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Diving experience and the aerobic dive capacity of muskrats: does training produce a better diver?

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Summary

We tested the hypothesis that the body oxygen stores, aerobic dive limit (ADL) and dive performance of muskrats can be enhanced by dive-conditioning in a laboratory setting. We compared several key variables in 12 muskrats trained to swim a 16 m underwater course to a feeding station ('divers') with those of 12 animals precluded from diving but required to travel identical distances in water to feed ('surface swimmers'). Acclimated muskrats assigned to each group were trained concurrently over a 9–11 week period. We observed significant gains in the haematocrit (P=0.0005) and blood haemoglobin concentration (P=0.015) of 'divers', but not 'surface swimmers'. The post-training blood O_2 store calculated for 'divers' (22.9 ml O_2 kg^{-1}) was nearly 26% higher than that (18.2 ml O_2 kg^{-1}) derived for 'surface swimmers' (P=0.03). Dive-conditioning had no apparent effect on lung volume, whole blood and plasma volumes, nor on the glycogen level and buffering capacity of skeletal muscles. Cardiac and skeletal muscle myoglobin levels were also similar in both test groups following training. The mean total body oxygen store of 'divers' (37.8 ml O_2 STPD kg^{-1}) was 13.5% higher (P=0.037) than for 'surface swimmers' (33.3 ml O_2 STPD kg^{-1}), an increase attributed entirely to the gain in blood O_2 storage capacity of the former group. However, owing to a slightly higher estimate of diving metabolic rate in dive-conditioned animals, the calculated ADL for this group (61.3 s) was indistinguishable from that of 'surface swimmers' (61.8 s). Few differences were observed in the post-training dive behaviour of 'surface swimmers' and 'divers', a finding consistent with the strong similarity in their calculated aerobic dive capacities.

Key words: dive conditioning, body oxygen store, dive behaviour, myoglobin, haemoglobin, muscle buffering capacity, metabolism, aerobic dive limit, muskrat, *Ondatra zibethicus*.

Introduction

A recent trend in vertebrate diving research has been the growing emphasis on studies directed at intraspecific variability in dive performance (see Burns and Castellini, 1996; Burns et al., 1997; MacArthur et al., 2001). To date, most of this research has focused on developmental changes in the diving behaviour and blood-muscle physiology of avian and mammalian divers (Horning and Trillmich, 1997; Ponganis et al., 1999; Noren et al., 2001). A conclusion emerging from several of these studies is that immature animals typically dive to shallower depths and remain submerged for shorter periods than adults. The superior diving ability of adults is often credited to their larger body oxygen reserves and reduced metabolic costs of diving, compared to juveniles (see Burns et al., 1997; McCafferty et al., 1998). Though allometry is often invoked to account for such differences, the gain in tissue oxygen reserves by growing young may also reflect their increased experience of diving (Ponganis et al., 1999; Noren et al., 2001). Noren et al. (2001) suggested that as young animals mature and gain experience of swimming underwater, the increased time spent in dive apnea may make an important contribution to the acquisition of adult levels of muscle myoglobin (Mb).

The possibility that increased encounters with diving hypoxia contribute to boosting the oxygen storage capacity of divers presents an intriguing hypothesis that is amenable to testing. Yet few experimental studies have specifically addressed the benefits of prior dive experience to any aspect of diving capacity. In a study of the tufted duck *Aythya fuligula*, Stephenson et al. (1989) compared several key respiratory variables of control birds with those of dive-conditioned ducks that were required to swim 6.0 m underwater in order to feed. Following a 6-month training period, total body oxygen stores were similar in both groups, though the partitioning of these stores was altered significantly. These authors reported a decline in the lung-air sac oxygen reserve of dive-conditioned ducks that was offset by a commensurate gain in their blood and muscle stores. In a parallel study of immature harbor seals *Phoca vitulina*, Kodama et al. (1977) compared several blood indices of seals raised in a dry enclosure ('non-divers') with those of animals

that were provided free access to a large seawater tank (‘divers’). At the end of the 10-month conditioning period, these authors reported significant gains in the blood haematocrit (Hct) and haemoglobin (Hb) levels of ‘divers’, which they attributed to hypoxia-induced erythropoiesis associated with intermittent diving.

The paucity of experimental studies linking underwater experience with physiological diving capability is surprising, given the considerable attention devoted to the potential benefits of altitude hypoxia in boosting the body oxygen reserves of terrestrial mammals – especially humans (see Böning, 1997; Rodríguez et al., 2000). Exercise physiologists have repeatedly demonstrated that training at altitude increases Hct, Hb and serum erythropoietin levels (Mairbäurl, 1994; Klausen et al., 1996; Böning, 1997). There is also evidence that altitude hypoxia in combination with exercise is more potent in stimulating erythropoiesis than is hypoxia alone (Mairbäurl, 1994). In a more recent study, Rodríguez et al. (2000) reported that brief, intermittent episodes of hypobaric hypoxia presented over a 3-week period were sufficient to stimulate erythropoiesis and boost red cell mass, Hct and Hb levels of humans. The effects of hypoxia conditioning on muscle oxygen reserves are equivocal, though Terrados et al. (1990) observed increased Mb levels in human leg muscle following hypobaric hypoxic training.

Thus, despite the considerable research effort focused on the physiological benefits of hypoxia training in terrestrial mammals, little is currently known about the ability of natural divers to modulate body oxygen reserves or other indices of dive capacity in response to chronic changes in diving activity. An excellent model for investigating this problem is provided by the semi-aquatic muskrat *Ondatra zibethicus* Link 1795. Earlier research of muskrats inhabiting a northern prairie marsh (MacArthur, 1990; MacArthur et al., 2001) established that the total body oxygen reserves of this rodent increase 29–42% between summer and winter. In both studies, significant gains were observed in the mass-specific blood volume, Hct, Hb and skeletal muscle Mb of winter-caught animals. The oxygen-binding affinity of muskrat blood is likewise elevated in winter compared to summer, and this increase appears to be linked to a reduction in red cell 2,3-diphosphoglycerate (2,3-DPG) content (MacArthur, 1984a). Winter-acclimatized muskrats also appear to be superior divers, exhibiting greater cumulative and average dive times and longer dive:pause ratios than adults tested in summer (MacArthur et al., 2001).

The underlying basis for these physiological and behavioural changes is currently unknown, though one possibility relates to the increased reliance on diving by muskrats after marshes freeze over, when most foraging and home range movements dictate the necessity for underwater travel (MacArthur, 1992). It is conceivable that the winter gains in body oxygen reserves, diving ability, and perhaps even whole blood oxygen affinity, develop in response to the increased dependence on diving during the ice-bound season.

The primary goal of this study was to test the hypothesis that an increased dependence on diving leads to phenotypic upregulation in the body oxygen stores, aerobic dive limit (ADL) and diving ability of muskrats. We also considered the possibility that dive conditioning could result in greater reliance on anaerobic pathways in the primary swimming muscles of this species. To address these questions, we compared several key variables in acclimated muskrats trained to swim an underwater course to a feeding station, with those of animals precluded from diving but required to travel identical distances in water in order to feed. The specific variables examined included lung, blood and muscle oxygen stores, resting and diving metabolic rates, ADL, muscle buffering capacity, red cell 2,3-DPG content, and selected behavioural indices of dive performance.

Materials and methods

Animals

24 adult and subadult muskrats *Ondatra zibethicus* Link 1795 of both sexes were live-trapped at Oak Hammock Marsh, Manitoba (50°06′N, 97°07′W), during the months of July (*N*=6), August (*N*=4), October (*N*=10) and December (*N*=4). Captured animals were transported to the Animal Holding Facility, University of Manitoba, and placed in a controlled-environment room set at 14±1°C with a 12 h:12 h L:D photoperiod. Each muskrat was housed in a separate wire cage (24 cm×24 cm×65 cm) with attached fibreglass-lined, wooden nest box (24 cm×23 cm×20 cm). All cages were mounted on a slight incline in a fibreglass tank (10 cm×76 cm×122 cm) supplied with running water (12±1°C). The lower half of each cage was immersed in the water to a depth of 3–5 cm. Muskrats were fed commercial rodent chow supplemented daily with fresh apples and carrots. All animals were acclimated to these laboratory holding facilities for a period of 4–6 weeks prior to assignment to test groups (see below). Though muskrats could move freely within the confines of their holding cages, they had insufficient water for swimming and diving, and tended to remain relatively sedentary during the acclimation period. Throughout their stay in captivity, muskrats were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care under the authorization of a University-approved animal research protocol.

Training tanks and testing protocol

Acclimated muskrats were randomly assigned to one of two identically constructed fibreglass-lined, plywood tanks (internal dimensions: 217 cm×126 cm×60 cm) installed in a controlled-environment room set at 14±1°C with a 12 h:12 h L:D photoperiod. Each tank was partitioned into eight interconnected swimming lanes by removable dividers, forming a 16 m-long maze. Detachable hardware cloth screens mounted on top of the dividers prevented subject animals from escaping.

A wire cage (53 cm×53 cm×48 cm) containing two nest boxes (see above) was positioned above the maze, in one corner of the tank. An opening in the cage floor provided access, *via* a ramp, to the swimming maze. At the opposite end

of the maze, a ramp provided access to a dry feeding station, consisting of a 53 cm×53 cm×48 cm cage mounted above the tank. Food consisted of *ad libitum* rations of rodent chow supplemented daily by fresh apples and carrots. In each training tank, animals could reach the feeding station only by traversing the 16 m maze separating the two cages.

The 'control' tank was filled to a depth of 10–12 cm with 14°C water, leaving an 18–20 cm air space between the water surface and the tank cover. This shallow depth precluded diving but required muskrats to swim at the surface while negotiating the maze. In the experimental ('diving') tank, the maze was completely submerged (water depth=32 cm) and muskrats could move between their nest box and feeding station only by diving. During the first 3–4 days following release of muskrats into the diving tank, the water level was reduced to provide a 2–3-cm air space beneath the tank cover. This was necessary to allow animals to become familiar with the maze before requiring them to dive, thereby minimizing stress and the risk of accidental drowning in the early stages of training. When we were confident that muskrats could negotiate the maze, the water level was raised to eliminate the breathing space beneath the screen, thereby limiting all movement in the tank to underwater swimming. Based on 32 timed dives recorded from five trained muskrats, the time required for animals to dive from nest box to feeding station was 43.8±2.4 s (mean ± 1 S.E.M.; range 26.0–76.1 s).

Over the 3–4 day period immediately before introducing subjects into their assigned tanks, a series of baseline (pre-training) measurements were recorded from each animal. These included determinations of blood Hb and Hct levels, resting rate of oxygen consumption ($\dot{V}_{O_2}$) in air, as well as voluntary dive times and $\dot{V}_{O_2}$ recorded from each animal during a 15 min diving trial (see below). Following baseline measurements, each muskrat was released into its assigned holding tank where it remained for a period of 9–11 weeks. In all tests, a pair of muskrats was held in each tank, thus every dive-training session was accompanied by a control run on an identical number of 'surface-swimming' muskrats. In total, 12 animals (7 males, 5 females) were tested in the control tank, and 12 animals (5 males, 7 females) in the diving tank. The initial (pre-training) body mass was similar in both groups ('surface swimmers' 813.0±38.5 g; 'divers' 787.6±37.7 g). Of the seven subadults included in the study (438–623 g), three were assigned to the control tank and four to the diving tank. Within each test group, we observed no differences between adults and subadults, hence data for both age classes were pooled.

Food rations were replenished daily and each tank was drained and cleaned at 2 day intervals. During the final 3–4 days of each training session, muskrats were removed from each tank for brief periods, in order to gather behavioural and metabolic data comparable to those collected prior to training. At the conclusion of testing, animals were killed and blood and tissue samples harvested for determination of relevant biochemical and respiratory measures (see below).

Metabolic and behavioural recordings in air and water

Pre- and post-training measurements of resting $\dot{V}_{O_2}$ were obtained from fasted muskrats at thermoneutrality (15±0.5°C) using positive pressure, open-circuit respirometry (MacArthur and Campbell, 1994). Short-term (15 min) diving trials were conducted in a fibreglass-lined, plywood tank (183 cm×175 cm×72 cm) housed in a controlled-environment room. The tank was filled to a depth of 68–70 cm with warm (29–30°C) water to minimize thermal stress for subjects. A wire screen cover secured to a frame 3 cm below water level prevented diving muskrats from surfacing at any point in the tank except in a 20.5 liter respirometry chamber (MacArthur and Krause, 1989). Animals could swim or float at the surface in the chamber but were prevented from leaving the water. At both the pre-training and post-training stage, each animal was tested twice (on separate days). In each case, the first trial provided a training session to familiarize the animal with the tank and only data for the second trial were used in analyses. The behavioural data gathered included frequency and duration of all exploratory dives, as well as cumulative dive time for the 15 min trial. Diving $\dot{V}_{O_2}$ was estimated following the procedure of MacArthur and Krause (1989).

Body oxygen stores

To obtain pre-training measures of blood Hb and Hct levels, blood samples were drawn by cardiac puncture from animals lightly anaesthetized with an inhalant anaesthetic (Halothane, MTC Pharmaceuticals). Following training and completion of all metabolic and behavioural testing, each animal was anaesthetized with an intramuscular injection of ketamine hydrochloride (Rogar/STB Inc.) given in combination with xylazine (Haver-Lockhart) and atropine sulphate. The left jugular vein was exposed, cannulated, and a blood sample drawn for Hb, Hct and red cell 2,3-DPG determinations (MacArthur, 1984a; 1990). Mean corpuscular Hb concentration (MCHC) was calculated from Hb and Hct measurements (Schalm et al., 1975). Blood volume was calculated from Hct and the plasma dilution of Evans Blue dye (Swan and Nelson, 1971; El-Sayed et al., 1995). Subsequently, the muskrat was euthanized and muscle samples and the intact lungs were removed from the carcass immediately. The ventricles and biceps brachii, biceps femoris and gastrocnemius muscles were dissected free from fat and connective tissue and immediately frozen at –70°C. Muscle Mb concentrations were subsequently measured according to the technique described by Reynafarje (1963). Lung volume, corrected to standard temperature and pressure (STPD), was determined gravimetrically (MacArthur, 1990).

Muscle oxygen stores were estimated from the mean Mb concentration of the three skeletal muscles sampled, assuming skeletal muscle constitutes 44% of the ingesta-free body mass of these rodents (MacArthur et al., 2001). The potential lung and blood oxygen stores of each individual were calculated in accordance with MacArthur (1990) and followed conventional protocol (Kooyman, 1989).

Muscle buffering capacity and glycogen content

Intracellular buffering capacities of forelimb (biceps brachii) and hind limb (biceps femoris) muscles were determined as described by Castellini and Somero (1981). A 0.5 g sample of frozen muscle was homogenized in 0.15 mol l^{-1} NaCl and titrated with 0.2 mol l^{-1} NaOH using a Corning model 360i pH meter equipped with an ISFET electrode (Fisher Scientific Canada, Whitby, ON, Canada). Buffering capacity, measured in slykes, is defined as the μmoles of base required to titrate the pH of 1 g wet mass of muscle by 1 pH unit, over the pH range 6 to 7 (Van Slyke, 1922). The glycogen content of these muscles was determined using a spectrophotometric procedure based on a calibration curve derived for glucose standards (Kemp and Kits Van Heijningen, 1954). Absorbances of standards and unknowns were read at 520 nm using a Spectronic 601 Spectrophotometer (Rochester, NY, USA).

Treatment of data

Mean values for 'surface swimmers' and 'divers' were compared using one- or two-tailed independent-samples *t*-tests (Zar, 1984). Comparisons of pre- and post-training values derived for the same individuals were made using paired *t*-tests. For analyses of muscle Mb, glycogen and buffering capacity, two-way analysis of variance (ANOVA) was used to evaluate sampling site and treatment effects. *Post hoc* testing for differences in Mb content between muscle sampling sites was made using Tukey's HSD test (Zar, 1984). All statistical procedures were performed using SPSS software (version 9, 1999), following Kolmogorov–Smirnov or Shapiro–Wilk tests for normality and Levene's test for homogeneity of variance (SPSS). In the few cases when data did not meet normality or homogeneity of variance assumptions, the data were either transformed prior to statistical testing or the non-parametric Mann–Whitney *U*-test was performed. Values are presented as means ±1 S.E.M.

Results

Baseline measurements of acclimated muskrats

Prior to training, 'surface swimmers' and 'divers' exhibited similar (P>0.05) mass-specific resting and diving metabolic rates (Table 1). In both groups, mean diving $\dot{V}_{O_2}$ was 2.4–3.0 × the resting $\dot{V}_{O_2}$ value. We detected no initial differences between groups in any of the behavioural indicators of dive performance, including dive frequency, cumulative dive time and mean duration of exploratory dives (Table 1) (P>0.05). MCHC, Hct level and blood oxygen capacity were also similar in both groups prior to testing (Fig. 1) (P>0.05).

We considered the possibility that pre-training Hb and Hct levels might differ for muskrats captured in different months (July–December), even though all animals were acclimated to the laboratory holding facilities for 4–6 weeks prior to testing. Results of a one-way ANOVA revealed that there was no relationship between month of capture and the initial (pre-training) blood Hb concentration of acclimated muskrats ($F_{3,20}$=1.923, P=0.158). However, the pre-training Hct levels of acclimated animals did vary with time of capture ($F_{3,20}$=5.516, P=0.006), averaging 45.60±1.14% for July (N=6), 41.88±1.21% for August (N=4), 47.28±1.03% for October (N=10) and 49.65±1.01% for December (N=4) captures.

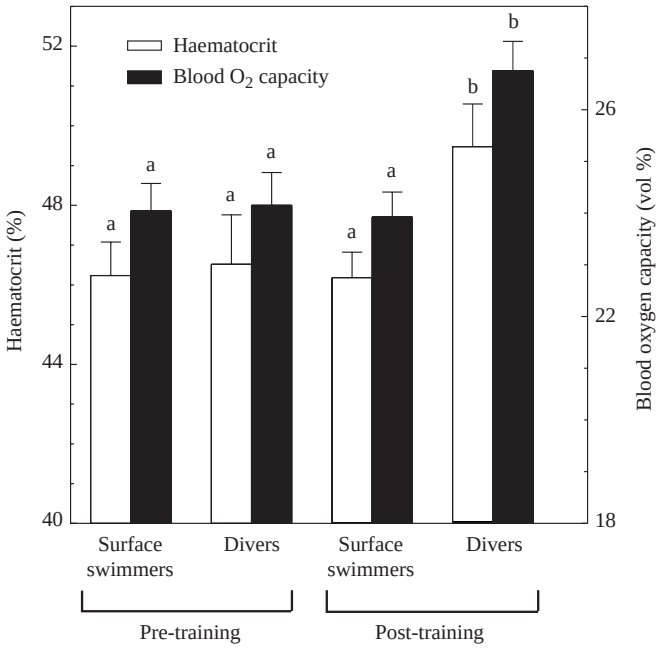

Fig. 1. Haematocrit and blood oxygen capacity of 12 surface-swimming (control) and 12 dive-conditioned muskrats prior to (pre-training) and following 9–11 weeks of training (post-training) in the laboratory (see text for details). Values are means + 1 S.E.M. For each variable, means sharing the same letter are not significantly different (P>0.05).

Training effects on metabolic rate and diving behaviour

Following the 9- to 11-week training session, mean mass-specific resting $\dot{V}_{O_2}$ values were 18.1% lower in 'surface swimmers' (t=4.07, d.f.=11, P=0.002) and 9.4% lower in 'divers' (t=1.49, d.f.=11, P=0.164), compared to their respective pre-training values (Table 1). Mean diving $\dot{V}_{O_2}$ values of 'surface swimmers' followed a similar trend (Table 1), and were 22.1% lower following training (t=2.67, d.f.=11, P=0.022). In contrast, diving $\dot{V}_{O_2}$ of 'divers' was not significantly affected by training (t=0.70, d.f.=11, P=0.498). It is noteworthy that body mass was similar for 'surface swimmers' and 'divers' prior to training, but animals in both groups experienced significant (P<0.05) mass gains during the training session. Mean mass gained by 'divers' was 16.9±3.41%, compared to 19.5±4.73% for 'surface swimmers'. However, these mass changes could not account for the observed training effects on resting and diving $\dot{V}_{O_2}$, since conversion of $\dot{V}_{O_2}$ to mass-independent units (ml O_2 $g^{0.67}$ h^{-1}) (Campbell and MacArthur, 1998) did not alter the observed metabolic trends. Post-training metabolic rates tended to be highest for the dive-conditioned animals, though only the increase observed in resting $\dot{V}_{O_2}$ in air was significant (Table 1).

Table 1. *Metabolic rates and dive performance of muskrats prior to and following 9–11 weeks acclimation to a holding tank that restricted aquatic activity either to surface swimming (controls) or to diving*

	Surface swimmers (*N*=12)		Divers (*N=12*)		
	Pre-training	Post-training	Pre-training	Post-training	*P*
Resting $\dot{V}_{O_2}$ in air (ml g^{-1} h^{-1})	0.83±0.04	0.68±0.02	0.85±0.03	0.77±0.04	0.040*
Diving $\dot{V}_{O_2}$ (ml g^{-1} h^{-1})	2.49±0.18	1.94±0.21	2.05±0.22	2.22±0.18	0.277*
Cumulative dive time (s)	430.6±35.5	441.0±44.3	486.0±23.8	431.7±37.7	0.438
Total number of dives	16.5±1.7	17.5±1.5	17.3±1.6	17.4±2.0	0.478
Mean dive duration (s)					
All exploratory dives	27.5±1.7	25.1±1.8	30.7±2.9	25.9±2.4	0.388
Five longest exploratory dives	40.0±2.2	37.4±2.4	48.8±4.8	39.1±4.2	0.369
Longest exploratory dive	56.4±6.0	46.9±2.8	90.3±22.9	54.4±10.5	0.295

Dive performance was based on a 15 min observation session in the diving tank (see text for details).

P values are for post-training comparisons of surface swimmers and divers. Asterisks denote two-tailed *P* values; all other *P* values are for one-tailed comparisons.

We found little evidence that our training regime altered the diving behaviour of muskrats in either test group. Post-training measures of cumulative dive time, total number of dives, and overall mean dive duration did not differ from the respective pre-training values in either test group (Table 1) ($P>0.05$). However, the two indices of maximal dive time tended to be lower following training, especially in 'divers' (Table 1). In this test group, the mean of the five longest and the single longest exploratory dive were reduced by 19.9% (t=2.179, d.f.=11, P=0.052) and 39.8% (t=1.951, d.f.=11, P=0.077), respectively, after training. We detected no differences in the dive behaviour of 'surface swimmers' and 'divers' following training (Table 1).

Training effects on blood, lung and muscle respiratory variables

The requirement for underwater swimming imposed on 'divers' clearly elevated the Hct and blood oxygen capacity of muskrats assigned to this test group (Fig. 1). Compared to initial (pre-training) values, blood Hct and Hb concentration of 'divers' were increased 10.8% (t=4.684, d.f.=11, P=0.0005) and 6.4% (t=2.483, d.f.=11, P=0.015), respectively, after training. This compares with differences of only 0.1–0.5% between pre- and post-training measures of Hct and Hb levels recorded from 'surface swimmers' ($P>0.05$) (Fig. 1). Final (post-training) Hct, Hb concentration, blood oxygen capacity and MCHC were significantly higher in 'divers' than in 'surface swimmers' (Fig. 1, Table 2). The mean blood oxygen store calculated for 'divers' (22.9 ml O_2 kg^{-1}) was nearly 26% higher than that (18.2 ml O_2 kg^{-1}) derived for 'surface swimmers' (t=1.990, d.f.=22, P=0.030) (Fig. 2).

The dive-training regime imposed on muskrats in this study had no apparent effect on lung volume, whole blood and plasma volumes, erythrocyte 2,3-DPG concentration (Table 2), nor on the glycogen level or buffering capacity of skeletal muscles (Table 3). Ventricular and skeletal muscle Mb levels were also similar in 'surface swimmers' and 'divers' following training (Tables 2 and 3). Consequently, the final lung and muscle oxygen stores calculated for each group were virtually identical (Fig. 2). However, owing to the significant gain in blood oxygen reserves, the mean total body oxygen store of 'divers' (37.8 ml O_2 STPD kg^{-1}) was 13.5% higher

Table 2. *Lung, blood and muscle characteristics of muskrats following 9–11 weeks acclimation to a holding tank that restricted aquatic activity either to surface swimming (controls) or to diving*

	Surface swimmers (*N*=12)	Divers (*N*=12)	*P*
Ingesta-free body mass (g)	914.5±42.3	845.2±53.2	0.319*
Total lung capacity (ml STPD kg^{-1})	52.19±1.72	51.11±2.99	0.758*
Blood volume (ml 100 g^{-1})	9.30±0.56	10.31±0.74	0.144
Plasma volume (ml 100 g^{-1})	4.82±0.30	5.04±0.39	0.331
Haematocrit (%)	46.18±0.64	49.47±1.07	0.0075
Haemoglobin (g 100 ml^{-1})	17.85±0.36	19.97±0.43	0.0005
MCHC (%)	38.69±0.66	40.44±0.74	0.046
Blood O_2 capacity (vol%)	23.92±0.48	26.76±0.57	0.0005
Erythrocyte 2,3-DPG (μmol ml^{-1} packed red cells)	5.45±0.41	5.58±0.29	0.399
Skeletal muscle Mb (mg g^{-1} wet tissue)	12.42±0.47	12.29±0.24	0.466

Asterisks denote two-tailed *P* values; all other *P* values are for one-tailed comparisons.

Table 3. *Muscle characteristics of muskrats following 9–11 weeks acclimation to a holding tank that restricted aquatic activity either to surface swimming (controls) or to diving*

	Surface swimmers (*N*=12)	Divers (*N*=12)	*P*
Myoglobin (mg g^{-1} wet tissue)			
Ventricles	9.29±0.35	8.92±0.29	0.212
Biceps brachii	11.82±0.60	12.00±0.37	0.398
Biceps femoris	12.43±0.55	12.03±0.42	0.285
Gastrocnemius	13.01±0.51	12.84±0.22	0.422
Glycogen (mg g^{-1} wet tissue)			
Biceps brachii	0.31±0.08	0.55±0.20	0.139
Biceps femoris	0.50±0.08	0.69±0.16	0.150
Buffering capacity (β)			
Biceps brachii	55.13±0.77	54.06±1.60	0.276
Biceps femoris	58.34±2.26	57.75±2.36	0.429

All *P* values are for one-tailed comparisons of surface swimmers and divers.

β=Slykes (μmoles of base required to titrate the pH of homogenized muscle by one pH unit).

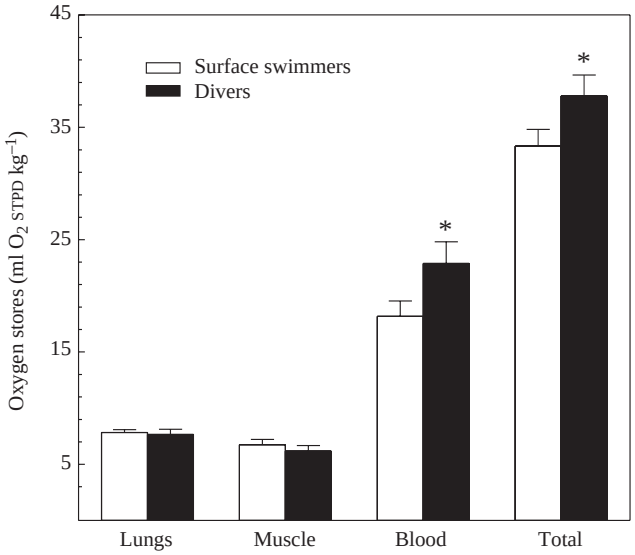

Fig. 2. Total and site-specific oxygen storage capacity of 12 surface-swimming (control) and 12 dive-conditioned muskrats following 9–11 weeks of training in the laboratory (see text for details). Values are means + 1 S.E.M. Asterisks denote comparisons in which mean values of 'divers' exceeded those of 'surface swimmers' (P<0.05).

(t=1.874, d.f.=22, P=0.037) than for 'surface swimmers' (33.3 ml O_2 STPD kg^{-1}; Fig. 2).

Site-specific variability in muscle buffering capacity and glycogen and Mb content

Although there was a tendency for glycogen level and buffering capacity to be higher in the hind limb swimming muscles (biceps femoris) than in the non-propulsive muscles of the forelimbs (b. brachii), these trends were not statistically significant (P>0.05). Results of a two-way ANOVA performed on the Mb data revealed a significant muscle site effect ($F_{1,88}$=30.12, P<0.0001), but no evidence of a test group effect ($F_{1,88}$=0.387, P=0.536) or a muscle site × test group interaction ($F_{3,88}$=0.194, P=0.900). Though Mb levels of the hind limb muscles, especially gastrocnemius, tended to slightly exceed those of the forelimbs (Table 3), *post hoc* comparisons (Tukey's HSD test) indicated no differences (P>0.05) in mean Mb content amongst any of the skeletal muscles sampled. By comparison, Mb content of the ventricles was consistently lower (P=0.006–0.0001) than levels recorded from any of the limb muscles, accounting for the muscle site effect observed with ANOVA.

Discussion

Dive conditioning and body oxygen reserves

This study was designed to test the hypothesis that the aerobic dive capacity and diving ability of muskrats can be enhanced by conditioning in a laboratory setting. We addressed this question by comparing the responses of two groups of muskrats housed in holding tanks that were identical in every respect, except that one (dive-training tank) precluded surface swimming by test animals. The latter set-up was intended to simulate winter field conditions when continuous ice cover in the marsh dictates that muskrats travel underwater.

The most striking observation was the significant gain in the blood oxygen storage capacity of dive-conditioned muskrats. Not only did Hct, Hb and blood oxygen capacity of 'divers' increase over the 9- to 11-week training period, but the final (post-training) values of this test group exceeded those of 'surface swimmers' by 7–12% (Fig. 1; Table 2). We attribute the gains in Hb and Hct by dive-conditioned muskrats to intermittent hypoxia associated with underwater swimming through the 16 m maze separating nest box from feeding station in the dive tank. This conclusion is strengthened by the absence of any change whatsoever in the blood parameters of control animals ('surface swimmers') held concurrently in an identical tank furnished only with shallow water (Fig. 1). Though we cannot preclude the possibility that these differences may also reflect a stronger exercise response by the dive-trained group, it was our impression that the effort required to negotiate the maze was similar, whether muskrats were diving or swimming at the surface.

The results of this study are consistent with the comparison by Kodama et al. (1977) of 'diving' (N=4) and 'non-diving'(N=3) juvenile harbor seals reared in captivity. Over a 10 month period, these authors reported modest gains in several blood parameters, including Hb and Hct levels, in 'divers' that had continuous access to an outdoor seawater tank. To our knowledge, the only other study that specifically addressed the benefits of dive conditioning in a natural diver was by Stephenson et al. (1989) on tufted ducks. These authors

reported no significant gains in the Hb content, blood oxygen capacity or red blood cell count of dive-conditioned ducks following a 6 month training period when birds were required to swim a distance of 6 m underwater in order to feed. However, their dive-conditioned ducks exhibited a 34% increase in plasma volume, leading these researchers to conclude that this hypervolemia was probably accompanied by dive-induced erythropoeisis, thereby avoiding the haemodilution response commonly observed in training.

Our results harmonize with the extensive literature in exercise physiology documenting elevated serum erythropoietin (EPO), Hb and Hct levels of mammals, especially humans, following training under hypobaric hypoxic conditions (see Böning, 1997; Rodríguez et al., 2000). Several of these studies have established that intermittent hypoxia, including that simulating sleep apnoea in humans, is sufficient to stimulate EPO synthesis and/or elevate Hb and Hct levels (Nattie and Doble, 1984; Knaupp et al., 1992; Rodríguez et al., 2000). Though EPO was not measured in this study, we assume that the recurrent apnoeic episodes associated with underwater swimming provided a sufficient hypoxic signal to stimulate renal EPO synthesis in dive-conditioned muskrats. It is well-established that this glycoprotein stimulates erythropoiesis by enhancing mitotic frequency and promoting iron uptake and Hb synthesis by erythroid-committed cells in bone marrow (Jelkmann, 1986; Kranz, 1991). Consequently, the observed gains in Hct, Hb and blood oxygen capacity of 'divers' probably reflect elevated EPO production by the kidneys of these animals.

The degree of hypoxia experienced by muskrats in this study is unknown, as is the level required to promote EPO synthesis. In an earlier study of controlled dives by restrained muskrats, MacArthur (1986) reported that the arterial partial pressure of oxygen (P_{aO_2}) fell to 3.06 kPa after 80–90 s of submergence. Though this is nearly twice the mean duration of voluntary dives by muskrats in the maze, the earlier study involved non-exercising rather than swimming muskrats, and the resultant end-dive P_{aO_2} was well below the P_{aO_2} (7.32–7.98 kPa) required to induce polycythemia in the laboratory rat (Nattie and Doble, 1984).

To our knowledge, there is no evidence to suggest that EPO might also account for the modest gain in MCHC of dive-trained animals (Table 2). Human studies, for example, have reported no change (Klausen et al., 1991) or even a drop (Richalet et al., 1994) in MCHC with hypoxia-induced increases in blood EPO level. Though plasma osmolality was not recorded in this study, it is possible that changes in this variable may have contributed to the observed differences in MCHC between 'surface swimmers' and 'divers'. Irrespective of the proximal cause, an elevated MCHC should theoretically enhance blood oxygen transport capacity while limiting the attendant rise in blood viscosity (Rodríguez et al., 1999). In this context, it is noteworthy that the MCHC of recently captured, winter-acclimatized muskrats (38.5%) is nearly 10% higher than for animals captured in summer (35.1%) (MacArthur, 1984a).

Despite the pre-eminent role often ascribed to muscle Mb in the maturation of diving proficiency in marine birds and mammals (see Ponganis et al., 1999; Noren et al., 2001), we found no evidence suggesting this variable is responsive to dive conditioning in muskrats (Tables 2 and 3). The Mb content of ventricles and fore- and hindlimb skeletal muscles were virtually identical in 'divers' and 'surface swimmers' (Table 3). Though Mb content tended to be higher in the hindlimb swimming muscles than in the forelimb muscles, a trend previously noted in field-acclimatized muskrats (MacArthur, 1990; MacArthur et al., 2001), the differences in this study were not statistically significant ($P>0.05$) (Table 3). The mean skeletal muscle Mb levels of 'divers' and 'surface swimmers' following training (12.3–12.4 mg g^{-1} wet tissue; Table 2) were similar to the level (12.1 mg g^{-1} wet tissue) reported for recently captured, summer-acclimatized adult muskrats (MacArthur et al., 2001). However, these post-training values were 10–11% lower than the mean muscle Mb level (13.8 mg^{-1} g wet tissue) of recently captured, winter-acclimatized animals (MacArthur et al., 2001).

In their investigation of tufted ducks, Stephenson et al. (1989) reported no change in myocardial Mb, but significant gains in the Mb content of the pectoralis and locomotor limb muscles of dive-conditioned birds. Though researchers have speculated that the intermittent hypoxia accompanying early dive experience may contribute to the upregulation of muscle Mb in young avian and mammalian divers (Ponganis et al., 1999; Noren et al., 2001), experimental support for this hypothesis remains limited (Stephenson et al., 1989; Terrado et al., 1990). In a previous investigation of developmental changes in body oxygen stores of wild-caught muskrats (MacArthur et al., 2001), we reported that skeletal muscle Mb content is strongly age- and mass-dependent in animals ranging from 254 to 600 g ($Mb=27.7\times Mass^{1.63}$, $r^2=0.82$). The present study suggests that as muskrats grow and gain experience swimming underwater, dive-induced hypoxia probably has minimal effect on the acquisition of adult levels of Mb.

Not surprisingly, lung oxygen stores were similar for 'divers' and 'surface swimmers'. Our finding that red cell 2,3-DPG concentration also remained unchanged (Table 2) suggests there may be little, if any, modulation in the oxygen binding properties of Hb by this organophosphate in response to dive conditioning. In an earlier study, MacArthur (1984a) reported that the affinity of muskrat blood for oxygen was greatest in winter and suggested that at least one contributing factor was the reduction in red cell 2,3-DPG concentration observed during this season. Whatever the proximal cause for the enhanced binding affinity of muskrat blood for oxygen in winter, this shift should, in theory, enable diving animals to extract a greater fraction of available oxygen from their lung reserves during under-ice excursions (Snyder, 1983; MacArthur, 1984a).

Metabolic and behavioural responses to training

An unexpected finding in this study was the tendency for resting and diving $\dot{V}_{O_2}$ of control animals to decline

significantly over the course of the training period. Metabolic rates of 'divers' appeared to be more stable over time, though resting $\dot{V}_{O_2}$ of this test group exhibited a slight, albeit non-significant decline (9.4%) during the training session (Table 1). We considered the possibility that these trends might reflect the tendency for animals in both groups to gain appreciable body mass during training sessions (19.5% in 'surface swimmers'; 16.9% in 'divers'). However, mass change alone cannot account for the observed depression in resting metabolic rate, since conversion of $\dot{V}_{O_2}$ to mass-independent units (Campbell and MacArthur, 1998) did not eliminate this effect. It is relevant to note that extensive subcutaneous and intra-abdominal fat depots were evident in most subject animals at the time of necropsy. We suspect that the observed gains in body mass were due mainly to fat accretion by muskrats maintained on a high plane of nutrition in captivity and that the increase in body lipid content may be implicated in the apparent decline in resting $\dot{V}_{O_2}$ (Hayward, 1965). Unfortunately, body composition analyses were not performed on any of the subjects, hence we cannot assess the extent to which variation in $\dot{V}_{O_2}$ between and within test groups may have arisen from differences in body lipid or protein content. For example, the finding that mean post-training diving $\dot{V}_{O_2}$ of 'divers' ($2.22\,ml\,g^{-1}\,h^{-1}$) was 14.4% higher than for 'surface swimmers' ($1.94\,ml\,g^{-1}\,h^{-1}$) could indicate a gain in relative muscle mass by the dive-trained muskrats.

While the difference in diving $\dot{V}_{O_2}$ between the two test groups was not statistically significant (Table 1), it nevertheless offset the 13–14% gain in total body oxygen stores by dive-conditioned muskrats. Consequently, the calculated ADL (MacArthur et al., 2001) of this group (61.3 s) was indistinguishable from that of control animals (61.8 s), a finding that may at least partially explain the observed similarities in diving behaviour of animals in the two test groups (Table 1).

Though we examined only two indices of anaerobic potential in skeletal muscle (glycogen content and buffering capacity), there was no evidence that either variable was upregulated in response to dive conditioning. The mean time required by muskrats to negotiate the underwater maze was 43.8 s, which is well within their calculated ADL, and it is doubtful that these animals routinely resorted to anaerobic metabolism during underwater swimming in the dive tank.

Concluding remarks

On balance, the results of this study demonstrate that the blood oxygen stores of muskrats can be improved with an enforced diving regime over a relatively short time course (9–11 weeks). Total body oxygen stores of dive-conditioned animals were boosted 13–14% beyond those of animals that were prevented from diving (Fig. 2). However, equally striking was our failure to detect any evidence whatsoever that the accessible oxygen reserves in lungs and muscles can be manipulated by dive training. In contrast, Stephenson et al. (1989) reported significant gains in both blood and muscle oxygen stores of dive-conditioned ducks, but at the expense of reduced oxygen availability in their respiratory system following training. On the basis of this study, we cannot conclude that an increase in diving activity is the sole, or even the primary, factor responsible for the marked elevation in body oxygen reserves of muskrats in winter (MacArthur, 1990; MacArthur et al., 2001). These earlier investigations established that total body oxygen stores of field-acclimatized muskrats increased 29–42% between summer and winter, with most of the increase accounted for by gains in blood oxygen capacity.

Several factors may have contributed to our failure to observe changes of similar magnitude in the laboratory. One is the possibility that 4–6 weeks was an insufficient period to fully acclimate field-caught muskrats prior to training. This argument is supported by the observation that pre-training Hct levels tended to be lowest for acclimated muskrats caught in July–August (41.9–45.6%, N=10) and highest for animals captured in October–December (47.3–49.6%, N=14), a similar trend to that reported for field-acclimatized animals (MacArthur et al., 2001). On the other hand, we found no evidence that month of capture significantly affected the extent to which Hct or Hb levels were elevated following dive conditioning. We similarly observed no relationship between time of capture and the post-training Hct, Hb or muscle Mb levels of muskrats assigned to either test group.

The absence of a stronger training response could also reflect the artificial nature of the dive-conditioning protocol that required acclimated muskrats to negotiate an underwater maze separating nest box from feeding station. This limitation notwithstanding, the average dive time of muskrats travelling between these sites in the dive tank (43–44 s) was consistent with average under-ice transit times calculated for free-living muskrats swimming between resting and feeding shelters in winter (20–42 s; MacArthur, 1992). However, we also recognize that other factors may be implicated in boosting body oxygen reserves of winter-acclimatized muskrats in nature, including exposure to low ambient temperatures (Deb and Hart, 1956; Morrison et al., 1966) and the hypoxic-hypercapnic atmospheres of the winter shelters frequented by these rodents (MacArthur, 1984b). Clearly, further research is needed to assess the relative contributions of each of these variables to the seasonal adjustments in body oxygen reserves and diving proficiency of muskrats inhabiting northern marshes.

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