediately prior to and during immersion in the chilling tank. This limited confinement restricted movement without unduly stressing animals. For control ("no chill") trials, muskrats were placed in the same live trap and tank setup as were "chilled" animals, but were immersed in warm (30 ± 1°C) water. Based on previous studies (MacArthur 1984), this water temperature ensured that control animals remained normothermic throughout the 1-h controlled immersion period.

Experiment 1: Cost of Thermal Recovery from Chilling

This experiment enabled me to quantify post-immersion rewarming costs of hypothermic muskrats in the absence of diving. Average $\dot{V}O_2$ was determined, as it reflects the combined costs of recovery from chilling and post-immersion grooming and comfort movements within the metabolic chamber. All muskrats (*n* = 12) participated in each "pre-chill" and "no chill" trial (assigned in random order), allowing each individual to serve as its own control. After 1 h immersion in either 6°C or 30°C water (see above), muskrats were introduced directly into a small, dry metabolic chamber (5.2 L, Fig. 1) where $\dot{V}O_2$ was measured continuously for 40-60 min post-immersion. This interval encompassed the

Figure 1. Schematic diagram of 5.22 L metabolic chamber used in diving studies. Note that effective chamber size is determined by chamber dimensions and water level. Note the presence of a hinged partition which blocks movement between the chamber and water when closed.

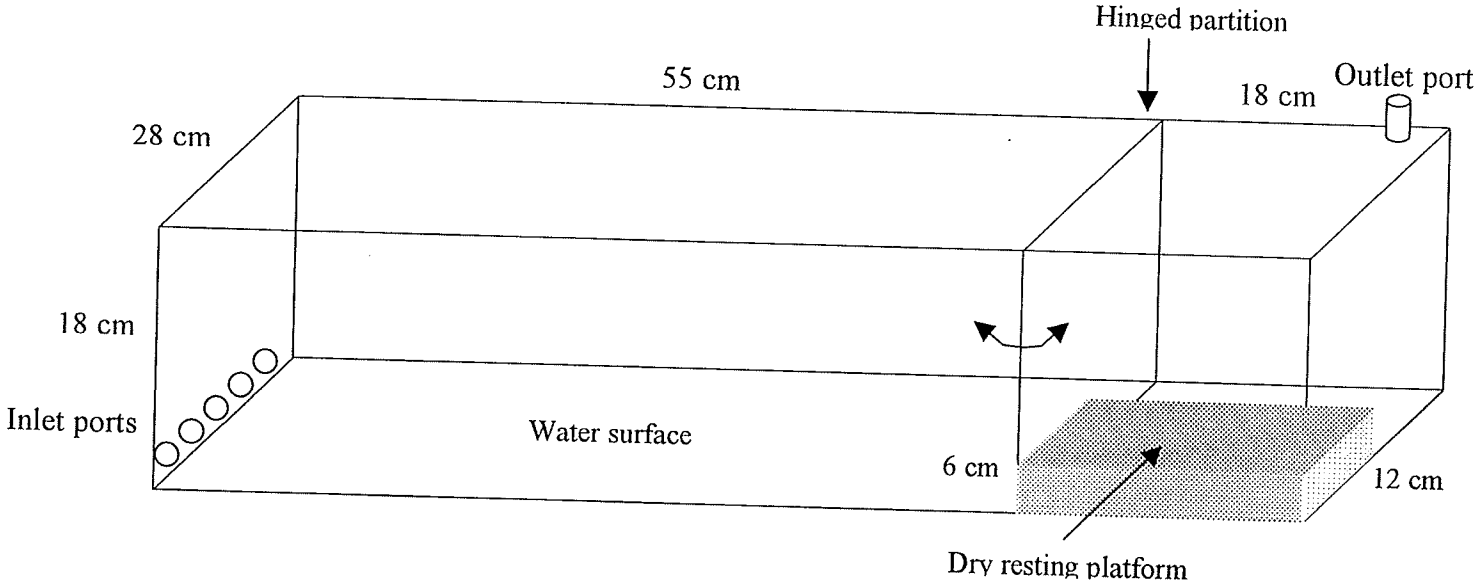

Outlet port
15 cm
Fan
17 cm
15 cm
Dry resting platform
10 cm
Trap Door
Inlet ports
1.5 cm
Water

period required for the T_b of all animals to recover to normothermic values (mean T_b recovery time = 27 ± 1 min).

Experiment 2: Free Diving during Continuous Immersion

In nature, muskrats may dive either from the water surface during periods of continuous immersion, or from a dry site such as a bank or the chamber of a dwelling or feeding shelter. Experiments 2 and 3 were designed to reflect these two natural dive patterns. In experiment 2, pre-chilled and non-chilled muskrats were introduced into a 2.1 × 1.2 × 0.6 m fiberglass-lined, plywood tank (MacArthur and Krause 1989) filled to a depth of 0.6 m with either 10°C or 30°C water (*n* = 10 animals; 4 treatments per animal). Submerged screens prevented diving muskrats from surfacing at any point other than in a purpose-built metabolic chamber (14.6 L, Fig. 2) situated at one end of the covered tank. A water temperature (T_w) of 30°C was selected for one set of trials, since it is a T_w known to induce minimal thermoregulatory costs in muskrats (MacArthur 1984). At this T_w, average $\dot{V}O_2$ during the run (see below) should incorporate the costs of exercise associated with diving and post-immersion grooming, as well as any thermal recovery costs associated with the pre-chilling treatment. Trials were repeated in 10°C water for two reasons. First, the possibility exists that chilled animals may at least partially rewarm during 15 min immersion in 30°C water. Colder water was used to ensure animals remained chilled for the duration of the free-dive period. A trial T_w of 10°C is also a closer approximation of marsh T_w during fall and winter and hence was necessary to make the behavioural data

Figure 2. Schematic diagram of 14.57 L metabolic chamber used in diving studies. Effective chamber size is determined by chamber dimensions and water level. Note the presence of a hinged partition which blocks movement between the dry resting platform and the water surface when closed.


Hinged partition
55 cm
18 cm
Outlet port
28 cm
18 cm
Inlet ports
Water surface
6 cm
12 cm
Dry resting platform

more ecologically relevant. As well, it has been noted previously that diving behaviour is affected by water temperature (MacArthur 1984). Therefore, use of only a single, high water temperature in this study could have introduced a systematic error.

$\dot{V}O_2$ and dive behaviour were monitored continuously over the 15-min period of continuous immersion. Several behavioural indices of dive performance were measured during this aquatic phase, including dive frequency, average and total dive time, and dive: surface ratio (ratio of surface recovery time to duration of preceding dive). The duration of the single longest exploratory dive and the average duration of the three longest exploratory dives were also documented.

Following 15 min of free diving during continuous immersion, muskrats were allowed access to the dry "platform" section of the metabolic chamber (Fig. 2). Post-immersion recovery of $\dot{V}O_2$ and T_b were monitored for an additional 40-60 min following emergence of the animals from water. In this case, average $\dot{V}O_2$ incorporated the energetic costs of recovery, including the costs of restoring T_b and eliminating end-products of anaerobic metabolism that accumulate during exercise, as well as costs associated with grooming and post-immersion comfort movements in the chamber. The influence of hypothermia on metabolic rate was assessed by plotting the regressions of average $\dot{V}O_2$ (associated with diving and all surface activity) during the immersion phase and calculated diving $\dot{V}O_2$ against percent time diving for pre-chilled and control animals, respectively. Based on the hypothesis that a decreased T_b allows muskrats to dive at lower cost, I

predicted that pre-chilled animals should exhibit a lower V_{O_2} with increased dive time, compared to non-chilled controls. Also tested was the strength of correlation between both average and diving V_{O_2} and ΔT_b of pre-chilled muskrats

Experiment 3: Free Diving from Platform

In this case, the set-up described for experiment 2 was slightly modified such that muskrats had access to a dry platform in the metabolic chamber (Fig. 2) throughout the 15-min free-dive period. This modification may also have provided a less stressful environment for the diving muskrats. Its inclusion is justified on the basis that animals observed to dive frequently during periods of continuous immersion (experiment 2) may, in fact, have been trying to escape from the water. Otherwise, the testing apparatus and experimental protocol adopted in experiment 3 were identical to those described for experiment 2 (n = 10; 4 treatments per animal).

Experiment 4: Dives of Controlled Duration

Using the same metabolic chamber (Fig. 1) and tank setup described previously (see experiments 1,2,3), muskrats performed single controlled dives of 0.5, 1, 1.5 and 2 min duration (n = 10, 8 treatments per animal). In each instance, a pre-chilled or non-chilled control muskrat entered the water directly from the metabolic chamber and dive duration was controlled by blocking the entrance to the metabolic chamber once the muskrat had initiated a dive. Following each dive, muskrats were confined to the metabolic chamber and post-

dive excess $\dot{V}O_2$ was calculated for a 40-60-min period. Trial T_w was kept constant at 30°C in this experiment.

Preliminary observations indicated that little rewarming of hypothermic animals occurs during the first 5 min of activity in 30°C water. On the other hand, I have observed the T_b of normothermic muskrats to begin declining soon after exposure to cold water. Thus a trial T_w of 30°C seemed most appropriate to minimize changes in T_b over the course of each dive. Post-dive excess $\dot{V}O_2$ was calculated for both pre-chilled and control muskrats after accounting for the metabolic costs of post-immersion recovery in the non-diving groups (experiment 1). Corrected estimates of post-dive excess $\dot{V}O_2$ for each dive time were compared in normothermic and hypothermic muskrats.

Experiment 5: Dive Trials on Juvenile Muskrats

Diving $\dot{V}O_2$ of normothermic and hypothermic juveniles were calculated in the same manner as for adults (see experiments 1-4). However, due to time constraints associated with working on young, rapidly growing animals, juveniles did not participate in the same number of treatments as adults. As well, the protocol was altered slightly to account for the smaller size of juveniles. Immersion time during the chilling procedure was reduced to 20-30 min, since the smaller animals were more susceptible to cooling than adults. Free-dive experiments (experiments 2 and 3) involving juveniles were conducted in 30°C water only. As muskrats in nature typically would not encounter 10°C water until

they have grown to at least the sub-adult stage, there was little ecological relevance to testing young animals at this colder T_w.

The youngest cohort (Juv1, n = 8) participated in experiments 2 and 3, and were allowed only a 10-min free-dive period. As these 200-400 g animals were considered too young for transmitter implants, T_b was taken rectally before and after the chilling or control immersion period to determine degree of hypothermia. Since it was not possible to monitor T_b recovery following diving, post-immersion $\dot{V}O_2$ was not recorded from Juv1 muskrats and these animals were removed from the chamber immediately following the free-dive period.

The older juvenile cohort (Juv2, n = 6) participated in experiments 1, 2 and 4. These animals were instrumented with intra-abdominal T_b transmitters and the experimental protocol followed that described for adults. Experiment 3 was omitted so that tests involving this single cohort could be condensed in order to minimize any confounding effects of growth on metabolic measurements. Experiment 2 was retained so that comparisons could be made between the two juvenile cohorts. In order to streamline experiment 4, controlled dives of only a single duration (30 s) were conducted on normothermic and hypothermic Juv2 animals.

Experiment 6: Heart Rate During Diving and Post-Immersion Recovery

All eight adults instrumented with ECG/T_b transmitters participated in each "pre-chill" and "non-chill" trial. Three replicate trials assigned in random order were conducted on each subject to ensure the animal performed several long

dives at each T_b. Following controlled immersion in water (T_w = 6 or 30°C, see above), instrumented muskrats entered the metabolic chamber (Fig. 1) and were allowed 15-min of free diving from the dry platform (T_w = 30°C). The metabolic chamber provided access to a diving tank that was partitioned into three inter-connected swimming lanes (180 × 12 × 30 cm per lane) to encourage long-duration dives.

$\dot{V}O_2$ was measured for the duration of each 15-min trial and for a subsequent 30-min recovery period. For each T_b treatment, mean diving heart rate recorded during the free-dive period was regressed on mean dive duration. Also tested was the degree to which mean diving heart rate correlated with diving $\dot{V}O_2$ and with ΔT_b of pre-chilled muskrats. Mean $\dot{V}O_2$ recorded during periods of steady-state activity in the post-immersion recovery period was regressed against mean heart rate recorded over the same period, in order to assess the degree of correspondence between these variables. The development of diving bradycardia was also compared between the two treatment groups using a time-series plot of heart rate calculated at 0.5-s intervals during selected dives. Representative heart rate recordings were also obtained for each trial when the animal was grooming and resting at the surface.

Statistical Analyses

Data were tested for normality using the Shapiro-Wilkes statistic and homogeneity of variance was confirmed using a modified Levene test. The absence of statistical outliers was confirmed using Bonferroni's outlier test.

Transformations were employed when necessary to meet normality assumptions for statistical tests. Behavioural and metabolic data for the adult cohort were analyzed using 2-sample ANOVA procedures to compare the effects of T_w and T_b. Paired *t*-tests were used in analyses of these data for juveniles, since these animals were exposed to only a single T_w. Least-squares regression analyses were employed to test for linear relationships between ΔT_b and specified behavioural and metabolic parameters. Regression analyses were also used to compare linear relationships between metabolic, heart rate and dive parameters. Linear relationships for chilled and non-chilled control animals were compared using *F*-tests for slopes and elevations. Repeated-measures ANOVA was employed to test for differences in time series or related data (*e.g.* dives of controlled duration). Bonferroni's correction factor for sequential/related tests (Rice 1989) was applied to *P*-values generated from groups of *t*-tests for parameters that were not independent of each other. Means are presented with ± 1 SEM.

RESULTS

Dive Behaviour of Normothermic Muskrats

Influences of Water Temperature and Diving Situation

During continuous immersion trials, adult muskrats generally dove more at T_w = 30°C than at T_w = 10°C. For adult muskrats diving from the water surface at T_w = 30°C, average dive duration was ca. 20% longer and cumulative dive time was elevated by more than 25%, compared to animals diving in T_w = 10°C (Table 1; $F_{3,39}$ = 6.237, P = 0.017 and $F_{3,39}$ = 4.146, P = 0.049, respectively). However, dive: surface (D:S) ratios of adult muskrats diving from either the water surface or a dry platform were not affected by T_w ($F_{1,39}$ = 0.15, P = 0.71 and $F_{1,39}$ = 0.21, P = 0.65, respectively). Normothermic adults diving from a dry platform at T_w = 30°C demonstrated an average dive time of 33.5 ± 2.8 s, a value ca. 50% greater than the 19.8 ± 1.2 s obtained for the same animals diving from the water surface (Tables 1, 2). However, in all trials access to a dry platform was accompanied by a decrease in the cumulative dive time of adults (Tables 1, 2). For example, at T_w = 10°C, normothermic adults dove from the water surface for a total of 284.7 ± 31.1 s compared to only 185.5 ± 27.4 s from a dry platform. Adults diving from a dry platform exhibited dive frequencies that were less than half those recorded from these same individuals during continuous immersion trials, regardless of T_w (Tables 1, 2). For example, dive frequencies recorded from normothermic muskrats diving at T_w = 30°C averaged 1.18 ± 0.09 dives·min^{-1} during continuous immersion and only 0.53 ± 0.09 dives·min^{-1} for platform diving.

Table 1. Comparisons of selected behavioural and metabolic dive parameters for adult (> 600g, *n* = 10) and juvenile (Juv2 = 400-600 g, *n* = 6; Juv1 = 200-400 g, *n* = 8) muskrats diving from the water surface at T_w = 10 and 30°C.

		T_w = 30°C		T_w = 10°C	
Variable		Pre-chilled	Control	Pre-chilled	Control
Mean dive time (s)					
	Adults	23.2 ± 1.8 ^{A}	19.8 ± 1.3 ^{AB}	17.5 ± 1.2 ^{B}	18.1 ± 1.5 ^{B}
	Juv2	14.3 ± 1.2 ^{A}	18.6 ± 1.8 ^{A}		
	Juv1	11.1 ± 0.3 ^{A}	14.6 ± 1.1 ^{B}		
Longest exploratory dive (s)					
	Adults	45.1 ± 2.7 ^{A}	41.1 ± 3.8 ^{AB}	32.6 ± 2.4 ^{C}	34.1 ± 2.1 ^{BC}
	Juv2	48.7 ± 7.0 ^{A}	39.8 ± 9.1 ^{A}		
	Juv1	21.6 ± 1.4 ^{A}	35.9 ± 6.0 ^{B}		
Mean of 3 longest exploratory dives (s)					
	Adults	39.1 ± 2.4 ^{A}	36.9 ± 3.0 ^{AB}	29.2 ± 2.1 ^{C}	30.6 ± 1.8 ^{BC}
	Juv2	35.8 ± 2.4 ^{A}	39.8 ± 4.8 ^{A}		
	Juv1	19.2 ± 1.2 ^{A}	25.8 ± 2.3 ^{B}		

Cumulative dive time (s)				
Adults	368.5 ± 40.2 ^{A}	354.2 ± 40.8 ^{A}	288.2 ± 34.1 ^{A}	284.7 ± 31.1 ^{A}
Juv2	363.2 ± 39.2 ^{A}	446.8 ± 36.0 ^{A}		
Juv1	138.4 ± 18.9 ^{A}	190.8 ± 22.3 ^{A}		
Dive Frequency (dives·min^{-1})				
Adults	1.11 ± 0.15 ^{A}	1.18 ± 0.09 ^{A}	1.14 ± 0.14 ^{A}	1.12 ± 0.14 ^{A}
Juv2	1.72 ± 0.17 ^{A}	1.63 ± 0.14 ^{A}		
Juv1	1.25 ± 0.17 ^{A}	1.26 ± 0.11 ^{A}		
Dive : Surface Ratios				
Adults	1.25 ± 0.29 ^{A}	1.20 ± 0.21 ^{A}	1.02 ± 0.15 ^{A}	1.30 ± 0.29 ^{A}
Juv2	1.44 ± 0.22 ^{A}	1.98 ± 0.36 ^{A}		
Juv1	0.71 ± 0.12 ^{A}	0.90 ± 0.13 ^{A}		
Diving $\dot{V}O_2$ (mL $O_2 \cdot g^{-1} \cdot h^{-1}$)				
Adults	2.31 ± 0.34 ^{AB}	2.37 ± 0.31 ^{AB}	3.33 ± 0.25 ^{A}	2.19 ± 0.24 ^{B}
Juv2	2.12 ± 0.24 ^{A}	1.87 ± 0.18 ^{A}		
Juv1	2.64 ± 0.49 ^{A}	3.32 ± 0.40 ^{A}		
Average $\dot{V}O_2$ during dive trial (mL $O_2 \cdot g^{-1} \cdot h^{-1}$)				
Adults	1.34 ± 0.07 ^{A}	1.33 ± 0.05 ^{A}	2.11 ± 0.07 ^{B}	1.76 ± 0.10 ^{C}
Juv2	1.54 ± 0.07 ^{A}	1.48 ± 0.08 ^{A}		
Juv1	2.37 ± 0.10 ^{A}	1.91 ± 0.07 ^{B}		

NOTE: Values are presented as means ± SE. Like means ($P > 0.05$) within any row are followed by the same superscript letter (statistically related groups were generated by paired *t*-tests or Tukey's pairwise comparisons).

Table 2. Comparisons of selected behavioural and metabolic dive parameters for adult (> 600 g, *n* = 10) and juvenile (Juv2 = 400-600 g, *n* = 6; Juv1 = 200-400 g, *n* = 8) muskrats diving from a dry platform at T_w = 10 and 30°C.

	T_w = 30°C		T_w = 10°C	
Variable	Pre-chilled	Control	Pre-chilled	Control
Mean dive time (s)				
Adults	31.5 ± 2.4 ^{A}	33.5 ± 2.8 ^{A}	27.8 ± 1.3 ^{A}	28.5 ± 2.2 ^{A}
Juv1	14.3 ± 0.9 ^{A}	15.7 ± 1.2 ^{A}		
Longest exploratory dive (s)				
Adults	44.9 ± 4.4 ^{AB}	51.3 ± 5.5 ^{A}	40.9 ± 2.9 ^{B}	40.3 ± 4.0 ^{B}
Juv1	28.5 ± 2.7 ^{A}	26.8 ± 3.6 ^{A}		
Mean of 3 longest exploratory dives (s)				
Adults	41.8 ± 4.6 ^{A}	45.9 ± 3.7 ^{A}	37.9 ± 2.6 ^{B}	35.2 ± 3.1 ^{B}
Juv1	22.6 ± 1.9 ^{A}	22.8 ± 2.5 ^{A}		
Cumulative dive time (s)				
Adults	252.7 ± 45.4 ^{A}	260.2 ± 42.3 ^{A}	201.9 ± 36.7 ^{A}	185.5 ± 27.4 ^{A}
Juv1	182.4 ± 21.6 ^{A}	144.5 ± 21.5 ^{A}		

Dive Frequency (dives·min^{-1})				
Adults	0.53 ± 0.10 ^{A}	0.53 ± 0.09 ^{A}	0.49 ± 0.08 ^{A}	0.45 ± 0.07 ^{A}
Juv1	1.36 ± 0.18 ^{A}	0.89 ± 0.10 ^{B}		
Dive : Surface Ratios				
Adults	1.14 ± 0.19 ^{A}	1.28 ± 0.24 ^{A}	1.57 ± 0.33 ^{A}	1.84 ± 0.60 ^{A}
Juv1	1.50 ± 0.12 ^{A}	1.57 ± 0.44 ^{A}		
Diving $\dot{V}O_2$ (mL $O_2 \cdot g^{-1} \cdot h^{-1}$)				
Adults	1.89 ± 0.20 ^{A}	2.48 ± 0.35 ^{A}	2.50 ± 0.31 ^{A}	2.46 ± 0.35 ^{A}
Juv1	1.52 ± 0.09 ^{A}	2.20 ± 0.37 ^{A}		
Average $\dot{V}O_2$ during dive trial (mL $O_2 \cdot g^{-1} \cdot h^{-1}$)				
Adults	1.55 ± 0.04 ^{A}	1.45 ± 0.05 ^{A}	1.88 ± 0.06 ^{B}	1.74 ± 0.06 ^{B}
Juv1	1.89 ± 0.04 ^{A}	1.75 ± 0.07 ^{A}		

NOTE: Values are presented as means ± SE. Like means ($P > 0.05$) within any row are followed by the same superscript letter (statistically related groups were generated by paired *t*-tests or Tukey's pairwise comparisons).

Age Effects

The average dive time of normothermic Juv2 muskrats tested in 30°C water (18.6 ± 1.8 s) was similar to that recorded for adults (19.8 ± 1.2 s; Table 1). However, normothermic Juv1 individuals tested at T_w = 30°C exhibited an average dive time of 14.6 ± 1.1 s, which was 35% lower than for adults. On average, normothermic Juv2 muskrats tested at T_w = 30°C in experiment 2 had cumulative dive times of 446.8 ± 36.0 s, a 20% increase over the 354.2 ± 40.8 s recorded for normothermic adults. Regardless of age, juveniles displayed higher dive frequencies at T_w = 30°C than did adults (Tables 1, 2). The mean dive frequency of adults diving from the water surface (1.18 ± 0.09 dives·min^{-1}) was surpassed by both Juv1 (1.26 ± 0.11 dives·min^{-1}) and Juv2 (1.63 ± 0.14 dives·min^{-1}) animals. Even when Juv1 young were diving from a dry platform, their mean dive frequency (0.89 ± 0.10 dives min^{-1}) was nearly 70% higher than for adults (0.53 ± 0.09 dives min^{-1}). The mean of the three longest exploratory dives of normothermic Juv2 muskrats diving from the water surface at T_w = 30°C (39.8 ± 4.8 s) was virtually identical to the value recorded for normothermic adults (36.9 ± 3.0 s). By comparison, the average of the three longest dives for normothermic Juv1 muskrats was only 25.8 ± 2.3 s (Table 1). Juv2 muskrats demonstrated D:S ratios that were elevated more than 30% beyond those of adults, while the average D:S ratio of normothermic Juv1 young diving from a platform at T_w = 30°C (1.57 ± 0.44; Table 2) was elevated beyond that of normothermic adults (1.28 ± 0.24). However, Juv1 muskrats diving from the water surface demonstrated a reduced D:S ratio (0.90 ± 0.13; Table 1).

Dive Behaviour of Hypothermic versus Normothermic Muskrats

The sole behavioural difference detected between normothermic and hypothermic adults was that pre-chilled muskrats tested in 10°C water with access to a dry platform spent more time engaged in diving and surface swimming (mean immersion time = 283.3 ± 22.1 s) than did control animals (246.3 ± 12.1 s; $F_{3,39}$ = 12.552, P = 0.006). Behavioural indicators of dive capacity, such as the longest exploratory dive and the average of the three longest exploratory dives, showed no changes related to T_b in adult muskrats diving either from the water surface (Table 1) or from a dry platform (Table 2). For instance, for adults diving from the water surface at T_w = 10°C, longest exploratory dive times were comparable for hypothermic (32.6 ± 2.4 s) and normothermic (34.1 ± 2.1 s) muskrats. Similarly, the longest dives observed in platform trials at T_w = 10°C were comparable for pre-chilled (40.9 ± 2.9 s) and control (40.3 ± 4.0 s) animals. Variables such as average dive time, cumulative dive time, dive frequency and D:S ratio were not affected by chilling in adults diving either from the water surface (Table 1) or from a dry platform (Table 2). Animals allowed access to a dry platform spent ca. 60% of each trial grooming, irrespective of T_b ($F_{3,39}$ = 0.04, P = 0.85).

Contrary to the situation in adults, several behavioural indicators of dive performance were affected by hypothermia in juvenile animals. In the oldest juvenile cohort tested (Juv2), an elevation (albeit non-significant) was observed in the average and cumulative dive times of normothermic compared to

hypothermic young (Table 1). Pre-chilling caused the average dive time of Juv2 animals to decline from 18.6 ± 1.8 s to 14.3 ± 1.2 s (t = 2.13, df = 5, P = 0.09), while their cumulative dive time decreased from 446.8 ± 36.0 s to 363.2 ± 39.2 s (t = 2.14, df = 5, P = 0.09). Additionally, Juv2 muskrats showed a slight reduction in D:S ratios when pre-chilled (Table 1; t = 2.43, df = 5, P = 0.06). It is noteworthy that despite their shorter pre-chilling times, juvenile muskrats experienced a greater degree of hypothermia than did the adults in this study (Figs. 3, 4).

The youngest (Juv1) cohort demonstrated the most profound behavioural changes associated with pre-chilling. For Juv1 animals diving from the water surface, both the single longest and the average of the three longest exploratory dives were reduced in pre-chilled individuals. For this cohort, the longest exploratory dive time decreased from 35.9 ± 6.0 s to 21.6 ± 1.4 s (t = 3.401, df = 7, P = 0.011), while the average of three longest dives was reduced from 25.8 ± 2.3 s to 19.2 ± 1.2 s with pre-chilling (t = 2.817, df = 7, P = 0.026). Hypothermia was also accompanied by a decrease in both average (14.6 ± 1.1 s to 11.1 ± 0.3 s; Fig. 1) and cumulative (190.8 ± 22.3 s to 138.4 ± 18.9 s; Table 1) dive times of Juv1 animals diving from the water surface (t = 2.808, df = 7, P = 0.026; t = 2.894, df = 7, P = 0.026, respectively). These behavioural changes were not observed when Juv1 animals were provided access to a dry platform. In fact, in these latter trials, dive frequency of Juv1 muskrats increased from 0.89 ± 0.10 to

Figure 3. Relationship between selected behavioural indices of dive performance and the extent of body cooling in pre-chilled adult and juvenile muskrats diving from the water surface during a 10- to 15-min period of continuous immersion. Data are presented for adults (> 600 g) diving at T_w = 30°C (closed circles), adults diving at T_w = 10°C (open circles), Juv2 cohort (400-600 g) diving at T_w = 30°C (closed triangles) and Juv1 cohort (200-400 g) diving at T_w = 30°C (open triangles).

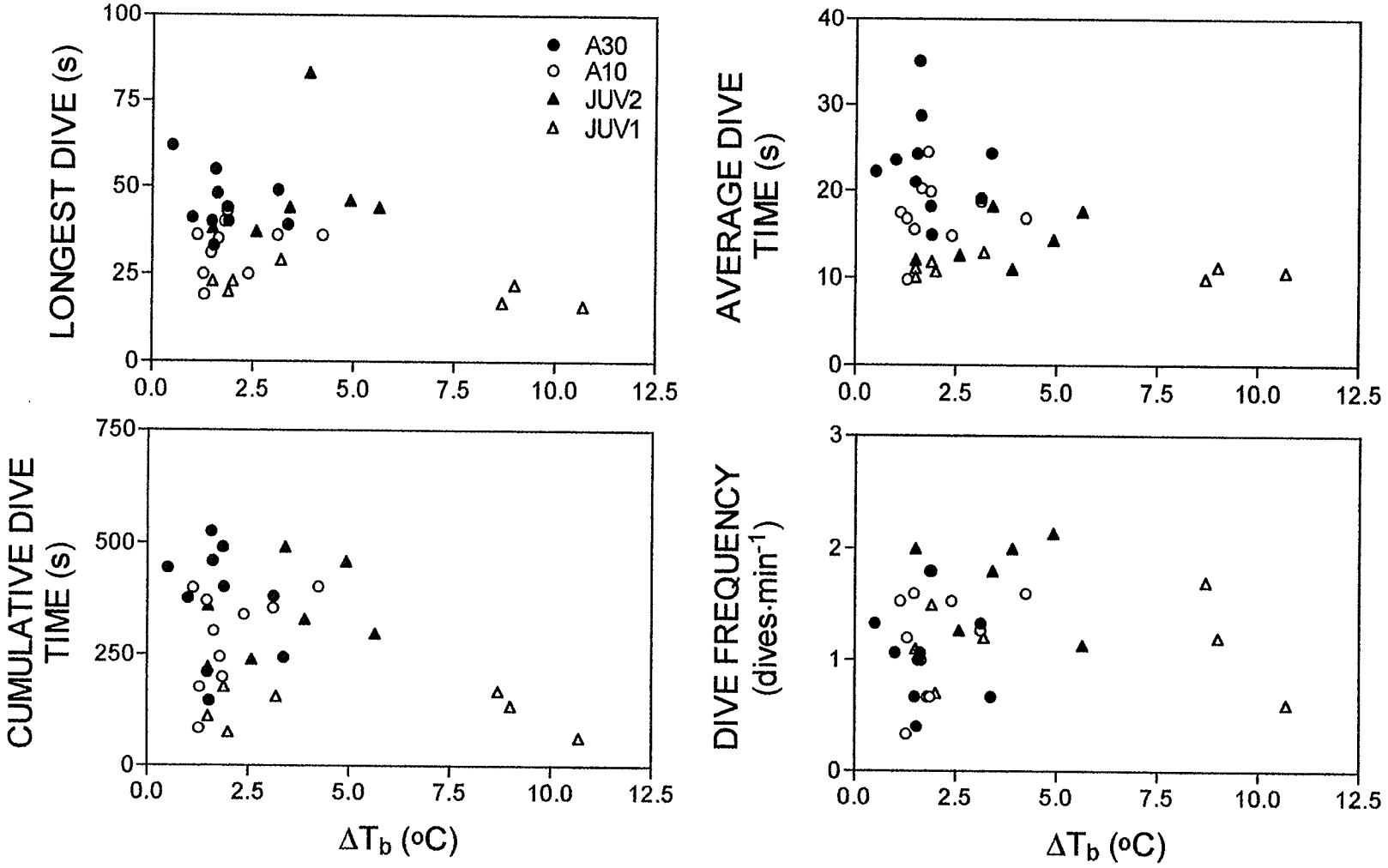
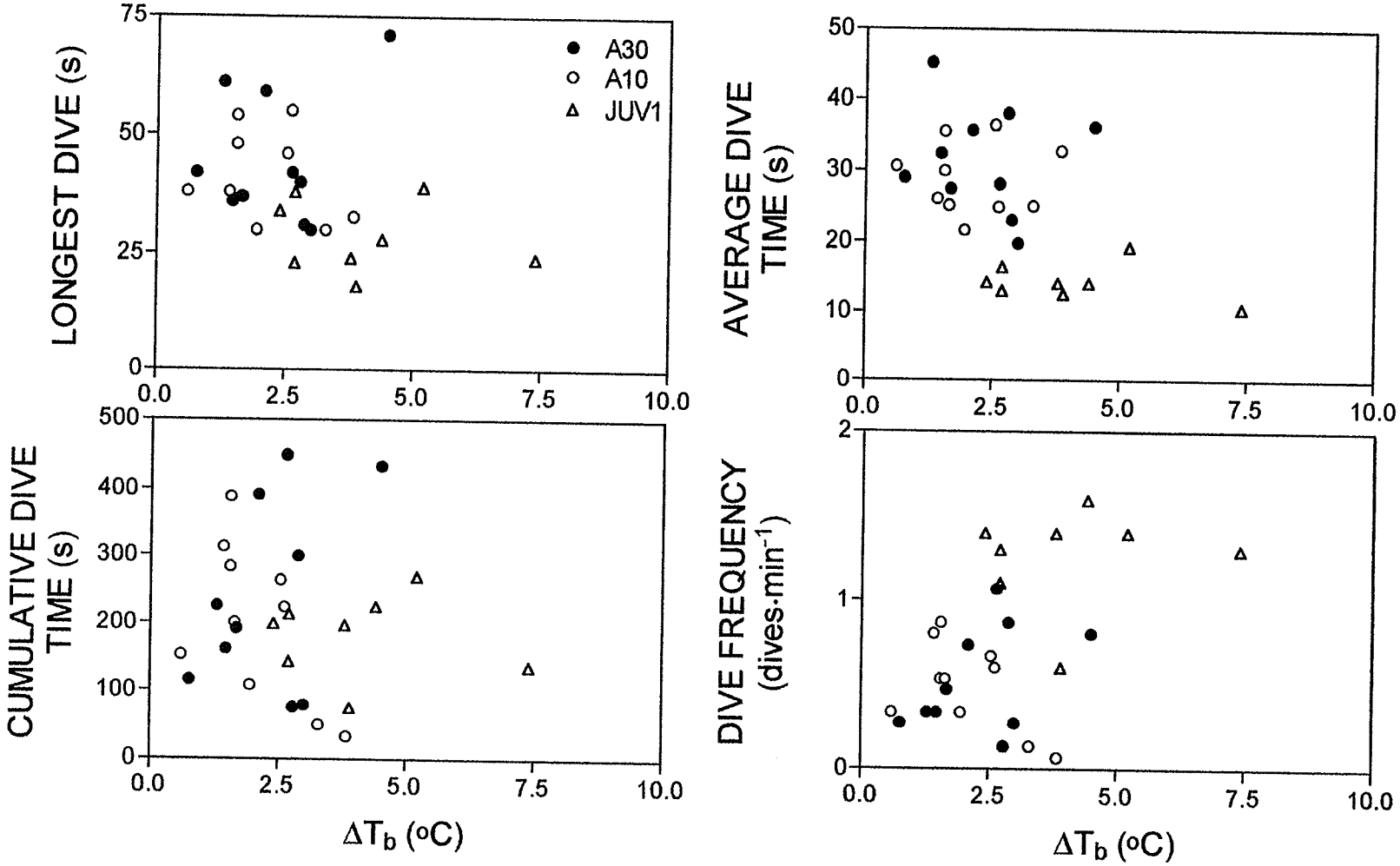

LONGEST DIVE (s)
AVERAGE DIVE TIME (s)
CUMULATIVE DIVE TIME (s)
DIVE FREQUENCY (dives·min-1)
ΔTb (oC)
ΔTb (oC)
A30
A10
JUV2
JUV1
0
25
50
75
100
10
20
30
40
250
500
750
1
2
3
0.0
2.5
5.0
7.5
10.0
12.5

Figure 4. Relationship between selected behavioural indices of dive performance and the extent of body cooling in pre-chilled adult and juvenile muskrats during 10- to 15-min period of diving from a dry platform. Data are presented for adults (> 600 g) diving at Tw = 30°C (closed circles), adults diving at T_w = 10°C (open circles) and Juv1 cohort (200-400 g) diving at T_w = 30°C (open triangles).


LONGEST DIVE (s)
AVERAGE DIVE TIME (s)
CUMULATIVE DIVE TIME (s)
DIVE FREQUENCY (dives·min⁻¹)
ΔTb (oC)
ΔTb (oC)
A30
A10
JUV1
0
25
50
75
10
20
30
40
100
200
300
400
500
1
2
0.0
2.5
5.0
7.5
10.0

1.36 ± 0.18 with pre-chilling (Table 2; t = 3.319, df = 7, P = 0.013). It is also noteworthy that no relationship was apparent for any of the experimental groups between degree of body cooling and selected variables of dive behaviour. This finding applied to muskrats diving from both the water surface (Fig. 3) and from a dry platform (Fig. 4).

In contrast to the situation for adults implanted with body temperature transmitters, adults instrumented with heart rate transmitters showed a general reduction in dive time in response to similar levels of pre-chilling (Table 3). While cumulative dive times and dive frequencies of these animals were not affected by body temperature, their average dive time was significantly reduced with hypothermia (Table 3, P = 0.033). As well, a slight reduction was observed in both the longest and mean of the three longest exploratory dives of pre-chilled animals instrumented with heart rate monitors (Table 3).

Metabolic Rates of Normothermic Muskrats

Influences of Water Temperature and Diving Situation

T_w did not affect the diving VO_2 of adult muskrats tested in either paradigm (*i.e.* diving from the water surface or from a dry platform; Tables 1, 2). However, diving in T_w = 10°C did result in an increase in the average VO_2 of adults associated with all activities during the dive trial (Tables 1, 2). Diving VO_2 of adults was similar for individuals diving from a dry platform and from the water surface (Tables 1, 2). However, at T_w = 30°C, average VO_2 of adults over the dive interval (including all surface activity and grooming) was higher when

Table 3. Behavioural indices of dive performance in pre-chilled and normothermic (control) muskrats implanted with heart rate transmitters (n = 8; 3 replicates) diving from a dry platform in T_w = 30°C. Mean (± SE) values were compared using paired t-tests.

Variable	Pre-Chilled	Control	df	t	P
Mean dive time (s)	19.1 ± 3.8	28.9 ± 4.5	7	2.643	0.033
Longest dive (s)	54.6 ± 6.1	91.6 ± 16.7	7	2.030	0.082
Mean of 3 longest dives (s)	45.6 ± 6.1	68.5 ± 10.1	7	2.108	0.073
Cumulative dive time (s)	450.7 ± 111.1	517.2 ± 95.2	7	0.843	0.427
Dive frequency (dives·min^{-1})	0.51 ± 0.08	0.43 ± 0.08	7	1.591	0.156
Diving $\dot{V}O_2$ (mL O_2·g^{-1}·h^{-1})	2.95 ± 0.48	3.16 ± 0.35	7	0.4715	0.652
Average $\dot{V}O_2$ (mL O_2·g^{-1}·h^{-1})	1.66 ± 0.05	1.47 ± 0.03	7	4.526	0.003

animals dove from the platform than from the water surface. Conversely, access to a platform reduced average $\dot{V}O_2$ of the Juv1 cohort (Tables 1, 2).

Age Effects

Juv2 muskrats diving from the water surface at T_w = 30°C displayed a mean diving $\dot{V}O_2$ (1.87 ± 0.18 mL $O_2 \cdot g^{-1} \cdot h^{-1}$) slightly lower than that obtained for adults (2.37 ± 0.31 mL $O_2 \cdot g^{-1} \cdot h^{-1}$). Diving $\dot{V}O_2$ of the Juv1 cohort during voluntary diving from a dry platform averaged 2.20 ± 0.37 mL $O_2 \cdot g^{-1} \cdot h^{-1}$ for normothermic animals (Table 2). Diving $\dot{V}O_2$ was highest for the youngest cohort (Juv1) diving from the water surface, when it averaged 3.32 ± 0.40 mL $O_2 \cdot g^{-1} \cdot h^{-1}$ (Table 1). At T_w = 30°C, average $\dot{V}O_2$ over the dive session varied inversely with body mass, regardless of body temperature.

Metabolic and Cardiac Responses of Hypothermic versus Normothermic Muskrats

Metabolic Rates of Animals Diving from the Water Surface

At T_w = 30°C, diving $\dot{V}O_2$ was not affected by pre-chilling in either adults (Table 1; Tukey pairwise comparison P = 0.999), or juveniles free-diving from the water surface (t = 1.35, df = 7, P = 0.22 for Juv1; t = 0.85, df = 5, P = 0.44 for Juv2). Average $\dot{V}O_2$ was also unaffected by pre-chilling in both the adult (Table 1; Tukey pairwise comparison P = 0.999), and Juv2 group (t = 0.59, df = 5, P = 0.58). While diving from the water surface at T_w = 30°C, only the youngest juvenile cohort displayed an elevated average $\dot{V}O_2$ when pre-chilled (Table 1; t = 4.224, df = 7, P = 0.004).

For adults tested in both 10°C and 30°C water, significant interaction between body and water temperatures was present for diving and average $\dot{V}O_2$ ($F_{3,39}$ = 4.307, P = 0.045 and $F_{3,39}$ = 4.555, P = 0.040, respectively). Though no effect of pre-chilling was observed for either $\dot{V}O_2$ variable at T_w = 30°C, both cases revealed significantly elevated metabolic rates for pre-chilled muskrats diving from the water surface at T_w = 10°C (Table 1; Tukey pairwise comparison P = 0.045 for diving $\dot{V}O_2$; P = 0.017 for average $\dot{V}O_2$).

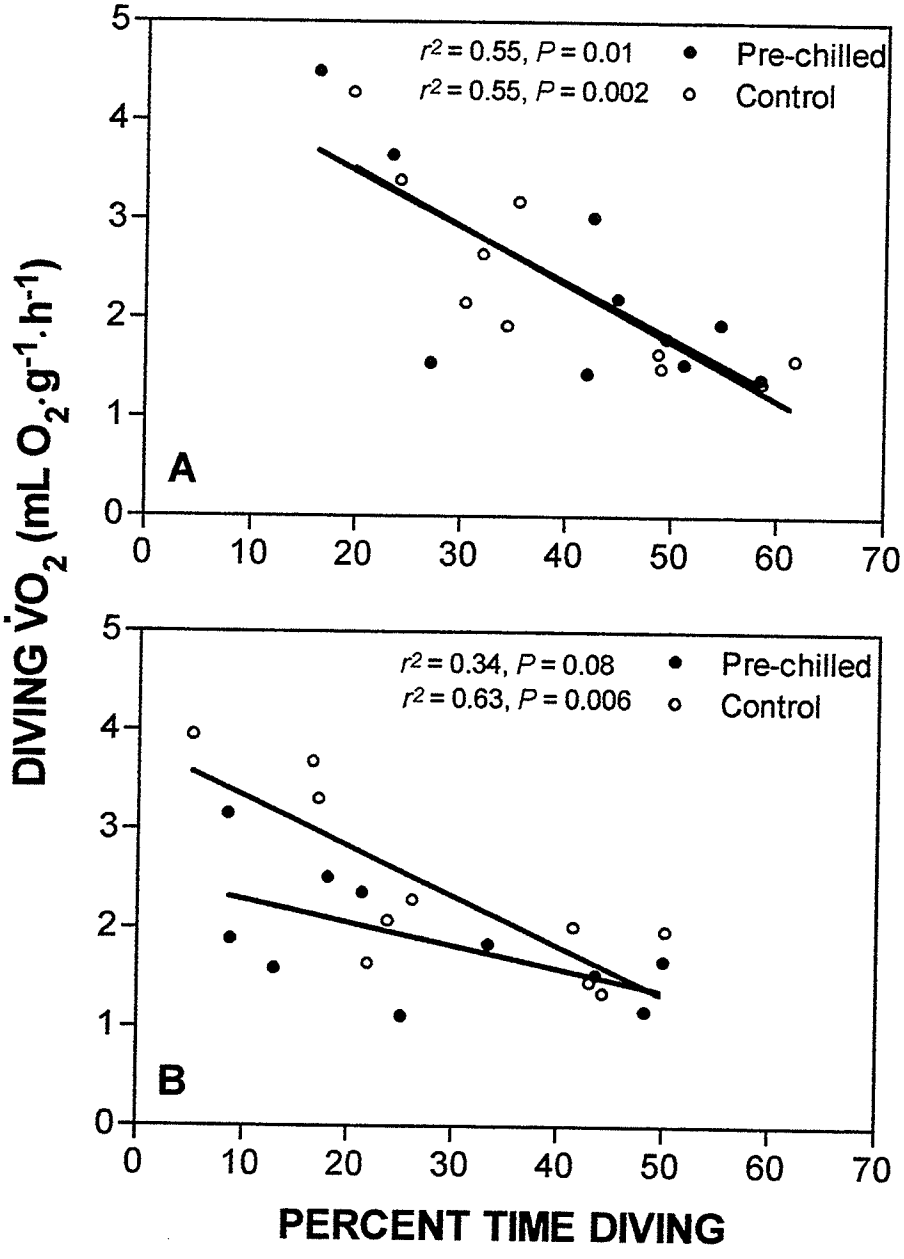
Metabolic Rates of Animals Diving from a Dry Platform

Pre-chilling had no discernable effect on the diving $\dot{V}O_2$ of adult muskrats during 15 min of voluntary diving from a dry platform, regardless of T_w (Table 2; $F_{3,39}$ = 1.19, P = 0.28). Diving $\dot{V}O_2$ of these adults was also not significantly affected by T_w ($F_{3,39}$ = 3.20, P = 0.08). Similar observations were made for 200-400 g (Juv1) muskrats diving from a dry platform (Table 2; t = 1.90, df = 7, P = 0.10). While average $\dot{V}O_2$ of Juv1 muskrats during platform diving was unaffected by pre-chilling (Table 2; t = 1.63, df = 7, P = 0.15), average $\dot{V}O_2$ of adults was elevated at both water temperatures for pre-chilled muskrats (Table 2; $F_{3,39}$ = 5.366, P = 0.026). As well, diving in 10°C, compared to 30°C water resulted in higher average $\dot{V}O_2$ for both hypothermic and normothermic adults ($F_{3,39}$ = 37.611, P < 0.0001).

Relationship between Submergence Time and Metabolic Rate

Diving $\dot{V}O_2$ was negatively correlated with percent time diving in pre-chilled and normothermic adults, whether animals dove from the water surface (Fig. 5A) or from a dry platform (Fig. 5B). This inverse relationship was also

Figure 5. Diving VO_2 versus percent time diving for 10 adult (> 600 g) muskrats diving over a 15-min period (T_w = 30°C) from either (A) the water surface during continuous immersion or (B) a dry platform. Responses of pre-chilled muskrats (closed circles) are compared with control values for the same individuals during normothermic diving (open circles).


$r^2 = 0.55$, $P = 0.01$
Pre-chilled
$r^2 = 0.55$, $P = 0.002$
Control
A
$r^2 = 0.34$, $P = 0.08$
Pre-chilled
$r^2 = 0.63$, $P = 0.006$
Control
B
DIVING $\dot{V}O_2$ (mL $O_2 \cdot g^{-1} \cdot h^{-1}$)
PERCENT TIME DIVING

apparent in the youngest (Juv1) cohort during a 10-min session of free-diving from the water surface (Fig. 6A). A similar, albeit weak trend was observed in Juv1 muskrats diving from a dry platform (Fig. 6B) and in Juv2 muskrats diving from the water surface (Fig. 7). Body temperature did not significantly affect the slope of this relationship in any of the groups tested. At T_w = 30°C, hypothermia affected the relationship between diving $\dot{V}O_2$ and percent time diving only in the youngest cohort (Juv1), in which it significantly decreased the elevation (Y-intercept) of the regression line (Fig. 6A; $F_{1,13} = 28.65$, $P = 0.0001$).

Unlike diving $\dot{V}O_2$, average $\dot{V}O_2$ showed little tendency to vary inversely with percent time underwater (Figs. 8-11). The only significant inverse relationship between average $\dot{V}O_2$ and percent time diving was observed in 200-400 g (Juv1) muskrats during 10 min of free-diving from the water surface (Fig. 11A). Though regression slopes were similar for pre-chilled and normothermic animals (Fig 11A; $F_{1,12} = 3.10$, $P = 0.10$), the regression intercept was significantly higher in hypothermic muskrats ($F_{1,13} = 11.20$, $P = 0.005$).

Heart Rate Responses of Adult Muskrats

When plotted against dive time, telemetered heart rate of both normo- and hypothermic adult muskrats followed a first-order exponential decay relationship (Fig. 12). Though regression slopes were similar ($F_{1,243} = 3.32$, $P = 0.07$), the intercept was significantly lower for chilled than for control animals ($F_{1,244} = 26.21$, $P < 0.0001$). However, only dives < 25 s were associated with more intense bradycardia in chilled animals (Table 4; $P = 0.0008$). For dives < 25 s, heart rate averaged 94.5 ± 4.6 beats·min^{-1} for hypothermic muskrats, and 109.4 ±

Figure 6. Diving VO_2 versus percent time diving for eight juvenile muskrats (Juv1; 200-400 g) diving over a 10-min period (T_w = 30°C) from either (A) the water surface during continuous immersion or (B) a dry platform. Responses of pre-chilled muskrats (closed circles) are compared with control values for the same individuals during normothermic diving (open circles).

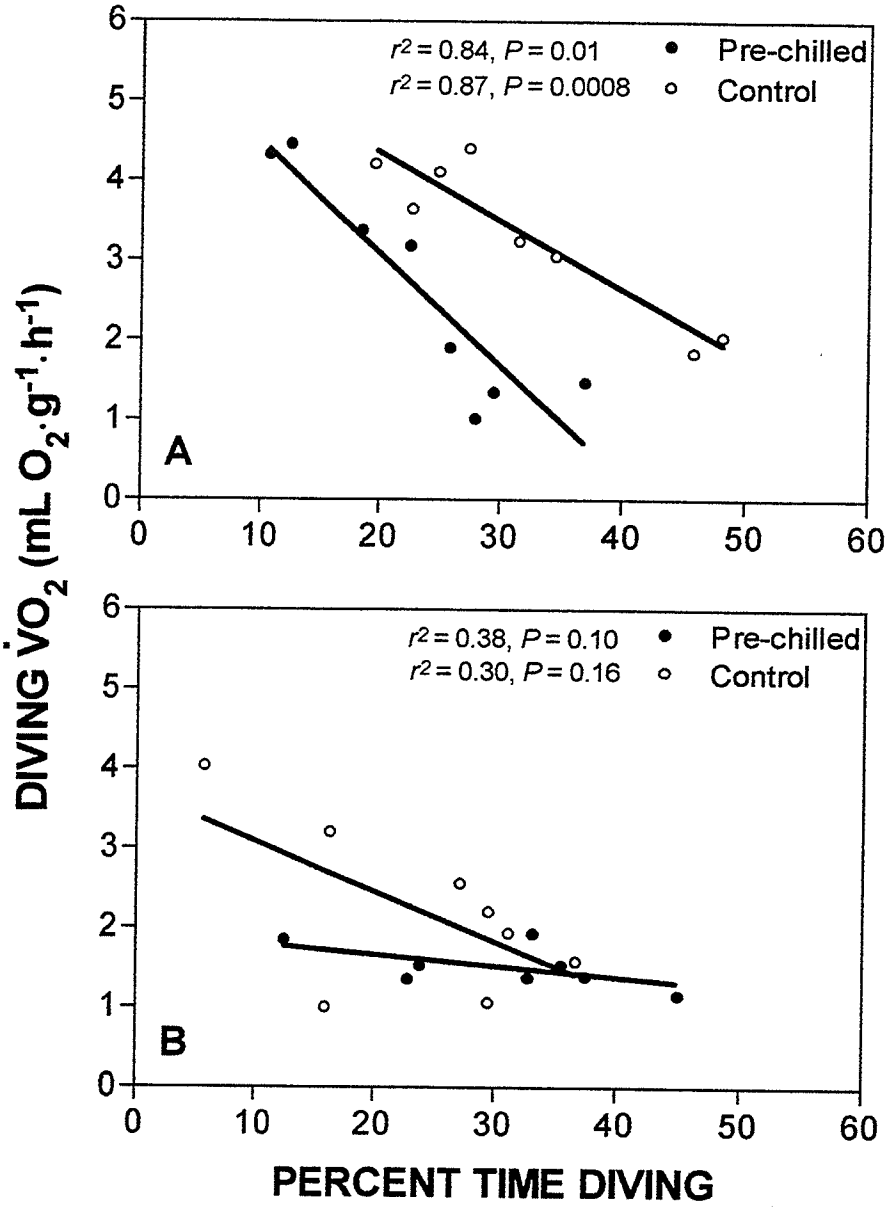

$r^2 = 0.84$, $P = 0.01$
Pre-chilled
$r^2 = 0.87$, $P = 0.0008$
Control
A
$r^2 = 0.38$, $P = 0.10$
Pre-chilled
$r^2 = 0.30$, $P = 0.16$
Control
B
DIVING $\dot{V}O_2$ (mL $O_2 \cdot g^{-1} \cdot h^{-1}$)
PERCENT TIME DIVING

Figure 7. Diving $\dot{V}O_2$ versus percent time diving for six juvenile muskrats (Juv2; 400-600 g) diving from the water surface (T_w = 30°C) during a 15-min period of continuous immersion. Responses of pre-chilled muskrats (closed circles) are compared with control values for the same individuals during normothermic diving (open circles).

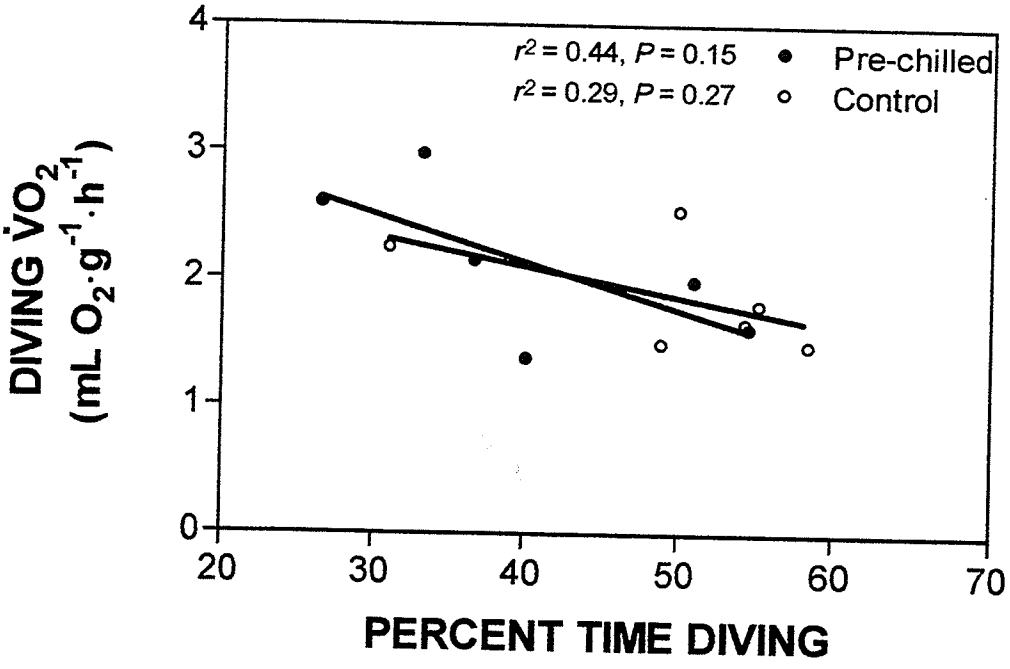

r^2 = 0.44, P = 0.15
r^2 = 0.29, P = 0.27
Pre-chilled
Control
DIVING $\dot{V}O_2$ (mL $O_2 \cdot g^{-1} \cdot h^{-1}$)
PERCENT TIME DIVING
0
1
2
3
4
20
30
40
50
60
70

Figure 8. Average VO_2 versus percent time diving for 10 adult (> 600 g) muskrats diving from the water surface during 15 min of continuous immersion at either (A) T_w = 30°C or (B) T_w = 10°C. Responses of pre-chilled muskrats (closed circles) are compared with control values for the same individuals during normothermic diving (open circles).

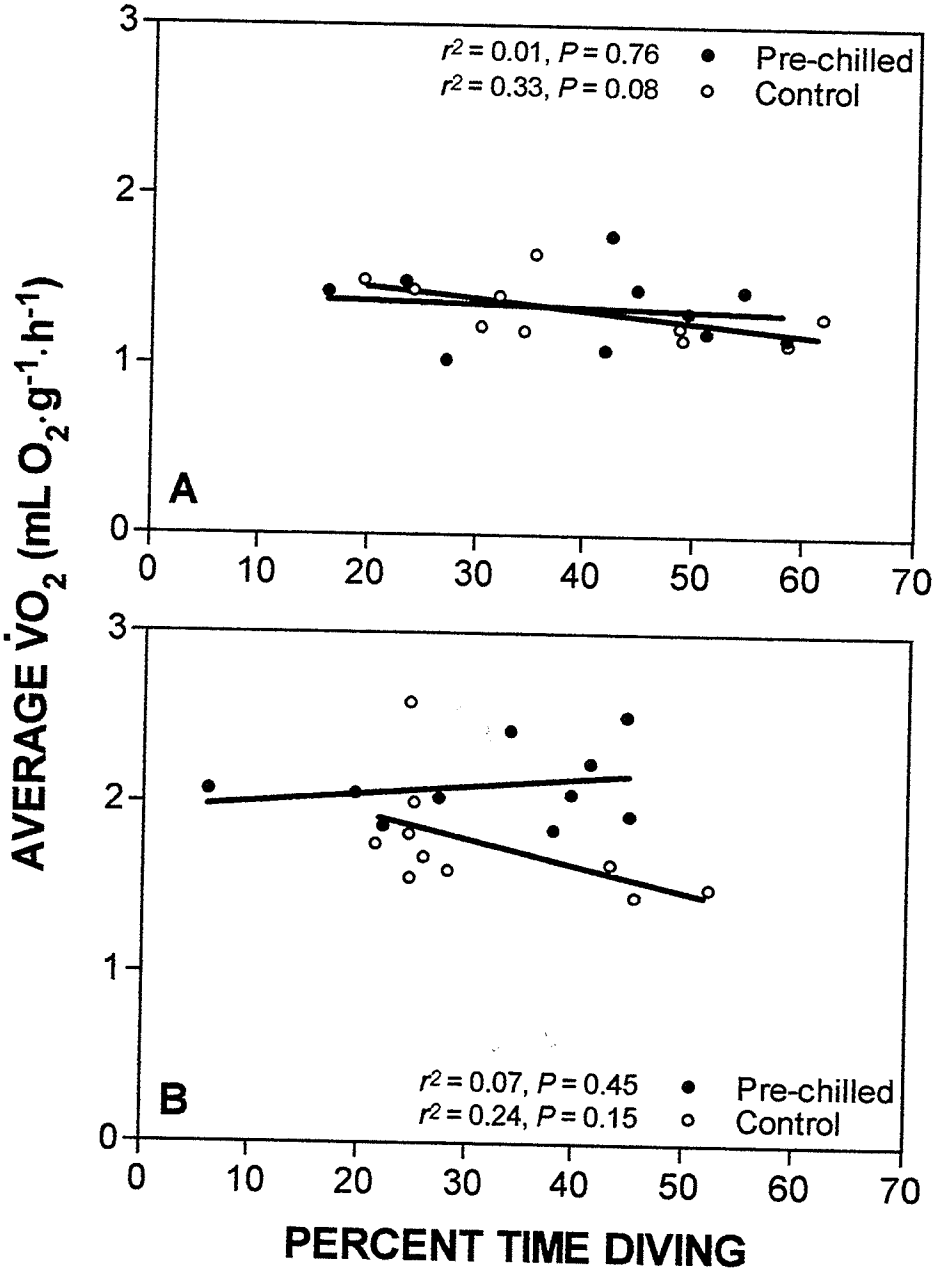

AVERAGE $\dot{V}O_2$ (mL $O_2 \cdot g^{-1} \cdot h^{-1}$)
PERCENT TIME DIVING
A
$r^2 = 0.01$, $P = 0.76$
$r^2 = 0.33$, $P = 0.08$
Pre-chilled
Control
B
$r^2 = 0.07$, $P = 0.45$
$r^2 = 0.24$, $P = 0.15$
Pre-chilled
Control
0
1
2
3
0
10
20
30
40
50
60
70

Figure 9. Average VO_2 versus percent time diving for 10 adult (> 600 g) muskrats diving over a 15-min period from a dry platform at either (A) T_w = 30°C or (B) T_w = 10°C. Responses of pre-chilled muskrats (closed circles) are compared with control values for the same individuals during normothermic diving (open circles).

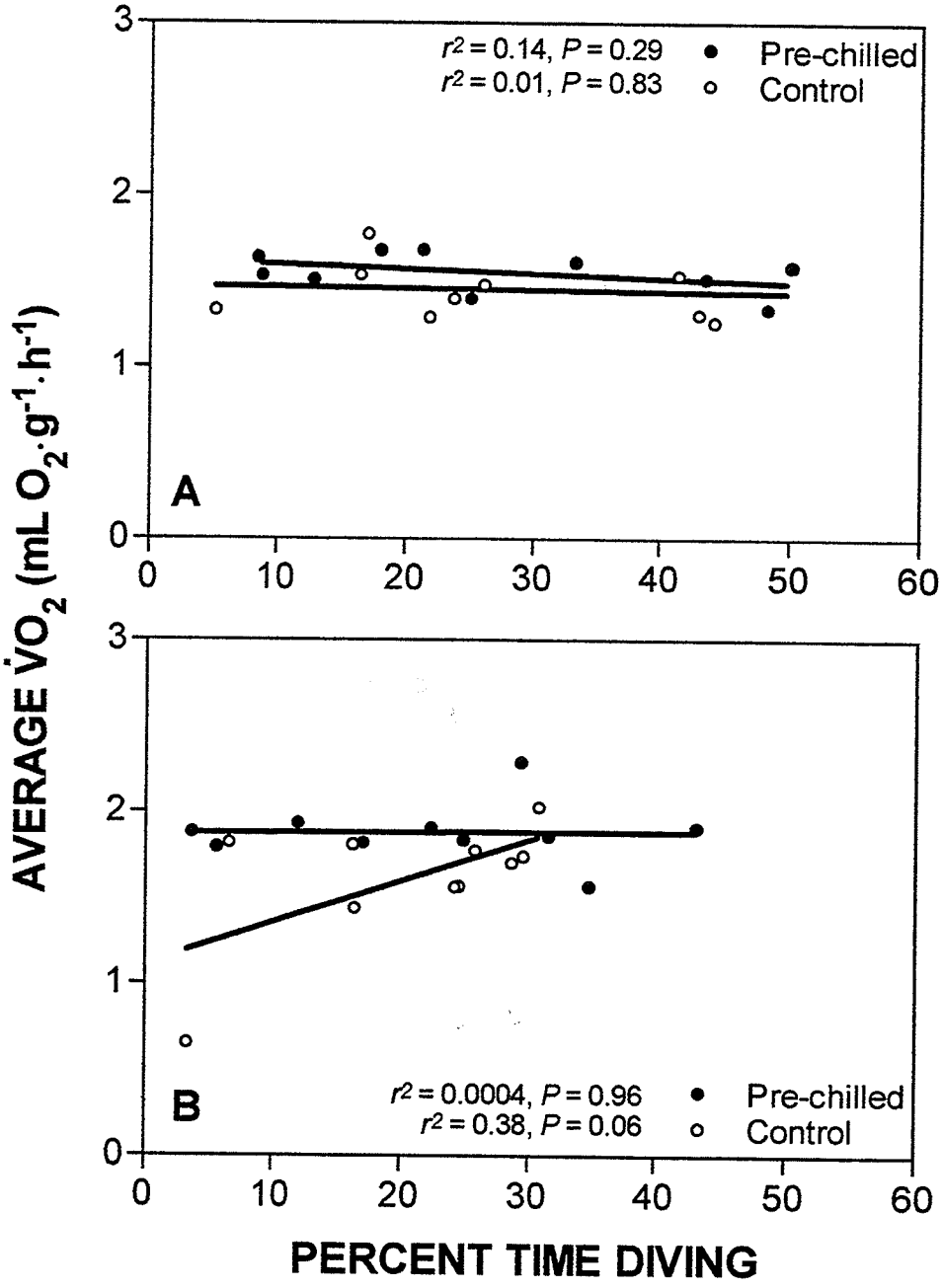

AVERAGE $\dot{V}O_2$ (mL $O_2 \cdot g^{-1} \cdot h^{-1}$)
PERCENT TIME DIVING
A
$r^2 = 0.14$, $P = 0.29$
$r^2 = 0.01$, $P = 0.83$
Pre-chilled
Control
B
$r^2 = 0.0004$, $P = 0.96$
$r^2 = 0.38$, $P = 0.06$
Pre-chilled
Control
0
1
2
3
0
10
20
30
40
50
60

Figure 10. Average $\dot{V}O_2$ versus percent time diving for six juvenile muskrats (Juv2; 400-600 g) diving from the water surface (T_w = 30°C) during 15 min of continuous immersion. Responses of pre-chilled muskrats (closed circles) are compared with control values for the same individuals during normothermic diving (open circles).

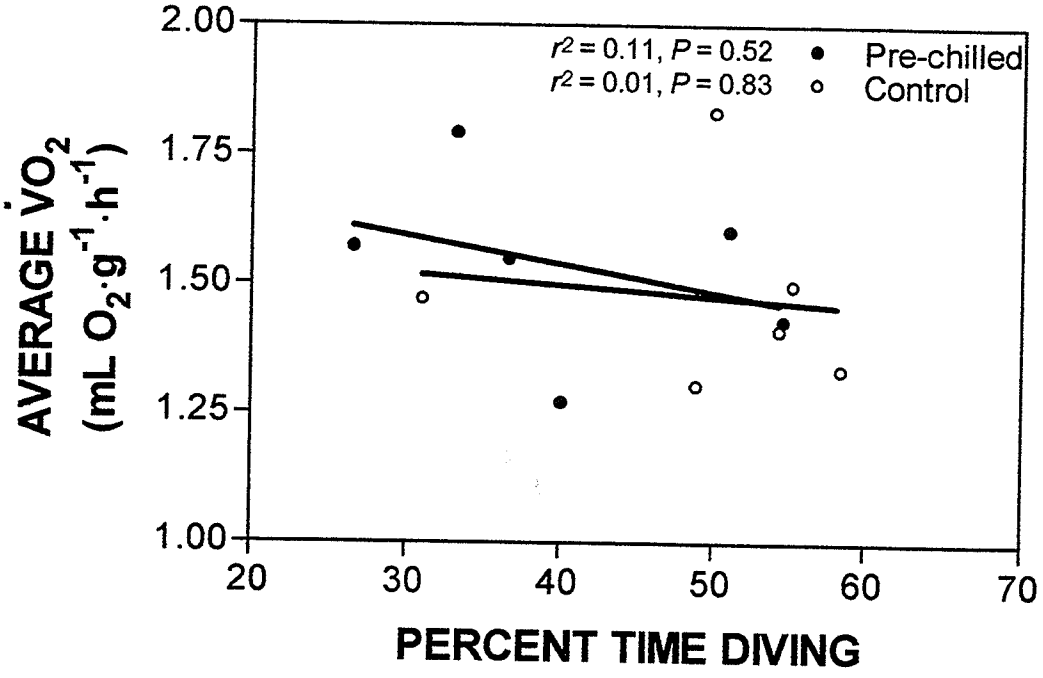

2.00
1.75
1.50
1.25
1.00
20
30
40
50
60
70
$r^2 = 0.11$, $P = 0.52$
$r^2 = 0.01$, $P = 0.83$
Pre-chilled
Control
AVERAGE $\dot{V}O_2$ (mL $O_2 \cdot g^{-1} \cdot h^{-1}$)
PERCENT TIME DIVING

Figure 11. Average VO_2 versus percent time diving for eight juvenile muskrats (Juv1; 200-400 g) diving for a 10-min period (T_w = 30°C) from either (A) the water surface during continuous immersion or (B) a dry platform. Responses of pre-chilled muskrats (closed circles) are compared with control values for the same individuals during normothermic diving (open circles).

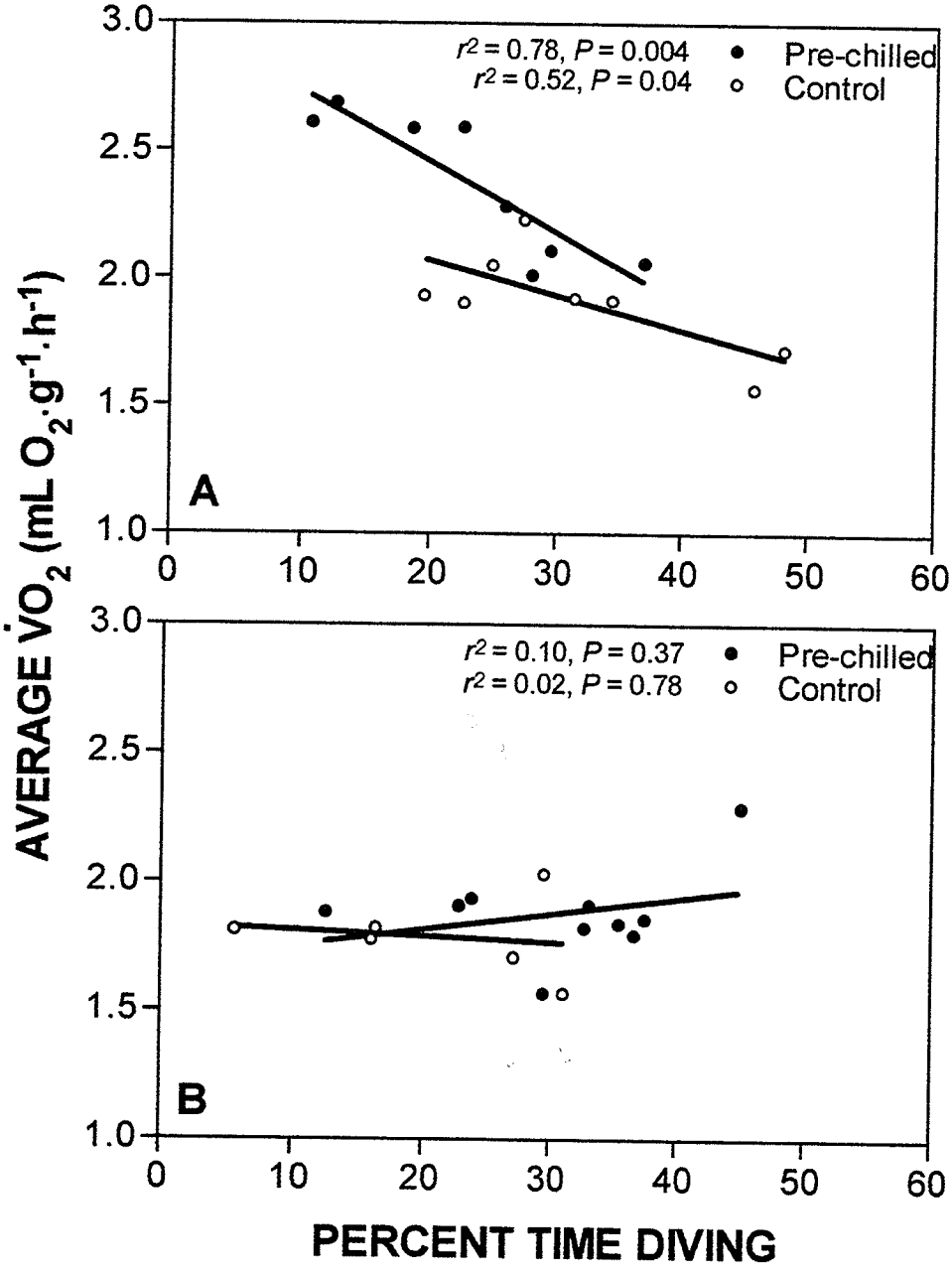
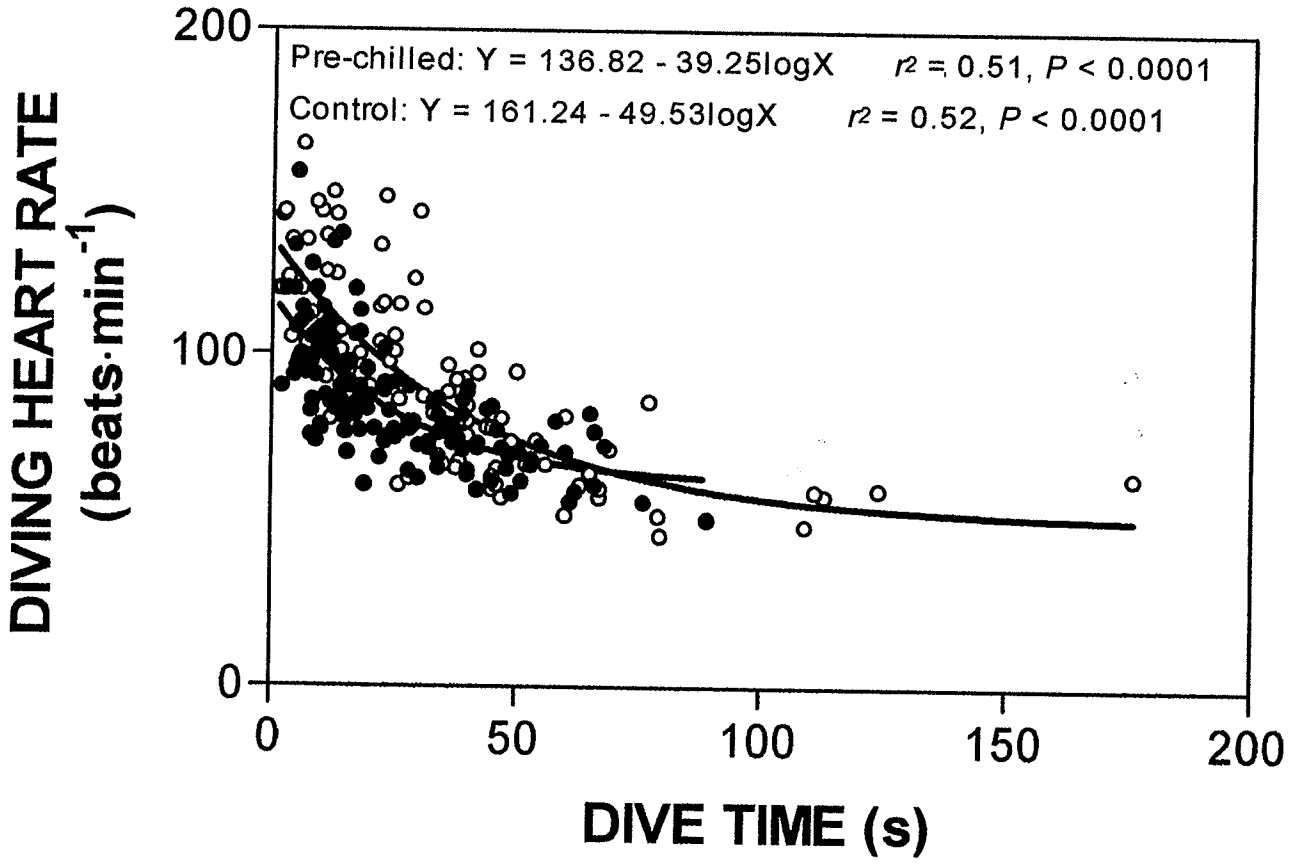

3.0
2.5
2.0
1.5
1.0
$r^2 = 0.78$, $P = 0.004$
$r^2 = 0.52$, $P = 0.04$
Pre-chilled
Control
A
0
10
20
30
40
50
60
$r^2 = 0.10$, $P = 0.37$
$r^2 = 0.02$, $P = 0.78$
Pre-chilled
Control
B
AVERAGE $\dot{V}O_2$ (mL $O_2 \cdot g^{-1} \cdot h^{-1}$)
PERCENT TIME DIVING

Figure 12. Mean diving heart rate versus dive duration for eight adult muskrats during a 15-min free-dive period from a dry platform (T_w = 30°C). Responses of pre-chilled muskrats (closed circles) are compared with control values for the same individuals during normothermic diving (open circles).


Pre-chilled: Y = 136.82 - 39.25logX r2 = 0.51, P < 0.0001
Control: Y = 161.24 - 49.53logX r2 = 0.52, P < 0.0001
DIVING HEART RATE (beats·min-1)
200
100
0
0
50
100
150
200
DIVE TIME (s)

6.2 beats·min^{-1} for normothermic animals. No differences in the intensity of bradycardia were apparent when data for all dive times (2 - 176 s) were pooled for pre-chilled (89.1 ± 3.9 beats·min^{-1}) and control (92.5 ± 5.4 beats·min^{-1}) animals, respectively (Table 4; $P = 0.435$).

Mean diving heart rate was not significantly correlated with degree of hypothermia in pre-chilled muskrats, even when only short (< 25 s) dives were analyzed (Fig. 13). As well, no trends were apparent in the development of diving bradycardia in pre-chilled, compared to control animals over the first 20 s of an average dive (Fig. 14). During resting periods at the surface prior to diving, heart rates of muskrats in both treatment groups were similar (Table 4; $P = 0.552$), averaging 279.9 ± 18.8 beats·min^{-1} in pre-chilled animals and 256.8 ± 14.4 beats·min^{-1} in controls. Heart rate during episodes of grooming that followed diving bouts was also not significantly affected by chilling in muskrats (Table 4).

Diving $\dot{V}O_2$ was significantly correlated with mean diving heart rate in both pre-chilled ($r^2 = 0.31$, $P = 0.01$) and control ($r^2 = 0.29$, $P = 0.01$) muskrats during 15 min of voluntary diving from a dry platform (Fig. 15B). Stronger correlations were observed between heart rate and $\dot{V}O_2$ when muskrats engaged in varying levels of activity in air (Fig.15; pre-chilled $r^2 = 0.82$, $P < 0.0001$; control $r^2 = 0.71$, $P < 0.0001$). Interestingly, the slope of the relationship for terrestrial activity was considerably steeper than for diving ($F_{1,132} = 197.117$, $P < 0.0001$).

Table 4. Mean (±SE) heart rates of eight pre-chilled and normothermic (control) muskrats associated with specific behaviours. In all cases, means were compared using paired *t*-tests.

	Heart rate (beats·min^{-1})				
	Pre-Chilled	Control	df	*t*	*P*
Short dives (< 25 s)	94.5 ± 4.6	109.4 ± 6.2	7	5.676	0.001
Long dives (≥ 25 s) [a]	72.4 ± 1.7	77.4 ± 2.7	6	2.003	0.092
All dives combined	89.1 ± 6.2	92.5 ± 5.4	7	0.828	0.435
Pre-dive baseline	279.9 ± 18.8	256.8 ± 14.4	7	0.625	0.552
Post-dive grooming	311.0 ± 17.4	305.6 ± 14.6	7	1.240	0.255

[a] Dives ≥ 25 s were recorded for n = 7 animals

Figure 13. Mean diving heart rate versus extent of body cooling in pre-chilled muskrats (n = 8) for (A) all dives combined and (B) only dives $\leq$ 25 s in length. T_b = drop in abdominal T_b over the pre-chilling immersion period.

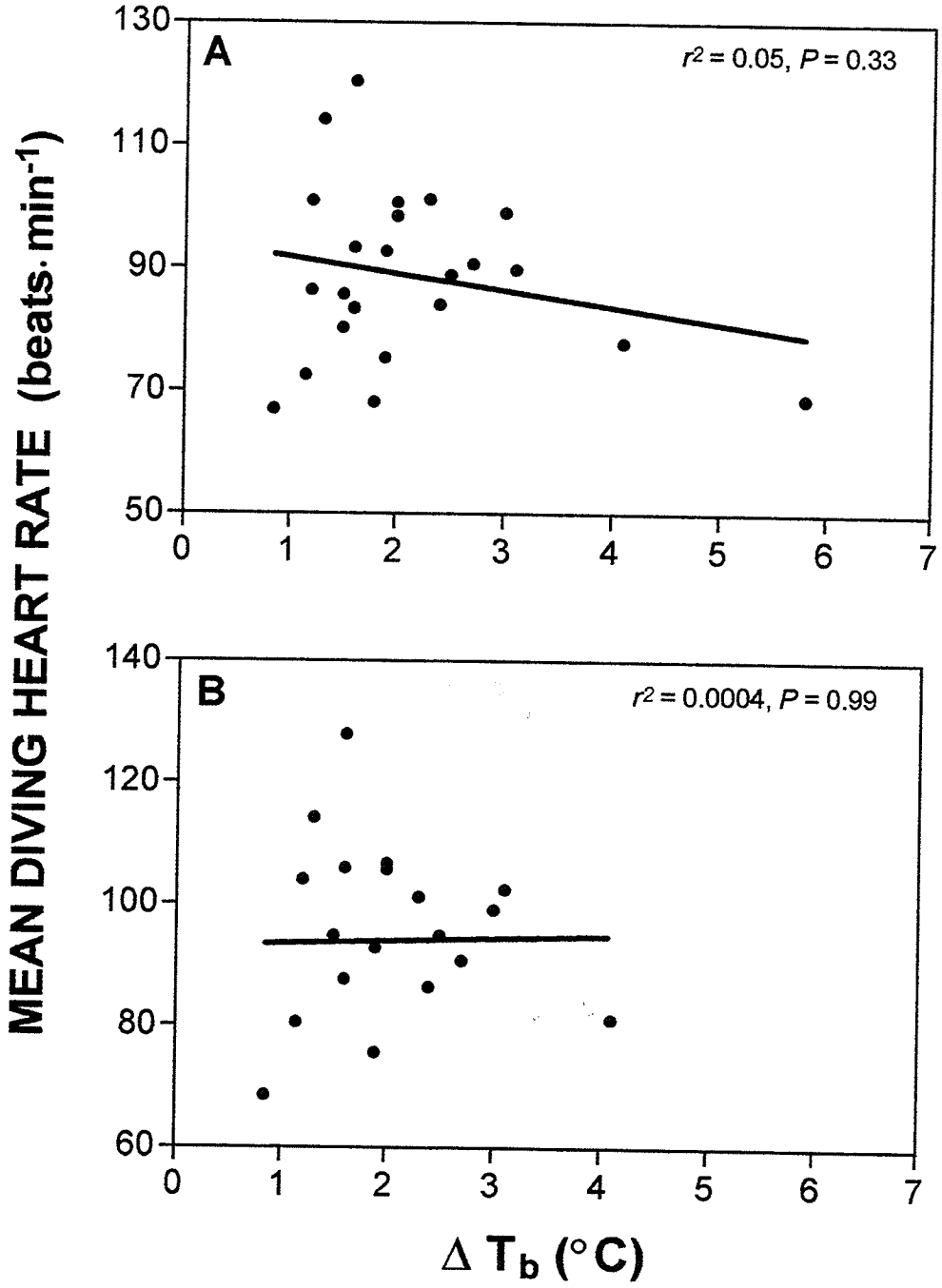

A
$r^2 = 0.05$, $P = 0.33$
B
$r^2 = 0.0004$, $P = 0.99$
MEAN DIVING HEART RATE (beats·min^{-1})
ΔT_b (°C)
130
110
90
70
50
140
120
100
80
60
0
1
2
3
4
5
6
7

Figure 14. Temporal changes in the mean (± SE) heart rate of eight muskrats during exploratory dives of varying duration (2.5 – 122 s). Heart rate was calculated for each 0.5 s interval of the dive, to a maximum of 20 s. Time of submergence = 0 s.

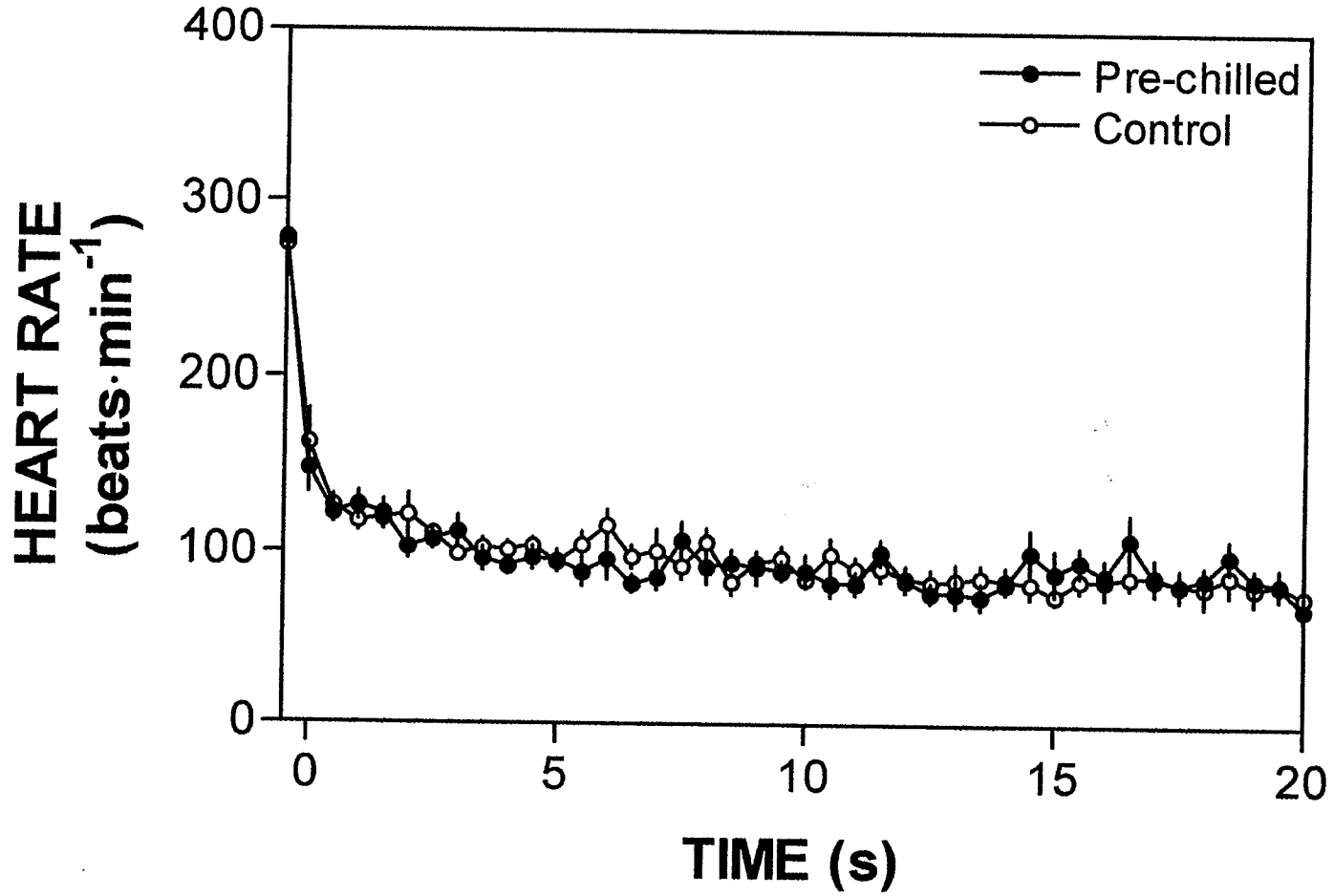
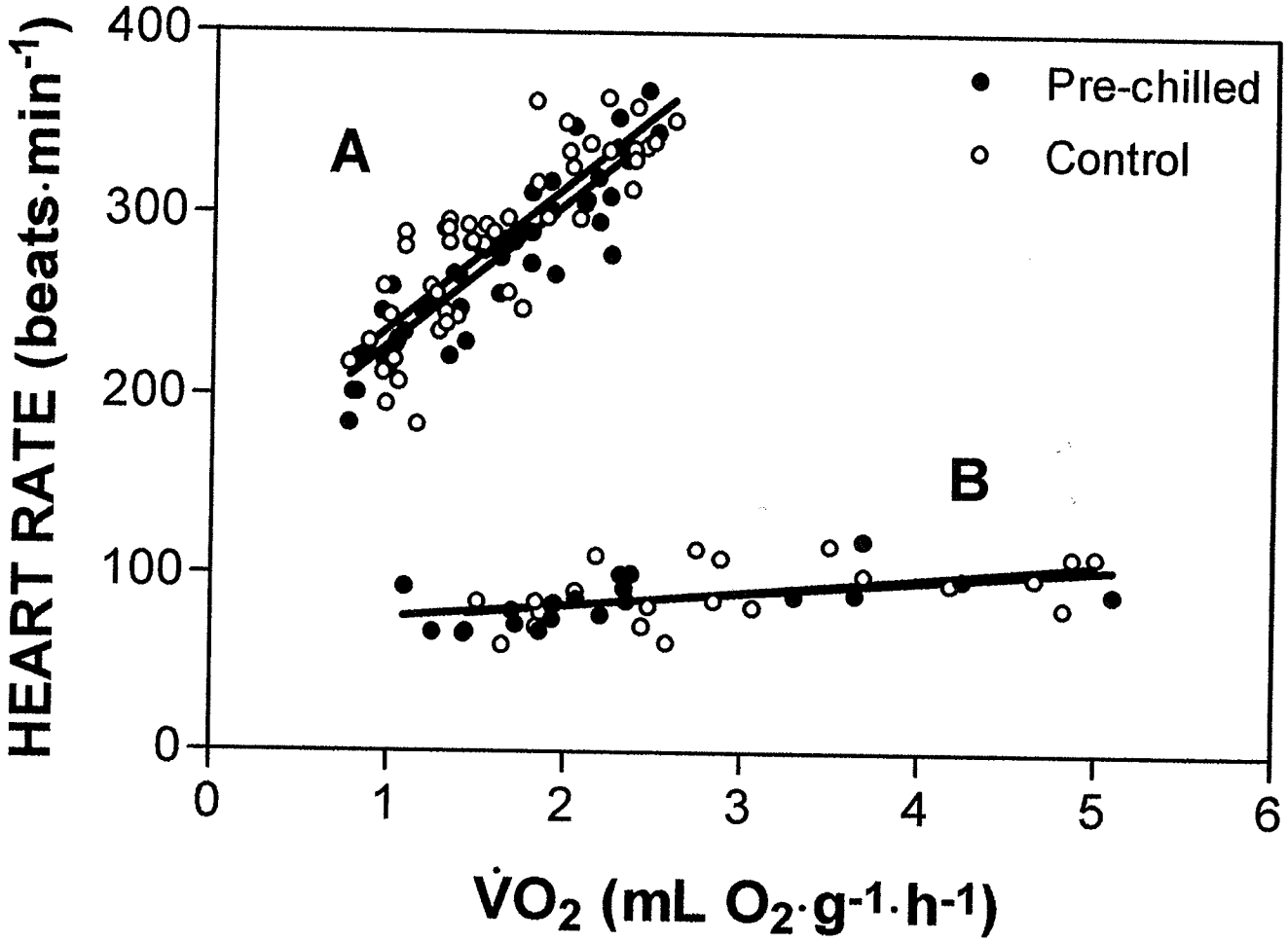

400
300
200
100
0
HEART RATE
(beats·min-1)
Pre-chilled
Control
0
5
10
15
20
TIME (s)

Figure 15. The relationship of heart rate to $\dot{V}O_2$ for eight adult muskrats during (A) varying levels of terrestrial activity following a diving trial and (B) during a 15-min period of voluntary diving from a dry platform. Diving $\dot{V}O_2$ was calculated after correction for the elevation in $\dot{V}O_2$ associated with inter-dive episodes of grooming (see text for details). Linear regressions were significant for terrestrial activity (control: heart rate = 78.8 $\dot{V}O_2$ + 159; r^2 = 0.71, $P < 0.0001$; pre-chilled: r^2 = 0.82, $P < 0.0001$) and for diving (control: heart rate = 8.2 $\dot{V}O_2$ + 66.3; r^2 = 0.29, P = 0.009; pre-chilled: r^2 = 0.31, P = 0.01; control).


HEART RATE (beats·min-1)
400
300
200
100
0
0
1
2
3
4
5
6
A
B
Pre-chilled
Control
V̇O2 (mL O2·g-1·h-1)

Post-Immersion Recovery of $\dot{V}O_2$ and T_b

Following a 30-s controlled dive, pre-chilled adult muskrats consumed, on average, an additional 73.9 ± 22.3 mL $O_2 \cdot kg^{-1}$ compared to normothermic controls (Fig. 16A). As dive length increased, post-dive excess $\dot{V}O_2$ associated with pre-chilling declined, reaching a minimum of 11.1 ± 38.1 mL $O_2 \cdot kg^{-1}$ for a 2-min submergence. On average, post-dive excess $\dot{V}O_2$ corrected for $\dot{V}O_2$ associated with non-diving post-immersion activities (*i.e.* grooming, comfort movements) as well as with T_b recovery, increased with increasing dive time in both pre-chilled and control muskrats (Fig. 16B). Overall, the corrected post-dive excess $\dot{V}O_2$ was significantly higher for pre-chilled than for normothermic adult animals (Fig. 16B; repeated measures ANOVA $F_{1,18} = 31.68$ $P < 0.0001$). For example, post-dive excess $\dot{V}O_2$ associated with a 30-s dive was 66.2 ± 28.6 mL $O_2 \cdot kg^{-1}$ for hypothermic adults, while it was only 3.1 ± 24.5 mL $O_2 \cdot kg^{-1}$ for normothermic controls. However, post-dive excess $\dot{V}O_2$ associated with pre-chilling was reduced with increasing dive length (Fig. 16A), accounting for the virtually identical corrected values obtained for hypothermic (201.9 ± 20.0 mL $O_2 \cdot kg^{-1}$) and normothermic (201.6 ± 34.7 mL $O_2 \cdot kg^{-1}$) adults following 2 min of submergence (Fig. 16B).

A series of paired *t*-tests with Bonferroni's α-level correction for sequential tests revealed that post-emergence recovery $\dot{V}O_2$ of chilled adults was elevated ca. 25% beyond that of control animals for 6 min. On the other hand, T_b of chilled adults following emergence required, on average, 27.2 ± 1.7 min to recover to pre-chill levels (Fig. 17). The same tests revealed that post-

Figure 16. Relationship between post-dive excess $\dot{V}O_2$ and dive time for pre-chilled compared to normothermic (control) muskrats (n = 10 in both cases). (A) Mean (±SE) post-dive excess $\dot{V}O_2$ of pre-chilled muskrats minus excess $\dot{V}O_2$ of the same individuals following normothermic diving (see text for details). (B) Mean (±SE) post-dive excess $\dot{V}O_2$ of pre-chilled and normothermic muskrats minus the excess $\dot{V}O_2$ of the same individuals following an equivalent period of non-diving immersion in water.

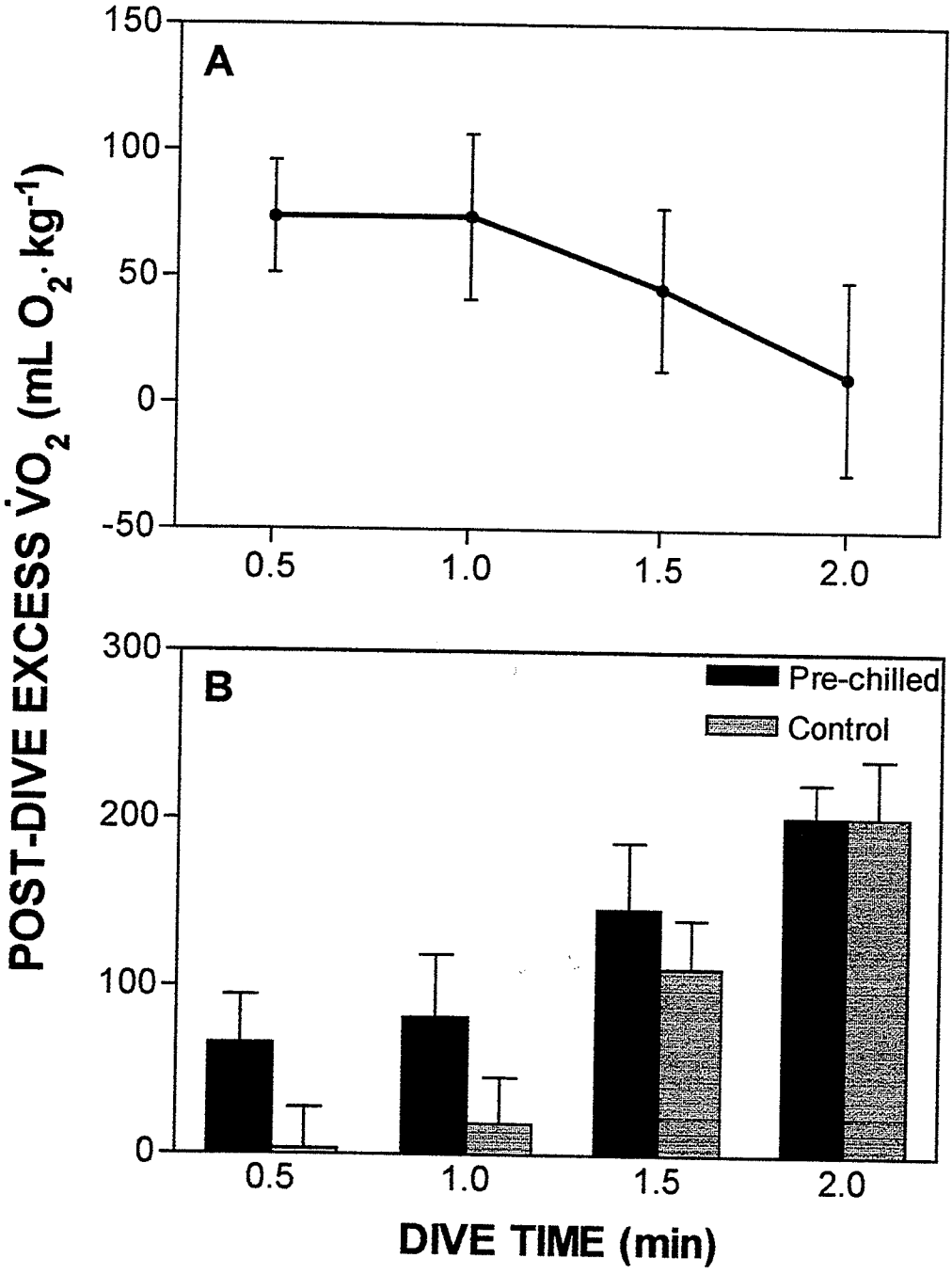
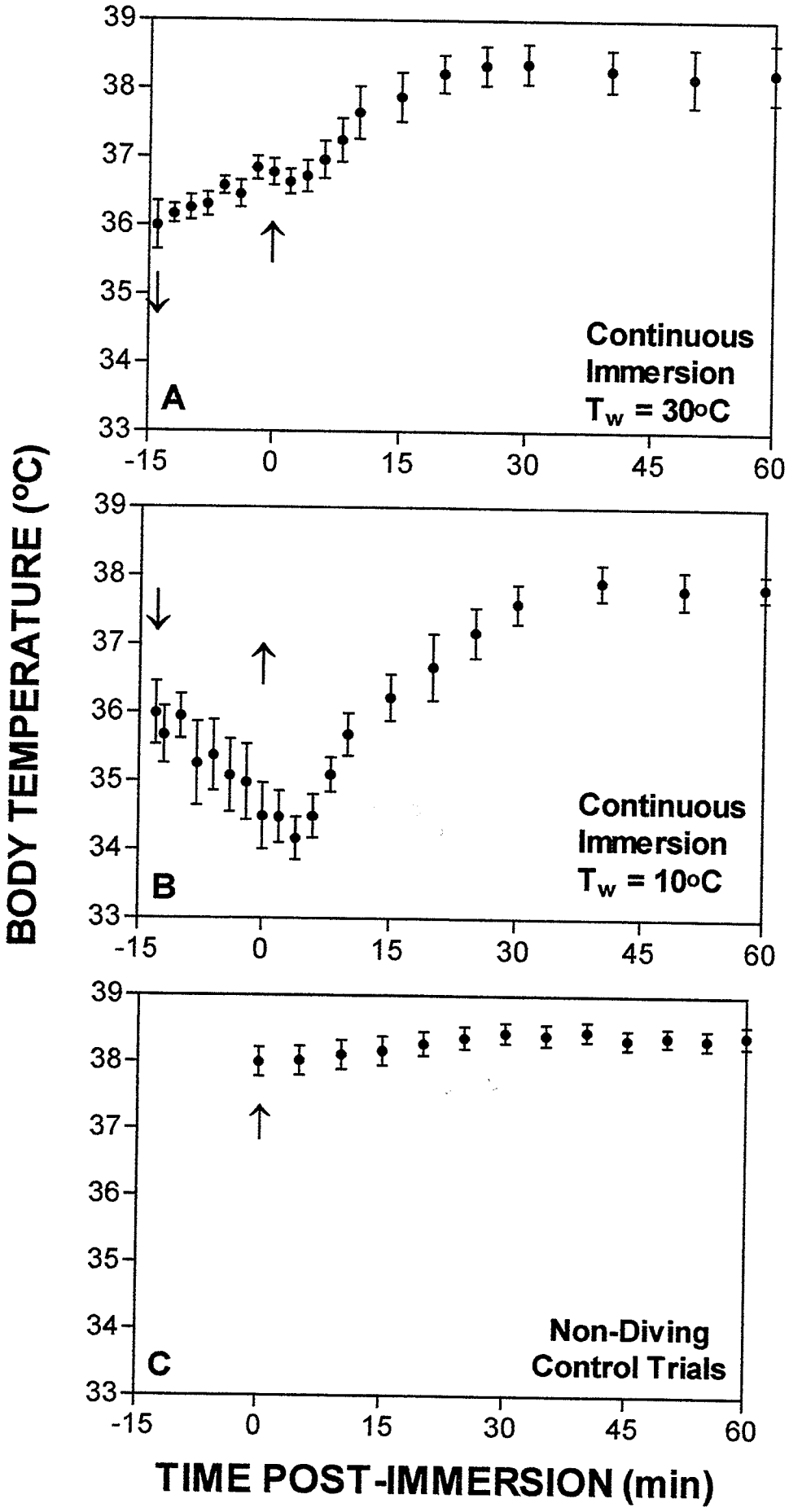
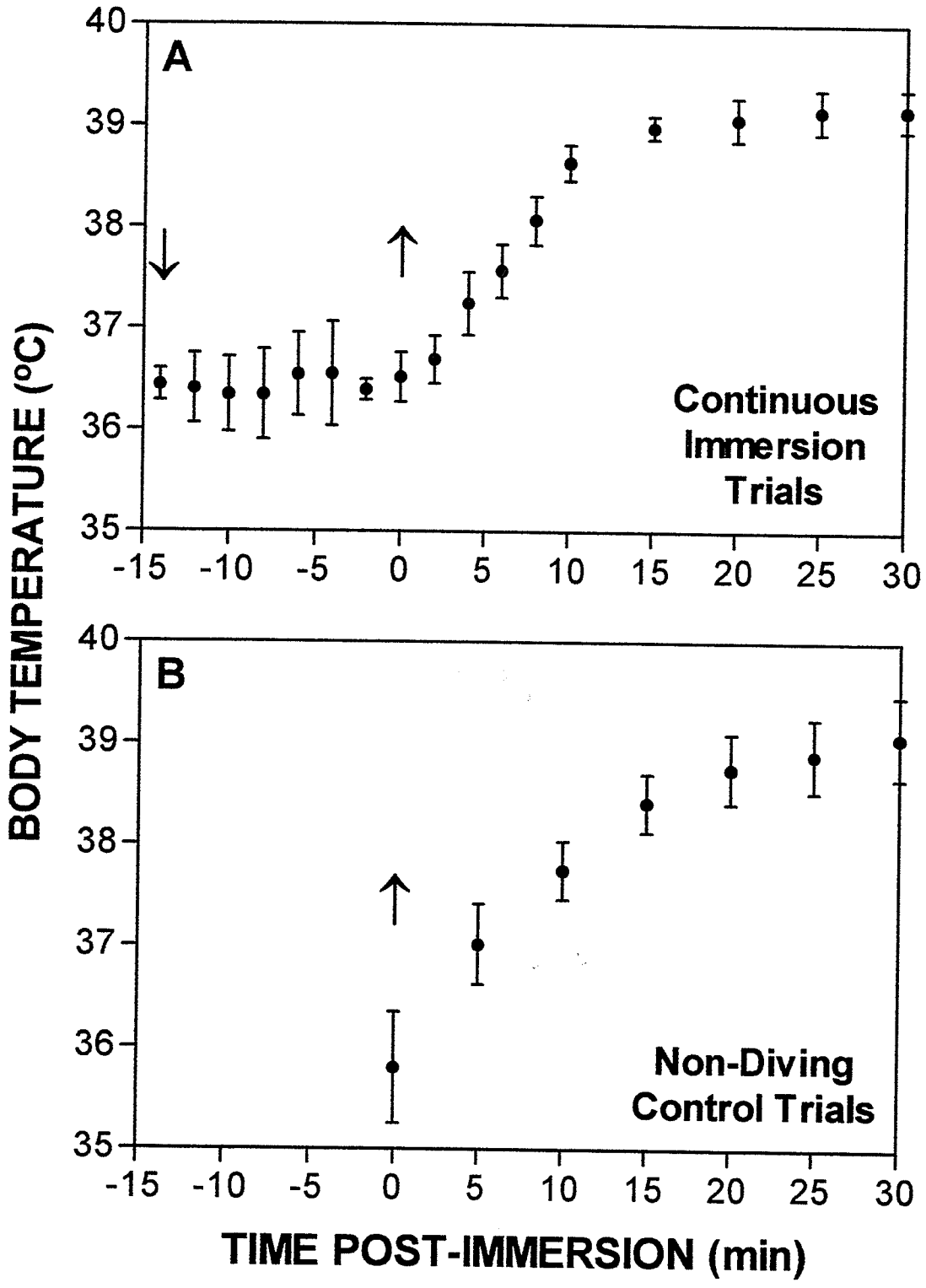

A
B
150
100
50
0
-50
300
200
100
0
0.5
1.0
1.5
2.0
Pre-chilled
Control
POST-DIVE EXCESS $\dot{V}O_2$ (mL $O_2 \cdot kg^{-1}$)
DIVE TIME (min)

Figure 17. Telemetered body temperature (mean ± SE) dynamics of 10 pre-chilled adult (>600 g) muskrats during and following a 15-min session of free-diving from the water surface at T_w = 30°C (A) and 10°C (B). Comparative data for non-diving, hypothermic control animals (n = 10) are also included (C). Arrows indicate times at which animals entered (↓) and left (↑) the water to recover on a dry platform.


BODY TEMPERATURE (°C)
39
38
37
36
35
34
33
-15
0
15
30
45
60
A
Continuous
Immersion
T_w = 30°C
B
Continuous
Immersion
T_w = 10°C
C
Non-Diving
Control Trials
TIME POST-IMMERSION (min)

emergence recovery $\dot{V}O_2$ of chilled Juv2 animals was elevated beyond that of control animals for only 2 min, and then by less than 10%. Additionally, T_b of juveniles demonstrated a more rapid recovery than was the case for adults (Fig. 18). T_b of adults did not remain fixed for the duration of the dive bout, as a gradual rise in T_b occurred during continuous immersion in T_w = 30 °C, contrasting with the gradual decline in T_b observed during continuous immersion in T_w = 10 °C (Fig. 17). Following transfer of the animal from the dive tank to the dry platform, a transient T_b decrease was observed (irrespective of T_w) prior to recovery to normothermic levels (Fig. 17A). However, no such T_b decrease was observed in non-diving adults (Fig. 17C) or in juveniles during continuous immersion (Fig. 18A).

Figure 18. Telemetered body temperature (mean ± SE) dynamics during and following a 15-min session of free-diving from the water surface (T_w = 30°C) in *n* = 6 pre-chilled juvenile (400-600 g) muskrats (A). Comparative data for non-diving, hypothermic control animals (*n* = 10) are also included (B). Arrows indicate times at which animals entered (↓) and left (↑) the water to recover on a dry platform.


A
B
Continuous Immersion Trials
Non-Diving Control Trials
BODY TEMPERATURE (°C)
TIME POST-IMMERSION (min)
40
39
38
37
36
35
-15
-10
-5
0
5
10
15
20
25
30

DISCUSSION

Central to the "adaptive hypothermia" hypothesis (Butler and Jones 1997) is the premise that hypothermia depresses whole-body $\dot{V}O_2$ via the Q_{10} effect. Additionally, if the diver suspends active thermoregulation, the attendant reduction in thermoregulatory costs would reinforce the hypometabolic state (Butler 2001). This hypothesis has gained prevalence in large part owing to the exceptional body cooling reported for free-ranging animals foraging in cold water. Abdominal and stomach temperatures of penguins for example, decrease significantly upon ingestion of cold prey (Wilson and Culik 1991; Handrich *et al.* 1997). Weddell seals display a considerable drop in the temperature of dorsal aortic blood during a dive bout (Kooyman *et al* 1980; Hill *et al.* 1987), implying a high rate of heat loss to the surrounding water. Both cases have been interpreted as reflecting an impressive overall drop in T_b, which likely impacts on the animal's dive performance. In fact, hypothermia has often been invoked to explain the low field metabolic rates (based on heart rate and doubly-labeled water methods) documented for many marine mammals and birds (see Butler 2001). Prominent examples of divers for which the hypothermia argument has been advanced to account for the observation that the majority of natural dives exceed the calculated ADL, are the gentoo (Bevan *et al.* In press) and king penguins (Handrich *et al.* 1997).

However, the bulk of published studies bearing on this question present only circumstantial evidence that hypothermia improves dive performance (Butler 2001). In response to the need for a more rigorous experimental approach to

this problem, I proposed to investigate the influences of mild hypothermia on the semi-aquatic muskrat diving in a controlled laboratory setting. By inducing mild hypothermia through cold-water immersion, my aim was to objectively determine the impact of hypothermia on selected behavioural and physiological indices of dive performance. Baseline data were collected from the same individuals during normothermic diving, in order to obtain essential control values as well as relate my findings to previous studies. Baseline behavioural, cardiac and metabolic data were collected to confirm that subject animals were behaving typically prior to investigating the effects of hypothermia on these variables.

Baseline Responses of Normothermic Muskrats

Analyses of diving behavioural parameters in adults revealed, as expected, changes related to water temperature. Consistent with the findings of MacArthur (1984), cumulative and average dive times were reduced for individuals diving in 10°C, compared to 30°C water (Tables 1, 2). In addition, this study also revealed that the maximum dive time and dive frequency of muskrats decline when animals dive in colder water. Also as expected (MacArthur 1984), average $\dot{V}O_2$ over the entire dive interval was elevated during trials in 10°C water, indicating increased thermoregulatory costs associated with cold-water immersion. In the absence of an elevated diving $\dot{V}O_2$, the observed increase in average $\dot{V}O_2$ of normothermic adults diving in cold water suggests that T_b recovery occurs predominately during the inter-dive surface interval. This conjecture is supported by MacArthur's (1986) finding that BAT of adult muskrats

diving in cold water is not thermogenically active during underwater swimming. The absence of active thermogenesis during submergence in adult muskrats diving in cold water can therefore be explained by increased thermoregulatory requirements that must be met at the surface.

These trends were most obvious for animals diving from the water surface during a period of continuous immersion (Tables 1, 2). When muskrats were provided access to a dry platform, their cumulative dive time decreased, regardless of T_w (Table 2), since animals spent more time on the platform grooming. Consequently, the costs of grooming obscured, to some extent, the differences in average $\dot{V}O_2$ between tests at the two water temperatures. Yet, there was little increase in either the mean or longest dive time of muskrats diving from a dry platform versus the waters surface, suggesting that these animals were under minimal stress during the continuous immersion trials. The similarity in calculated diving $\dot{V}O_2$ for animals tested in the two diving situations also indicates that both scenarios promoted natural diving responses from these muskrats.

It is well established that dive behaviour of many mammals and birds reflects their capacity for aerobic metabolism, as defined by their calculated ADL (Butler and Jones 1997). Thus, any observed differences in the diving behaviour of the three cohorts of normothermic muskrats in this study should, in theory, reflect differences in their respective body oxygen stores and rates of oxygen consumption underwater (*i.e.* diving $\dot{V}O_2$). Since the allometric scaling of oxygen stores and metabolic rate predict a shorter ADL for a smaller diver (Calder 1984;

Schreer and Kovacs 1997), it was not surprising that the youngest cohort of juvenile muskrats exhibited the shortest dive times in this study (Tables 1, 2). This was true whether animals dove from the water surface or from a dry platform. Consistent with allometric predictions, the youngest cohort displayed the highest diving $\dot{V}O_2$ of any group tested, averaging 3.32 ± 0.40 mL $O_2 \cdot g^{-1} \cdot h^{-1}$ when diving from the water surface. This finding is at variance with MacArthur *et al.*'s (2001) observation that young (254-360 g) muskrats have anomalously low diving $\dot{V}O_2$ (1.15 ± 0.5 mL $O_2 \cdot g^{-1} \cdot h^{-1}$). On the other hand, estimates of diving and average $\dot{V}O_2$ for adult muskrats were consistent with the results of previous studies (Fish 1982; MacArthur 1984; MacArthur and Krause 1989).

The oldest juvenile cohort (Juv2) displayed mean and longest dive times comparable to those of adults (Table 1). As well, of the three cohorts tested, the Juv2 group demonstrated the highest cumulative dive time and dive frequency during continuous immersion trials. This group also had a mean D:S ratio approximately two times greater than those of the other two groups examined. Given that the calculated mean diving $\dot{V}O_2$ of normothermic animals was lowest for Juv2 muskrats (1.87 ± 0.18 mL $O_2 \cdot g^{-1} \cdot h^{-1}$), it appears that this group had the lowest diving costs. For each cohort, resting mass-specific $\dot{V}O_2$ measured at the surface increased with decreasing body size. This explains the higher average $\dot{V}O_2$ observed in the younger cohorts. The use of these higher resting values in diving $\dot{V}O_2$ calculations for each cohort may also explain the lower estimates of diving $\dot{V}O_2$ obtained in this study compared to those reported by MacArthur *et al.*

(2001), in which a single estimate of resting $\dot{V}O_2$ determined for adult muskrats was used in their equation for calculating diving $\dot{V}O_2$ of all cohorts.

In this study, diving $\dot{V}O_2$ of muskrats in warm water became progressively lower as the proportion of time spent diving increased (Figs. 5-7). This supports previous studies in which a similar reduction in $\dot{V}O_2$ was associated with increased dive duration in tufted ducks (Bevan *et al.* 1992) and several pinniped species (Costello and Whittow 1975; Castellini *et al.* 1992; Hurley and Costa 2001). It is generally assumed that as dive length increases, oxygen conservation becomes more critical than the oxygen demands of exercise, thus strengthening the dive response. This is supported by the observed intensification of bradycardia with increased dive duration (Fig. 12), which suggests a more pronounced vasoconstrictor response.

For adult muskrats, mean diving heart rate was inversely related to duration of submergence, consistent with the trend reported by Drummond and Jones (1979) and MacArthur and Karpan (1989). Drummond and Jones (1979) provided evidence that the diving bradycardia response of muskrats is triggered by input from nasal receptors responding to submergence and by lung receptors responding to lung deflation related to apnea. As a result, the onset of bradycardia is immediate upon submergence, with degree of bradycardia intensifying for longer dives. The latter suggests little involvement of arterial chemoreceptors in modulating bradycardia during the course of a dive. Development of a more intense bradycardia with longer dives also has been observed in free-diving Weddell seals, and has been related to heightened

peripheral vasoconstriction in preparation for a prolonged episode of diving apnea (Kooyman and Campbell 1972).

Because it is a variable that is relatively easy to monitor, yet one also critical for assessing cardiovascular status, heart rate is often considered to be a global indicator of metabolic rate (Butler 1993). An interesting finding of this study was that heart rate showed significant linear correlation with $\dot{V}O_2$ during terrestrial (grooming) activity and also during submergence. Heart rate is known to correlate generally with $\dot{V}O_2$ during steady-state metabolism, accompanying, for example, terrestrial exercise. This has been validated previously for several species of diving birds and mammals during exercise in a water flume or on a treadmill (see Butler 1993 for review). In earlier diving studies, heart rate was shown to vary linearly with $\dot{V}O_2$ when both parameters are averaged over an entire dive cycle (*e.g.* Webb *et al.* 1998). In this study, however, mean heart rates recorded during individual dives by muskrats were correlated, in each case, with the corresponding diving $\dot{V}O_2$ (Fig. 13). Further, these correlations remained significant when data for all eight individuals were pooled.

The observed tight correspondence of diving heart rate with $\dot{V}O_2$ indicates that heart rate is indeed tracking $\dot{V}O_2$ during submergence, and therefore appears to be a viable index of diving costs in muskrats. Theoretically, diving does not represent a steady-state situation, which should preclude the use of heart rate as an overall metabolic indicator. However, advocates for the use of heart rate as a metabolic indicator have suggested that in the absence of appreciable lactate production, the aerobic metabolism occurring underwater can

be considered steady-state at the tissue level (Butler 1993). This will be the case if working tissues maintain aerobic metabolism and do not completely exhaust their oxygen supply prior to surfacing. Indeed, Davis and Kanatous (2001) have recently published a model for tissue-oxygen delivery during diving, which suggests that working muscles receive a continuous oxygen supply underwater. Since aerobic diving likely predominates in muskrats (MacArthur *et al.* 2001), heart rate is strongly indicated as a predictor of diving $\dot{V}O_2$ in these animals, and therefore provides an independent measure of metabolic status during voluntary dives. The finding that heart rate clearly tracks $\dot{V}O_2$ in muskrats provides further support for adopting this species to test the hypothermia hypothesis. Additionally, the inverse relationships of $\dot{V}O_2$ and heart rate to dive time indicate that muskrats are capable of modulating diving metabolic rate according to demands, permitting longer dives at a reduced rate of O_2 depletion.

Does Hypothermia Enhance Dive Performance?

Hypothermia did not appear to alter the behaviour or metabolic rate of adult muskrats during aquatic activity in warm (30°C) water (Tables 1, 2). The absence of hypothermia-related changes in dive performance suggests that muskrats do not exploit hypothermia to maximize submergence time during routine diving. However, these findings do indicate a striking behavioural and metabolic resistance of adult muskrats to hypothermia. On the other hand, hypothermia in juveniles caused some reduction in diving and, in the case of the Juv1 cohort, an increased metabolic rate (Tables 1, 2). Behavioural indices of

dive performance, such as average and cumulative dive times, were decreased during hypothermia in both cohorts of juvenile muskrats. The younger cohort also demonstrated a reduction in the longest exploratory dive following pre-chilling. These behavioural changes notwithstanding, similarities in diving VO_2 between the hypothermic and normothermic juveniles imply that chilling does not affect their capacity for diving. It is important to note that the degree of hypothermia experienced by juvenile muskrats far exceeds that experienced by adults. Small body size and reduced thermal inertia make juvenile muskrats highly susceptible to aquatic cooling (MacArthur and Humphries 1999), and may explain why they demonstrated a greater response to hypothermia than did adults. However, it should also be emphasized that T_b recorded from hypothermic juveniles in this study fell well within the range reported for muskrats of the same size in nature (MacArthur and Humphries 1999).

During continuous immersion in cold (10°C) water, hypothermic adult muskrats demonstrated some elevation of diving and average VO_2, indicating elevated costs of underwater swimming in addition to greater costs associated with rewarming following cold-water dives. However, no changes in diving or average VO_2 were apparent as a result of T_b change when animals were allowed to dive from a dry platform. As mentioned previously, since time spent on the platform was spent grooming nearly exclusively, it seems likely that any thermoregulatory costs associated with diving when chilled or diving in cold water were obscured by the metabolic costs of grooming and comfort movements on the platform. The sole behavioural change related to chilling in adults was the

observation that hypothermic individuals with access to a dry platform spent significantly more time in the 10°C water (both diving and surface swimming) than did their normothermic counterparts. Perhaps this indicates an aversion to cold water in normothermic adult muskrats. However, it is also possible that the hypothermic individuals may have become pre-adapted to the cold water as a result of their pre-chilling immersion (Sawada *et al.* 2000; Leppaluoto *et al.* 2001).

Unlike the adults implanted only with abdominal T_b transmitters, those implanted with heart rate transmitters showed significant behavioural changes associated with hypothermia. Similar to the responses of juvenile muskrats, longest, mean and cumulative dive times declined in pre-chilled animals instrumented with ECG/T_b transmitters (Table 3). Though there is no obvious explanation for this finding, it is due perhaps to stress effects from either the more intensive surgical procedure or the larger transmitter and associated subcutaneous leads required for the heart rate study. However, despite these behavioural differences, transmitter type had no influence on the metabolic responses of adult muskrats to hypothermia.

During diving, muskrats displayed lower mean diving heart rates when hypothermic (Fig. 12). However, this finding was noted only for shorter dive durations ($\leq$ 25 s). There appeared to be no difference in the onset of bradycardia between the two experimental groups, just as there was no difference in the onset of bradycardia between individuals embarking on long versus short dives. The explanation that lower bradycardia at the start of the

dive is related to degree of vasoconstriction (Kooyman and Campbell 1972) seems reasonable in both cases.

The strong similarity in heart rate of the two experimental groups during longer dives (Table 4) suggests that other components of the dive response (*e.g.* peripheral vasoconstriction) should also be equivalent. Conversely, the observation that hypothermia elicits a more profound bradycardia in short dives suggests that chilling intensifies vasoconstriction associated with diving. In an earlier study, MacArthur and Karpan (1989) reported that heart rate of unrestrained muskrats diving voluntarily in the absence of any alarm response did not decrease below 45 beats·min^{-1}, no matter how long the dive duration. My data suggest that there exists some maximal degree of vasoconstriction and bradycardia for these animals during unrestrained diving that can be attained by hypothermic animals during shorter dives than is the case for normothermic individuals.

This study indicates that hypothermia does influence the dive response in a way that could potentially extend dive time through oxygen conservation. However, the bradycardia response of hypothermic and normothermic muskrats quickly becomes indistinguishable as dive length increases. Consequently, the observed cardiac response to hypothermia was not strong enough to yield a relationship between mean diving heart rate and degree of pre-chilling (ΔT_b). As well, heart rates of muskrats resting at the surface did not differ between hypothermic animals floating in cold (6°C) water and normothermic animals resting in warm (30°C) water. Presumably hypothermic muskrats experienced a

greater vasoconstrictor response since abdominal T_b was lower. If so, failure to detect an attendant reduction in heart rate implies muskrats may readily tolerate hypertension during immersion.

No differences were observed between the two T_b groups for the regression of diving $\dot{V}O_2$ and percent time diving when muskrats dove from the surface during a period of continuous immersion (Figs. 5, 7). If the dive response becomes comparable in both groups after a given period of diving, and assuming no contribution of non-shivering thermogenesis (NST) underwater, then diving $\dot{V}O_2$ as well as heart rate should be similar in normothermic and hypothermic muskrats. Although diving $\dot{V}O_2$ correlated well with percent time diving, no relationship was observed between amount of diving and average $\dot{V}O_2$ (Figs.6-9). This suggests that any potential energy savings during underwater swimming is masked by oxygen consumption at the water surface.

A significant limitation of this study was the inability to confirm a true hypothermic state, specifically in the core region. Recent studies of both birds and mammals have contradicted previous reports of dramatic T_b decline associated with foraging in cold water (see above). Instead, recent data suggest that immersion hypothermia acts to cool a diver's peripheral shell, leaving the body core (Ponganis *et al.* 2001) and swimming muscles (Ponganis *et al.* 1993) at, or above the pre-dive temperature. Muskrats are known to adopt regional heterothermy, with appendage and tail temperatures approaching the ambient in situations outside of thermoneutrality (Fish 1979). Though we assume that a measured decline in abdominal temperature reflects a drop in core T_b, more likely

this reflects an average decline in whole-body T_b, where greater cooling is experienced in peripheral tissues.

Assessing the Post-Immersion Costs of Hypothermic Diving

Costs associated with post-immersion T_b recovery must also be considered in order to gain a meaningful ecological perspective of the impact of hypothermia on the diving energetics of muskrats. Though muskrats do not appear to engage NST via brown adipose tissue during submergence, intrascapular brown fat is an important site of heat production following diving in cold water (MacArthur 1986). As well, the amount of heat generated by this thermogenic tissue is reflected in $\dot{V}O_2$. Thus, immersion hypothermia not only has the potential to influence dive performance, but may also have a bearing on the long-term dive patterns of these animals.

In this study, post-dive excess $\dot{V}O_2$ increased as controlled dive time was extended, confirming the previous findings of MacArthur (1984). This presumably reflects greater total dive costs that accompany periods of longer submergence. In general, post-dive excess $\dot{V}O_2$ was higher for the hypothermic group even though they dove in warm water (Fig. 16A), indicating that the oxygen requirement of T_b recovery can be significant for these divers. Post-immersion excess $\dot{V}O_2$ was also elevated for hypothermic muskrats that were immersed in cold water but not permitted to dive. However, this increase was significant only for the 6-min period immediately following emergence, whereas T_b recovered at a much slower rate (Fig. 17).

Interestingly, the discrepancy in post-dive excess $\dot{V}O_2$ between hypothermic and normothermic groups was most obvious during the shortest (30 s) controlled dives (Fig. 16). As dive duration was increased, the elevation in post-dive excess $\dot{V}O_2$ for hypothermic muskrats beyond that of the normothermic controls declined, until after 2 min of diving, the difference was negligible (Fig. 16). A possible explanation for this trend is that the costs of T_b recovery comprised a smaller component of total dive costs in longer dives since the absolute oxygen requirements associated with this type of dive should theoretically be higher. Yet this does not completely explain the identical $\dot{V}O_2$ values obtained for pre-chilled and control animals following the longest (2 min) controlled dives (Fig. 16B). It is unlikely that the oxygen requirements of NST are obscured completely by the oxygen deficit of the dive. This may possibly indicate a lack of reliance on NST for rewarming in this experimental situation, or may indicate only a short burst of NST immediately following emergence. Since the thermal environment of the metabolic chamber platform is well above the lower critical temperature of the thermoneutral zone of these animals, perhaps hypothermic muskrats were passively rewarming, (*i.e.* by adjusting whole-body conductance through selective vasoconstriction). This passive rewarming may also occur through heat released incidental to grooming activity (MacArthur 1986). Controlled release of vasoconstriction and passive rewarming may also explain why T_b recovery is delayed compared to the post-dive elevation of $\dot{V}O_2$. If this is the case, hypothermic diving is truly associated with only minor post-diving energetic penalties.

CONCLUSIONS

Overall, this study documented few behavioural or metabolic changes in dive performance related to hypothermia in adult muskrats. While implying there is no energetic benefit to muskrats diving with a reduced T_b, this finding also suggests an impressive resistance to the effects of hypothermia in these animals. An elevation in average $\dot{V}O_2$ for hypothermic individuals implies that adult muskrats may defer rewarming to surface intervals, which is consistent with MacArthur's (1986) earlier study of brown fat thermogenesis in muskrats. The apparent ability of hypothermic adult muskrats to dive normally is impressive, especially compared to the younger animals which experience more pronounced body cooling and demonstrated reduced diving activity. Consequently, my findings suggest that in this species development of hypothermia tolerance increases with body size.

Heart rate data obtained in this study also suggest no overt response to hypothermia in adults. Though heart rate of pre-chilled animals was reduced with respect to controls in shorter dives, this reduction did not persist throughout the duration of longer dives, nor did it influence the development of diving bradycardia. These data may indicate that hypothermia promotes a peripheral vasoconstriction comparable to that of the dive response, and that this aspect of cardiovascular adjustment is promoted by hypothermia. However, the absence of a persistently lower heart rate in the pre-chilled muskrats, coupled with the absence of an obvious depression in diving $\dot{V}O_2$, suggests that reductions in tissue metabolism due to the Q_{10} effect were either negligible or absent.

From an ecological perspective, the energetic costs of T_b recovery should reflect the penalty associated with hypothermic diving. Recovery costs must be weighed against potential benefits during submergence to gain an ecological perspective of hypothermia. In the case of muskrats, the penalty associated with T_b recovery appears to be minor. In fact, the time required to restore T_b far exceeded the period of excess O_2 consumption following diving. This finding suggests that passive rewarming and selective vasoconstriction may play a more critical role in the post-dive recovery of T_b than facultative thermogenesis. The overriding conclusion to emerge from this study is that hypothermia in muskrats exerts a negligible effect on dive performance, as it influences neither submergence nor post-immersion metabolic costs.

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