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ESTIMATION OF TOTAL BODY WATER IN HARBOR SEALS: HOW USEFUL IS BIOELECTRICAL IMPEDANCE ANALYSIS?

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ABSTRACT

We evaluated bioelectrical impedance analysis (BIA) as a means of rapidly and inexpensively estimating total body water (TBW) of harbor seals (*Phoca vitulina*). Deuterium oxide dilution was used to estimate TBW in 17 adult females and 16 of their pups between birth and late lactation. Isotope dilution was also used to determine TBW in 12 adult males early and 10 of these males late in the breeding season. At the same time, resistance (*Rs*) and reactance (*Xc*) measurements were taken using a tetrapolar, impedance plethysmograph (Model 101A, RJL Systems). Seals were sedated with diazepam prior to taking BIA measurements. Within-day duplicate *Rs* measurements on pups and adults, taken 2–240 min apart, differed by an average of 3.0% ± 1.4% (n = 42, CV = 102%). Movement of the seal during BIA measurements caused variability in both *Rs* and *Xc* values. BIA measurements were generally poor predictors of TBW. *Rs* was significantly correlated with TBW in pups only (*Rs* = 0.93, P = 0.001, n = 11). Bioelectrical conductor volume (length2/*Rs*) was significantly correlated with TBW only in adult females (*Rs* = 0.63, P = 0.02, n = 14). We conclude that BIA is not a reliable method of estimating TBW in wild harbor seals.

Key words: bioelectrical impedance, total body water, deuterium, harbor seal, *Phoca vitulina*.

Changes in body composition are routinely used to study energy expenditure, nutrient storage, and physical condition of pinnipeds (*e.g.*, Costa *et al.*

1986, Costa and Gentry 1986, Oftedal *et al.* 1987, Boyd and Duck 1991, Bowen *et al.* 1992, Rea and Costa 1992, Muelbert and Bowen 1993, Boyd *et al.* 1993, Iverson *et al.* 1993, Arnould and Boyd 1995, Oftedal *et al.* 1996, Reilly *et al.* 1996). Although direct chemical analysis of body composition is often used in these types of studies in small animals and domestic livestock, indirect methods are generally used in wild pinnipeds owing to their large size. Indirect methods rely on estimates of total body water (TBW) of an animal and relationships between TBW, lean body mass, and fat to determine body composition.

Hydrogen isotope dilution has gained wide use as a method of estimating TBW in pinnipeds (Costa 1987, Bowen and Iverson 1998). Dilution methods have been shown to yield accurate and precise estimates of TBW which can be used with equations such as those developed by Reilly and Fedak (1990) to estimate protein, fat, and energy content. However, there are several potential disadvantages of isotope dilution methods. First, these methods are somewhat time consuming in that typically the animal must be held for 2–3 h to allow the isotope to equilibrate with TBW. Second, the cost of isotopes and subsequent quantitative analysis can become significant. As both of these serve to limit the number of animals that can be studied, investigators have sought other methods that are fast, accurate, inexpensive, and can be reliably used in fields studies.

One such contender is bioelectrical impedance analysis (BIA). This method is based on the conduction of an applied electric current that penetrates both intra- and extracellular fluids of the body (reviewed by Lukaski 1987). The method has been used with good results in humans (Lukaski *et al.* 1986, Kushner *et al.* 1990), bears (Farley and Robbins 1994), and swine (Swantek *et al.* 1992). Gales *et al.* (1994) were the first to use BIA to estimate TBW in a pinniped. Based on measurements from several captive harp (*Phoca groenlandica*) and ringed (*Phoca hispida*) seals, they concluded that BIA had considerable potential as a reliable technique for estimating body composition of phocid seals. However, the method has recently been evaluated in adult female Antarctic fur seals (*Arctocephalus gazella*) and found to be of limited value in predicting TBW of individual animals (Arnould 1995). Based on the promising results of Gales *et al.* (1994) and the value of a rapid, reliable method of estimating TBW in field studies, we attempted to validate BIA as a field tool in the harbor seal (*Phoca vitulina*).

Materials and Methods

The study was conducted during the May–June breeding seasons of 1992 and 1993 on Sable Island, a 40-km-long crescent-shaped, vegetated sandbar located about 160 km east of Nova Scotia, Canada (43°90′N 60°00′W). A total of 17 female harbor seals and 16 of their pups ranging in age from birth to late lactation and 12 adult males were studied. Each day all newborn pups in the study area were marked with an individually numbered jumbo Rototag, placed in the webbing of the hind flipper, to provide accurate information on pup age and hence the lactation stage of the female. Pups were captured by hand. We captured adult females and adult males using a net as previously described (Bowen *et al.* 1992, Walker and Bowen 1993). At initial capture all seals were individually marked using a fast-drying, fluorescent paint (Lenmar, Baltimore) to permit rapid identification from a distance. Adults and pups, respectively, were weighed to the nearest 0.5 kg and 0.1 kg on Salter spring balances. Seals were sedated with diazepam (0.2–0.3 mg/kg) just prior to taking length and BIA measurements. Length was measured as the straight line distance from nose to the tip of the tail.

Total body water (TBW) was estimated using deuterium oxide (D_2O) dilution following the methods described in Oftedal and Iverson (1987). A preweighed quantity of D_2O (99.8 atom % excess, Atomic Energy of Canada) was administered by syringe and gastric tube at a dose of 1 g/kg body mass. After administration, mothers and their pups were held in separate but adjacent pens constructed at the capture site to allow the isotope to equilibrate with body fluids. Adult males were held individually in similar pens. Seals were bled twice from the extradural vein to provide a basis for establishing that equilibration had occurred. The time of initial bleeding varied from 2 to 4 h after administration, with the second sample being taken 20–30 min later.

Blood samples (about 8 ml) were collected in Vacutainers without additives. Serum from centrifuged samples was transferred to cryovials and stored at −20°C. Total water was recovered from serum by heat distillation and assayed for D_2O concentration with infrared spectrophotometry (Oftedal and Iverson 1987). TBW was estimated from a regression equation of D_2O dilution space on TBW (Bowen and Iverson 1998). In the absence of data on adult harbor seals, components of body composition were estimated from TBW using the equations in Reilly and Fedak (1990) developed for gray seals.

Resistance (Rs) and reactance (Xc) were measured using a tetrapolar impedance plethysmograph (BIA; model 101A, RJL Systems, Detroit, MI). An 800-μA current was applied at 50 kHz through the two outer electrodes. The voltage drop between the two inner electrodes was measured with a high-input impedance amplifier. We used the same electrode construction, configuration, and placement on the seal as described by Gales *et al.* (1994). Briefly, the electrodes were 2.5-cm vacutainer needles attached to banana plugs on the RJL leads. Posterior electrodes were inserted into muscle on the lateral midline, 4 cm anterior to the ankle joint. Anterior electrodes were inserted into muscle along the mid-dorsal line, 4 cm behind the ears. In both cases electrodes were oriented anterior-posterior. Electrodes remained in place until readings of Rs and Xc stabilized, usually <30 sec.

Biological impedance (Z), Rs, and Xc vary inversely with the volume and composition of the body (Nyboer *et al.* 1943, Lukaski *et al.* 1986). Impedance is defined as Z (ohms) $= (Rs^2 + Xc^2)^{0.5}$. Impedance is related to bioelectrical conductor volume as follows: Vol (cm$^2/\Omega$) $= L^2/Rs$, where L = length in mm and Rs = resistance in ohms.

Standard error is reported as the measure of variability about the mean.

Results

Twelve males were equilibrated early in the breeding season, and 10 of these males were re-equilibrated near the end of the breeding season. Of these 22 isotope administrations, reliable estimates of TBW were obtained in 19 cases (Table 1). Estimates of TBW were also obtained from 17 adult females and 16 pups from birth to late lactation (Table 1). Among the three groups, TBW ranged from about 5.8 to 69.1 kg and percent TBW from 40.1 to 68.1, providing a wide range of body compositions against which BIA could be evaluated.

Repeatability of Rs *measurements*—Duplicate BIA readings were obtained from 16 females, 6 pups, and on 21 occasions from the 12 males. Generally, the interval between readings was less than 12 min, but two readings were taken 25 min apart and another five readings were taken approximately 4 h apart. In these latter five cases the seal was again sedated with diazepam before the second measurements were taken. In only five cases was there no difference in duplicate readings. Of the remaining 38 cases, there was a marginally significant tendency for the second reading to be greater than the initial reading (Binomial test $P = 0.049$). The mean difference between 43 duplicate readings relative to the initial reading was 3.0% ± 1.4%. The difference in *Rs* readings was not significantly correlated with the time interval between readings ($r = 0.20$, $P = 0.21$, $n = 43$, log transformed).

Although sedated, most seals reacted to the placement of the electrodes and exhibited some movement during the period of measurement. Movement of the seal affected the stability of *Rs* values. Therefore, we assigned measurements to one of three stability classes, with 1 indicating stable readings and 3 representing quite variable readings.

Relationships between BIA measurements and TBW—In all age-sex groups studied, measured *Rs* was so much larger than *Xc* that *Xc* could be effectively ignored. Relatively stable readings (*i.e.*, quality 1 and 2) were obtained on 18 of 19 occasions in which an isotope-dilution estimate of TBW was available in males, in 14 of the 17 females and in 11 of 16 pups. Using only these quality readings, Rs was not significantly correlated with either TBW or percentage TBW of adult males (Spearman rank correlation $rs = -0.12$ and -0.35 , $P = 0.63$ and 0.16, respectively, $n = 18$) or adult females ($rs = 0.48$ and -0.52, $P = 0.08$ and 0.06, respectively, $n = 14$; Fig. 1), but was highly correlated with TBW and percentage TBW in pups ($rs = 0.93$ and -0.87, $P = 0.001$ and 0.001, $n = 11$; Fig. 2). Combining data from adult males and females resulted in a significant negative correlation between *Rs* and both TBW ($rs = -0.52$, $P = 0.002$, $n = 32$) and percent TBW ($rs = 0.45$, $P = 0.01$, $n = 32$).

Bioelectrical conductor volume (*i.e.*, L^2/Rs) is strongly correlated with TBW in several species and thus has the potential to be a better predictor of TBW than *Rs*. In adult males, volume was not significantly correlated with TBW ($Rs = 0.14$, $P = 0.63$, $n = 18$; Fig. 3) or percent TBW ($Rs = -0.20$, $P = 0.43$, $n = 18$). In adult females, volume was significantly correlated with

Table 1. Body mass, length, TBW (from isotope dilution), and percentage TBW of harbor seals used in study.

		Body mass (kg)	Length (cm)	TBW (kg)	% TBW
Adult males	Mean ± SE	107.1 ± 1.75	153.9 ± 1.32	62.6 ± 0.94	58.6 ± 1.11
	Range	91.5–120.5	144.0–168.0	53.7–69.1	52.7–68.1
	n	19	19	19	19
Adult females	Mean ± SE	76.0 ± 2.31	141.4 ± 1.48	41.1 ± 0.65	54.7 ± 1.45
	Range	59.0–97.5	133.0–156.0	37.3–47.3	43.9–64.9
	n	17	17	17	17
Pups	Mean ± SE	16.9 ± 1.86	83.9 ± 2.00	8.8 ± 0.62	54.9 ± 1.96
	Range	9.3–29.8	71.0–96.0	5.8–12.9	40.1–64.7
	n	16	14	16	16

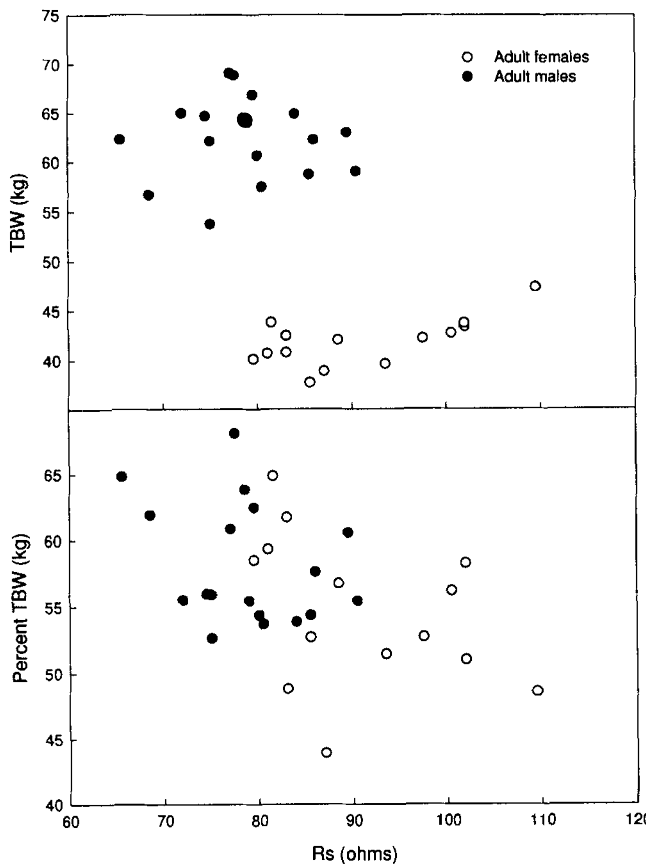

Figure 1. Relationships between Rs and TBW and percentage TBW of adult male and female harbor seals.

TBW (Rs = 0.63, P = 0.02, n = 14; Fig. 3), but was not correlated with percent TBW (Rs = −0.07, P = 0.81, n = 14). Volume was not significantly correlated with either TBW (Rs = 0.37, P = 0.29, n = 11) or percent TBW (Rs = −0.44, P = 0.20, n = 11) in pups.

Stepwise multiple regression was used to construct models to predict TBW from a combination of *Rs*, *Xc*, *Vol* and body mass and length. In adult males only body length was a significant predictor of TBW, but even here the regression explained just 42% of the variation in TBW (Eq. 1, Table 2). Bioelectrical conductor volume was the only significant predictor of TBW in adult females (Eq. 2, Table 2). In pups, *Rs* was a significant predictor of TBW (Eq. 3, Table 2), but was eliminated from the regression when body mass was entered. Body mass alone accounted for 93% of the variation in pup TBW. The addition of body length to the model increased the explained variation in pup TBW to 97% (Eq. 4, Table 2).

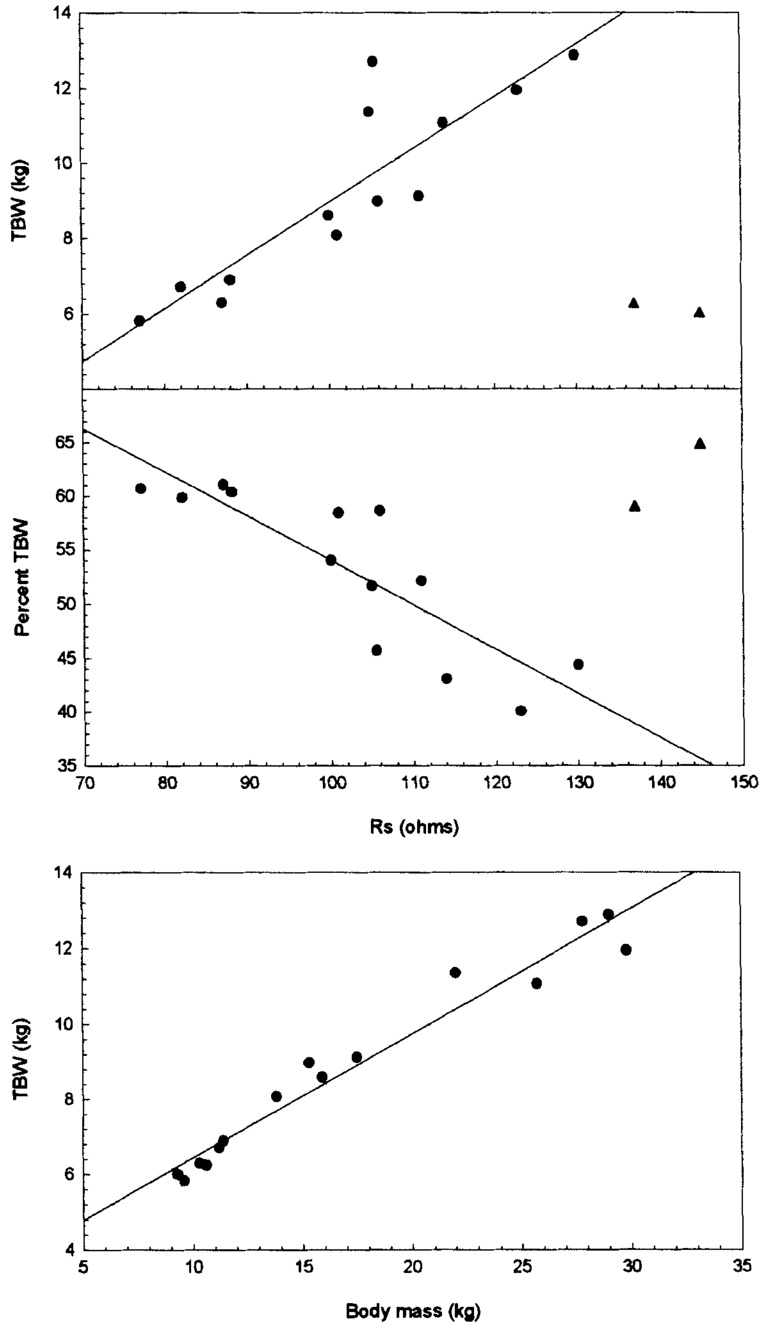

Figure 2. Regressions of TBW and percentage TBW in pups on *Rs* and of TBW in pups on body mass. Two outliers (▲) omitted from analyses.

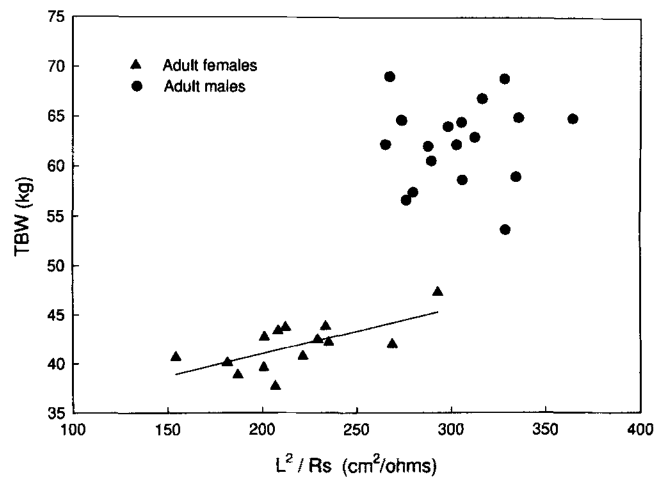

Figure 3. Relationships between Vol and TBW in adult males and adult females.

Discussion

BIA is a useful method of predicting TBW and body composition in humans (Lukaski *et al.* 1986, Kushner *et al.* 1990, Suprasongsin *et al.* 1995) and several species of domestic animals (Swantek *et al.* 1992, Berg and Marchello 1994). Two previous studies have evaluated the use of BIA to estimate TBW in pinnipeds. Based on 10 measurements of captive harp seals, Gales *et al.* (1994) concluded that BIA had "considerable potential as an inexpensive, rapid, and reliable technique." By contrast, Arnould (1995) concluded that BIA showed limited value in the estimation of TBW in 52 wild, adult female Antarctic fur seals. Rs and Vol accounted for 9% and 56%, respectively, of the variation in TBW among fur seal females. We found that BIA measurements were generally poor predictors of TBW in wild harbor seals over a wide range of ages and body sizes. Only *Rs* and bioelectrical conductor volume (*Vol*) in pups and adult females, respectively, provided any basis for the prediction of TBW. *Rs* as the sole independent variable accounted for 80% of the variation in TBW of pups, but *Vol* accounted for only 43% of the variation in adult females. Given that we took care to duplicate the procedures used by Gales *et al.* (1994), the poor performance of BIA on harbor seals is puzzling.

Two factors may have contributed to the differences between our results and those reported by Gales *et al.* (1994): namely, movement of the seal during the measurement period and the body composition of the seals studied. Even though sedated with diazepam, the wild harbor seals used in our study reacted to placement of the needle electrodes by moving or contracting body musculature. In most cases the reaction appeared to be transient and animals seemed relaxed while the measurements were taken. However, some seals continued to react to gentle restraint and the electrodes while measurements were taken. In extreme cases (*i.e.*, quality 3 readings), *Rs* values continued to vary such that only an estimated average value could be determined. The fur seals

Table 2. Relationships for predicting TBW in harbor seals.

Eq.	Group	Predictive equation	r^2	SE	*P*	*n*
1	Adult males	TBW (kg) = $-11.3 + 0.478 \pm 0.140 \cdot L$ (cm)	0.42	3.25	0.004	18
2	Adult females	TBW (kg) = $31.9 + 0.046 \pm 0.015 \cdot \mathrm{Vol}$ (L^2/Rs)	0.43	1.92	0.01	14
3	Pups	TBW (kg) = $-5.0 + 0.136 \pm 0.019 \cdot Rs$ (Ω)	0.85	0.88	0.0001	11
4	Pups	TBW (kg) = $-7.9 + 0.169 \cdot L$ (cm) $+ 0.150 \cdot M$ (kg)	0.97	0.44	0.0001	11

studied by Arnould (1995) were not sedated but were restrained. However, females were allowed to completely relax before any BIA measurements were taken (J. Arnould, personal communication) and duplicate measurements generally differed by <5%. Thus, movement of the female is unlikely to have significantly contributed to the poor performance of BIA in this case. The captive seals used by Gales *et al.* (1994) appeared relaxed during placement of the electrodes and while measurements were taken, even though they were not sedated or restrained. Despite the apparent difference in the behavior of seals during BIA measurements, variability in repeated Rs readings was <5% in both our study and the Gales *et al.* (1994) study.

The second factor which may have contributed to the difference between our study and that of Gales *et al.* (1994) is the difference in the range of body composition of the seals studied. Inspection of Figure 3 in Gales *et al.* (1994) shows that the range of percentage TBW in their captive seals was much greater than that found in the wild harbor seals in our study. In fact, all but one of their values of percentage TBW was lower than anything we observed in wild harbor seals. The greater contrast in TBW of the seals studied by Gales *et al.* (1994) may have provided a greater opportunity to detect relationships between BIA measurements and TBW. This also may partly explain why our Rs readings were considerably lower (65–129 ohms in adults) than those given in Gales *et al.* (1994) of 102–180 ohms.

Electrode placement in the animal can have large effects on both the magnitude and repeatability of *Rs* and *Xc* readings (Farley and Robbins 1994, Gales *et al.* 1994, Arnould 1995). We eliminated this source of variability by using the same electrode construction, configuration, and placement in the seal as that used by Gales *et al.* (1994).

Changes in hormone concentrations during estrus can also influence the performance of BIA (Adam *et al.* 1981). Pietraszek and Atkinson (1994) found that whole-body BIA values in a captive female Hawaiian monk seal (*Monachus schauinslandi*) varied in a pattern similar to estrone sulphate concentrations during the estrous cycle. Thus, some of the BIA variability we observed in adult harbor seals may have been caused by changes in the concentrations of hormones that occurred over the course of the breeding season.

Deuterium estimates of TBW have been validated in several phocid species (Reilly and Fedak 1990; Oftedal *et al.* 1993, 1996). Variation in the quantity of stomach water may contribute to variability in estimates of TBW in wild seals compared to captive animals because the quantity of food in the stomach of wild seals will not generally be known. However, stomach water will be a small fraction of TBW and thus variation in stomach water is unlikely to account for the poor correlation between TBW and BIA measurements in our study.

Another troubling feature of our BIA data is the lack of consistency in the relationships among BIA variables and TBW across age and sex groups. None of the BIA variables was significantly correlated with TBW in adult males, only bioelectrical conductor volume was correlated with TBW in adult females, and only *Rs* was correlated with TBW in suckling pups. In contrast, no significant difference was found in the BIA equations used to predict TBW in men and women (Lukaski *et al.* 1986).

We conclude that BIA cannot be used to estimate TBW in sedated, wild harbor seals. Improved results may depend on anesthetizing seals prior to taking BIA measurements such that the seal is completely relaxed during the measurement period. A recent study on three species of bears obtained good results with BIA on anesthetized animals (Farley and Robbins 1994). However, other investigators have obtained good BIA results on non-anesthetized, restrained lambs (Berg and Marchello 1994) and swine (Swantek *et al.* 1992).

Costa *et al.* (1989) and Arnould (1995) found that body mass alone was a good predictor of TBW in adult female Antarctic fur seals. Although this was true for the harbor seal pups we studied, it was not the case in adult males or females during the breeding season. Body mass explained only 10% and 24% of the variation in TBW in males and females, respectively. Thus, isotopic tracers will continue to be the method of choice for studies on the body composition of wild harbor seals.

Impedance of an animal is related to conductor length and configuration volume, its cross-sectional area, and signal frequency (Lukaski 1987). The body shape of phocid seals differs substantially from that of the other mammals, including humans, in which BIA has been successfully applied. It may be that this difference in shape is partly responsible for the poor performance of BIA in phocid seals. The poor electrical conductance of the blubber layer that covers the body of pinnipeds may also degrade the quality of data that can be obtained from BIA measurements. However, the needle electrodes used in our study should have greatly reduced this problem by delivering current to the muscle. Notwithstanding these observations, additional studies are needed to confirm the results of Gales *et al.* (1994), which are based on only 10 readings. Our results also suggest that the predictive equations of Gales *et al.* (1994) for estimating TBW in phocid seals from BIA measurements may have limited generality.

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