

Body Oxygen Stores, Aerobic Dive Limits, and the Diving Abilities of Juvenile and Adult Muskrats (*Ondatra zibethicus*)

Robert A. MacArthur^{1,*}

M. M. Humphries²

G. A. Fines¹

K. L. Campbell³

¹Department of Zoology, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada; ²Department of Biology, McGill University, Montreal, Quebec H3A 1B1, Canada; ³Danish Centre for Respiratory Adaptation, Zoophysiology Department, Institute for Biological Sciences, Aarhus University, DK-8000 Aarhus C, Denmark

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ABSTRACT

Intraspecific variability in body oxygen reserves, muscle buffering capacity, diving metabolic rate, and diving behavior were examined in recently captured juvenile and adult muskrats. Allometric scaling exponents for lung ($b = 1.04$), blood ($b = 0.91$), and total body oxygen storage capacity ($b = 1.09$) did not differ from unity. The concentration of skeletal muscle myoglobin scaled positively with mass in 254–600-g juveniles ($b = 1.63$) but was mass-independent in larger individuals. Scaling exponents for diving metabolic rate and calculated aerobic dive limit (ADL) were 0.74 and 0.37, respectively. Contrary to allometric predictions, we found no evidence that the diving abilities of muskrats increased with age or body size. Juveniles aged 1–2 mo exhibited similar dive times but dove more frequently than summer-caught adults. Average and cumulative dive times and dive : surface ratios were highest for fall- and winter-caught muskrats. Total body oxygen reserves were greatest in winter, mainly due to an increase in blood oxygen storage capacity. The buffering capacity of the hind limb swimming muscles also was highest in winter-caught animals. Several behavioral indicators of dive performance, including average and maximum duration of voluntary dives, varied positively with blood hemoglobin and muscle myoglobin concentration of muskrats. However, none of the behavioral measures were strongly correlated with the total body oxygen reserves or ADLs derived for these same individuals.

* Corresponding author; e-mail: rmacarth@ms.umanitoba.ca.

Introduction

It is widely acknowledged that the breath-hold endurance of endothermic divers depends critically on the accessible oxygen reserves and the rate at which these reserves are depleted underwater (Kooyman 1989; Butler and Jones 1997; Schreer and Kovacs 1997; Kooyman and Ponganis 1998). This dependency is supported by interspecific comparisons that repeatedly demonstrate a positive relation between diving capability and oxygen storage potential in both avian (Weber et al. 1974; Keijer and Butler 1982) and mammalian (Ridgway and Johnston 1966; Lenfant et al. 1970) divers. However, only in recent years have researchers begun to seriously examine intraspecific variability, including ontogenetic changes, in behavioral, metabolic, and respiratory indices relevant to dive performance (Thorson and LeBoeuf 1994; Burns and Castellini 1996; Burns et al. 1997; Horning and Trillmich 1997a, 1997b; Costa et al. 1998; Ponganis et al. 1999). Such studies are vital to assessing phenotypic plasticity and developing appropriate intraspecific scaling functions for key metabolic and respiratory variables. They also are essential to confirm a functional relationship between diving ability and physiological indicators of dive endurance in the same individuals (Bennett 1987). Though underwater endurance is often linked to the oxygen storage capacity of tissues, especially in cross-species comparisons, remarkably few studies have specifically tested for such a relationship in vertebrate divers (Hudson and Jones 1986; Thorson and LeBoeuf 1994; Burns and Castellini 1996; Costa et al. 1998). The paucity of such studies may at least partially reflect the traditional focus on marine species and, consequently, the considerable costs and effort involved in acquiring sufficient numbers of animals.

To address these needs, we investigated the lung, blood, and muscle oxygen reserves, diving metabolic rate, and diving behavior of field-acclimatized, juvenile, and adult muskrats (*Ondatra zibethicus*) captured in different seasons. Though the diving capabilities of this rodent are modest compared with most marine birds and mammals (Kooyman 1989), the muskrat is a common, easily caught, semiaquatic species that is ideally suited to studies of interindividual physiological variation (Bennett 1987). Not only can these rodents be obtained in large numbers at relatively low expense throughout the year, but muskrats are also accomplished divers by the age of 3 wk (Er-

rington 1939) and, hence, are well suited to studies of developmental change in diving behavior and physiology.

In an earlier investigation, MacArthur (1990) reported that the total oxygen storage capacity of muskrats increased from 25.2 mL O₂ kg⁻¹ in July to 35.7 mL O₂ kg⁻¹ in December. Assuming that the cost of diving is similar in both seasons, this represents a 42% gain in the theoretical aerobic dive limit (ADL) of muskrats in winter, a season when their habitat is ice-covered and the animals are most dependent on diving. However, our recent finding that the basal metabolic rate (BMR) of muskrats in the same population increased 31% during winter (Campbell and MacArthur 1998) raises the possibility that the absolute metabolic cost of diving may also increase during the ice-bound season. If so, it might be argued that the increased oxygen storage capacity observed in winter-acclimatized muskrats is a compensatory response to a higher rate of tissue oxygen consumption ($\dot{V}O_2$) during diving (Snyder 1983). Also, the MacArthur (1990) study demonstrated only that the oxygen storage capacity of muskrats is elevated in winter; no evidence was provided that demonstrated that animals with increased oxygen stores actually exploit those reserves to dive longer.

This study has two main objectives. First, we wished to examine phenotypic variability in the diving abilities, oxygen storage capacity, and diving $\dot{V}O_2$ of muskrats inhabiting a northern prairie marsh, including the influence of seasonal and mass- and age-dependent factors. We were especially interested in comparing adults and juveniles, given an earlier report (Errington 1939) that recently weaned muskrats are expert divers capable of prolonged periods (2–4 min) of voluntary submergence. This observation is at variance with expectations based on allometry (Hudson and Jones 1986; Schreer and Kovaks 1997) and on several previous studies that have underscored the limited diving capabilities of immature animals (Thorson and LeBoeuf 1994; Burns and Castellini 1996; Burns et al. 1997; Horning and Trillmich 1997a, 1997b; Costa et al. 1998; Ponganis et al. 1999). Sufficient data were gathered over a 9-yr period to establish allometric functions for several lung, blood, and muscle variables, as well as diving $\dot{V}O_2$ and calculated (“theoretical”) ADL of wild-caught muskrats. Previous estimates of the ADL of muskrats (MacArthur 1990) have been refined by measuring all input variables (including diving $\dot{V}O_2$) in the same individuals and by incorporating more accurate estimates of the muscle mass of these rodents. Refined estimates of muscle oxygen stores are vital, given that elevated myoglobin tends to be the most prominent and consistent physiological correlate of underwater endurance in vertebrate divers (Kooyman 1989; Kooyman and Ponganis 1998; Ponganis et al. 1999). As an index of the potential for anaerobic metabolism (Castellini and Somero 1981; Mill and Baldwin 1983), the buffering capacity of the primary swimming muscles of juvenile and adult muskrats also was examined. Our second major goal was to test for statistical correlation between measured indices

of aerobic capacity and the diving behavior of the same, recently caught individuals. To our knowledge, such a relationship has not been confirmed for any population of vertebrate divers studied to date. Specifically, we tested the hypothesis that increased tissue oxygen reserves and calculated ADL are accompanied by increased voluntary dive times of field-acclimatized muskrats.

Material and Methods

Animals

Weaned juvenile and adult muskrats of both sexes were live-trapped at Oak Hammock Marsh, Manitoba (lat. 50°06'N, long. 97°07'W) between July 1987 and December 1996. Animals were immediately transported to the Animal Holding Facility, University of Manitoba, and housed individually at 14° ± 1°C with a 12L : 12D photoperiod (MacArthur 1979).

Ages of juveniles were estimated from growth curves derived for captive animals (Virgl and Messier 1995; MacArthur and Humphries 1999). To facilitate analyses, animals were assigned to one of five cohorts based on age and season of capture (summer, S; fall, F; winter, W): SJUV1 = 254–360 g (4.5–6-wk-old) juveniles caught July 17–21, 1996 (*n* = 8); SJUV2 = 390–525 g (6.5–9-wk-old) juveniles caught July 14–August 11, 1993 (*n* = 10); FJUV = 492–620 g (8.5–13-wk-old) juveniles caught September 13–October 27, 1995 (*n* = 9); SADULT = 749–1,104 g adults caught June 9–August 12, 1993 (*n* = 31); WADULT = 513–898 g adults and subadults caught December 6–21, 1988, 1996 (*n* = 18). Since juveniles enter a growth diapause during winter (Virgl and Messier 1995), it was not possible to estimate ages of winter-caught young, nor could they easily be discriminated from small adults in this season. Consequently, all immature muskrats caught in December were designated as “subadults” (Errington 1963; Campbell and MacArthur 1998) and no attempt was made to separate these from winter-caught adults in any of the analyses. It should be noted that the SADULT and WADULT cohorts included previously reported (MacArthur 1990) oxygen stores data for summer (*n* = 9) and winter (*n* = 8) animals, revised to incorporate new estimates of muscle mass. To increase sample sizes for scaling of lung, blood, and muscle variables, data for an additional 26 adults (768–1,127 g) were included in allometric analyses. These were muskrats caught in summer and fall (1995, 1996) for another, unrelated study, and all were held in captivity for at least 6 wk before testing. All of the remaining animals (*n* = 76) were tested 2–7 d after capture. Preliminary tests revealed no differences between sexes, hence, data for males and females were pooled in all subsequent analyses.

Diving Trials

Behavioral and metabolic data were gathered from 59 of the field-acclimatized muskrats trapped in summer, fall, and winter.

Observations were made of animals free-diving for a 30-min period in a large fiberglass-lined plywood tank (Dyck and MacArthur 1993) filled to a depth of 68 cm with 29°–30°C water. This temperature was chosen to minimize thermal stress on subjects, especially immature animals (MacArthur 1984a; MacArthur and Krause 1989). 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 purpose-built, 20.5-L respirometry chamber (MacArthur and Krause 1989). Animals could swim or float at the surface in the chamber but were prevented from leaving the water. Each animal was tested twice (on separate days) and, except where noted otherwise, statistical procedures were performed on mean values calculated for both trials. The behavioral data gathered included frequency and duration of all exploratory dives, cumulative dive time, and interdive surface periods. Three measures of maximal voluntary submergence time were recorded: (1) longest exploratory dive, (2) mean of five longest exploratory dives, and (3) longest “alarm” dive. The latter was obtained at the end of the second trial, after the animal had returned to its holding cage through an opening in the screen, by applying a sharp blow to the side of the tank. In most instances, the animal responded by immediately diving underwater and hanging motionless beneath the screen for a variable period before slowly returning to its cage. This disturbance was repeated twice, with ca. 5–10-min recovery between tests. Diving $\dot{V}O_2$ was recorded following the procedure of MacArthur and Krause (1989).

Body Oxygen Stores and ADLs

In 1987–1988, blood volume was calculated from hematocrit (Hct) and the plasma dilution of iodinated (^{125}I) bovine serum albumin (MacArthur 1990). In all subsequent measurements, Evans blue dye (T-1824) was substituted for ^{125}I -labeled albumen to determine circulating plasma volume (Swan and Nelson 1971). Briefly, a preweighed quantity of dye was injected via a jugular catheter into an anesthetized muskrat. The catheter was then flushed repeatedly with blood withdrawn from the animal and finally with a known quantity of heparinized saline. Serial 2–3-mL blood samples were withdrawn at 10, 20, and 30 min postinjection and immediately centrifuged to separate red cells from plasma. Absorbance of plasma samples was read at 605 nm with a Spectronic 601 Spectrophotometer, using a pre-injection plasma sample as a blank. Absorbance values were compared to a calibration curve prepared from Evans blue diluted with muskrat plasma. The equilibrium concentration of Evans blue in the animal’s circulation was back-calculated using a standard regression procedure (Gillet and Halmagyi 1970; El-Sayed et al. 1995). Blood volume was calculated from the equation: blood volume = plasma volume \times 100/100 – Hct. Hct and hemoglobin (Hb) concentrations were determined from 3–4-mL blood samples drawn before dye injection, as described by MacArthur (1984b).

Following postinjection blood sampling, the muskrat was killed and muscle samples immediately harvested for myoglobin (Mb) determinations. Samples of the ventricles, biceps brachii, biceps femoris, and gastrocnemius 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, was determined gravimetrically (MacArthur 1990).

Muscle oxygen stores were estimated from the mean Mb concentration of the three skeletal muscles sampled. Total skeletal muscle mass was determined for eight juvenile (270–328 g) and eight adult (560–1,106 g) muskrats. In each case, as much tissue as possible was trimmed from the skinned, eviscerated carcass. The remaining muscle was digested from the skeleton by immersing the carcass in a heated ($70^\circ\text{--}80^\circ\text{C}$) solution containing the enzyme papain, Bio-Ad detergent, and EDTA, for 12–24 h. We found that, on average, skeletal muscle accounted for 43.25% and 44.4% of the ingesta-free body mass of juvenile and adult muskrats, respectively. The difference between age groups was not significant ($P > 0.05$) and an overall mean value (\pm SE) of $43.8\% \pm 0.47\%$ was used to estimate muscle oxygen stores. It should be noted that this muscle content is close to that (48%) estimated from carcass protein levels (Campbell and MacArthur 1998) but is considerably higher than the 30% previously assumed by MacArthur (1990).

The muskrat has a large, complex GI tract adapted for processing high-fiber plant diets (Campbell and MacArthur 1996) and the digestive contents can account for 4%–20% of the total body mass of these herbivores (Virgl and Messier 1993). Consequently, all mass-specific values reported in this study, including body oxygen stores and diving $\dot{V}O_2$, are presented on an ingesta-free basis. Ingesta mass was calculated by subtracting the total mass of gut + contents from empty gut mass at the time of tissue sampling.

The potential lung and blood oxygen stores of a diving muskrat were calculated in accordance with MacArthur (1990) and followed conventional protocol (Kooyman 1989). The maximum “theoretical” ADL (s) were calculated as total body oxygen stores (mL O_2 , STPD) divided by the diving $\dot{V}O_2$ (mL O_2 s^{-1}) recorded for each animal. Implicit in this calculation is the assumption that all oxygen reserves are fully exploited during submergence (Kooyman 1989).

Muscle Buffering Capacity

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 M NaCl and titrated with 0.2 M NaOH using a Corning model 360i pH meter equipped with a ISFET electrode. Buffering capacity (β) in slykes is defined as the μmol of base required to titrate the pH

of 1 g wet mass of muscle by 1 pH unit, over the pH range of 6 to 7.

Treatment of Data

The relationship of measured variables to body mass was assessed using linear least squares regression analysis on \log_{10} -transformed data to generate power functions of the form: $Y = aM^b$, where Y = variable of interest, M = body mass (kg), a = mass coefficient, and b = mass exponent. In the case of myoglobin, a distinct transition was apparent in this relationship, and continuous two-phase regression analysis (Nickerson et al. 1989) was used to establish the threshold body mass.

Mean values were compared with one- and two-way ANOVA and Tukey's Studentized range test (SPSS 1999) for post-hoc multiple comparisons. Two-sample comparisons of means were made with Student's t -test or paired t -tests. Significance was set at the 5% level and means are presented ± 1 SE.

Results

Allometric Relationships

Allometric equations for lung, blood, and muscle variables were highly significant in all cases ($P < 0.0001$; Table 1). Mass exponents for lung volume ($b = 1.04$), lung oxygen stores ($b = 1.04$), and blood oxygen stores ($b = 0.91$) did not differ from unity ($P > 0.05$). However, the exponent for blood volume ($b = 0.84$) was significantly less than 1.0 ($P > 0.05$). The highest scaling exponent ($b = 1.85$) was obtained for muscle O_2 stores. A strongly linear relationship ($r^2 = 0.82$, $df = 38$, $P < 0.0001$) was apparent between \log_{10} skeletal muscle Mb content and \log_{10} body mass of muskrats, varying in size from 254 to 600 g (Fig. 1). Over this mass range, the scaling exponent ($b = 1.63$, $SE_{\text{slope}} = 0.125$) exceeded unity ($P < 0.05$). Beyond a body mass of 600 g, skeletal muscle Mb content was independent of

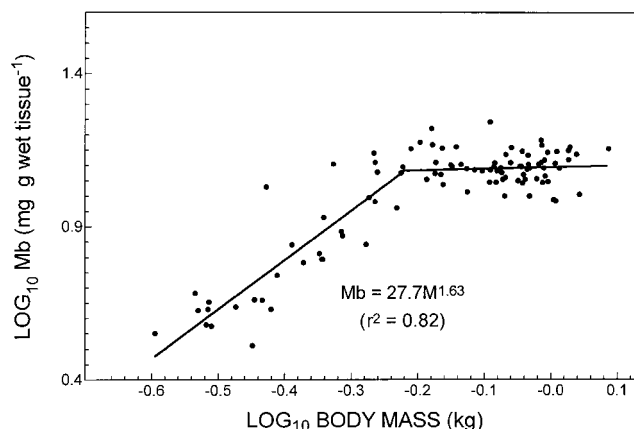


Figure 1. \log_{10} concentration of skeletal muscle myoglobin versus \log_{10} body mass of juvenile and adult muskrats. Lines were fitted using a continuous two-phase regression procedure (Nickerson et al. 1989). Over a mass range of 254–600 g, the relationship between these variables is described by the allometric equation: $Mb = 27.7M^{1.63}$ ($F = 169.8$, $df = 1, 38$, $P < 0.0001$).

body size ($r^2 = 0.004$, $df = 62$, $P > 0.05$; Fig. 1). The mass exponent for total body oxygen stores ($b = 1.09$), which combines lung, blood and muscle estimates, did not differ from unity ($P > 0.05$; Table 1).

Measurements of diving $\dot{V}O_2$ were obtained for a total of 59 field-acclimatized juvenile and adult muskrats. Metabolic data for animals in the youngest cohort (SJUV1, $n = 8$) appeared unusually low and inconsistent with measurements of larger muskrats and, hence, were not included in allometric analyses (Fig. 2). For the remaining animals, a regression of \log_{10} diving $\dot{V}O_2$ against \log_{10} body mass was highly significant ($r^2 = 0.72$, $df = 51$, $P < 0.0001$), yielding an allometric equation with a mass exponent of 0.74 (Table 1; Fig. 2). This value is similar

Table 1: Parameter estimates and statistics for allometric power equations relating respiratory, blood, and metabolic variables to body mass in muskrats (0.25–1.22 kg)

Variable	n	a	b	Sb	$S_{y,x}$	r^2	P
Lung volume (mL; STPD)	102	54.1	1.04	.043	.072	.86	<.0001
Blood volume (mL)	102	93.3	.84	.057	.096	.69	<.0001
Lung O_2 stores (mL; STPD)	102	8.1	1.04	.043	.072	.86	<.0001
Blood O_2 stores (mL)	102	17.3	.91	.076	.128	.59	<.0001
Muscle O_2 stores (mL)	102	8.3	1.85	.059	.100	.91	<.0001
Total body O_2 stores (mL; STPD)	102	33.7	1.09	.051	.084	.82	<.0001
Skeletal muscle Mb (mg g wet tissue $^{-1}$)	102	14.2	.85	.059	.100	.67	<.0001
Diving $\dot{V}O_2$ (mL O_2 min $^{-1}$)	51	1,908.8	.74	.065	.067	.72	<.0001
Calculated aerobic dive limit (s)	51	61.4	.37	.092	.091	.25	<.0002

Note. The model is $Y = aM^b$, where M is body mass (kg) and Y is the covariate in the units indicated. Statistical parameters were obtained by linear least squares regression on \log_{10} -transformed data. n = number of muskrats examined; a = mass coefficient; b = mass exponent; Sb = standard error of the slope; $S_{y,x}$ = standard error of the residuals (log).

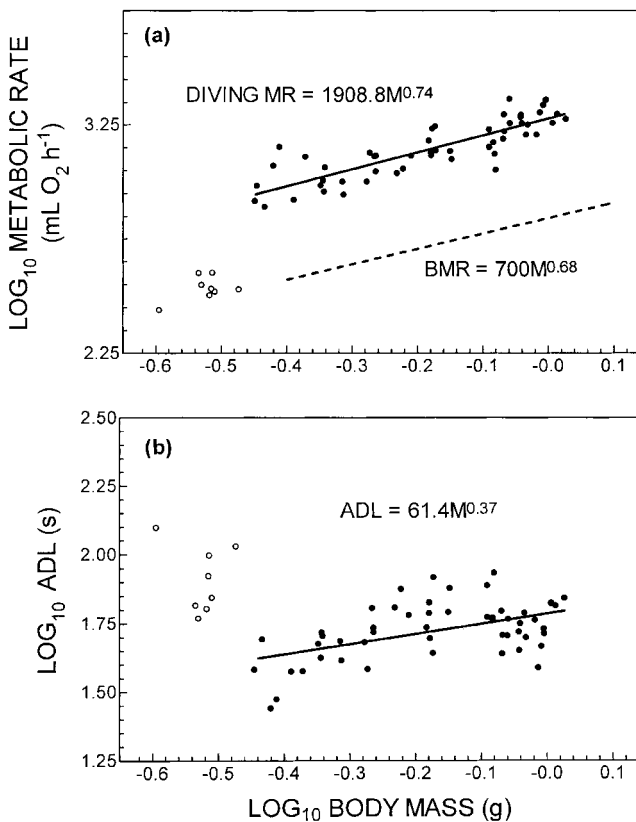


Figure 2. Relationship of \log_{10} metabolic rate (a) and \log_{10} aerobic dive limit (b) to \log_{10} body mass of seasonally acclimatized muskrats. Allometric equations were derived for animals ranging in mass from 390 to 1,104 g (solid points); muskrats in the youngest cohort, SJUV1 (254–360 g; open circles) were excluded from the regression analyses. Also presented is the regression (dashed line) and allometric equation for basal metabolic rate of wild-caught (461–1,241 g; $n = 125$) muskrats reported by Campbell and MacArthur (1998).

($P > 0.05$) to the exponent ($b = 0.68$) previously reported by Campbell and MacArthur (1998) for the BMR of field-acclimatized muskrats (461–1,241 g, $n = 125$ animals) sampled from the same population. However, the proportionality (mass) coefficient for diving $\dot{V}O_2$ is 2.73 times that of BMR (Fig. 2). Interestingly, the diving $\dot{V}O_2$ of the youngest (SJUV1) muskrats conforms more closely to the allometric prediction for basal than for diving $\dot{V}O_2$ of older animals (Fig. 2). Excluding SJUV1 data, the calculated ADL of muskrats scales with the 0.37 power of body mass (Table 1; Fig. 2). Surprisingly, none of the behavioral indicators of diving capacity, including mean and maximum dive times, scaled to body mass ($r^2 = 0.062$ – 0.0001 , $P > 0.05$).

Age and Seasonal Effects

Compared to summer-caught adults, juveniles captured in July–August had similar Hct and Hb levels, blood O_2 capacity,

and mass-specific lung capacity and blood volume (Table 2). Myoglobin levels and buffering capacities of skeletal muscles of these juveniles were significantly lower than in summer-caught adults (Tables 2, 3). Though not statistically significant ($P > 0.05$), most of the above variables were lowest in the youngest cohort, SJUV1 (Tables 2, 3). Except for muscle, the mass-specific oxygen stores of summer-caught adults did not exceed those of any of the juvenile cohorts (Fig. 3). Mass-specific diving $\dot{V}O_2$ was lowest for the 4.5–6-wk-old muskrats that compose the SJUV1 group ($1.15 \text{ mL } O_2 \text{ g}^{-1} \text{ h}^{-1}$) and highest for the slightly older (6.5–9-wk-old) SJUV2 animals ($2.54 \text{ mL } O_2 \text{ g}^{-1} \text{ h}^{-1}$; Table 2). Diving $\dot{V}O_2$ was similar for summer-caught adults and 8.5–13-wk-old juveniles tested in fall (Table 2). Owing to an exceptionally low diving $\dot{V}O_2$, the calculated ADL of the SJUV1 cohort was higher than that of any other group examined, including winter-caught adults and subadults (Table 2). Conversely, the high diving costs of SJUV2 animals resulted in the lowest calculated ADL in this study (Table 2).

Compared to summer-caught adults, winter-acclimatized muskrats (WADULT cohort) exhibited higher blood volume, Hct, Hb, blood O_2 capacity, and skeletal muscle Mb (Table 2). Total body oxygen reserves of winter-caught animals were the highest of any group tested, with the gain in blood oxygen stores accounting for most of the seasonal increase (Fig. 3). It is noteworthy that the Hct, Hb, and blood O_2 capacity of fall-caught juveniles also tended to exceed the corresponding values for summer-acclimatized adults (Table 2). Although the mean diving $\dot{V}O_2$ and calculated ADL of the WADULT group were 8.6% and 12.1% higher than for summer-caught adults, these differences were not significant ($P > 0.05$; Table 2). The buffering capacity of skeletal muscles also tended to be higher in winter, though only the increase in β for the biceps femoris was significant (Table 3). Myoglobin levels were typically highest in the gastrocnemius swimming muscles of the hind limbs, while buffering capacity varied little between fore- and hind limb muscles (Table 3).

Average dive times, including those calculated for maximum duration exploratory and alarm dives, were similar for summer-caught adults and juveniles (Fig. 4). For these cohorts, the overall mean dive time (based on all exploratory dives) varied from 19.2 to 22.0 s, compared with mean times of 51.4–86.2 s and 121.5–224.2 s for the longest recorded exploratory and alarm dives, respectively. Juveniles, especially the SJUV1 cohort, dove more frequently and spent proportionately more time underwater than the adults tested in summer (Fig. 5). Overall mean dive times were greatest for fall- and winter-caught muskrats (FJUV = 33.2 s, WADULT = 29.9 s; Fig. 4). The mean duration of the five longest exploratory dives also was higher for the FJUV (59.3 s) and WADULT (62.4 s) cohorts than for the SADULT group (48.6 s; Fig. 4). Likewise, cumulative dive time and percent time underwater were greater for muskrats tested in fall and winter than for those examined in summer

Table 2: Respiratory, blood, and metabolic characteristics of field-acclimatized juvenile and adult muskrats

Variable	Juvenile			Adult	
	SJUV1 (<i>n</i> = 8)	SJUV2 (<i>n</i> = 10)	FJUV (<i>n</i> = 9)	SADULT (<i>n</i> = 31) ^a	WADULT (<i>n</i> = 18) ^a
Ingesta-free body mass (g)	300.1 ^A ± 8.08	403.8 ^B ± 12.39	536.5 ^C ± 21.2	914.0 ^D ± 17.1	681.0 ^E ± 25.2
Total lung capacity (mL STPD kg ⁻¹)	49.25 ^A ± 2.27	54.11 ^A ± 2.98	55.10 ^A ± 2.05	54.57 ^A ± 1.42	58.20 ^A ± 2.78
Blood volume (mL 100 g ⁻¹)	11.32 ^A ± 1.66	10.44 ^A ± .58	9.91 ^A ± .70	9.65 ^A ± .28	11.70 ^B ± .53
Hematocrit (%)	39.57 ^A ± .65	41.22 ^A ± .60	46.57 ^B ± 1.16	39.10 ^A ± .66	46.76 ^{BC} ± .67
Hemoglobin (g 100 mL ⁻¹)	14.81 ^A ± .31	15.65 ^{AB} ± .29	16.76 ^{BC} ± .61	15.36 ^{AB} ± .24	17.97 ^C ± .38
Blood O ₂ capacity (vol%)	19.84 ^A ± .41	20.97 ^{AB} ± .39	22.46 ^{BC} ± .82	20.59 ^{AB} ± .32	24.08 ^C ± .51
Skeletal muscle Mb (mg g wet tissue ⁻¹)	4.15 ^A ± .15	5.41 ^A ± .37	9.43 ^B ± .74	12.12 ^C ± .24	13.76 ^D ± .40
Diving $\dot{V}O_2$ (mL O ₂ g ⁻¹ h ⁻¹)	1.15 ^A ± .05	2.54 ^B ± .18	2.20 ^{BC} ± .11	1.97 ^{CD} ± .06	2.14 ^{CD} ± .07
Calculated ADL (s)	86.5 ^A ± 8.56	41.3 ^B ± 2.84	51.9 ^{BC} ± 3.12	59.3 ^{CD} ± 2.35	66.5 ^{CD} ± 3.55

Note. SJUV1 = juveniles tested July 17–21; SJUV2 = juveniles tested July 14–August 11; FJUV = juveniles tested September 13–October 27; SADULT = adults tested June 9–August 12; WADULT = adults and subadults tested December 6–21. Values are presented as means ± SE. Within each row, means sharing the same letter are not significantly different ($P > 0.05$).

^a For diving $\dot{V}O_2$ and calculated aerobic dive limit, $n = 22$ (SADULT) and $n = 10$ (WADULT).

(Fig. 5). The proportion of dives longer than the calculated ADL was highly variable among juvenile cohorts, ranging from 0.1% to 20% (Fig. 5). In comparison, only 4%–6% of all dives by adults exceeded their calculated ADL (Fig. 5).

Interdive surface intervals were calculated for SJUV1, SADULT, and WADULT cohorts and plotted against corresponding dive times (Fig. 6). Despite considerable variability, surface time tended to increase with length of the preceding dive in all three groups (Fig. 6). However, there was no indication that the relationship between these variables changed when dive times approached or even exceeded the calculated ADL. The mean dive : surface ratio was 0.91 ± 0.082 for SADULT, 1.43 ± 0.048 for SJUV1, and 1.64 ± 0.089 for WADULT animals ($F = 25.53$, $df = 2, 1,061$, $P < 0.0001$). This ratio was higher for winter-caught adults/subadults than for either summer-caught adults ($t = 6.059$, $df = 651$, $P < 0.0001$) or fall-trapped juveniles ($t = 2.086$, $df = 436$, $P = 0.038$).

Oxygen Storage Capacity and Diving Ability

Sufficient data were obtained to test for a relationship between physiological indices of aerobic dive capacity and the diving behavior of 59 field-acclimatized muskrats ranging in size from 254 to 1,104 g. The mean dive duration of these animals was positively correlated with their blood Hb concentration (Fig. 7). Blood Hb also varied positively with duration of the longest exploratory dive ($r^2 = 0.112$, $P = 0.0098$) and with mean duration of the five longest exploratory dives recorded in the diving trials ($r^2 = 0.163$, $P = 0.0015$). No relationship ($P > 0.05$) was detected between blood Hb level and length of the longest alarm dive, dive frequency, or cumulative dive time. A

significant, albeit weak, correspondence was observed between mean skeletal muscle Mb content and mean dive duration (Fig. 7). Skeletal muscle Mb also varied positively with mean duration of the five longest exploratory dives ($r^2 = 0.085$, $P = 0.025$), with cumulative dive time ($r^2 = 0.082$, $P = 0.028$) and, most strongly, with dive frequency ($r^2 = 0.383$, $P < 0.0001$).

Despite these trends, we found no evidence that the dive performance of muskrats was strongly affected by interindividual variability in overall oxygen storage capacity. Of the behavioral variables monitored, only dive frequency correlated significantly ($r^2 = 0.113$, $P = 0.009$) with total body oxygen stores, though here the relationship was negative, not positive (Fig. 8). None of the behavioral indices of dive performance, including frequencies and durations of voluntary dives, correlated with the calculated ADL ($r^2 = 0.032$ – 0.0004 , $P > 0.05$). Dive frequency and percent time diving both varied inversely with body mass (Fig. 8). However, all other measures of dive time, including mean dive duration (Fig. 8), were mass-independent ($r^2 = 0.049$ – 0.000 , $P > 0.05$).

Discussion

Size- and Age-Dependent Correlates of Diving

As expected (Calder 1984; Hudson and Jones 1986), the total body oxygen stores of muskrats scaled to the first power of body mass ($b = 1.09$). This can be explained by the observation that the two principal stores of oxygen, lungs and blood (Fig. 3), were linearly proportional to body mass ($b = 0.91$ – 1.04 ; Table 1). The allometric equation derived for lung volume of muskrats ($Y = 54.1M^{1.04}$; Table 1) was virtually identical to that established for eutherian terrestrial mammals ($Y = 53.5M^{1.06}$;

Stahl 1967). On the other hand, the equation obtained for total blood volume ($Y = 93.3M^{0.84}$; Table 1) yielded a mass-predicted volume for a 900-g muskrat that was 1.45 times the value calculated from Stahl's (1967) interspecific equation for eutherians ($Y = 65.6M^{1.02}$). Mass-specific blood volumes of adult muskrats (96.5–117.0 mL kg⁻¹; Table 2) were comparable to those previously reported for the sea otter, *Enhydra lutris* (91 mL kg⁻¹; Lenfant et al. 1970), northern fur seal, *Callorhinus ursinus* (109 mL kg⁻¹; Lenfant et al. 1970), and California sea lion, *Zalophus californianus* (96 mL kg⁻¹; Ponganis et al. 1997) ut were considerably lower than for Phocid seals (132-210 mL kg⁻¹; Kooyman 1989; Kooyman and Ponganis 1998). As reported for the tufted duck (Hudson and Jones 1986), the scaling exponent for total blood volume of muskrats was significantly less than 1.0.

Elevated muscle Mb is a hallmark of accomplished divers (Kooyman 1989; Reed et al. 1994; Kooyman and Ponganis 1998), and several researchers have documented age-dependent changes in Mb levels that parallel developmental changes in diving ability of birds and mammals (Thorson and LeBoeuf 1994; Burns and Castellini 1996; Ponganis et al. 1999). In muskrats, skeletal muscle Mb clearly varied with age and appeared to be strongly mass-dependent from 250 to 600 g (Fig. 1). We are not aware of any previous study that has reported a strong positive allometry for Mb concentration, either in diving or nondiving endotherms. Beyond 600 g, a mass typical of a fall- or winter-caught juvenile, muscle Mb concentration was clearly independent of body size (Fig. 1). Though higher than in many terrestrial eutherians, the concentrations of skeletal muscle Mb in adult muskrats (11.7-14.4 mg g⁻¹; Table 3) were well below levels previously reported for marine birds and mammals (27–81 mg g⁻¹; Kooyman 1989; Kooyman and Ponganis 1998).

For muskrats exceeding 360 g (>6 wk old), the metabolic

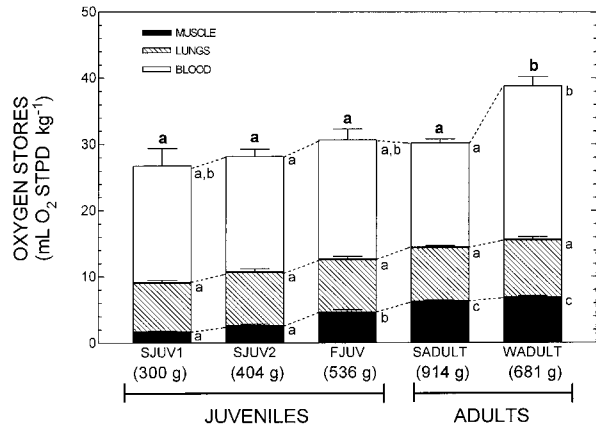


Figure 3. Total and site-specific oxygen storage capacity of field-acclimatized muskrats. Mean body mass of each cohort is indicated in parentheses. Means sharing the same letter for each site and for total stores (*bold type*) are not significantly different ($P > 0.05$). Vertical lines denote 1 SEM.

cost of diving scaled similarly to BMR ($b = 0.74$ vs. 0.68). This finding is at variance with DeLeeuw's (1996) observation that the metabolic scaling exponent tends to be lower during diving than when animals are resting at the surface. The mass-predicted $\dot{V}O_2$ during diving is ca. 2.7 times that of BMR (Fig. 2), an increase comparable to that reported by MacArthur and Krause (2.8; 1989). Based on the allometric scaling of total body oxygen stores and diving $\dot{V}O_2$ (Table 1), the diving capacity of muskrats should be proportional to $M^{1.09}/M^{0.74}$, or $M^{0.35}$ (Calder 1984; Hudson and Jones 1986; Schreer and Kovaks 1997). This prediction concurs with the scaling exponent ($b = 0.37$),

Table 3: Muscle characteristics of field-acclimatized juvenile and adult muskrats

Variable	Juveniles			Adults	
	SJUV1 (n = 8)	SJUV2 (n = 10)	FJUV (n = 9)	SADULT (n = 31) ^a	WADULT (n = 18) ^a
Myoglobin (mg g wet tissue ⁻¹):					
Ventricles	6.47 ^{AB} ± .20	5.80 ^A ± .25	8.28 ^C ± .27	7.11 ^B ± .16	8.25 ^{CD} ± .19
Biceps brachii	4.15 ^A ± .19	5.67 ^A ± .38	8.94 ^B ± .76	11.66 ^C ± .32	13.62 ^D ± .44
Biceps femoris	4.05 ^A ± .26	4.71 ^A ± .36	9.58 ^B ± .72	11.94 ^C ± .31	13.27 ^C ± .51
Gastrocnemius	4.27 ^A ± .19	5.82 ^A ± .57	9.76 ^B ± .92	12.63 ^C ± .26	14.38 ^D ± .60
Buffering capacity (β): ^b					
Biceps brachii	46.00 ^A ± 1.22	...	52.81 ^B ± .96	51.5 ^B ± 1.41	56.39 ^B ± 1.49
Biceps femoris	45.39 ^A ± 1.26	...	52.44 ^B ± 1.23	50.81 ^{BC} ± .89	56.86 ^D ± 1.23

Note. SJUV1 = juveniles tested July 17–21; SJUV2 = juveniles tested July 14–August 11; FJUV = juveniles tested September 13–October 27; SADULT = adults tested June 9 August 12; WADULT = adults and subadults tested December 6–21. Values are presented as means ± SE. Within each row, means sharing the same letter are not significantly different ($P > 0.05$).

^a For muscle buffering values, n = 8 (SADULT) and n = 10 (WADULT).

^b β = Slyke (μmoles of base required to titrate the pH of homogenized muscle by 1 pH unit).

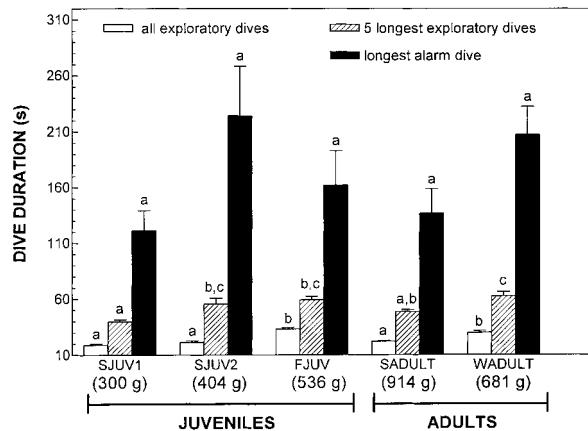


Figure 4. Mean durations of voluntary laboratory dives by seasonally acclimatized muskrats. Mean body mass of each cohort is indicated in parentheses. For each dive category, means sharing the same letter are not significantly different ($P > 0.05$). Vertical lines denote 1 SEM.

derived statistically by regressing \log_{10} ADL against \log_{10} body mass (Fig. 2b).

Surprisingly, we found no evidence that the diving abilities of muskrats increased with age or body size. Not only was average dive time independent of mass, but dive frequency and percentage time underwater actually declined with increasing body size of muskrats (Fig. 8). These findings contrast with the numerous field studies of marine birds (Ponganis et al. 1999) and mammals (Kooyman et al. 1983; Le Boeuf 1994; Thorson and LeBoeuf 1994; Burns et al. 1997), demonstrating improved diving proficiency as animals mature and gain experience swimming underwater. According to Errington (1939), preweanling, 3-wk-old muskrats “dive with facility” when pursued and are capable of hiding beneath submerged vegetation on the marsh bottom for periods of 2–4 min. He documented similar behavior in 4–6-wk-old muskrats (the age of the SJUV1 cohort), noting that these animals often displayed a preference for swimming below the surface when traveling to and from their summer dens. Thus, the diving precocity of juvenile muskrats may, in apparent deference to allometric scaling, be an integral component of the antipredator strategy of this species. Interestingly, the ringed seal, *Phoca hispida*, also develops diving skills at an early age, presumably as an evolutionary response to predation pressure from polar bears, *Ursus maritimus* (Lydersen and Hammill 1993). As in this study, Lydersen and Hammill (1993) observed little correspondence between average dive times and the ages of immature animals.

The precocious diving abilities of muskrat young implies that, compared with adults, juvenile stages are endowed with (1) low rates of tissue O_2 uptake underwater, (2) high O_2 storage capabilities of respiratory organs and tissues, or (3) greater capacities for anaerobic metabolism, including a high tolerance

to anaerobic end products. Evidence for a low metabolic cost of diving was obtained only for muskrats <360 g (SJUV1 cohort). The basis for the unusually low diving $\dot{V}O_2$ of these juveniles is unclear. One possibility is that they were mildly hypothermic compared with older animals, even though all trials were conducted in 29° – $30^{\circ}C$ water and despite the fact that in air, muskrat young are fully homeothermic at ca. 60 g, or 10–11 d of age (MacArthur and Humphries 1999). At 3 wk, juveniles are adept swimmers and divers but are prone to immersion hypothermia (MacArthur and Humphries 1999). Susceptibility to cooling also may apply to the 4.5–6-wk-old young tested in this study, and it is unfortunate that body temperatures were not recorded.

Several researchers have speculated that naturally occurring hypothermia could effectively extend the ADL of endothermic divers by reducing tissue demands for O_2 (see Boyd and Croxall 1996; Butler and Jones 1997). Boyd and Croxall (1996), for example, reported a distinct difference in the allometric scaling of dive duration for pinnipeds and seabirds, with the smaller avian divers achieving longer relative dive times. They proposed that the enhanced dive performance of seabirds may be linked to a hypothermia-induced depression in diving metabolism. Whether or not regional hypothermia accounted for the low metabolic cost of diving of SJUV1 muskrats, their calculated ADL surpassed that of all other cohorts (Table 2; Fig. 2b) and may have contributed to the impressive diving abilities of these young.

Predictably, the mass-specific diving $\dot{V}O_2$ of older juveniles (SJUV2 and FJUV) was higher than for adults (Table 2) and clearly provided no diving advantage to these animals. Similarly,

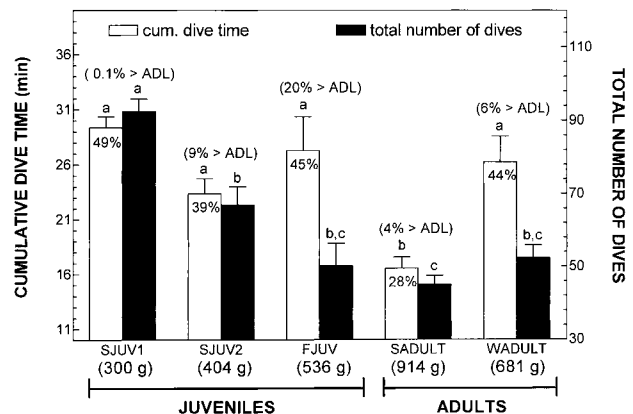


Figure 5. Cumulative dive times and total number of dives recorded from seasonally acclimatized muskrats during two 30-min laboratory diving trials. Mean body mass of each cohort is indicated in parentheses. Fraction of available time spent diving is indicated by percentage values within open bars. The percentage of total dives exceeding the calculated aerobic dive limit (ADL) is also included for each group. Vertical lines denote 1 SEM.

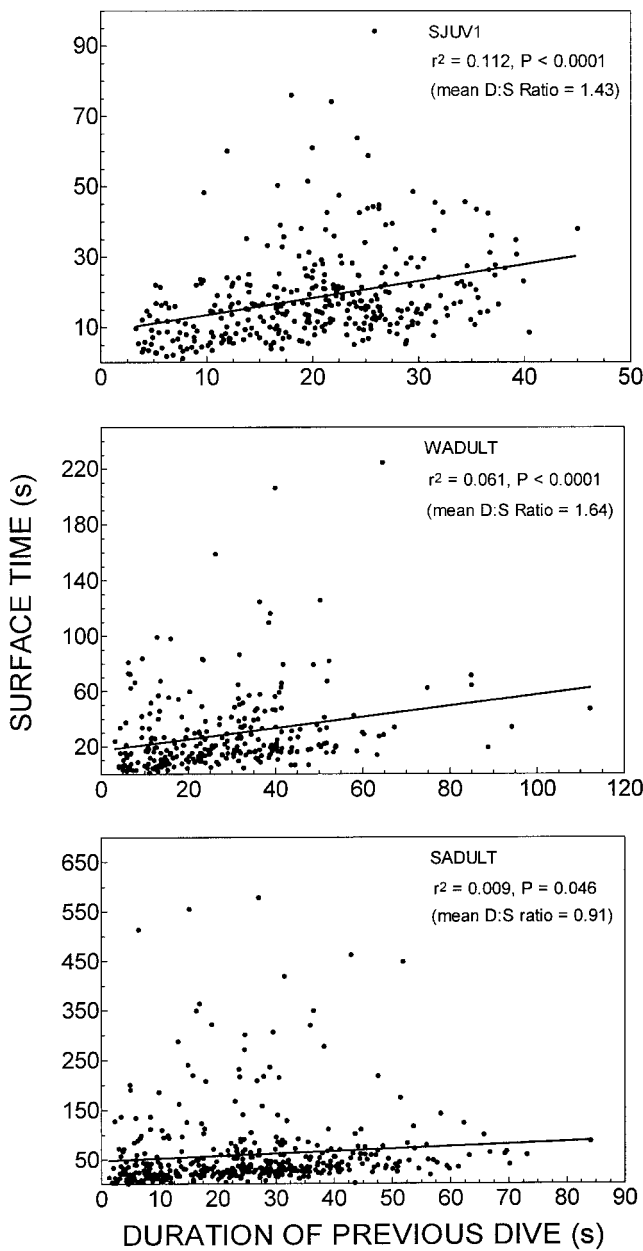


Figure 6. Interdive surface period versus length of preceding dive during bouts of voluntary diving by fall-caught juvenile (*FJUV*), summer-caught adult (*SADULT*), and winter-caught adult/subadult (*WADULT*) muskrats. Regression lines were fitted by the method of least squares. D : S ratio = dive : surface ratio.

we saw no evidence that the oxygen storage capacity of any body compartment was disproportionate in juveniles compared with adults (Table 2; Fig. 3). In the absence of disproportionate O_2 stores or a reduction in $\dot{V}O_2$, young muskrats likely are more dependent than adults on anaerobic pathways during underwater swimming. This conclusion is consistent with the rela-

tively high proportion of dives exceeding the calculated ADL of SJUV2 and, especially, FJUV cohorts (Fig. 5), as well as our findings that juveniles exhibited a higher dive : pause ratio (Fig. 6), dove more frequently, and spent proportionately more time underwater than adults tested in summer (Fig. 5). The tendency for smaller, younger individuals to dive more frequently and exceed the ADL more often than larger, more mature animals has also been observed in free-diving pinnipeds (Kooyman et al. 1983; Burns et al. 1997). If, in fact, juvenile muskrats are more prone than adults to dive anaerobically, this trend was not apparent from muscle buffering capacities; neither the β values of forelimb or hind limb skeletal muscles were elevated in juveniles, compared with adults (Table 3). The low buffering values of juvenile limb muscles were consonant with the low Mb levels of these tissues (Castellini and Somero 1981).

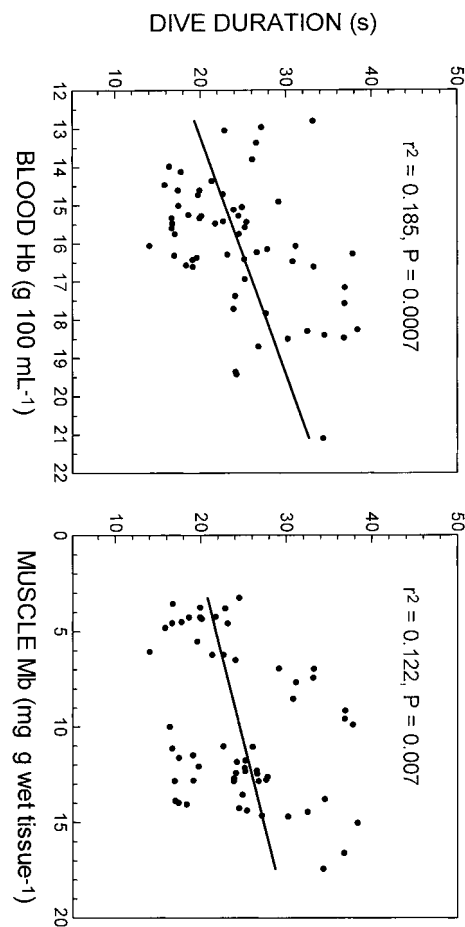


Figure 7. Relationship of dive time to blood hemoglobin and skeletal muscle myoglobin levels of seasonally acclimatized muskrats. Regression lines were fitted by the method of least squares.

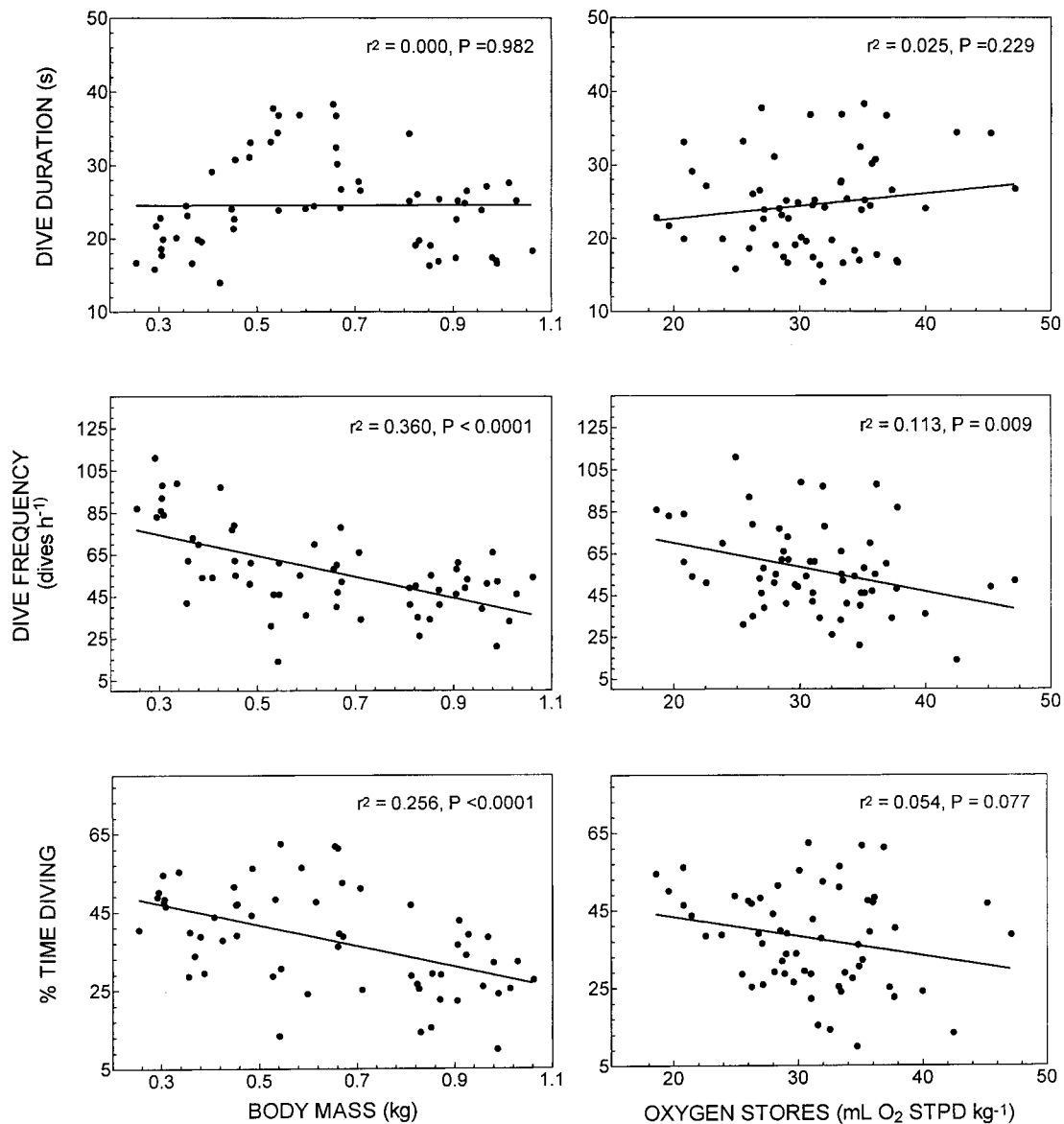


Figure 8. Relationships of mean dive time, dive frequency, and percentage of time underwater to body mass and total oxygen stores of seasonally acclimatized muskrats. Regression lines were fitted by the method of least squares.

Seasonal Correlates of Diving

The winter increases in mass-specific blood volume, Hct, Hb, and skeletal muscle Mb of field-acclimatized muskrats (Table 2; Fig. 3) corroborate previous studies of this rodent (MacArthur 1984b, 1990). Whereas MacArthur (1990) reported a 41.6% increase in total body O₂ reserves of muskrats between summer and winter, we observed a more modest winter gain of 28.6% in the larger sample of animals examined in this study (Fig. 3). In both investigations, elevated blood O₂ stores were mainly responsible for boosting body O₂ reserves in December.

This trend may, to a large extent, reflect a greater emphasis on diving by muskrats after marshes freeze over, when most foraging and home range movements necessitate underwater travel (MacArthur 1992). Diving hypoxia has been implicated as a factor stimulating erythropoiesis in marine mammals, including “dive-trained” harbor seals, *Phoca vitulina* (Geraci 1971; Kodama et al. 1977). Moreover, in a recent study that compared muskrats trained to swim an underwater course to a feeding station with animals precluded from diving, the major response noted was a chronic rise in the Hct and Hb levels of “dive-trained” muskrats (R. A. MacArthur, K. L. Campbell, and

G. Weseen, unpublished data). As pointed out by MacArthur (1990), the observed increases in blood volume, Hb, and Mb concentrations of muskrats in winter could also occur in response to seasonal changes in microclimate and/or gaseous composition of muskrat dwelling shelters. In this context, it is noteworthy that the Hct and Hb levels of juveniles caught before freeze up in September–October, when ambient temperatures and photoperiod were declining, exhibited higher Hct and Hb levels than summer-trapped adults (Table 2).

Compared with adults tested in summer, winter-caught adults/subadults appeared to be superior divers. Average and cumulative dive times and dive : pause ratios were highest for the WADULT group, a pattern that was consistent with the observed differences in blood and muscle oxygen reserves (Table 3; Fig. 3). The enhanced buffering capacity of the hind limb swimming muscles of winter-caught animals (Table 3) implies a greater tolerance to lactic acidemia and perhaps an increased dependence on anaerobic pathways in these tissues. Yet only 6% of all exploratory dives by WADULT animals exceeded the calculated ADL, compared with 4% for SADULT muskrats. On the other hand, the brief interdive recovery period of winter-caught animals may indicate a tendency to cumulate metabolic end products until the end of a diving bout, though it may also reflect a heightened capacity to rapidly recharge body oxygen stores at the surface. Elevated Hb levels and enhanced binding affinity of Hb for O₂ (MacArthur 1984b) could both contribute to shortening interdive surface periods during winter.

Results of this study indicate that the 31% gain in BMR previously observed in winter-trapped muskrats (Campbell and MacArthur 1998) is not matched by a commensurate increase in diving $\dot{V}O_2$. Nonetheless, the marginal (8.6%) rise in diving $\dot{V}O_2$ of WADULT muskrats (Table 2) offsets, to some extent, the greater O₂ storage capacity of these animals, with the result that their calculated ADL was not significantly greater than for the SADULT cohort (Table 2).

Do Oxygen Reserves or $\dot{V}O_2$ Dictate Dive Performance of Individuals?

A key objective of this study was to test for a statistical relationship between physiological measures of O₂ storage capacity and rate of O₂ depletion underwater, and behavioral measures of dive performance in the same individuals. The aforementioned summer-winter comparisons imply a causal link between the diving abilities of muskrats and their capacities to store O₂ and buffer metabolic acids. Indeed, two of the key determinants of oxygen-loading potential, blood Hb, and muscle Mb concentration, each varied positively ($P < 0.05$) with several indices of dive performance, including mean length of exploratory dives (Fig. 7). However, in no case did the Hb or Mb level account for more than 40% of the observed variation in diving behavior of individual muskrats. Even weaker was the corre-

spondence between overall body O₂ stores or calculated ADL and the diving behavior of individuals. Total O₂ stores correlated most strongly with dive frequency but varied negatively and accounted for only ca. 11% of the observed variability in this behavioral measure (Fig. 8). To our knowledge, one of the few other studies to statistically test for a correspondence between physiological or biochemical indices of diving capability (excluding $\dot{V}O_2$) and diving behavior, is Burns et al.'s (1997) investigation of yearling Weddell seals, *Leptonychotes weddelli*. Of several variables examined by these authors, including blood Hct and Hb concentration, only body mass correlated strongly with the diving behavior of seals.

Our findings did not indicate that the diving behavior of muskrats is delimited by the oxygen storage capacity of respiratory organs and tissues. It must be stressed, however, that this is a conclusion based on studies of recently captured muskrats tested in a laboratory setting at a single water temperature (30°C). The extent to which the age- and season-specific patterns of dive behavior observed in this study reflect dive performance of muskrats in nature remains to be tested. It is unknown, for example, whether regional hypothermia encountered by muskrats diving at cooler temperatures in the field enhances dive performance by reducing tissue metabolism. However, it is noteworthy that in an earlier study, MacArthur (1992) reported that underwater transit times between winter dens and feeding stations only occasionally exceeded the calculated ADL of winter-acclimatized muskrats with estimated mean dive times (19.9–41.7 s) comparable to those observed in captive, winter-caught muskrats (Fig. 4).

It should be noted also that in this study we have calculated only the potential of these animals to store O₂ before diving and, in calculating ADL, have tacitly assumed that this capacity is fully exploited by a diving muskrat. Few, if any divers can achieve this efficiency (Kooyman 1989; Kooyman and Ponganis 1998), and perhaps more critical to diving endurance is the lowest tolerable O₂ tension in body tissues, or the extent to which “on-board” O₂ reserves can be drawn down during the course of a dive. If so, animals may switch to anaerobiosis well before reaching their “theoretical” ADL, in which case muskrats may be far more dependent on anaerobic pathways than our findings suggest. This conjecture is supported by the relatively high buffering value of muskrat swimming muscles, especially in winter (Table 3). The β values of muskrat skeletal muscle are higher than those reported for the platypus, *Ornithorhynchus anatinus*, and several species of terrestrial birds and mammals (Castellini and Somero 1981; Evans et al. 1994) but are similar to β values reported for the swimming muscles of the little penguin, *Eudyptula minor* (Mill and Baldwin 1983). That diving muskrats readily deploy anaerobic as well as aerobic metabolic pathways is suggested also by the observation that pharmacological blockade of the O₂-conserving bradycardia and vasoconstrictor responses does not suppress diving behavior of this rodent (Signore and Jones 1995).

Clearly, further studies are required, including assessment of plasma lactate dynamics, to clarify the extent to which this rodent relies on anaerobic pathways underwater and to define more precisely the physiological and biochemical constraints on voluntary diving. Knowledge of lactate kinetics is also critical to determine how closely theoretical ADLs based on calculated oxygen stores and diving metabolic rate match physiological limits defined on the basis of plasma lactate changes (Kooyman 1989). The data reported herein provided a foundation for such comparisons and hopefully will encourage investigation of the possible role of regional hypothermia in reducing tissue metabolism of diving muskrats. This species provides an ideal test model for the adaptive hypothermia hypothesis (Butler and Jones 1997) because it routinely experiences abdominal cooling during aquatic activity and shows little evidence of active thermogenesis when diving (MacArthur 1984a, 1986).

Acknowledgments

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