**TECHNICAL COMMENT**

# Validation of Water Flux and Body Composition in Glaucous Gulls (*Larus hyperboreus*)

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## ABSTRACT

Water influx rates (WIR) measured with tritiated water dilution were compared with direct measures of water and energy intake in glaucous gulls (*Larus hyperboreus*). Total body water (TBW) measured isotopically was also compared with TBW determined by body composition analysis (BCA) of the same birds. Seventeen wild gulls were captured and studied in outdoor enclosures at Ny-Ålesund, Svalbard, in July 2002. Gulls were hand-fed known quantities of Arctic cod (*Boreogadus saida*) or given water on the basis of one of four experimental treatments: (A) fasting, (B) fish only, (C) water only, or (D) fish and water. Water and energy content of Arctic cod was also determined. WIR of gulls (after subtracting metabolic water production) in treatments A, B, C, and D were 0, $101 \pm 5$, $62 \pm 19$, and $122 \pm 21$ SD g $d^{-1}$, respectively. Measured water intake in each group was 0, $111 \pm 2$, $64 \pm 3$, and $134 \pm 15$ SD g $d^{-1}$, respectively. On average, WIR underestimated measured water intake in each group. Errors were lowest but most variable for gulls fed water only ($-2.2\% \pm 32.8\%$) compared with gulls fed fish only ($-9.0\% \pm 5.4\%$) or fish and water ($-9.0\% \pm 7.0\%$). Compared with measured water intake, errors in WIR were relatively low overall ($-6.9\% \pm 17.4\%$) and comparable to previous validation studies. The difference in TBW determined by BCA versus isotopic dilution ranged between $-1.02\%$ and $+8.59\%$ of mass. On average, TBW measured isotopically ($632 \pm 24$ g $kg^{-1}$) overestimated true body water by a factor of 1.033.

## Introduction

The turnover rate of isotopically labeled water (e.g., $^3H_2O$) has widely been used to evaluate net water flux, overall water balance, and food or water consumption in free-ranging animals (e.g., Nagy and Costa 1980; Hui 1981; Nagy et al. 1984; Williams and Nagy 1985; Adams et al. 1986; Costa 1987; Nagy and Peterson 1988; Lea et al. 2002). Despite its widespread application (see Nagy and Peterson 1988), only a modicum of validation studies have quantified the errors in water turnover measured isotopically compared with direct measures of water intake (Nagy and Costa 1980; Degen et al. 1981; Costa 1987; Gales 1989; Robertson and Newgrain 1992; Lea et al. 2002; Salatas et al. 2002; Gessaman et al. 2004). Fewer validation studies have measured water flux when animals were allowed to consume both food and water (Degen et al. 1981; Costa 1987; Gessaman et al. 2004). Most validations studies also measured water flux across a fairly narrow range of water intake rates. Therefore, it is difficult to predict how water flux measurements are affected by wide ranges in water intake and/or by the form of water consumed (i.e., food or free water).

Water flux measurements rely on the ability to quantify changes in total body water (TBW; Lifson and McClintock 1966). When using labeled water, TBW is evaluated by the dilution of isotopic water at the beginning and ideally at the end of the measurement interval (Lifson and McClintock 1966; Nagy and Costa 1980; Speakman 1997). However, reevaluation of TBW by injecting animals with a second dose of labeled water may not be feasible or practical, given the study conditions (e.g., small subject size, handling stress). Therefore, it is common practice to estimate final TBW ($TBW_f$) by multiplying the initial relative TBW (percent of mass) by the final body mass of the animal (e.g., Adams et al. 1986; Costa et al. 1986; Green and Brothers 1989; Visser et al. 2000; Shaffer et al. 2001a; Salatas et al. 2002). This assumes that relative TBW remains constant over the study period. Violation of this assumption can lead to errors in estimates of TBW and water flux (Nagy and Costa 1980; Speakman 1997). Therefore, it seems prudent to test this directly by validating the assumption

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that relative initial and final body water pools remain constant over the measurement interval.

The dilution of isotopic water in an animal's body water pool generally predicts true body water from carcass desiccation within 3%–5% (see reviews in Nagy and Costa 1980; Speakman et al. 2001). Although there is an abundance of measurements comparing both methods in mammals, fewer studies have quantified the errors between methods in birds (e.g., Degen et al. 1981; Crum et al. 1985; Green and Brothers 1989). This is surprising, given that body water content has been measured by whole body desiccation or complete homogenization of carcasses in several bird species (Mahoney and Jehl 1984; Parker and Holm 1990; Ellis and Jehl 1991; Janes 1997). Moreover, bird feathers are not part of the exchangeable water pool and thus do not equilibrate with tritiated water (HTO; Degen et al. 1981). Hence, this may lead to overestimates in TBW determined with isotope dilution. Therefore, further quantification of errors between methods to estimate TBW in birds is needed.

The primary objective of this study was to quantify the error in water flux rates measured isotopically compared with direct measures of food and water intake in wild-caught glaucous gulls (*Larus hyperboreus*). Glaucous gulls have a generalist diet and feed by scavenging on fish, carrion, crustaceans, eggs, chicks, or adult birds (Løvenskiold 1964). They are ubiquitous throughout Svalbard, and populations are reasonably stable (Bakken and Tertitski 2000). Also, glaucous gulls occasionally eat snow and possibly drink water when bathing (G. W. Gabrielsen, personal observation). Therefore, these seabirds were a model system to validate water flux measurements using four experimental conditions: (A) a control group that fasted without water, (B) a group that received fish only, (C) a group that received water only, and (D) a group given both fish and water. This experimental design allowed us to test whether errors in water flux were greater when birds consumed food and/or water. It also allowed us to vary widely the water intake rates between treatment groups in order to quantify the errors attributed to variable water fluxes. We also chose to hand-feed known quantities of fish and water rather than allow the birds to eat or drink ad lib. to accurately measure total water intake. A second objective was to evaluate TBW at the start and end of an experimental period using isotopically labeled water. These measurements allowed us to test whether relative TBW remained constant over a measurement interval. Our third objective was to compare TBW determined with isotopic water dilution versus conventional body composition analysis on the same birds.

## Material and Methods

### Study Species and Location

The study was conducted at the Norwegian Polar Institute research station in Ny-Ålesund, Svalbard, Norway (79°N, 19°E) in June–July 2002. Seventeen glaucous gulls were captured at the Ny-Ålesund refuse dump using a cannon net (5 m × 4 m) triggered by a remote controlled unit 200–300 m from the birds. Once captured, gulls were transported to the lab in wooden crates and weighed to the nearest gram. Each bird was given a colored identification band placed around the tarsus before they were released into a sheltered outdoor enclosure. Gulls were then left undisturbed for 12–15 h, and food and water were withheld to ensure that birds were in a fasted state at the start of the experiment. The outdoor enclosure was divided into two runs (4 m long × 3 m wide × 2.5 m high per run), with a maximum of five gulls in each run. The experiment was conducted in two phases, with two study groups examined in each phase. All protocols employed in this study were approved by the Institutional Animal Care and Use Committee at University of California, Santa Cruz (UCSC; Cost00.03), the Governor of Svalbard (2002/00483-2 a. 512/2), and the Norwegian Animal Research Authority (S 1030/02).

### Experimental Design, Treatment Groups, and Procedures

The study had four treatment groups, and gulls were chosen randomly and assigned to a group for the remainder the experiment. Three gulls were assigned to group A, which received no food or water. They served as controls and provided a measure of metabolic water production (MWP) for fasting glaucous gulls. Five gulls were assigned to group B, which received food only. Four gulls were assigned to group C, which received water only. Five gulls were assigned to group D, which received both food and water. Gulls in groups B and D were hand-fed ∼75–100 g of thawed juvenile Arctic cod (*Boreogadus saida*; caught locally; 10–12 cm snout to fork length) at each feeding trial ($N = 4$). Prefrozen cod was thawed at room temperature without water 10–12 h before each feeding trial. During a feeding trial, a bird was removed from its run and weighed before it was fed a preweighed quantity ($\pm 1$ g) of fish. Any fish not eaten was weighed and subtracted from the total. After being fed, birds were returned to their runs and observed for several minutes to ensure that no fish (or water for groups C and D) was regurgitated. Gulls in groups C and D were given ∼50 mL (mass determined gravimetrically) of fresh water at each feeding trial ($N = 4$ for group C and $N = 3$ for group D). Water was administered using gastric intubation with a syringe and flexible tube (30 cm long × 1 cm diameter). After water was fed, gulls were held for an additional 30–60 s to ensure that no water was regurgitated before being returned to their runs. Group D, which was given fish and water, received fish in the morning and water in the early evenings (usually 8–10 h apart). Between water feedings for each bird, the syringe and tube were flushed five to six times in clean fresh water. The first feeding trials (fish or water) began 3–4 h after initial TBW had been evaluated with isotopic water. Similarly, the final feeding (fish or water) was conducted 8–10 h before $TBW_f$ was evaluated.

*Isotopic Water Administration, Sampling, and Analysis*

At the beginning of each experimental period, gulls were removed from a run, weighed, and given a 1-mL injection of sterile HTO containing 4.74 MBq mL$^{-1}$ (128 $\mu$Ci mL$^{-1}$) of isotope and 0.9% NaCl. Injections of HTO were administered into the breast muscle of each bird with a 1-mL syringe and 22-gauge needle. The mass of each volume injected was determined gravimetrically on a platform balance accurate to within $\pm 0.1$ mg. After the injection, each bird was released back into its respective run so isotopic water could equilibrate with the bird's body water. Approximately 150 min later, each gull was removed from its run, and 2–3 mL of blood was collected from an intertarsal vein. This blood sample was used to evaluate initial TBW (TBW$_i$). After blood sampling, the bird was then placed back into its run. Previous research has shown that 120 min is sufficient for HTO to equilibrate with body water in birds (Degen et al. 1981; Gales 1989; Kirkwood and Robertson 1997; Shaffer et al. 2001b). Therefore, we were confident that isotopic water had completely mixed with the gulls' body water. Approximately 3 d later, a second blood sample was collected from each gull for evaluation of water influx and efflux. TBW$_f$ was then reevaluated following the same procedures used to evaluate TBW$_i$. Immediately following the collection of the postequilibration blood sample, each bird was fully anesthetized with 5% isofluorine gas mixed with pure oxygen, and the neck of the gull was dislocated. Final body mass was measured ($\pm 1$ g), and then carcasses were double-bagged and frozen until compositional analyses were performed at the Norwegian Polar Institute in Tromsø, Norway, in March 2003.

Blood samples were centrifuged for 10 min at 1,000 g, and serum was transferred to plastic screw cap vials and frozen at $-20°C$ until analyses were performed at UCSC in August 2003. Specific activity of tritiated body water was determined in triplicate by scintillation spectrometry (Beckman LS 6500, Beckman Coulter, Fullerton, CA) of water obtained from serum using the freeze-trap method (Ortiz et al. 1978). TBW (g) was calculated from the initial and final dilution of isotopic water injected into a bird (Degen et al. 1981). Total water influx (TWI) and efflux (TWE) were calculated from the turnover of isotopic water using Equations (4) and (6) of Nagy and Costa (1980), assuming a linear change in body mass. Water flux measurements were also adjusted for fractionation due to evaporative water loss (Lifson and McClintock 1966; Nagy and Costa 1980; Speakman 1997). We assumed that evaporative water loss accounted for 25% of water efflux (Speakman 1997) and that the pooled fractionation factor for tritium was 0.9179 (pooled correction factor with equilibrium and kinetic exchanges between water liquid and vapor contributing 3 : 1, respectively; Speakman 1997, Table 7.1). Water efflux (and subsequently influx) was corrected using Equation (7.6) of Speakman (1997). Mean MWP estimated from the fasting gulls

in group A was also deducted from measurements of TWI for each bird in all groups before comparison to measured water intake.

*Body Composition Analysis*

To measure body composition of each gull, frozen carcasses were thawed and reweighed, and a quick dissection was performed. This was done in order to determine the sex of each bird and the mass of the heart, kidneys, and liver. These organs were saved for other analyses. Therefore, we assumed that the heart, kidneys, and liver had a similar compositional makeup (i.e., percent fat, protein, water, etc.) as the rest of the body. The remainder of the carcass was homogenized in a food grinder. Five preweighed aliquots of each homogenate (22.5 $\pm$ 2.82 g) were dried in an oven at 55°C until the mass of the homogenate remained constant ($\sim$16 h). The TBW$_f$ (g) of each bird was then calculated by multiplying the proportion of water evaporated from each homogenate times the body mass of each bird after being euthanized. These results were compared with body water determined with isotopic water.

*Proximate Composition of Fish Fed in Feeding Trials*

Arctic cod from six feeding sessions were collected and refrozen for analyses of proximate composition. Each batch ($\sim$100 g, which was similar to the amount of cod fed to gulls) was double-sealed in plastic ziplock freezer bags and stored at $-20°C$ until compositional analyses were performed at UCSC in July 2003. At the time of analysis, each batch was thawed, weighed, and homogenized in a food processor. Duplicate aliquots of each homogenized batch were then weighed and freeze-dried to a constant mass to determine the average water content of the cod (78.7% $\pm$ 0.7% water). The total energy content of freeze-dried cod (19.8 $\pm$ 0.2 kJ g$^{-1}$ dry mass of cod) was also determined in triplicate using a ballistic bomb calorimeter with benzoic acid as a standard (Lieth 1975).

*Weather and Environmental Conditions*

During the experimental period, daily weather conditions were monitored four times a day. At approximately 3–5-h intervals between 0800 and 2200 hours local time, temperature, barometric pressure, and relative humidity were recorded at the animal enclosures.

*Statistics*

Statistical analyses were performed using SYSTAT 11 (SPSS, Chicago) with a significance level of $P \leq 0.05$ for all statistical tests (t-tests, ANOVAs, and general linear models [GLMs]). Because of unequal variances of the means in water flux between treatment groups, inferential statistical tests were per-

formed on $\log_{10}$-transformed data. Pairwise comparisons of means within ANOVAs or GLMs were performed with a 1-df test using the Specify feature in SYSTAT. In addition, proportional data were arcsine transformed for statistical comparisons. All calculations and comparisons of water were expressed in grams because all measurements were determined gravimetrically. All data are presented as means ± 1 SD.

## Results

### Feeding Trials

Experimental trials lasted 2.90–3.15 d for all study groups. All birds receiving fish (groups B and D) consumed their food, except for two birds in group D. These birds ate only partial meals during the first two feeding trials. Thereafter, all birds consumed everything that was fed to them. Birds receiving water (groups C and D) consumed everything at each feeding. At no time did we observe a bird regurgitating fish or water. Also, we did not observe any visible signs that regurgitations occurred inside the enclosures when birds were not observed.

On average, all birds in group B consumed the greatest amount of fish. However, birds in group D consumed more water overall because they received both fish and water (Table 1). Birds receiving water only consumed just over half the total quantity of water consumed when compared with birds receiving fish and water (134 ± 15 g $d^{-1}$; Table 1).

### Body Mass, Initial Total Body Water, and Water Flux Rates

The initial mean body mass for all birds was 1,373 ± 190 g (range 1,084–1,778 g). Although gulls in study groups B and D received approximately 100 g of fish per day, birds lost mass at an average rate of −1.85% ± 0.68% per day. Birds that were fasting (group A) lost mass at a rate of −5.78% ± 0.20% of body mass per day. Birds that received water only lost mass at an average rate of −3.51% ± 1.14% of body mass per day. The rates of mass loss were statistically different between groups (ANOVA, $F_{3,13}$ = 12.9, $P < 0.001$), with the exception of birds in groups B and D, which were nearly identical. The rates of mass loss for birds in groups A and C were statistically different from each other and from groups B and D ($P \leq 0.02$ for all comparisons).

$TBW_i$ varied from 737 to 1,048 g and was tightly coupled to the variations in body mass (Fig. 1). Mean $TBW_i$ for each group was not statistically different (ANOVA, $F_{3,12}$ = 3.26, $P = 0.059$) between treatment groups. Therefore, the mean $TBW_i$ for all birds combined was 870 ± 107 g (or 631 ± 21 g $kg^{-1}$). Relative $TBW_i$ (percent of body mass) was not statistically different (ANOVA, $F_{3,12}$ = 0.880, $P = 0.480$) between treatment groups (Table 2).

TWE (g $d^{-1}$) was lowest for gulls in group A and highest for gulls in group D (Table 1; Fig. 2), and the differences between all groups were significant (ANOVA, $F_{3,12}$ = 17.0, $P = 0.001$). Further comparisons showed that TWE was not statistically different between gulls in groups B and D. However, TWE of gulls in group D was significantly greater than TWE of gulls in groups A and C (e.g., $P < 0.005$ for comparisons of D vs. A or C). TWE of gulls in group C were statistically higher than the rates for gulls in group A ($F_{1,12}$ = 12.9, $P = 0.004$). TWE of all birds were significantly greater than their corresponding water influx rates (WIR; TWI in g $d^{-1}$; paired $t$-test, $t = -4.73$, df = 15, $P < 0.001$). This is consistent with losses

Table 1: Mean measured food and water intake, water influx rates, and the error differences between the two measurements for glaucous gulls in four treatment groups

|  | Fasting | Fish Only | Water Only | Fish and Water |
|---|---|---|---|---|
| No. individuals | 3 | 5 | 4 | 5 |
| Duration (d) | 2.90 ± .01 | 2.95 ± .00 | 3.20 ± .15 | 3.15 ± .01 |
| Gull body mass (g) | 1,271 ± 35 | 1,276 ± 113 | 1,555 ± 226 | 1,424 ± 175 |
| Total fish fed (g) | 0 | 416 ± 6 | 0 | 344 ± 60 |
| Preformed water in fish (g) | 0 | 327 ± 5 | 0 | 270 ± 47 |
| Total water fed (g) | 0 | 0 | 203 ± 1 | 152 ± 1 |
| Cumulative water intake (g) | 0 | 327 ± 5 | 203 ± 1 | 422 ± 47 |
| Daily water intake (g $d^{-1}$) | 0 | 111 ± 2 | 64 ± 3 | 134 ± 15 |
| TWI (g $d^{-1}$) | 37 ± 3 | 138 ± 5[a] | 99 ± 19 | 159 ± 21 |
| TWI − MWP (g $d^{-1}$) | 0 | 101 ± 5 | 62 ± 19 | 122 ± 21 |
| Water influx − water intake (g $d^{-1}$) | 0 | −10 ± 6 | −2 ± 21 | −12 ± 8 |
| Error (%) | 0 | −9.0 ± 5.4 | −2.2 ± 32.8 | −9.0 ± 7.0 |

Note. See "Material and Methods" for details on treatments. Preformed water in fish (Arctic cod) was 78.7% ± 0.7% of wet mass of cod. Metabolic water production (MWP) was assumed to be equivalent to the total water influx (TWI) of fasting gulls (37 ± 3 g $d^{-1}$). Errors (%) were calculated by [(water influx − water intake)/(water intake)] × 100. All data are presented as means ± 1 SD.

[a] Mean of four birds only.
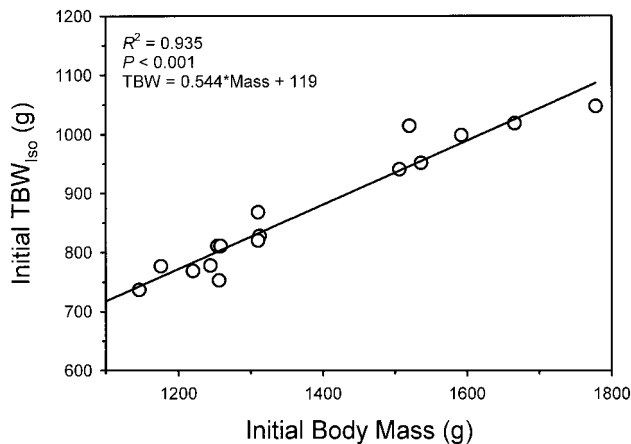
$R^2 = 0.935$
$P < 0.001$
TBW = 0.544*Mass + 119

Figure 1. Initial total body water (TBW) as a function of body mass in glaucous gulls. TBW was measured by isotopic dilution.

in body mass exhibited by all birds. TWI was significantly different between all groups (ANOVA, $F_{3,12} = 88.8$, $P < 0.001$). TWI was similar and not statistically different ($F_{1,12} = 2.52$, $P = 0.139$) between gulls in groups B and D. TWI of gulls in group C was significantly greater ($F_{1,12} = 97.3$, $P < 0.001$) than the rates of gulls in group A. However, the rates of gulls in group C were also significantly lower than TWI of gulls in groups B and D (e.g., $F_{1,12} = 14.4$, $P = 0.003$ for B vs. C).

After accounting for MWP (Table 1), water influx underestimated measured water intake by an average of 6.9% ± 17.4% (minimum: −34%; maximum: 32%). This difference was statistically significant (paired $t$-test, $t = -2.30$, df = 12, $P = 0.040$).

The mean body WIR, expressed as a proportion of body mass per day, were 5.6% ± 0.6%, 18.1% ± 0.4%, 11.4% ± 1.1%, and 17.1% ± 1.6% for gulls in groups A, B, C, and D, respectively. A comparison between gulls in each group revealed significant differences in mean influx rates (ANOVA, $F_{3,11} = 136$, $P < 0.001$). However, the influx rates between gulls in groups B and D were similar and not statistically different ($F_{1,11} = 1.17$, $P = 0.303$). The rates of gulls in groups A and C were significantly lower than the rates of gulls in groups B and D ($P < 0.001$ for comparisons of B and D vs. A or of B and D vs. C). Body WIR for gulls in group C were also significantly greater than rates of gulls in group A ($F_{1,11} = 105$, $P < 0.001$).

## Comparison of Initial and Final Total Body Water

Mean $TBW_i$ and $TBW_f$ of gulls in each group are presented in Table 2. The difference in body water at the start and end of each study period ranged from 0 to −188 g. A repeated-measures ANOVA was used to examine the significance of declines between $TBW_i$ and $TBW_f$ on the basis of all individuals using treatment group as a factor. Mean $TBW_f$ (g) was signif-

icantly lower than mean $TBW_i$ for all individuals ($F_{1,11} = 60.6$, $P < 0.001$). The effect of treatment group and the interaction between TBW and treatment group was also significant (group: $F_{3,11} = 6.25$, $P = 0.010$; interaction term: $F_{3,11} = 8.74$, $P = 0.003$). In contrast, TBW compared on a relative basis (percent of mass) did not differ between initial and final estimates within individuals ($F_{1,11} = 0.018$, $P = 0.895$). Also, the effect of treatment group and the interaction between individuals and treatment groups did not significantly affect the model (group: $F_{3,11} = 1.92$, $P = 0.185$; interaction term: $F_{3,11} = 1.32$, $P = 0.317$).

## Total Body Water: Isotopically Measured versus Body Composition Analysis

Mean $TBW_f$ estimated with isotopically labeled water and BCA are presented in Table 2. A general linear model was used to test for differences in mean TBW determined with each method (i.e., isotopic vs. BCA), using treatment group as a factor in the model. Comparisons of TBW (g) revealed no significant difference in the means between these two methods ($F_{1,30} = 1.07$, $P = 0.310$). However, there were significant differences in the means between groups ($F_{3,30} = 12.3$, $P < 0.001$). The interaction between method and group was not significant. Conversely, relative $TBW_{BCA}$ was significantly lower than $TBW_{Iso}$ ($F_{1,27} = 8.20$, $P = 0.008$). The effect of treatment group was also significant ($F_{3,27} = 7.51$, $P = 0.001$), whereas the interaction was not. For all gulls, the difference in TBW between methods ranged between −10 and +54 g or between −1.02% and +8.59% of mass (Table 2). Overall, $TBW_{Iso}$ (632 ± 24 g kg$^{-1}$) was greater than $TBW_{BCA}$ (613 ± 19 g kg$^{-1}$) in all but one gull (Fig. 3).

## Weather and Environmental Conditions

The weather for each day was cloudy to partly cloudy (≥50% cloud cover), but no rain was recorded. A roof covered the animal enclosures, so gulls were never exposed to direct sunlight. The average temperature, barometric pressure, and relative humidity were 6.6° ± 3.9°C, 1,010.8 ± 5.7 mbar, and 73.9% ± 7.2%, respectively.

## Discussion

### Water Flux Errors and Validation

Our comparison between water influx and measured water intake reveals reasonably good agreement on average for gulls in all treatment groups combined (Table 1; −6.9% ± 17.4%), despite the wide range in water intake between groups. The overall error is comparable to water flux validation studies on other birds and mammals (Nagy and Costa 1980; Degen et al. 1981; Costa 1987; Gales 1989; Robertson and Newgrain 1992; Vignault et al. 1996; Salatas et al. 2002), which range between −9%

and +13% of measured water intake. It was clear from our study, however, that water feedings, whether combined with fish or fed separately, increased the variability of errors between individuals within a treatment group (Table 1; Figs. 2, 4). The range in absolute errors was greatest among birds that received water only (−32% to +34% of measured water intake). However, birds receiving both water and fish exhibited more variation in errors than birds fed fish only (Fig. 4). Therefore, the ingestion of "unbound" water influenced the errors in our comparison between water influx and water intake.

The exact physiological mechanisms that resulted in the discrepancies between water influx and water intake were not the primary focus of this study. However, a number of explanations could apply. For example, cyclical water influx modeled in rabbits was shown to cause oscillations in water flux of 5% (Nagy and Costa 1980). This is analogous to our feeding schedule where birds received near instantaneous water input once per day. However, we sampled blood 8–10 h after birds received their final feeding (fish or water) to minimize the effect on water flux rates. Visser et al. (2000) also showed that ingested water in food can equilibrate rapidly with isotopically labeled body water of red knots (*Calidris canutus*). Evaporative water loss from breathing was also assumed to be 25% of water efflux (Speakman 1997). In contrast, Gessaman et al. (2004) found lower errors in water flux when evaporative water loss was assumed to be 45% of water efflux in poultry chicks. Given that arctic air is cold and relatively dry, it is conceivable that the amount of water lost to breathing dry air was underestimated. However, dry air also minimizes the input of unlabeled water from vapor exchange across the lung surfaces, which may cancel or reduce the errors attributed to evaporative water loss

(Nagy and Costa 1980). Biological fractionation (i.e., difference in activity of labeled body water vs. feces or urine) is another potential source of error that was not accounted for. Previous studies show that biological fractionation may or may not be a significant source of error (Nagy and Costa 1980; Speakman 1997; Visser et al. 2000). Although Visser et al. (2000) reported significant errors from biological fractionation due to diet in red knots, these fractionation effects did not influence water flux rates. Last, no rainfall was measured during our study period, nor did we observe any bird regurgitating fish or water. Therefore, we do not believe our results were affected by appreciable amounts of exogenous water beyond that which was fed to the gulls.

Another potential source of error in our comparison between water influx and water intake could have resulted from our application of MWP from fasting gulls (i.e., group A). We used the mean TWI of three birds as an estimate of MWP for the remaining gulls in the other treatment groups. The estimated MWP varied by as much as 8% between individuals in group A. This variation could have directly influenced the comparison with measured water intake because MWP was subtracted from the TWI of each bird (Table 1). For example, if the minimum MWP (33 g $d^{-1}$) was used instead of the mean, the overall error between water influx and measured water intake would have been −2.7% rather than −6.9%. The MWP of gulls receiving water and food could also have been different because of activity levels that would influence metabolic rates and thus MWP. Although we did not monitor activity levels of the gulls in each group, it is conceivable that fasting gulls minimized activity to conserve energy, which would have resulted in lower MWP compared with more active birds.

Table 2: Mass change and initial (TBW_i) and final (TBW_f) total body water of glaucous gulls in four treatment groups

|  | Fasting | Fish Only | Water Only | Fish and Water |
|---|---|---|---|---|
| No. individuals | 3 | 5 | 4 | 5 |
| Initial body mass (g) | 1,271 ± 35 | 1,276 ± 113 | 1,555 ± 226 | 1,424 ± 175 |
| Mass change (% $d^{-1}$) | −5.78 ± .20 | −1.84 ± .80 | −3.51 ± 1.14 | −1.86 ± .62 |
| Isotopically measured: |  |  |  |  |
| $\quad$ TBW_i (g) | 803 ± 22 | 786 ± 41[a] | 959 ± 138 | 907 ± 87 |
| $\quad$ TBW_i (% mass) | 63.2 ± 1.1 | 63.8 ± 3.8[a] | 61.7 ± 3.5 | 63.9 ± 2.1 |
| $\quad$ TBW_f (g) | 653 ± 30 | 784 ± 57 | 863 ± 106 | 894 ± 85[a] |
| $\quad$ TBW_f (% mass) | 60.8 ± 3.3 | 65.0 ± .8 | 62.2 ± 1.9 | 63.8 ± 1.8[a] |
| Body composition analysis: |  |  |  |  |
| $\quad$ TBW_f (g) | 630 ± 10 | 748 ± 59 | 848 ± 114 | 842 ± 95 |
| $\quad$ TBW_f (% mass) | 58.8 ± 1.2 | 61.9 ± 1.1 | 61.0 ± .9 | 62.8 ± 1.8 |
| Difference in methods: |  |  |  |  |
| $\quad$ Water (g) | 22.5 ± 27.5 | 40.2 ± 18.4 | 16.0 ± 18.5 | 21.6 ± 9.7 |
| $\quad$ Water (% mass) | 3.6 ± 4.3 | 5.5 ± 2.5 | 2.0 ± 2.2 | 2.4 ± .9 |

Note. See "Material and Methods" for details on treatments. TBW_f was determined using isotopic water and then by proximate composition of the same bird. All data are presented as means ± 1 SD.

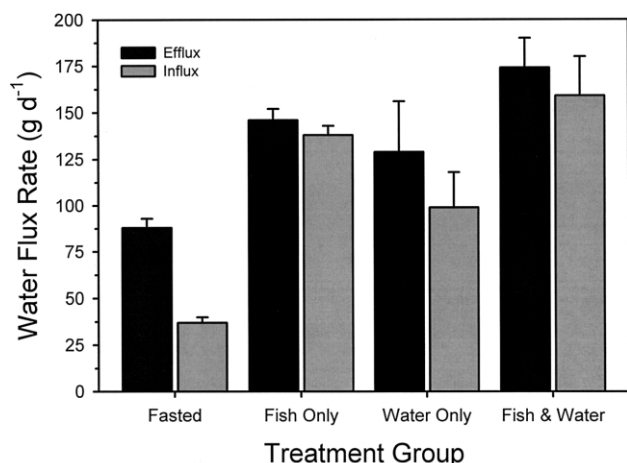[a] Mean of four birds only.

Figure 2. Daily water flux in fasted and fed glaucous gulls. Daily water efflux and influx for fasted gulls ($N = 3$) was significantly lower than for gulls in all other treatments. Neither water efflux nor influx was statistically different between gulls given fish ($N = 5$) or fish and water ($N = 5$). Gulls given water only ($N = 4$) had significantly lower influx rates than gulls given fish or fish and water, but efflux rates were similar to gulls given fish and significantly lower than gulls given fish and water. All data are presented as means ± 1 SD of birds in each treatment group.

To date, we are unaware of any study that has measured WIR in free-ranging glaucous gulls. Therefore, in order to evaluate how realistic our experiments were in comparison to what might be measured in the field, we used allometric equations to predict water influx on the basis of the average final body mass of glaucous gulls in our study (1,254 g). Nagy and Peterson (1988) developed a series of allometric equations to predict WIR on the basis of measurements from studies of wild and captive birds, mammals, reptiles, amphibians, fish, and an assortment of invertebrates. Using Equation (16) (Nagy and Peterson 1988), derived from studies on free-ranging seabirds, glaucous gulls are predicted to have a WIR of 168 mL d$^{-1}$. Similarly, Equation (11) (Nagy and Peterson 1988), derived from studies on a variety of captive birds, predicts a WIR of 123 mL d$^{-1}$. Measured WIR for glaucous gulls in our study that were fed fish or fish and water ranged from 138 to 159 mL d$^{-1}$ (mL = g). Thus, WIRs of our birds were within the upper and lower limits predicted by allometric equations for birds of a comparable body mass, suggesting that our results are realistic for comparison to free-ranging gulls.

Food consumption rates based on water influx measurements rely on the assumption that either animals do not drink freestanding water or that the amount of free water ingestion can be quantified (Costa 1987; Gales 1989; Robertson and Newgrain 1992). Our results show that even when birds consume up to 33% of their daily water intake as "free water" (i.e., not metabolic water or preformed water intake), errors between water influx and measured water intake are within 3% or up

to 9% when birds consumed both "free" and preformed water from food. Thus, HTO can be used indirectly to measure food or water consumption, provided that exogenous water sources can be quantified. In our study, the errors in WIR were relatively low compared with measured water intake because we were able to account for all major sources of water consumption. However, this may be difficult or nearly impossible to do in field studies on large mobile animals without the use of a second isotope (e.g., $^{22}$Na) that measures ion flux rates simultaneously (Gales 1989; Green and Brothers 1989; Robertson and Newgrain 1992).

### Changes in Total Body Water

The results of our analysis show that relative TBW in glaucous gulls did not change significantly over the course of our measurement interval (∼3 d). For all birds combined, relative TBW$_{Iso}$ (percent of mass) changed by an average of 0.1% ± 2.6%. However, it was clear that absolute body water decreased by an average of 8.4% ± 7.0% of mass, indicating that the glaucous gulls in our study were in negative water balance. These results suggest that when it is not possible to reevaluate TBW$_{Iso}$ directly, it is reasonable to assume that relative TBW$_{Iso}$ remains constant and that final body water can be estimated by the change in mass multiplied by the initial relative TBW$_{Iso}$.

Are the TBW results determined in glaucous gulls applicable to other animal species, particularly species that undergo large changes in body composition (e.g., pregnant or gravid females,
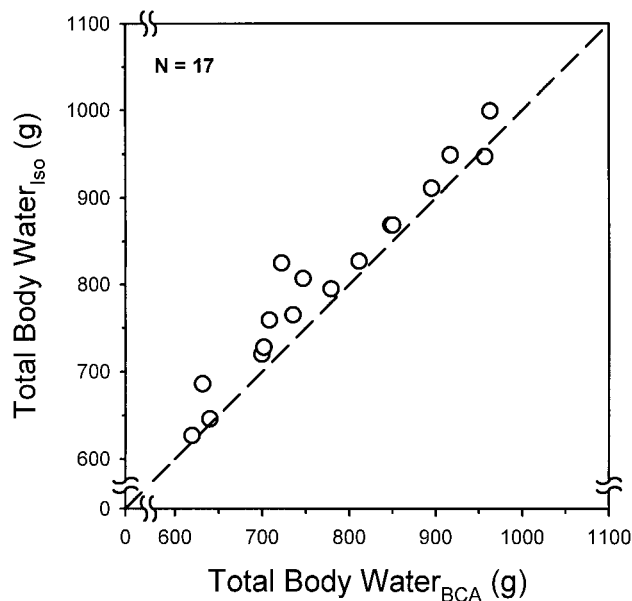


Figure 3. Comparison of total body water in glaucous gulls determined first by isotopically labeled water (Iso) dilution and then by body composition analysis (BCA). The dashed line represents the equality in methods (i.e., a slope equal to 1.0 that passes through the origin).
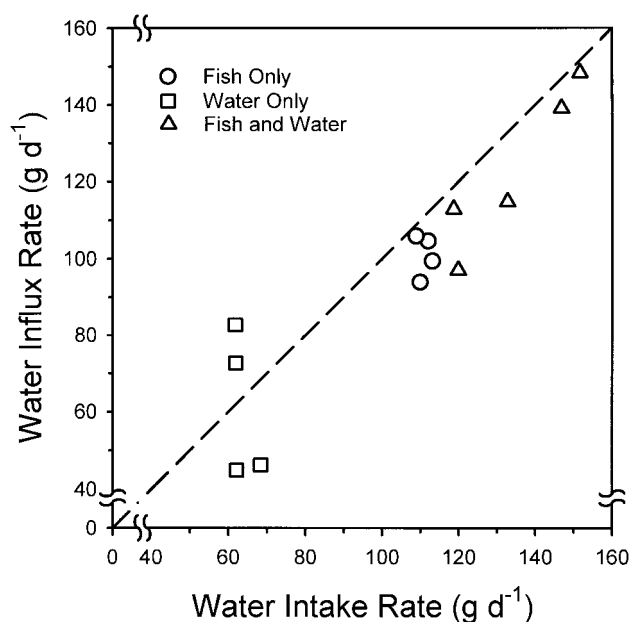
Figure 4. Water influx rate versus measured water intake in glaucous gulls. Water influx was determined by the dilution of isotopically labeled water (Nagy and Costa 1980; Degen et al. 1981) and subtracting metabolic water production (see Table 1 for details). Water intake was evaluated by measuring daily fish and/or water intake. Gulls were divided into four experimental treatment groups: fasting (not shown), fed fish only, fed water only, and fed both fish and water. The dashed line represents the equality in water intake/flux (i.e., a slope equal to 1.0 that passes through the origin).

migrating animals)? Given that our study was conducted under a variety of conditions, where animals fasted or received food and water, it is reassuring to see that the magnitude of change in relative $TBW_{Iso}$ was minimal. Ellis and Jehl (1991) determined that relative TBW can vary markedly within a species, particularly when TBW is below 60% of body mass. These variations in TBW can be correlated with large variations in total body fat or lean body mass relative to bird body mass (Ellis and Jehl 1991). The total body fat of gulls in our study was 3.6% ± 2.6% of body mass (or 46.3 ± 38.4 g; S. A. Shaffer and G. W. Gabrielsen, unpublished data), much leaner than the birds studied by Ellis and Jehl (1991). Hence, the low proportion of body fat of gulls in our study may account for the relatively low variability in TBW (±1.9% of mass; CV = 3.2%) observed. Perhaps a larger variation in body composition between individuals would have been required to alter relative TBW in glaucous gulls in this study.

*Total Body Water: Isotopically Measured versus Body Composition Analysis*

Relative TBW of glaucous gulls, measured either isotopically or by BCA, was consistent with values (57%–67% of mass)

reported for other seabirds (Mahoney and Jehl 1984; Gabrielsen et al. 1987; Hughes et al. 1987; Gales 1989). The exception are measurements for albatrosses, which are generally lower and range between 48% and 58% of mass (Costa and Prince 1987; Pettit et al. 1988; Ellis and Jehl 1991; Shaffer et al. 2001b, 2004).

Our results also show that isotopically measured TBW overestimates absolute water space by a factor of 1.033 (on average for all birds combined; Fig. 3). Previous studies on birds show that isotopic water dilution (using $^2$H- or $^3$H-labeled water) estimates TBW to be 0.973–1.180 times that of TBW determined by desiccation (reviewed in Speakman et al. 2001). This range was based on only four bird species, none of which were seabirds. Hughes et al. (1987) compared body water measured with isotopic dilution and carcass desiccation in nestling glaucous-winged gulls (*Larus glaucescens*). They found that isotopic dilution overestimated TBW by nearly 6% on average. However, this comparison was based on nestlings of different body size and composition, which influenced the degree of difference in TBW between methods (see Fig. 3 in Hughes et al. 1987). Green and Brothers (1989) also report that HTO dilution overestimated true water space by 3% in two seabird species (common diving petrel *Pelecanoides urinatrix* and fairy prion *Pachyptila turtur*). Data from 26 mammal species reveal that isotopic water dilution (using $^2$H- or $^3$H-labeled water) overestimates TBW by 1.046 times compared with whole body desiccation (reviewed in Speakman et al. 2001). Therefore, our results are consistent with other studies on birds and mammals.

Although we did not specifically investigate the cause for the overestimates in TBW by isotopic dilution, several sources address this issue more fully (Nagy and Costa 1980; Degen et al. 1981; Crum et al. 1985; Speakman et al. 2001). One potential source particular to birds is the amount of water contained in feathers that is not measured by isotopic dilution (Degen et al. 1981). Crum et al. (1985) determined that feathers can contain an additional 2% of body water. Our BCAs accounted for this because carcasses were homogenized with feathers intact. Therefore, our measurements of body water from BCA included any water that may have been contained in feathers. We also assumed that the heart, liver, and kidneys (which were saved for contaminants analyses) had a water content that was similar to the rest of the body (~61%). In order to determine the relative impact that this assumption could have on our results, we modeled the effect by varying the contribution of water from these organs (±10%) to TBW determinations. Overall, the effect of variation in organ water content on TBW was negligible (±0.7%).

In summary, the results of our study show that WIR in glaucous gulls measured by isotopically labeled water are within ~7% of actual water intake. However, the ingestion of water, either alone or in combination with fish, increased the variability of the error. The results also reveal that WIR showed a close agreement (on average) over a wide range of measured water intake rates. Our results also confirm that TBW measured

as a percentage of body mass (i.e., relative TBW) did not change even though absolute body water decreased over the measurement period. Last, TBW measured with isotopic dilution overestimates body water space by 3.3%, comparable to that determined for birds and mammals in other studies.

## Acknowledgments

## Literature Cited

Adams N.J., C.R. Brown, and K.A. Nagy. 1986. Energy expenditure of free-ranging wandering albatrosses *Diomedea exulans*. Physiol Zool 59:583–591.

Bakken V. and G.M. Tertitski. 2000. Glaucous gulls (*Larus hyperboreus*). Pp. 94–96 in T. Anker-Nilssen, V. Bakken, V. Bianki, A. Golovkin, H. Strøm, and L. Tatarinkova, eds. The Status of Marine Birds Breeding in the Barents Sea Region. Norsk Polarinstitutt Rapportserie, Tromsø.

Costa D.P. 1987. Isotopic methods for quantifying material and energy intake of free-ranging marine mammals. Pp. 43–66 in A.C. Huntley, D.P. Costa, G.A.J. Worthy, and M.A. Castellini, eds. Approaches to Marine Mammal Energetics. Allen, Lawrence, KS.

Costa D.P., P. Dann, and W. Disher. 1986. Energy requirements of free ranging little penguin, *Eudyptula minor*. Comp Biochem Physiol 85A:135–138.

Costa D.P. and P.A. Prince. 1987. Foraging energetics of grey-headed albatrosses *Diomedea chrysostoma* at Bird Island, South Georgia. Ibis 129:149–158.

Crum B.G., J.B. Williams, and K.A. Nagy. 1985. Can tritiated water-dilution space accurately predict total body water in chukar partridges? J Appl Physiol 59:1383–1388.

Degen A.A., B. Pinshow, P.U. Alkon, and H. Arnon. 1981. Tritiated water for estimating total body water and water turnover rate in birds. J Appl Physiol 51:1183–1188.

Ellis H.I. and J.R. Jehl Jr. 1991. Total body water and body composition in phalaropes and other birds. Physiol Zool 64:973–984.

Gabrielsen G.W., F. Mehlum, and K.A. Nagy. 1987. Daily energy expenditure and energy utilization of free-ranging black-legged kittiwakes. Condor 89:126–132.

Gales R. 1989. Validation of the use of tritiated water, doubly labeled water, and $^{22}$Na for estimating food, energy, and water intake in little penguins, *Eudyptula minor*. Physiol Zool 62:147–169.

Gessaman J.A., K. Newgrain, and B. Green. 2004. Validation of the doubly-labeled water (DLW) method for estimating $CO_2$ production and water flux in growing poultry chicks. J Avian Biol 35:71–96.

Green B. and N. Brothers. 1989. Water and sodium turnover and estimated food consumption rates in free-living fairy prions (*Pachyptila turtur*) and common diving petrels (*Pelecanoides urinatrix*). Physiol Zool 62:702–715.

Hughes M.R., J.R. Roberts, and B.R. Thomas. 1987. Total body water and its turnover in free-living nestling glaucous-winged gulls with a comparison of body water and water flux in avian species with and without salt glands. Physiol Zool 60:481–491.

Hui C.A. 1981. Seawater consumption and water flux in the common dolphin *Delphinus delphis*. Physiol Zool 54:430–440.

Janes D.N. 1997. Energetics, growth, and body composition of Adelie penguin chicks, *Pygoscelis adeliae*. Physiol Zool 70:237–243.

Kirkwood R. and G. Robertson. 1997. The energy assimilation efficiency of emperor penguins, *Aptenodytes forsteri*, fed a diet of Antarctic krill, *Euphausia superba*. Physiol Zool 70:27–32.

Lea M.-A., F. Bonadonna, M.A. Hindell, C. Guinet, and S.D. Goldsworthy. 2002. Drinking behaviour and water turnover rates of Antarctic fur seal pups: implications for the estimation of milk intake by isotopic dilution. Comp Biochem Physiol 132A:321–331.

Lieth H. 1975. The measurement of caloric values. Pp. 119–129 in H. Lieth and R. Whittaker, eds. Primary Productivity of the Biosphere. Springer, New York.

Lifson N. and R. McClintock. 1966. Theory of use of the turnover rates of body water for measuring energy and material balance. J Theor Biol 12:46–74.

Løvenskiold H.L. 1964. Avifauna svaldensis. Nor Polarinst Skr 129:1–460.

Mahoney S.A. and J.R. Jehl Jr. 1984. Body water content in marine birds. Condor 86:208–209.

Nagy K.A. and D.P. Costa. 1980. Water flux in animals: analysis of potential errors in the tritiated water method. Am J Physiol 238:R454–R465.

Nagy K.A. and C.C. Peterson. 1988. Scaling of Water Flux Rate in Animals. University of California Press, Berkeley.

Nagy K.A., W.R. Siegfried, and R.P. Wilson. 1984. Energy utilization by free-ranging jackass penguins, *Spheniscus demersus*. Ecology 65:1648–1655.

Ortiz C.L., D.P. Costa, and B.J. Le Boeuf. 1978. Water and energy flux in elephant seal pups fasting under natural conditions. Physiol Zool 51:166–178.

Parker H. and H. Holm. 1990. Patterns of nutrient and energy

expenditure in female common eiders nesting in the high Arctic. Auk 107:660–668.

Pettit T.N., K.A. Nagy, H.I. Ellis, and G.C. Whittow. 1988. Incubation energetics of the laysan albatross. Oecologia 74: 546–550.

Robertson C.J.R. and K. Newgrain. 1992. Efficacy of the tritiated water and $^{22}$Na turnover methods in estimating food and energy intake by emperor penguins *Aptenodytes forsteri*. Physiol Zool 65:933–951.

Salatas J.H., P.C. Frederick, K.A. Nagy, and G.E. Williams. 2002. Validation of the labeled-water method for estimating food consumption in nestling herons. Auk 119:551–556.

Shaffer S.A., D.P. Costa, and H. Weimerskirch. 2001*a*. Behavioural factors affecting foraging effort of breeding wandering albatrosses. J Anim Ecol 70:864–874.

———. 2001*b*. Comparison of methods to evaluate energy expenditure of incubating wandering albatrosses. Physiol Biochem Zool 74:823–831.

———. 2004. Daily energy expenditures of black-browed albatrosses during foraging and incubation. J Avian Biol 35: 551–558.

Speakman J.R. 1997. Doubly Labeled Water: Theory and Practice. Chapman & Hall, London.

Speakman J.R., G.H. Visser, S. Ward, and E. Król. 2001. The isotope dilution method for the evaluation of body composition. Pp. 56–98 in J.R. Speakman, ed. Body Composition Analysis of Animals: A Handbook of Non-Destructive Methods. Cambridge University Press, Cambridge.

Vignault M.-P., M. Saboureau, and C. Grenot. 1996. Water turnover evaluated by the tritiated water method and by the nutritional method in European hedgehog (*Erinaceus europaeus* L.). Comp Biochem Physiol 115A:187–194.

Visser G.H., A. Dekinga, B. Achterkamp, and T. Piersma. 2000. Ingested water equilibrates isotopically with the body water pool of a shorebird with unrivaled water fluxes. Am J Physiol 279:R1795–R1804.

Williams J.B. and K.A. Nagy. 1985. Water flux and energetics of nestling savannah sparrows in the field. Physiol Zool 58: 515–525.