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Ecological context determines the choice between prey of different salinity

Lay summary

Seawater is too salty for most land animals, but many marine birds and reptiles can cope with it owing to flexible cephalic "salt" glands that excrete excess salt from the bloodstream. We show that red knots without access to freshwater prefer prey with relatively low salt content when their salt glands are small, but this preference is lost after they enlarge their salt glands and regain access to freshwater.

Summary

Food choice has profound implications for the relative intakes of water and salts, and thus for an animal's physiological state. Discrimination behaviors with respect salt intake have been documented in a number of vertebrate species, but few studies have considered the ecological context in which they occur. Here, we report on the results of a two-choice experiment designed to examine the influence of dietary salt content and freshwater availability in food discrimination behaviors in red knots *Calidris canutus* (Aves: Scolopacidae) that feed on mud snails *Peringia ulvae* (Gastropoda: Hydrobiidae) whose body fluids have either relatively low (25‰) or high (42‰) salinity. Birds ate more and spent longer time foraging on low-salinity mud snails when their salt gland sizes—an indicator of excretory capacity—were relatively small and when they were deprived of freshwater. However, as they enlarged salt glands—following a prolonged exposure to salty diet without access to freshwater—and regained access to freshwater their preference for low-salinity prey disappeared.

Such a change of preference illustrates the context-dependency of discrimination. As the birds were able to maintain salt-water balance—inferred from plasma sodium concentration—under all conditions, changes in salinity preferences may occur without measurable physiological signs of osmotic stress. Our results highlight the importance of ecological context for understanding foraging responses. We argue that areas with high salinities could act as refuges for euryhaline invertebrates and fish from top vertebrate predators.

INTRODUCTION

Seawater is toxic to most terrestrial vertebrates due to its high salt content. However, many secondarily marine vertebrates such as snakes, turtles, birds and mammals live in marine and estuarine environments where they typically feed on food that is in osmotic and ionic equilibrium with the surrounding water (Schmidt-Nielsen 1997; McNab 2002). To cope with the excess salt and to maintain fluid homeostasis, these animals possess specialized organs (e.g. reniculate kidneys, cephalic '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; Ortiz 2001; Bentley 2002; McNab 2002). Among them, cephalic salt glands are one of the best-documented examples of physiological adaptation to marine life in non-mammalian vertebrates. Most birds and reptiles from marine environments have cephalic salt glands that extract salt ions from the bloodstream, producing a highly concentrated salt solution that is discarded through ducts that open into the nostrils (birds and lizards), eye (turtles), or tongue (snakes and crocodiles) (Peaker and Linzell 1975; Schmidt-Nielsen 1997; Bentley 2002; McNab 2002). This affords them the capacity to eat salty food and retain osmotically-free water (Schmidt-Nielsen 1960; Peaker and Linzell 1975; Schmidt-Nielsen 1997; McNab 2002).

Although various facets of vertebrate osmoregulation have been investigated exhaustively (Peaker and Linzell 1975; Skadhauge 1981; Schmidt-Nielsen 1997; Goldstein and Skadhauge 2000; Ortiz 2001; Bentley 2002), behavioral mechanisms leading to a decrease in salt intake have received only limited attention (Wolcott and Wolcott 2001; Brischoux et al. 2012; Gutiérrez 2014). For instance, it has been suggested that toothed whales whose diet consist mainly of hyperosmotic prey (osmoconforming invertebrates) derive a 'water bonus' by also eating (the osmoregulating) bony fish whose osmotic concentration resembles their own (Wolcott and Wolcott 2001). Likewise, it was recently found that captive Australian pelicans *Pelecanus conspicillatus* consumed pieces of elasmobranchs and squid (both osmoconformers) at substantially lower frequencies than bony fish (osmoregulators) (Troup and Dutka 2014). Moreover, coastal ducks *Aythya* spp. that forage in energy-rich and salty estuaries regularly move to inland freshwater ponds to rest and re-hydrate (Woodin 1994; Adair et al. 1996). In reptiles, it has been suggested that the abilities of sea kraits *Laticauda* spp. to acquire fresh water on land and tolerate dehydration at sea, determine their environmental tolerances and geographic distributions (Brischoux et al. 2013). Clearly, behavioral osmoregulation plays a large part in the maintenance of the osmotic balance in many marine and estuarine air-breathing vertebrates.

Shorebirds (Charadriiformes, suborders Charadrii and Scolopaci) provide excellent material to investigate how osmotic concentration of prey affects food discrimination behaviors in different environmental contexts. In estuarine and intertidal environments, both shorebirds and their prey may be subjected to abrupt changes in the osmotic environment. For these organisms, fast and flexible behavioral responses are essential in meeting osmotic challenges (Gutiérrez et al. 2011; Gutiérrez et al. 2012; Gutiérrez et al. 2013; Gutiérrez 2014; Gutiérrez et al. 2015). In particular, sandpipers of the genus *Calidris* have extensive arrays of taste buds (Gerritsen et al. 1982; Nebel et al. 2005), and several species (red knots *C. canutus*, purple sandpipers *C. maritima*, sanderlings *C. alba*, and dunlin *C. alpina*) can discriminate between 'clean' sand and sand that had contained prey, which suggests that they are able to use taste substances excreted by a particular prey for food detection (Gerritsen et al. 1982; van Heezik et al. 1983). For these reasons it seems plausible that they can use the salinity of prey and surrounding water to adjust their salt intake and avoid osmotic stress. Indeed, NaCl-sensitive taste buds found in chickens, pigeons and parrots react to 0.2 M and higher concentrations of NaCl (Kitchell et al. 1959; Duncan 1962; Matson et al. 2000). A recent study based on relevant gene sequences associated with taste buds showed that penguins (order Sphenisciformes) have evolutionarily lost receptors for detecting sweet, umami, and bitter tastes, but still possess those for detecting salty tastes (Zhao et al. 2015); this would enable them to adjust their salt intake.

In this study, we investigated whether prey salt content and freshwater accessibility influence food-discrimination behaviors using captive red knots that fed on mud snails *Peringia ulvae* whose body fluids had either relatively low (25‰) or high (42‰) salinity. Molluscivore shorebirds face the dilemma of having to conserve free water while consuming hard-shelled prey with high seawater content and relatively little flesh (Gutiérrez et al. 2012; Gutiérrez et al. 2015). Specifically, maintaining the osmotic balance is a major challenge for red knots, as they may process several times their body mass in seawater each day (Visser et al. 2000) with limited or no access to freshwater in some of their main nonbreeding areas (Wolff and Smit 1990; van de Kam et al. 2004). To establish whether birds really display foraging preferences, and whether these depend on the ecological context, birds were simultaneously offered low-salinity and high-salinity diets with and without previous access to freshwater. Then, the choice between diets was recorded as the food intake and time spent foraging from each diet. To assess whether their choice pattern was related to their physiology, we also measured different indices of osmoregulatory state. (i) Hematocrit is known to increase with dehydration in birds (Hannam et al. 2003; Fair et al. 2007) and might indicate whether our treatments affected hydration state. (ii) Plasma sodium concentration is another extensively studied hydration state parameter that serves as a good indicator of salt-water balance (Skadhauge 1981). (iii) The size of the salt glands positively correlates with the concentration and rate of their secretion, which in turn determines the amount of osmotically-free water they can retain for other physiological processes (Schmidt-Nielsen 1960; Staaland 1967).

We predicted that birds would prefer the low-salinity diet over the high-salinity diet to minimize salt intake and avoid osmotic stress; our null hypothesis was a lack of preference. Additionally, we predicted that preference for low-salinity food would be stronger when birds have small salt glands, and when they are deprived of freshwater since under such conditions the birds would not be able to deliberately 'dilute' dietary salt; our null hypotheses would be lack of differences.

MATERIALS AND METHODS

Subjects and Housing

Eight adult (four male and four female) red knots of the *islandica* subspecies (Nebel et al. 2000) were caught in the western Dutch Wadden Sea (53°31'N, 6°23'E) in August-September 2013 and kept in outdoor free-flight aviaries (4.5 m × 1.5 m surface × 2.5 m height) with unlimited access to trout pellets (Vézina et al. 2006; Gutiérrez et al. 2015). Birds had free access to a freshwater tray (60 cm x 40 cm surface x 5 cm height) for drinking and an artificial mudflat flooded with running seawater for probing. The floor of the aviaries was also flushed with running seawater to help prevent infections and skin lesions caused by dry feet (see Milot et al. 2014). In January 2015, birds were transferred to two separate indoor 'group' aviaries (4 birds per aviary) with similar characteristics to the outdoor aviaries and fed a diet composed exclusively of 2-4 mm mud snails *Peringia ulvae* collected by dredging in the Wadden Sea (Vézina et al. 2006; Gutiérrez et al. 2015). Outside the experiments, mud snails were presented to the birds in two trays (60 cm x 40 cm surface x 5 cm height) with running seawater taken directly from the sea (salinity ≈25‰; temperature ≈ 12°C).

In the intertidal zone, mud snails frequently dominate the benthic fauna numerically and in terms of biomass, and form an important constituent of the diet of shorebirds (Evans et al. 1979; Britton 1985; van Gils et al. 2003). Indeed, this gastropod species is one of the main prey for red knots along the East Atlantic flyway (Moreira 1994; van Gils et al. 2003; van den Hout 2010). *P. ulvae* can live in a salinity range of 6–85‰ (Komendantov and Smurov 2009) and is isosmotic to seawater (Todd 1964), meaning that red knots inevitably consume large amounts of salt when they ingest *P. ulvae* whole (Gutiérrez et al. 2015). Captive red knots were kept in these indoor aviaries with water available every day (salinity depending on the sessions; see below). The housing conditions were maintained under a 10:14 light-dark cycle with a 20-min period of dawn:dusk ramp, similar to ambient conditions during this period, and under indoor ambient temperature (12±0.5 °C). After the experiment, the birds were released at the same site from which they were caught.

Preparation of prey

Freshly collected mud snails were stored frozen. Frozen mud snails remained in their shells, so the birds had to crush the shells in their gizzard in order to digest the flesh (Vézina et al. 2006; Gutiérrez et al. 2015). Unlike alive mud snails, dead (frozen-thawed) mud snails cannot moderate their exposure to variation in salinity by withdrawing and closing their operculum (Berger and Kharazova 1997). Observations on dead snails from the stock offered to the birds showed that most (96%; N = 200) individuals had opened or lost their operculum when presented to birds. Therefore, we could easily modify the salt concentration of their body fluids (Gutiérrez et al. 2015). To do this, freshly (thawed) portions were placed in 90-L plastic containers with seawater of high (41.51 ± 0.22‰, N = 18) or low (24.92 ± 0.48‰, N = 18) salinity and maintained at 12 ± 0.5°C for approximately 12 h, which ensured that snails had enough time to become isosmotic with the surrounding seawater (see Supplementary Figure S1). At the end of this period, snails were removed from their tanks and visible water was removed using a sieve (1 mm mesh).

Throughout the experiment the body water content of high-salinity (50.86 ± 0.23%) and low-salinity (51.22 ± 0.28%) snails was similar (paired-*t* test: t_{71} = -1.20, *P* = 0.23; Supplementary Figure S2). Water salinity was measured in the tanks daily with a portable multi-parameter instrument (Delta Ohm, HD2156.1, Benelux B. V.). The salt concentration of snails' body fluids was determined by inductively coupled plasma mass spectrometry (Thermo Scientific iCAP Q ICP-MS, Thermo Fisher Scientific GmbH, Bremen, Germany) following Gutiérrez et al. (2015).

Experimental protocol

After three weeks of acclimation to mud snails, which ensures that red knots had enough time to adjust to a diet of hard-shelled mollusc prey (Piersma et al. 1993), we moved on to the experimental sessions. The phase consisted of three experimental sets on the basis of freshwater availability, starting with 'access to freshwater' (sessions 1–2), followed by 'no access to freshwater' (sessions 3–6) and finally 're-access to freshwater' (sessions 6–9). This sequence was used to manipulate the size of the glands (they are enlarged in birds fed salty diets and deprived of freshwater; Gutiérrez et al. 2015, JSG pers. obs.) and thus ensured that birds encountered different environmental contexts (i.e., access/no access to freshwater) with a range of salt gland sizes. As it was logistically impossible to measure and record all the individuals simultaneously, each individual was given one session every other day; therefore, the experimental period lasted 18 consecutive days. Birds were starved overnight before each experimental session (i.e., every other day) to get them motivated and eager to eat. Despite this regular fasting period, birds maintained a constant body mass throughout the experiment (Supplementary Figure S3). To avoid repetitive blood sampling and its potential effects on hematocrit and plasma sodium, we only took blood samples at the end of each experimental set (i.e., sessions 2, 6 and 9). Seawater (salinity ≈25‰; temperature ≈ 12°C) was available at all times throughout the study, except during the 3-h experimental sessions; freshwater trays were available during both the pre-experimental period and the 'access to freshwater' and 're-access to freshwater' experimental sets (see above) when not in experimental procedures.

Every day of the 18-day experimental period, we removed four birds from of one of the two indoor 'group' aviaries just before the start of the trials at 10:00 hours, weighed them (to the nearest 0.1 g) and scored their salt glands (Gutiérrez et al. 2015; see below). Then, we transferred each bird to identical indoor 'individual' aviaries (same characteristics as the indoor 'group' aviares) where two trays containing the same amount (*c*. 200 g) of low- and high-salinity mud snails were offered (Fig. 1a, b). Wet snails were offered in excess in identical plastic trays with no water to prevent birds from making a choice based only on water salinity without tasting the prey (Fig. 1a, b). It is important to note a similar situation can be encountered in the wild when red knots intercept prey near the receding water line of mudflats.

After each session, birds were returned to their indoor 'group' aviary and the food trays were removed and reweighed to determine the amount of food eaten (see below). In order to avoid the presence of potential visual and olfactory cues, the trays and floor of the aviaries were thoroughly cleaned after each trial. Moreover, the trays were reversed on a daily basis to avoid position-effect biases. All sessions were videotaped to code birds' behavior (see below).

Food intake

We measured food intake over each 3-hr session. Using food intakes in indoor aviaries during the pre-experimental period, we estimated average daily food intake at *c*. 250 g of wet mud snails per bird —this crude estimate represents the average food intake consumed in the 'group' aviaries per day divided by the number of birds in the aviaries. To minimize food depletion-related issues, birds were provided with 200 g of wet mud snails in each tray, which represents eight times the average amount of food a single bird would eat assuming a constant intake rate from only one tray (31.25 g; estimated as 250 g of daily food intake divided by 24 h of food access and multiplied by 3 h of experimental session). Every day, we sieved freshly thawed mud snails to remove all visible water and we then took two subsamples (10 g each) of food from this stock. In each session, we gave a pre-weighed amount of food from the same stock to the birds in the two trays. After the 3-hr session, the birds were returned to their 'group' aviaries and the food trays were removed from the aviaries. Droppings were carefully removed from the trays if present and mud snails adhered to them were separated and returned to their respective trays before weighing them for the second time to calculate food intake. Control (uneaten) portions of diet, weighed before and after trials, showed that water loss was negligible (on average 1%) and did not differ by diet (paired-*t* test: t_{13}= -0.38, P = 0.71). Nonetheless, we corrected for water losses because even such small mass losses can bias the results (i.e. with respect to the response ratios and relative food intake rates).

Birds occasionally fed onto the feeding trays (instead of walking around and taking food from them) and kicked out some snails from the tray onto the aviary floor —after correcting for water losses they consumed an average of 25.37 ± 2.22 g of food per session and spilled only 0.21 ± 0.09 g (0.84%). We assumed that snails spilled on the aviary floor belonged to the tray placed on that half of the aviary. Video recordings corroborated that birds did not transported food from one half of the aviary to the other. Therefore, we feel confident that food transport bias did not affect the results of this study.

Hematocrit and plasma sodium concentration

At the end of each set of trials, blood was taken from a wing vein into two heparinized capillary tubes (75 µl) per bird and centrifuged immediately for 10 min at 10,000 rpm. Hematocrits were read immediately after centrifugation using a microhematocrit capillary tube reader. The value reported herein for each bird was the mean of the two tubes. Plasma was saved to determine sodium concentration, which was determined by inductively coupled plasma mass spectrometry (Thermo Scientific iCAP Q ICP-MS, Thermo Fisher Scientific GmbH, Bremen, Germany) using a standardized procedure (Long and Vetter 2002). All samples were collected after food deprivation to ensure a post-absorptive condition.

Salt gland scores

We estimated salt gland scores for each individual using sensory evaluation (Gutiérrez et al. 2015). Briefly, we scored the thickness of the salt glands at the postorbital ridge by sliding a finger across a a smooth polyvinylchloride plate prepared with five increasing thicknesses (0–0.8 mm) at regular distances from each other, to then compare these thicknesses with those of the postorbital salt gland ridge.

Videotaping

Videocameras were placed outside the cages and were focused through a one-way mirror so that so that they did not interfere with the birds' activity (Fig. 1). We coded behavior using the software CowLog 2.0 (Hänninen and Pastell 2009). Behavior of each animal was categorized into foraging (from left or right tray), moving (i.e., walking and flying), and resting (i.e., standing, sleeping and preening). We then calculated the frequencies, bout durations, and total durations of the coded behaviors. Finally, we calculated the proportion of time each bird spent foraging from each tray and also noted which tray was visited first. Videos were examined by one person (J.S.G.) who was blind to the position (left/right) of the low-salinity and high-salinity trays.

Statistical analyses

Data were analyzed using linear mixed models (package 'nlme') in R (Team 2013). In choice trials, the intake of the two diets may not be independent, so response ratios of individual birds were used as a measure of preference (Martin and Bateson 1983). Response ratios were calculated as:

$$\frac{\text{amount of high-salinity snails eaten}}{\text{amount of high-salinity snails eaten} + \text{amount of low-salinity snails eaten}}$$

If an individual only ate the high-salinity mud snails, its response ratio would be 1.0; conversely, if it only ate the low-salinity mud snails, its score would be 0.0. The chance level of response is 0.5. Linear mixed models were then performed on the response ratio to analyze whether individuals differentiated between, and showed any preference for, low-salinity or high-salinity diets. The responses ratio was also calculated for the time foraging in the two diets. The response ratio (proportional non-binomial data) was logit-transformed prior to analyses in order to fulfill linear assumptions (Warton and Hui 2010). Freshwater availability (access/no access) was included in the model as a fixed factor, salt gland scores were included as a covariate, and individual and session were included in the model as random factors. Body mass remained stable during the experiment (session effect: t_{49}= -0.517, P = 0.61; Supplementary Figure S3), so we did not consider it as a covariate. We always started with the full model and simplified it using backwards elimination based on ANOVA test with $P < 0.05$ as the selection criterion until reaching the minimal adequate model. Model assumptions were checked using the residuals of the final model. In addition, we used paired *t*-tests to test whether the mean difference in food consumption was significantly different between diets.

We performed linear regressions to explore relationships between food intake and foraging time (both for overall and diet-specific intakes). To test for potential diet-specific differences in food intake rate (g of mud snails min^{-1}), we also performed a linear mixed model with food intake rate as a response, diet and freshwater access as factors, and individual and session as random factors.

We further explore the relationship between response ratios and salt gland scores. To do this, we first considered all data pooled together and then separately pooled by sets on the basis of freshwater availability and time: 'access to freshwater' (sessions 1-2), 'no access to freshwater' (sessions 3-6) and 're-access to freshwater' (sessions 7-9). We distinguished between the two sets with access to freshwater as previous experience with saline treatments could affect osmoregulatory abilities (e.g. salt gland size; see below) and yield different outcomes (Gutiérrez et al. 2011). Potential differences in hematocrit and plasma ion concentration recorded at the end of each set of sessions (sessions 2, 6 and 9) were examined using repeated measures analyses with hematocrit or plasma osmolality as response, session as a fixed factor, and individual as a random effect.

One individual refused to eat during all the experimental sessions and was excluded from food preference analyses. In addition, six cases where another bird (always the same individual) refused to eat were excluded from these analyses.

RESULTS

Food intake was positively correlated with foraging time, both when considering overall intake and time ($F_{1,55}$ = 64.52, $P < 0.001$; Fig. 2a) and when they were pooled by diets (high salinity: $F_{1,55}$ = 78.69, $P < 0.001$; low salinity: $F_{1,55}$ = 104.80, $P < 0.001$; Fig. 2b). Diet salinity had no significant effect on food intake rate ($F_{1,56}$ = *2.61, P= 0.11*; see Fig. 2b), indicating that birds ate high-salinity and low-salinity prey just as fast.

Salt gland scores had significant effects in the response ratio of both food intake and foraging time (Table 1a, b; see also Fig. 3), whereas freshwater availability only marginally affected food intake (Table 1b; see also Fig. 3). Salt gland scores did not interact significantly with freshwater availability and thus their interaction was not included in the minimum adequate models (Table 1a, b).

When considering all data pooled, the relationship between food intake's response ratio and salt gland scores was not significant ($F_{1,55}$= 2.69, P = 0.107). Neither did we find any significant relationship between these traits for the periods of 'access to freshwater' ($F_{1,12}$= 0.68, P = 0.424) or 'no access to freshwater' ($F_{1,23}$ = 0.001, P = 0.98). However, food intake's response ratio and salt gland scores positively correlated during the 're-access to freshwater' period ($F_{1,16}$ = 15.77, P = 0.001), meaning that birds with higher salt gland scores showed a lower preference for high-salinity diet.

Hematocrit did not differ between experimental sets (access to freshwater = 52.08 ± 0.67%; no access to freshwater= 51.88 ± 0.86%; and re-access to freshwater = 50.56 ± 0.42; set effect: $F_{2,14}$ = 2.18, P = 0.15). Neither did we find any significant changes of plasma sodium concentration (access to freshwater = 155.36 ± 5.36 mmol L^{-1}; no access to freshwater = 161.36 ± 4.37 mmol L^{-1}; and re-access to freshwater = 156.41 ± 3.20 mmol L^{-1}; treatment effect: $F_{2,14}$ = 0.54, P = 0.59). In addition, we found no correlation between these two blood parameters and salt gland scores either when data were pooled or when data were analyzed for each of the sets separately (always $P > 0.45$).

DISCUSSION

Our study demonstrates that red knots prefer prey with relatively low salt content when their salt glands are small (following a prolonged access to freshwater) and when they have no access to freshwater. This preference is lost after they enlarge their salt glands (following a prolonged exposure to salty diet without access to freshwater) and regain access to freshwater. This finding is consistent with the notion that behavior is dependent upon an animal's state (Houston and McNamara 1999), and that foraging responses are context-specific (Hurly and Oseen 1999; Chatelain et al. 2013; Halpin et al. 2014).

Salt glands are the major organs for salt excretion in many birds and reptiles (Peaker and Linzell 1975), including red knots (Staaland 1967; Gutiérrez et al. 2012). There is ample evidence that their size and activity change over short time-spans (Peaker and Linzell 1975; Shuttleworth and Hildebrandt 1999; Hildebrandt 2001; Gutiérrez et al. 2015). These rapid and flexible changes in the salt glands could be correlated with short-term changes in behavior. The negative relationship between preference for low-salinity prey (i.e. food intake's response ratio) and salt gland scores observed at the end of the experiment indicates that birds became less selective; that is, birds with larger salt glands, and thus higher concentrating ability (Schmidt-Nielsen 1960; Staaland 1967), consumed more high-salinity prey than birds with smaller salt glands. However, as larger salt glands require larger maintenance costs, these should also increase with salt gland size (Gutiérrez et al. 2011). Why then did birds not minimize energy expenditure by choosing low-salinity prey under all conditions?

We can think of two explanations. On one hand, captive birds with nearly unlimited access to food and freshwater might have offset osmoregulatory costs with no difficulty. This would explain why birds of several species manifested indifference to saline solutions at low concentrations in two-bottle drinking preference tests conducted after unlimited access to freshwater (Harriman and Kare 1966; Harriman 1967). A second, non-exclusive, explanation is related to potential trade-offs with osmoregulation; that is, higher salt loads result in larger osmoregulatory costs but also more efficient salt glands, so eating high-salinity prey could protect individuals from short-term physiological costs under variable osmotic environments (Gutiérrez 2014). This could partly explain why some pelagic seabirds show preference for saltwater over freshwater (Harriman and Kare 1966). In any case, birds did not show signs of osmotic stress or dehydration during the experiment. Both the hematocrit and plasma sodium concentration values reported varied little and sit comfortably within the range of values expected for mollusk-eating captive red knots (Piersma et al. 2000; Gutiérrez et al. 2015).

Interestingly, although red knots are able to quickly develop a preference for the less salty diet when they had to (i.e., when they had small salt glands and no freshwater source), they normally continue probing the high-salinity diet (Supplementary Figure S4). This could be interpreted either as rapid forgetting of food information or as natural inclination to make strategic ‘mistakes’ to explore and verify whether alternative reward rules have come into fashion (Piersma et al. 1998). Because probe-feeding red knots mainly feed in tight flocks on notoriously variable and patchy intertidal flats (van Gils et al. 2005; van Gils et al. 2006), they are expected to share public information about resource quality (van Gils et al. 2006; Bijleveld et al. 2015) rather than to remember the precise locations within intermittently available patches.

These findings suggest that free-ranging animals experiencing varying salinities can use discriminatory behaviors to adjust salt intake. For instance, they may select among microenvironments differing, spatially or temporally, in osmotic characteristics. Where osmotic characteristics of food are spatially variable, food-selection and handling behaviors can contribute to osmoregulation by maximizing input of required water and/or minimizing salt intake (Mahoney and Jehl 1985; Nyström and Pehrsson 1988; Brischoux et al. 2013; Troup and Dutka 2014). Moreover, they may exploit diel differences in water potential, restricting activity (e.g. foraging) to times when temperature is lowest (Zwarts et al. 1990). Such behaviors could be especially important for many bird species, including the red knot, that spend the winter in (sub)tropical intertidal sites without regular access to freshwater (Wolff and Smit 1990; van de Kam et al. 2004). Likewise, coping with salt may become particularly severe for tropical marine snakes during (and following) periods of high oceanic salinity with very limited access to freshwater (Brischoux et al. 2012; Brischoux et al. 2013) as well as for other marine and estuarine reptiles (e.g. turtles and crocodiles) that rely on the extraction of osmotically-free water (*via* salt glands) from food items of relatively low salt content or on periodic access to fresh or brackish drinking water (Schmidt-Nielsen and Fange 1958; Taplin and Grigg 1981; Mazzotti and Dunson 1989; Cramp et al. 2008). Under these circumstances, behavioral osmoregulation may be crucial to maintaining osmotic balance.

In addition, salinity has important direct and indirect effects on habitat structure and predator-prey interactions in aquatic systems (Ysebaert et al. 2000; Herbst 2001; Ravenscroft and Beardall 2003; Ysebaert et al. 2003). Though not without cost, more salt-tolerant invertebrates and fish might escape potential predators by using areas of high salinity. One might expect that, if birds and other secondarily marine air-breathing vertebrates avoid areas of high salinity, potential prey would reduce predation risk in what effectively would be saline 'refuges'. In this vein, it has been suggested that red knots do not feed extensively on brine shrimps *Artemia* spp. at supratidal salinas (salinity: 100–150‰) to avoid of osmotic stress (Masero 2002). Thus, it is plausible that selection of saline refuges by euryhaline species enable individuals to better survive than individuals at lower salinities but higher predation risk. Ultimately, osmoregulatory costs may affect selection pressures acting on both predators and prey. Reduced salinity tolerance at high ambient temperatures has been reported in red knots feeding on mud snails (Gutiérrez et al. 2015); this could lead to selection for less salty prey in environments where osmoregulatory costs would increase substantially: in warm climates.

In summary, discrimination behaviors with respect salt intake are a function of ecological context and physiological state, meaning that the decisions that birds make when they are osmotically challenged will be different from when they have an efficient osmoregulatory machinery and/or access to freshwater. We suggest that under osmotically stressful environments dietary salt may act as discriminative stimuli for foraging responses in birds and other secondarily marine vertebrates such as snakes, turtles, birds and mammals. Studies investigating how foraging decisions change with salinity and temperature should help us understand how climate change could affect predator-prey dynamics and animal populations.

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Table 1. Statistics and coefficients of models for the response ratios of (a) food intake and (b) foraging time. Predictors included in the final model are in bold; values for excluded predictors refer to the step before their exclusion.

Response variable	Predictors	Coefficients	s.e.m.	*d.f.*	*t*-value	*P*-value
(a) Food intake	**intercept**	**-4.222**	**1.067**	**47**	**-3.958**	**0.0003**
	Freshwater access[a]	0.833	0.397	7	2.099	0.074
	Salt gland scores	**0.809**	**0.330**	**47**	**2.454**	**0.018**
	FW access x SGS	0.986	0.706	46	1.396	0.168
(b) Foraging time	**intercept**	**-4.590**	**1.329**	**47**	**-3.453**	**0.001**
	Freshwater access[a]	0.563	0.495	7	1.136	0.293
	Salt gland scores	**0.921**	**0.411**	**47**	**2.242**	**0.029**
	FW access x SGS	0.362	0.894	46	0.404	0.688

[a] Reference category is ‘no access’

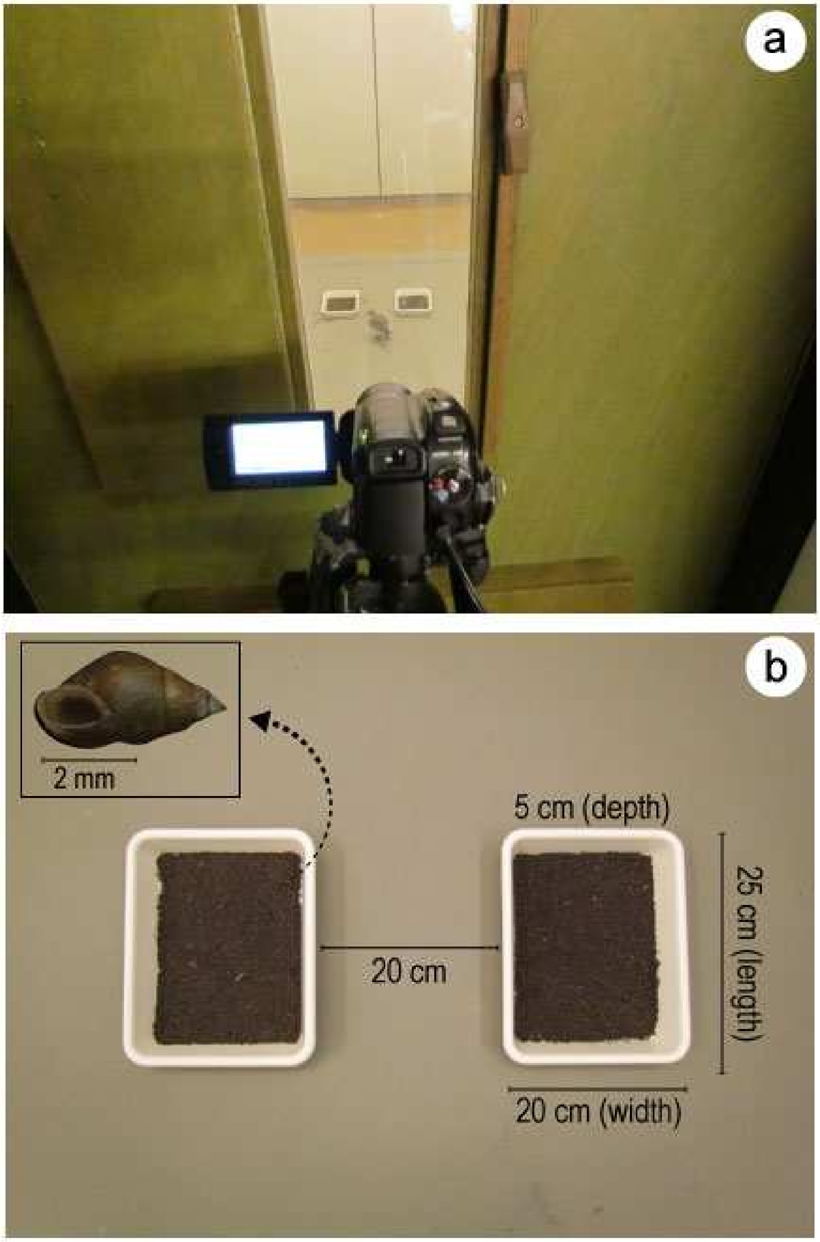
Legends to figures

Figure 1. The experimental arena. (a) Frontal view showing aviary, video camera and tray locations, and a focal bird; (b) plan view showing tray dimensions and offered food (inset box in top left corner shows a prey item under a zoom binocular microscope).

Figure 2. The relationships between (a) overall food intake and foraging time; and (b) between diet-specific food intake and foraging time. Note that individual data points refer to individual sessions and birds (all pooled) and thus show the between indidividual and experimental variation (see text for further details).

Figure 3. (a) The mean ± SE amount (in grams) of high- and low-salinity mud snails eaten by red knots during the nine 3-h experimental sessions; asterisks indicate significant differences between diets at each session (paired *t*-tests; $^{*}P<0.05$; $^{**}P<0.01$). (b) The response ratio for the same sessions; the horizontal dashed line depicts the chance level of response (0.5), so that values < 0.5 indicates preference for low-salinity diet and values > 0.5 indicate preference for high-salinity diet. (c) The salt gland scores during the experiment. The shaded areas depict access to freshwater prior to the experimental session. Note that each individual was given one session every other day (see text for further details).

554 **Figure 1**



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557 **Figure 2.**

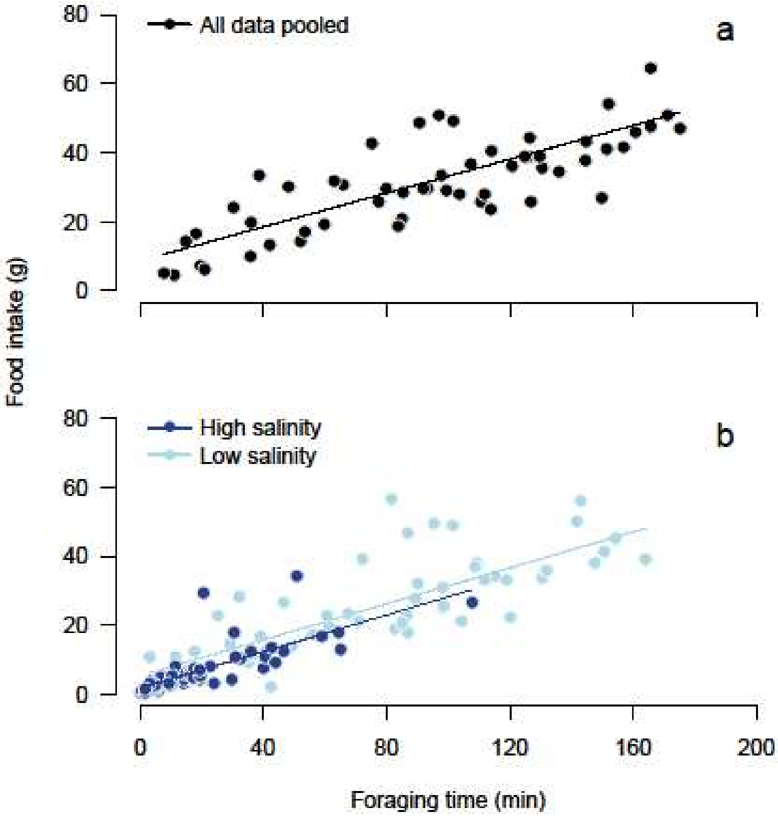

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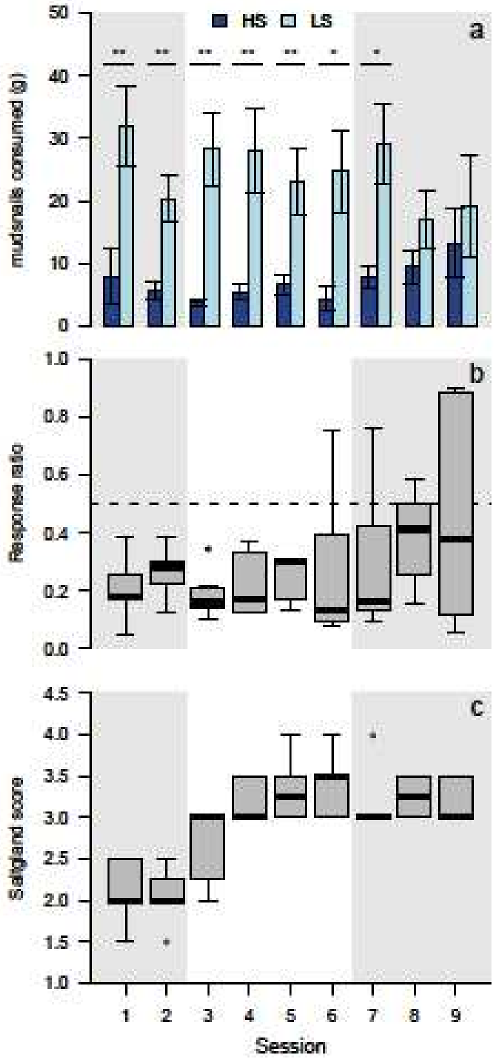
Figure 3.


HS
LS
a
mudsnails consumed (g)
50
40
30
20
10
0
** ** ** ** ** * *
b
Response ratio
1.0
0.8
0.6
0.4
0.2
0.0
c
Saltgland score
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
1
2
3
4
5
6
7
8
9
Session

Lay summary

Seawater is too salty for most land animals, but many marine birds and reptiles can cope with it owing to flexible cephalic “salt” glands that excrete excess salt from the bloodstream. We show that red knots without access to freshwater prefer prey with relatively low salt content when their salt glands are small, but this preference is lost after they enlarge their salt glands and regain access to freshwater.