RESEARCH ARTICLE

Understanding the energetic costs of living in saline environments: effects of salinity on basal metabolic rate, body mass and daily energy consumption of a long-distance migratory shorebird

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SUMMARY

Many migratory vertebrates typically move between habitats with varying salinities during the annual cycle. These organisms clearly exhibit a remarkable phenotypic flexibility in their 'osmoregulatory machinery', but the metabolic consequences of salinity acclimatization are still not well understood. We investigated the effects of salinity on basal metabolic rate (BMR), body mass and daily energy consumption of a long-distance migratory shorebird, the dunlin (*Calidris alpina*), outside the breeding season. Mass-corrected BMR and daily energy consumption increased significantly by 17 and 20% between freshwater (0.3‰ NaCl) and saltwater (33.0–35.0‰ NaCl), respectively. Body mass in both captive and wild dunlins was lower (9–16%) in saline than in freshwater environments. These changes on BMR and body mass were quickly reversed by returning the birds to freshwater, suggesting that metabolic adjustment to saltwater and metabolic readjustment to freshwater are both processes that occur in a few days. Our findings support empirically that the processes of developing and maintaining an active osmoregulatory machinery are energetically expensive, and they could help to explain diet and/or habitat selection patterns along the flyway. Finally, we discuss whether body mass loss in saltwater may be a strategy to reduce maintenance cost in osmotically stressful conditions such as overwintering in marine habitats, and raise some methodological implications for studies of BMR-related outcomes using captive birds captured in saline environments.

Key words: annual cycle, basal metabolic rate, *Calidris alpina*, dunlin, energetic cost, migration, osmoregulation, phenotypic flexibility, shorebird.

INTRODUCTION

Salinity has played an important role in the evolution of vertebrates (Bentley, 2002). Numerous species of fishes, birds, reptiles, marine mammals and some amphibians perform annual migrations or dispersions in which they are subject to large changes in the salinity of their environment. Osmoregulation is a major physiological challenge for these vertebrates (Peaker and Linzell, 1975; Skadhauge, 1981; Sabat, 2000; Bentley, 2002), and most of them possess the ability to osmoregulate in dynamic environments by means of physiological and behavioural adaptations (Schmidt-Nielsen, 1960; Peaker and Linzell, 1975; Shoemaker and Nagy, 1977; Shuttleworth and Hildebrandt, 1999; Sabat, 2000; Hildebrandt, 2001; Ortiz, 2001; Bentley, 2002). Apart from kidneys, non-mammalian vertebrates possess accessory organs (e.g. specialized salt glands, gills or urinary bladder) that can adjust in size and/or function to cope with changes in environmental salinity (Peaker and Linzell, 1975; Hildebrandt, 2001; Bentley, 2002). This phenotypic flexibility of the organs responsible for maintaining osmotic balance is present in many species that move over a wide range of salinities (e.g. Piermarini and Evans, 2000; Bentley, 2002; Sabat et al., 2004), and probably involves significant energy costs. Indeed, oxygen consumption studies have shown that acclimation to different salinities requires an energy surplus to maintain osmotic homeostasis (Bentley et al., 1967; Katz et al., 1973; Peaker and Linzell, 1975; Nelhs, 1996; Tseng and Hwang, 2008), although the nature of these energy costs remains poorly understood.

The basal metabolic rate (BMR) of a homoeothermic vertebrate is the sum of metabolic activities during the inactive period of the day in a thermoneutral environment and post-absorptive state (McNab, 1997; Hulbert and Else, 2000; Turner et al., 2004). Avian BMR is considered to be a highly flexible physiological trait and can be reversibly adjusted over short time scales (McKechnie et al., 2006; Jetz et al., 2008; McKechnie, 2008). In energy metabolism studies, it is an important physiological parameter for a number of reasons, including its use to estimate the energy demands in animals within a variety of ecological contexts (McNab, 2009). Peaker and Linzell estimated theoretically that the energy cost of salt gland secretion in ducks represented seven percent of their metabolic rate (Peaker and Linzell, 1975). In this context, it is reasonable to hypothesize that an increase in size and/or metabolic activity of the organs and tissues responsible for osmoregulation should contribute to increasing the organism's BMR. Time-constrained migrating vertebrates have narrow time windows in which to satisfy their high energy demands, so a salinity-linked increase in BMR could play a significant role in the individual's energy budget, affecting, for example, the patterns of habitat selection along the migration route. However, to the best of our knowledge, there are no empirical studies testing the effects of salinity on the BMR of homoeothermic vertebrates that regularly migrate between habitats differing strongly in their environmental salt concentrations (e.g. freshwater and marine environments). The environmental variables that influence energy and mass balance constitute significant axes of any organism's

fundamental niche (Kearney and Porter, 2004), so that any novel insights gained from such research will help in the understanding of an organism's distribution and abundance, resulting in greater confidence in extrapolations of new circumstances such as climate change (Kearney and Porter, 2004).

In birds, body mass loss has been considered a strategy to reduce maintenance cost during energy-costly activities such as reproduction (Freed, 1981; Norberg, 1981; Cavitt and Thomson, 1997; Bech et al., 2002), locomotion (Deerenberg et al., 1998; Elliott et al., 2008) and moulting (Portugal et al., 2007). Several studies have reported body mass losses in different species of aquatic birds during the adjustment period to saltwater (e.g. Purdue and Haines, 1977; Klaassen and Ens, 1990; Bennett et al., 2003), and they all attributed these body mass losses to osmotic stress. However, maintaining a low body mass could be an important source of energy savings to cope with hyperosmotic environments.

Many long-distance migratory shorebirds (charadriiforms) switch seasonally from freshwater habitats during the breeding season to marine habitats during migration and the winter period. This group of aquatic birds is considered to be 'osmotic generalists' (Blakey et al., 2006). To cope with saline environments, they have evolved paired orbital salt glands that secrete excess ingested salt *via* the nostrils (Fänge et al., 1958; Schmidt-Nielsen et al., 1958; Schmidt-Nielsen, 1960; Staaland, 1967), and they also present some behavioural adaptations (Mahoney and Jehl, 1985a; Mahoney and Jehl, 1985b; Nyström and Pehrsson, 1988). We here describe a laboratory and field study in which we investigated the effect of different saltwater regimes on the BMR, daily energy consumption and body mass of one of these long-distance migratory shorebirds, the dunlin (*Calidris alpina*). Because the avian BMR is a highly flexible physiological trait influenced by environmental factors, we predicted that phenotypic flexibility in BMR would be a significant component of short-term acclimation to saline environments. Specifically, we predicted that when dunlins are relying on saltwater habitats they have higher BMRs than when they are relying on freshwater habitats. It has been suggested that the processes of developing and maintaining active salt glands are energetically expensive (McArthur and Gorman, 1978; Burger and Gochfeld, 1984; Nyström and Pehrsson, 1988; Klaassen and Ens, 1990; Dosch, 1997). On this basis, we also predicted that the dunlins' BMR in a saltwater regime would remain high until the birds were moved back to a freshwater regime. A potential increase in energy demands associated with osmoregulation might result in increased food intake, so we predicted that dunlins in saline environment would increase their daily energy consumption relative to that in freshwater. Finally, we also predicted that body masses of both captive and free-ranging dunlins would be significantly lower in saline environments, and we addressed the question of whether body mass loss could be a strategy to reduce maintenance cost at marine habitats.

MATERIALS AND METHODS
Captive animals
Nine non-moulting adult *Calidris alpina* Linnaeus 1758 in non-breeding plumage were caught with mist nets on freshwater habitats (rice fields) in Extremadura, SW Spain (39°00′N, 5°57′W), in winter 2009. According to reports of ringed dunlins in Extremadura, they belonged to the subspecies *C. alpina alpina*. Prior to the metabolic experiment (see below), the birds were acclimated to an outdoor aviary (5×2.5×2 m) at the University of Extremadura for 2 weeks. During this period, they were provided with fly larvae (*Protophormia terraenovae*) and freshwater *ad libitum*. Daily temperature ranged between 10 and 23°C.

Experimental protocol
Although small calidrine sandpipers such as the dunlin principally use marine habitats during migration, they frequently also take advantage of brackish and freshwater habitats when they are available (Skagen, 2006; Piersma, 2007). The dunlins in the experiment were therefore maintained consecutively under three salinity regimes: first freshwater (FW; 0.3±0.0‰ NaCl; 7 days), followed by brackish water (BW; 10.4±0.5‰ NaCl; 10 days) and finally saline water similar to seawater (SW; 33.1±2.1‰ NaCl; 10 days). Prior to performing the experiment, we tested that mass-corrected BMR of dunlins acclimated to saltwater (following the protocol described below) did not vary after 2 weeks (comparison of mass-corrected BMR of dunlins at 2 and 4 weeks: $N=8$; period: $F_{1,14}=0.79$, $P=0.61$; log body mass: $F_{1,14}=5.25$, $P=0.06$; see Statistical analyses section for analysis type).

On the last day of each regime, we measured their BMR (see below) and then took blood samples to measure the plasma concentrations of Na$^+$ and Cl$^-$ ions. This electrolyte analysis (see below) was carried out to determine whether the birds were able to maintain the osmotic balance during the different salinity regimes.

During each treatment, water for drinking and bathing was provided daily *ad libitum* in three 6-l plastic trays (5 cm water depth). Fly larvae (body water: 73.3±0.5% of fresh body mass, $N=10$) killed by freezing were provided daily *ad libitum* in several plastic trays (2 cm depth) with water, ensuring that they were always surrounded by water so as to resemble natural feeding conditions. The water in the food trays had the same salt concentration as that in the drinking trays. The salinity of the water in the trays was measured twice a day with a portable multi-parameter instrument (WTW MultiLine P4 SET, Weilheim, Germany) to control for potential changes in salinity due to evaporation. The body water fraction of the dead larvae provided as food was similar in all three regimes (for example, the body water of larvae killed by freezing and kept in SW for 24 h was 71.0±0.2%, $N=10$).

Lastly, all the birds were returned to FW to study the readjustment period, measuring their BMR every 2 days. Unfortunately, during this readjustment period, four individuals were measured below the thermoneutral zone because of technical problems with the temperature-controlled cabinet. These measurements were therefore discarded from the analysis. Body mass was measured at 2-day intervals throughout the experiment.

BMR measurements
The BMR was measured in terms of oxygen consumption ($\dot{V}_{O_2}$) using a computerized flow-through respirometer. Since dunlins mainly forage during daylight, all measurements were made at night during the resting period of their daily cycle (Aschoff and Pohl, 1970). The birds were fasted for at least 3.5 h to create a post-absorptive condition (see Castro et al., 2008), and were then weighed (±0.1 g) before being placed in a polymethyl methacrylate (Perspex) metabolic chamber (effective volume=4.6 l; the floor was covered with indoor carpeting to prevent the birds from slipping). The metabolic chambers were in complete darkness and located in a temperature-controlled room at a constant temperature of 27°C, within the thermoneutral zone of dunlin (Kendeigh et al., 1977; Kelly, 2000). Temperatures inside the chambers were monitored continuously using a calibrated thermistor probe (±0.001°C). The metabolic chambers received atmospheric air at a rate of 11 min^{-1} from calibrated mass flow controllers (MFS-5; Sable Systems, Las Vegas, NV, USA), which allowed us to measure the air flow upstream. Water vapour was removed from the air stream immediately downstream from the metabolic chambers using

Drierite® columns, followed by a multiplexer (TR-RM4; Sable Systems), which allowed automatic switching between four channels. A subsample of the air was taken at 150 ml min⁻¹ using a subsampler mass flow meter unit (SS-3; Sable Systems), and the oxygen concentration was determined using a gas analyzer (FC-10 Oxygen Analyzer; Sable Systems) calibrated weekly using pure nitrogen as the low reference and a certified mixture of 21% O_2 as the high reference. The oxygen concentration was logged at a 1 Hz sampling rate on a computer using ExpeData software (v. 1.1.25; Sable Systems) and a UI2 converter. Each sampling sequence started with logging ambient baseline air for 10 min, followed by sampling each chamber for 10 min, with the system being flushed for 2 min between samples to remove latent gases. This sequence was repeated four times, so that there were four records per bird per night. At the end of the metabolic measurements the birds were weighed again and released into the aviary. The body mass reported for BMR analysis was taken to be the mean of the initial measurement and this final measurement. To make successive BMR measurements fully comparable, individuals were always measured in the same order and at the same time of day. Oxygen consumption was calculated according to steady-state equations [eqn 2 of Hill (Hill, 1972)] on the basis of the lowest 5-min average of O_2 consumption. The respiratory quotient (RQ) used was 0.72 assuming protein and lipid catabolism, and the metabolic rate was calculated using an energy equivalent of 20 kJ l⁻¹ O_2 (e.g. Kersten and Piersma, 1987; Kersten et al., 1998; Kvist and Lindtröm, 2001).

Plasma ion concentrations

After each BMR measurement, and therefore in a post-absorptive condition, blood samples were taken from the birds using brachial venipuncture with a 25-gauge needle. Blood was collected in heparinised capillary tubes, and centrifuged at 4600 g for 10 min to separate plasma from cells. The plasma was drawn off and stored at –20°C until assay. Plasma Na⁺ and Cl⁻ ion concentrations were determined using an electrolyte analyzer with ion-specific electrodes (SPOTCHEM EL SE-1510, Menarini, Milan, Italy).

Body mass patterns in wild dunlins

Overwintering dunlins were caught with mist nets in February 2005–2007 in Extremadura rice fields (freshwater habitat; N=75) and Cádiz Bay Natural Park, Spain (marine habitat, 36°23′N, 6°8′W; N=54). Both habitats are used by thousands of dunlins during winter (mostly *C. alpina alpina*) but Cádiz Bay supports greater numbers than Extremadura. Within 1–1.5 h of capture, each bird was fitted with an individual ring, aged (adult or juvenile), weighted (±0.1 g), and body morphometrics (bill, tarsus and wing length) were measured.

Daily energy consumption

A further eight dunlins (non-moulting adults) were caught in Extremadura rice fields during winter, and they were caged individually at 20°C under a photoperiod simulating the natural light cycle (13 h:11 h L:D). The floor of each cage (90×40×30 cm) was covered with sand, and fly larvae and water were offered in separate trays following the protocol described above. Birds were maintained for 7 days consecutively in FW (0.3‰ NaCl) and 7 days in SW (35.0‰ NaCl) for acclimation to each regime before measurements. Two individuals did not adjust to the indoor-caged conditions during the SW regime (body mass loss >20%) so they were returned to outdoors aviaries (freshwater) and excluded from further analysis. Daily food consumption was estimated in both regimes by offering a known amount of wet food to each experimental dunlin and

collecting the remains 24 h later. After an acclimation period of 7 days to the FW regime (0.3‰ NaCl), the body masses of the caged dunlins were stable, and we started the experiment. The daily food consumption of each individual in the FW regime was calculated for 3 days consecutively. We then changed the birds to the SW regime (35.0‰ NaCl) and measured daily food consumption for 3 days consecutively, after an acclimation period of 7 days. Body mass was measured (0.1 g) each day at 8:00 h throughout the experiment, and we took advantage of this monitoring to replenish water, remove prey remains and provide new food.

Wet mass of fly larvae was converted to dry mass using calculated wet:dry ratios. Finally, we converted dry mass to kilojoules assuming a value of 18.92 kJ g⁻¹ dry mass (*Protophormia terraenovae*) (Hoffmann, 1976).

Statistical analyses

A general linear mixed model (GLMM) was used to compare BMR and daily energy consumption between treatments, with log BMR or log daily energy consumption as the dependent variable, individual and treatment as random and fixed factors, respectively, and log body mass as covariate. The term 'treatment × log body mass' was included into the models to test specifically the influence of body mass. Differences in body mass between FW and SW dunlins were tested using a general linear model (GLM), with body mass as dependent variable and habitat type as fixed factor (two levels), including date of capture and body size as covariates to control for any confounding seasonal and body size effects, respectively. The body size was estimated from external measurements (bill, wing and tarsus lengths) and indexed by principal component analysis (e.g. Battley et al., 2004). Finally, a repeated measures analysis of variance (RM ANOVA) was used to compare plasma ion concentrations under the different salinity regimes. Tukey's test was used in *post hoc* comparisons. Data are presented as means ± s.e.m. Analyses were performed using SAS 9.0 (SAS Institute Inc., Cary, NC, USA) and Statistica 7.0 (StatSoft Inc., Tulsa, OK, USA).

RESULTS
Effect of salinity on BMR

Values of log BMR and log body mass were significantly and positively correlated in all salinity regimes (FW: log BMR=–2.02+0.51 log body mass; BW: log BMR=–1.86+0.50 log body mass; SW: log BMR=–2.26+0.56 log body mass). Whole-organism BMR did not differ significantly between regimes (Fig. 1A). However, mass-corrected BMR was 5 and 17% higher in BW and SW, respectively, than in FW ($F_{2,16}$=7.73, P<0.005; Fig. 1C, Table 1).

Effect of salinity on body mass and plasma ion concentrations

The body mass of captive dunlins decreased with increasing salinity. In particular, relative to the FW value, the body mass of captive dunlins decreased by 4.4% in BW and by 15.8% in SW (Fig. 1B). Similarly, the body mass of wild dunlins overwintering in the marine habitat was 9.3% lower than in the freshwater habitat ($F_{1,126}$=52.3, P<0.0001; covariates were not significant). The plasma ion concentrations did not differ between salinity regimes (Table 2).

Readjustment period

The mass-corrected BMR was found to be quickly reversed on returning the birds to FW (Fig. 2C). The mass-corrected BMR remained significantly different during the first 4 days, relative to the FW basal values ($F_{4,16}$=6.28, P<0.005), but these differences
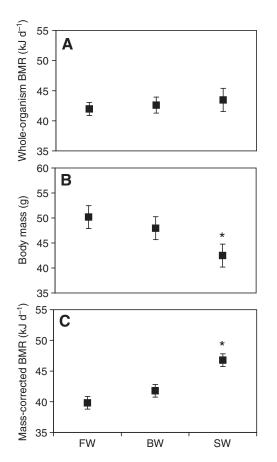
Fig. 1. (A) Whole-organism basal metabolic rate (BMR), (B) body mass and (C) mass-corrected BMR of dunlins under different salinity regimes (freshwater, FW; brackish water BW; and saltwater, SW). *$P<0.05$ by Tukey's *post hoc* test. $N=9$ for all time points. Data subjected to log transformation are shown as back-log-transformed least-square means.
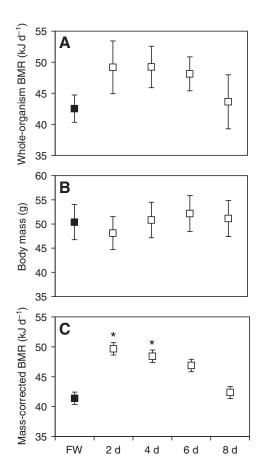
Fig. 2. (A) Whole-organism basal metabolic rate (BMR), (B) body mass and (C) mass-corrected BMR of dunlins returned to FW. Asterisks indicate $P<0.05$ by Tukey's *post hoc* test. $N=5$ for all time points. Data subject to log-transformation are shown as back-log-transformed least-square means. BMR was measured every 2 days (open squares) during the readjustment period. Initial FW values (filled squares) are shown for comparison.

became non-significant after 6 days (Fig. 2C). In particular, the mass-corrected BMR had returned to FW basal levels in just over a week. The differences in body mass were not significant during the readjustment period ($F_{4,16}=2.53$, $P=0.08$; Fig. 2B).

Daily energy consumption
Daily energy consumption of the SW group (130.37 ± 8.97 kJ day^{-1}) was 20% higher than that of the FW group (103.72 ± 12.06 kJ day^{-1}; $P<0.05$; Table 1). The body mass of dunlins in SW decreased by 8.9% relative to that in FW ($P<0.05$).

DISCUSSION
A significant increase was found in the BMR of dunlins as the salinity was increased, and energy intake also increased despite a

concurrent decrease in body mass. These results support empirically that the processes of developing and maintaining an active osmoregulatory machinery are energetically expensive (McArthur and Gorman, 1978; Burger and Gochfeld, 1984; Nyström and Pehrsson, 1988; Klaassen and Ens, 1990; Dosch, 1997). If the BMR is the sum of the metabolic rates of all the organs and tissues (Hulbert and Else, 2000; Turner et al., 2004), then the significant increase in BMR could be due to increased size and metabolic intensity of the organs and tissues involved in salt excretion. Indeed, it has been demonstrated that the salt glands of birds exposed to prolonged osmotic stress undergo a series of physiological adjustments (hyperplasia, hypertrophy and differentiation) accompanied by increased Na$^+$/K$^+$-ATPase enzyme activity (Hildebrandt, 2001). Therefore, these physiological adjustments that occur during the first

Table 1. Mixed general linear model analysis testing for effects of treatment on basal metabolic rate and daily energy consumption

	Covariate	Fixed factors		Random factor
	log body mass	Treatment	Treatment $\times$ log body mass	Individual
Log BMR	$F=5.57$ $P=0.02$	$F=6.04$ $P<0.01$	$F=0.28$ $P=0.76$	$z=0.61$ $P=0.27$
Log DEC	$F=12.13$ $P<0.05$	$F=5.55$ $P<0.05$	$F=0.50$ $P=0.48$	$z=0.65$ $P<0.001$

BMR, basal metabolic rate; DEC, daily energy consumption.
Interaction terms were not statistically significant and were not retained in final models.

Table 2. Plasma Na$^+$ and Cl$^-$ concentrations (mmol l^{-1}) of dunlins under different salinity regimes

	FW	BW	SW	F	P
Na$^+$	168.67±6.34	159.56±2.62	172.00±3.42	1.61	0.23
Cl$^-$	151.56±9.03	148.22±5.31	148.00±5.68	0.11	0.90

BW, brackish water; FW, freshwater; SW, seawater.

days of exposure to saline in order to increase the organism's salt-secretion capacity could be at least partially responsible for the significant increase in BMR with increasing salinity.

The readjustment period results also suggest that coping with excess salt requires significant energy expenditure. The changes in BMR with increased salinity were reversed quickly (within a week) when the birds were returned to freshwater. These results are consistent with those reported by Fletcher et al. and Holmes and Stewart who demonstrated in mallards (*Anas platyrhynchos*) that both the size and the activity of the salt glands decrease rapidly once the birds that had been acclimated to saline water were returned to freshwater (Fletcher et al., 1967; Holmes and Stewart, 1968). In sum, the metabolic adjustment to saltwater and metabolic readjustment to freshwater are both processes that occur in a few days, supporting the idea that the phenotypic flexibility of the BMR of small migratory birds allows large, reversible changes over short time scales (McKechnie et al., 2006; Jezt et al., 2008; McKechnie, 2008).

Because of this our experimental birds spent a relatively short period of time in saltwater, so it could be argued that we measured short-term adjustments to salinity more than inherent costs of living in a marine environment. However, the pre-experimental test showed that mass-corrected BMR of dunlins after 4 weeks in saltwater was similar to that reported after 2 weeks (see Materials and methods). The salt gland is the major organ for salt excretion in shorebirds such as dunlins (see Staaland, 1967) and, to date, all experiments performed on waterbirds showed that the maximum secretory ability per unit mass of glandular tissue occurred in less than a week of salt exposure (Fletcher et al., 1967; Holmes and Stewart, 1968; Hanwell and Peaker, 1975; Peaker and Linzell, 1975; Shuttleworth and Hildebrandt, 1999). Accordingly, we suggest that we measured the inherent costs of living in marine environments, although more studies are needed to clarify this issue.

In our experiment, dunlins had to cope with abrupt osmotic changes, and they could not have anticipated such changes (at least from freshwater to brackish water, and from saltwater to freshwater). Each year, millions of waterbirds, including dunlins, leave the breeding grounds (freshwater habitats) and arrive suddenly in marine habitats (non-breeding grounds) and *vice versa*. Although Burger and Gochfeld suggested that Franklin's gull (*Larus pipixcan*) could anticipate these annual osmotic changes by adjusting their osmoregulatory machinery, this still remain unclear (Burger and Gochfeld, 1984). It must be note that small migratory shorebirds, such as dunlins, have an opportunistic foraging behaviour and they can take advantage of temporary superabundance of food (Piersma, 1996). This opportunistic foraging behaviour leads them to move

Table 3. Summary of body mass changes of different species of aquatic birds maintained on saltwater regimens

Common name	Species	Age	% Body mass change (N)	Salinity range (‰)	Regimen duration (days)	Food source	Source
Eared grebe	*Podiceps nigricollis*	A	−10 (7)	90	2	WF	1
White ibis	*Eudocimus albus*	Y	D* (28)	–	21	WF	2
Black swan	*Cygnus atratus*	A	U (4)	0–SW	56	DF	3
		Y	−20* (8)	0–23	84	DF	3
Mallard	*Anas platyrhynchos*	A	−15* (30)	0–26	42	DF	4
		A	−13 (6)	17–28	21	WF	5
		A	−5* (10)	17	30	WF	6
		Y	−15 (12)	17	28	WF	7
		Y	−27* (20)	0–30	140	DF	8
		Y	−24* (14)	49	45	DF	9
		Y	−23* (123)	10–12 (+FW)	15	DF	10
Mottled duck	*Anas fulvigula*	Y	D* (79)	0–18	60	DF	11
Common eider	*Somateria mollissima*	Y	D* [9–42](120)	0–33	6.5	DF	12
American avocet	*Recurvirostra americana*	Y	−31* (67)	0–32	3	DF	13
Kentish plover	*Charadrius alexandrinus*	A	D (8)	18	3–11	DF	14
Killdeer	*Charadrius vociferus*	A	D (7)	6–12	3–11	DF	14
Red knot	*Calidris canutus*	A	D (12)	32–42	5–6	DF	15
Sanderling	*Calidris alba*	A	D (5)	42	5–6?	DF	15
Semipalmated sandpiper	*Calidris pusilla*	A	D (3)	18	3–11	DF	14
Dunlin	*Calidris alpina*	A	−16 (9)	10–33	17	WF	16
Glaucous-winged gull	*Larus glaucescens*	A	U (13)	SW	30	WF	17
		Y	−21 (?)	SW	–	–	18

A, adult; D, decrease; DF, dry food; FW, freshwater; SW, seawater; U, unaffected; WF, wet food, Y, young.

*Difference in mean body mass between experimental (SW) and control birds (FW). Those values with no asterisks indicate that the body mass change occurred in birds acclimated to SW.

Source: 1, Mahoney and Jehl, 1985a; 2, Johnston and Bildstein, 1990; 3, Hughes, 1976; 4, Bennett et al., 2003; 5, Fletcher and Holmes, 1968; 6, Fletcher et al., 1967; 7, Holmes and Steward, 1968; 8, Schmidt-Nielsen and Kim, 1964; 9, Ruch and Hughes, 1975; 10, Ellis et al., 1963; 11, Moorman et al., 1991; 12, DeVink et al., 2005; 13, Hannam et al., 2003; 14, Purdue and Haines, 1977; 15, Klaassen and Ens, 1990; 16, This study; 17, Walter and Hughes, 1978; 18, Holmes et al., 1961.

abruptly between salinities, for example between marine water and hypersaline water (Masero et al., 2000; Masero, 2002) or between marine water and freshwater of ephemeral continental wetlands (see Skagen, 2006). In these situations, they cannot anticipate such abrupt osmotic changes, so we think that our experiment reflects the real situation free-living dunlins face.

The rapid rate at which BMR decreases during the readjustment to freshwater may have methodological implications for studies of BMR-related outcomes using birds captured in saline environments but acclimated to freshwater for short time periods prior to measurements. The BMR of captive shorebirds captured in marine habitats, for example, has been measured after a period of several days or even weeks in captivity with freshwater available *ad libitum* (e.g. Kersten et al., 1998). These studies, therefore, might fail when they extrapolate BMR results to wild conditions, because these captive individuals would have lower BMR than free-ranging birds. Consequently, it is essential to note the salinity conditions under which the BMR is measured, especially when metabolic comparisons are made between animals at different places in the course of their annual cycle (Kvist and Lindström, 2001).

During migration, shorebirds and other waterbirds need to meet their high energy demands in short time periods, facing energetic 'bottlenecks' (Piersma, 2002; Buehler and Piersma, 2008). The increase in BMR and food intake in saline environments may play a significant role in the individual's energy budget, and could help explain diet and/or habitat selection patterns along the flyway (Nyström and Pehrsson, 1988; Adair et al., 1996; Woodin et al., 2008). Routine movements of several species of diving ducks (*Aythya* spp.) between saltwater wetlands (foraging grounds) and freshwater wetlands (resting grounds) during the non-breeding season, for example, have been related to osmoregulation (Woodin, 1994; Adair et al., 1996). According to our results, this pattern of habitat use could be explained by the high energy requirements associated with feeding in saltwater wetlands, which would be reduced by drinking freshwater. We stress the role of salinity in determining the energy demands of migratory organisms, so this abiotic factor must be included in studies of an organism's energy budget as well as in patterns of habitat selection along migration routes.

Reductions in body mass have also been reported in waterbird species during the period of adjustment to saltwater (Table 3). In our case, the body mass loss of captive dunlins was progressive, and the plasma ion concentration analysis indicated that the individuals were able to osmoregulate successfully throughout the experiment. The mean body mass (42.5±2.7g; post-absorptive state) of the captive birds at the end of the saltwater regime was similar to the mean body mass of overwintering dunlins (subspecies *alpina*) in their main wintering area on the west coast of Africa (42.0±0.5g in the Banc d'Arguin) (Zwarts et al., 1990). It has been suggested that a reduction in body mass may reduce maintenance costs (Freed, 1981; Norberg, 1981; Cavitt and Thompson, 1997; Deerenberg et al., 1998). Although the body mass of free-ranging shorebirds may vary according to several factors such as predation risk (van de Hout et al., 2010) or adverse weather (Yasué et al., 2003), the loss of body mass of dunlins coping with saltwater could be considered as a strategy to reduce the high energy costs of living in a saline environment. In the case of shorebirds, previous studies have suggested adaptive body mass adjustments in overwintering birds (Mitchell et al., 2000; Piersma and Jukema, 2002; Piersma et al., 2003). Mitchell et al. proposed that redshanks *Tringa totanus*, for example, optimize their body mass during winter to reduce

maintenance costs and risks of predation (Mitchell et al., 2000). We suggest that a reduction in body mass might be part of the response maximizing energy saving in shorebirds coping with osmotically challenging environments such as estuaries and marine coasts.

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