



Does saline water consumption affect feeding and fuel deposition rate of a staging, long-distance migrating passerine?

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To accomplish their enduring journeys, migrating birds accumulate fuel consisting mainly of lipids in stopover sites located throughout their migration routes. Fuel deposition rate (FDR) is considered a key parameter determining the speed of migration and thereby bird fitness, and recent studies have demonstrated the positive effects of fresh water consumption on the FDR of migrating blackcaps *Sylvia atricapilla*. Sewage water reservoirs, characterized by higher water salinity than fresh water, were extensively built in different parts of the world and are used by birds during their travel, but their effects on wildlife and specifically on migrating birds have been largely overlooked thus far. We experimentally examined the effects of water salinity on blackcap FDR during migration. We captured birds in an autumn stopover site, transported them to the laboratory and provided them with fruits, mealworms and water of different salinity levels (0.3, 4.5 and 9‰ NaCl) for several days. We examined the effects of water salinity on the blackcaps' diet, water consumption and FDR and found that FDR was mainly affected by fruit consumption rate and not by the water salinity levels. Water salinity nevertheless caused elevated water consumption as the birds consumed almost 3 times more saline water than fresh water per consumed fruit mass. Our work is the first to explore the consequences of saline water consumption on migrating passerines, specifically suggesting that anthropogenic alterations of habitats by sewage water treatment facilities may modulate bird nutrition and diet.

Billions of migrating birds undertake enduring journeys that are usually intermittent and include stopovers in a variety of habitats. To complete their journey, migrants must deposit fuel consisting mainly of lipids before their departure. The birds' fuel stores are used during flight and when staging in areas devoid of food resources, like deserts (Moreau and Dolp 1970, Bairlein 1985, Schaub and Jenni 2000a, Rubolini et al. 2002). Since a certain amount of fuel is required for traveling, a high rate of fuel accumulation may allow the birds to more rapidly complete the journey. Hence, it has been argued that fuel deposition rate (hereafter FDR) determines the speed of migration, a key fitness component of migratory birds (Alerstam and Lindström 1990, Lindström and Alerstam 1992). FDR is calculated by determining the surplus of daily metabolized energy intake after considering the daily energy expenditure (Lindström 1991, Sapir et al. 2011). Consequently, FDR is affected by food availability (Schaub and Jenni 2000b, Johnson and Sherry 2001), competition (Moore and Yong 1991, Newton 2006), stopover duration (Moore and Yong 1991, Schaub et al. 2008), day length (Kvist and Lindström 2000) and ambient environmental conditions (Newton 2006).

An important environmental factor determining FDR is water availability (Sapir et al. 2004, Tsurim et al. 2008, Mizrahy et al. 2011). Body mass and FDR, as well as diet, are strongly affected by water availability in migrating blackcaps *Sylvia atricapilla*, and it has been proposed that digestion tract build-up is hampered in water deprived birds (Mizrahy et al. 2011). An important, though largely overlooked, factor that might also affect FDR is the bird's daily salt intake. High salt consumption may affect the body's osmolarity and may lead to often lethal dehydration (Sabat 2000, Bollinger et al. 2005). In addition, it may decrease the bird's immune response and might incur high energy costs due to intense kidney activity (Gutiérrez et al. 2013, Gutiérrez 2014). Consequently, birds employ different solutions to avoid excessive salt intake. Some birds, living in saline-rich environments, have been documented to choose prey with a lower salt content than other available prey (Nyström and Pehrsson 1988, Hughes and Winkler 1990). Other birds avoid drinking from saline water sources and instead acquire most of their water from relatively less saline food, or by putting special efforts into finding more diluted water sources (Sabat 2000). A different solution for avoiding high salt intake is employed

by several South American passerine species that breed in coastal habitats. These species undertake a short inland migration after the end of the breeding season, presumably to access fresh water sources (Sabat 2000). Thus, bird behavior, diet and seasonal habitat occupancy may be adjusted to avoid high salt intake.

To prevent dehydration, birds osmoregulate their body fluids by excreting salts (Shoemaker 1972, Sabat 2000, Bentley 2002). Extraction of concentrated salt fluids is performed by the kidneys. In addition, some bird species have special salt glands located on the birds' skull that excrete fluids containing very high salt concentrations (Holmes and Phillips 1985, Sabat 2000, Bentley 2002). Functional salt glands are known to occur in seabirds, shorebirds, some birds of prey and species that regularly live in saline and arid environments (Holmes and Phillips 1985, Sabat 2000, Gutiérrez 2014). Passerines do not have salt glands and consequently must osmoregulate their body using only their kidneys, which are much less effective in salt excretion relative to salt glands (Sabat 2000, Bentley 2002). The physiological and morphological attributes of the renal system and its response to high salt concentrations in birds have been described in a number of studies (Lavery and Wideman 1989, Casotti and Braun 2000, Bentley 2002). It is also known that osmoregulation is associated with a high metabolic cost (Nehls 1996, Gutiérrez 2014), and a few recent studies have specifically quantified this cost in birds. These studies have shown a substantial increase in the basal metabolic rate (BMR) of both shorebirds and passerines, with a 17% increase in the BMR of dunlins *Calidris alpina* (Gutiérrez et al. 2011) and a 30% increase in the BMR of rufous-collared sparrows *Zonotrichia capensis* (Peña-Villalobos et al. 2013).

Animals inhabiting arid and semi-arid regions are particularly prone to excessive salt intake because these environments are typically characterized by high salinity (Hannam et al. 2003), and because access to fresh water sources is usually limited in these areas due to their scarcity. The availability of sewage water facilities located in these regions may consequently offer an important source of water for wildlife (Fuller and Glue 1980, Hamilton et al. 2005, Murray and Hamilton 2010, Hsu et al. 2011, Orlowski 2013). Sewage water sources are nevertheless often saline. For example in Israel, the salinity of various water sources available to migrating birds, including sewage treatment facilities, is substantially higher than the salinity of fresh water (Fig. 1). Indeed, it is well known that some of the best places to find migrating birds in Israel, a global hotspot of bird migration (Shirihai 1996), are around sewage facilities where birds stop to drink and eat. One of the biggest concentrations of passerines in Israel is found in the bird park of Eilat, with an annual average of more than 2800 ringed blackcaps during 2008–2013 (Israel bird ringing center pers. comm.). In this location, the available drinking water sources are either seawater or a wastewater pond consisting of 4‰ NaCl saline water (T. Tzemah pers. comm.). Moreover, birds consume water from agriculture irrigation during their migratory staging throughout Israel. This water source is also mostly saline, since most of the water utilized for agriculture in Israel is reclaimed wastewater (Kalavrouziotis et al. 2013).

Although bird response to high (11 to 35‰ NaCl) water salinity has been documented in the past in different species

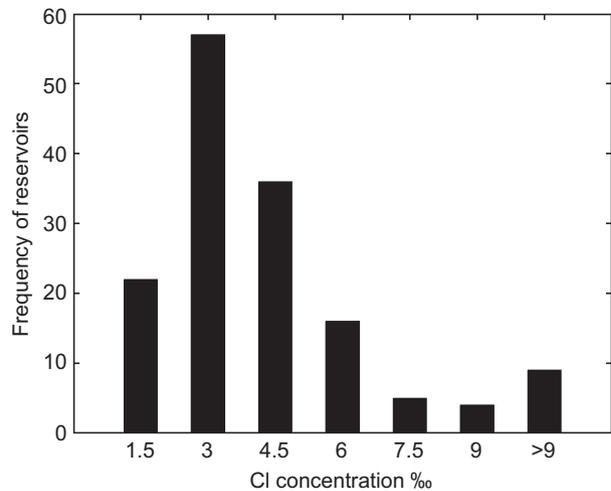


Figure 1. Frequency distribution of NaCl concentration in 149 open reservoirs in Israel that are available for water consumption by migrating birds (data obtained from an unpublished report of the Israeli Water Authority for the year 2008).

(Davies 1982, Sabat et al. 2004, Gutiérrez et al. 2011, Peña-Villalobos et al. 2013, Troup and Dutka 2014), no study thus far has investigated the consequences of saline water consumption on migrating passerines during stopover, using lower NaCl concentrations that passerine birds are likely to encounter during their staging (Fig. 1). Therefore, the goal of our current study was to experimentally examine the effects of saline water consumption on migrating blackcaps about to cross the Sahara Desert during autumn migration. We specifically examined the following research questions: 1) does water salinity affect food and water intake in migrating blackcaps? 2) Does water salinity affect the diet of migrating blackcaps? 3) Does water salinity affect FDR in migrating blackcaps?

We hypothesize that when consuming relatively low-salinity water (but still substantially higher than fresh water), FDR in birds will be reduced due to the high energy expenditure required for the excretion of excessive salt through the kidneys. Nevertheless, we hypothesize that food and water intake and bird diet will not be affected.

Material and methods

Study species and area

We studied the blackcap, a long distant migrant, abundant in Israel (Shirihai 1996) and many other parts of the western Palearctic region (Cramp and Simmons 1998). Blackcaps and the vast majority of the birds that pass through Israel in autumn subsequently cross the Sahara Desert and spend the winter in tropical Africa (Cramp and Simmons 1998). The species is known to feed on both fruits and arthropods (Cramp and Simmons 1998, Aymí et al. 2013), consuming a diet that consists about 70% fruits during autumn migration stopovers (data from a habitat similar to the trapping site of the present study, Sapir 2002). The latter work also reported a mean daily FDR of 4% during stopover through

five consecutive autumns for this species. During their passage through Israel in autumn, blackcaps weigh between 14 (relatively lean birds) and 28 (relatively fat birds) grams (Israel ringing center pers. comm.).

The present study was conducted during the autumn of 2013 in the eastern Galilee region of northeastern Israel (33°11'N, 35°55'E). Blackcaps were captured in a 0.4 ha area of open forest containing fruiting Mt Atlas mastic trees *Pistacia atlantica*. This site is located about 200 km north of the Negev Desert, which comprises the northern fringes of the 1800 km wide Sahara Desert. Consequently, after a single night of migration from the eastern Galilee stopover site, the birds enter a wide desert that they must cross to reach their over-wintering quarters.

Experimental design

We trapped birds using mist nets over five days at the end of September 2013. After trapping, each bird was marked with an individually numbered aluminum leg ring, its flat wing chord was measured, sex and age (first calendar year or older) were determined according to plumage, and body mass was determined to the nearest 0.1 g using a digital scale (sa-500, Satrue, Taiwan). Birds with body mass < 16 g were immediately released at the site of trapping, to avoid inclusion of birds with deteriorated body condition in the experiment. Captured birds were transported 8 km to the north east and kept in the facilities of the Northern Israel Regional Research and Development Center (33°09'N, 35°37'E). A daily light:dark cycle of 11.5:12.5, similar to regional, environmental conditions was maintained during this period, as was ambient temperature (mean ± SD: 20.4 ± 7.7°C). Each bird was kept in an individual cage (length × width × height of 28.5 × 21 × 30 cm) and was provided ad libitum with tap water, fresh mealworms *Tenebrio molitor* and *P. atlantica* fruits collected at the capture site. Mealworms contain 62% water, and their dry weight is comprised of 35% fat and 49% protein (Finke 2002). *Pistacia atlantica* fruits contain 35% water, and their dry weight is comprised of 53% fat and 10% protein (Sapir 2002). Every morning, between 8:00 and 10:00 am local time (UTC + 3), each bird was weighed, its food and water were replenished and its consumption was measured. We weighted 30 fruits and 30 mealworms and calculated their average weight, and then we calculated the daily food intake by subtracting the number of fruits and mealworms left from those given and multiplying this number by the average weight. The birds were kept in these conditions until they were able to maintain or increase their body mass for at least two consecutive days. Birds that lost any weight for more than two consecutive days or reached a weight of or below 16 g were excluded from the experiment and subsequently released at the trapping site.

All birds (n = 20, 11 males, 9 females) included in the experiment that lasted three days following the initial feeding period were randomly assigned to three experimental groups. The first group (hereafter termed control group; n = 6) was provided with tap water with a salinity of 0.3‰ NaCl (Israel water authority pers. comm.). The second group (hereafter termed 4.5 NaCl group; n = 7 birds) was provided with 4.5‰ NaCl water. The third group (hereafter termed

9 NaCl group; n = 7 birds) was provided with 9‰ NaCl water. In all three groups, three birds were female and all but one were first calendar year birds. Birds of all groups were provided with mealworms and *P. atlantica* fruits ad libitum throughout the experiment.

Every morning for the three days of the experiment, we measured each bird's body mass, the number of consumed fruits and mealworms and the mass of water consumed. To measure evaporation, every day three water containers were placed in the room where the birds were kept, one for each of the three NaCl concentrations. We calculated the birds' net water intake by weighting the water remaining in the bird's cage and accounting for evaporated water. A diet index (*DI*) for each bird was calculated daily by dividing fruit mass (*F*, in grams) by the sum of fruit mass and mealworm mass (*M*, in grams):

$$DI = \frac{F}{F + M} \quad (1.1)$$

such that a DI of one indicates 100% fruit consumption, and a DI of zero implies a mealworm-only diet.

Following Ellegren and Fransson (1992), we used the formula described in Sapir et al. (2004) to calculate lean body mass (LBM) for every individual according to its wing length. We then used LBM to calculate every bird's daily FDR (in %): a bird's current body mass (m_b) was subtracted from its body mass the previous day (m_{b-1}), and divided by LBM:

$$FDR = \frac{m_b - m_{b-1}}{LBM} \times 100 \quad (1.2)$$

In addition, we determined each bird's fuel load, a proxy of body condition (Lindström 1991, Ellegren and Fransson 1992), as the ratio between bird body mass (m_b) and LBM:

$$Fuel\ load = \frac{m_b}{LBM} \quad (1.3)$$

At the end of the experiment, before being released at the trapping area, the 4.5 NaCl group birds were provided with tap water for one day, and the 9 NaCl group birds were given 4.5‰ NaCl for one day and tap water for another day. The control group was not released at the end of the experiment, and were kept in captivity for one additional day for an experiment that is not described in this manuscript.

Data analysis

First, we analyzed the data to test for differences in accumulated FDR between the three treatment groups using one-way ANOVA. Accumulated FDR is the rate of fuel accumulation from the start to the end of the experiment, as opposed to the daily FDR. We also examined the correlation between accumulated FDR and bird fuel load at the start of the experiment using Pearson's correlation. The effects of consumed water, fruit and mealworm mass, bird fuel load (independent covariates) and treatment (independent categorical variable) on bird daily FDR (dependent variable) were analyzed using ANCOVA and multiple linear regression. Then, we tested for differences in daily FDR, water, DI and fruit and mealworm consumption between the three treatment groups, using one-way ANOVA and further

tested these relationships using ANCOVA, while including bird fuel load as an independent covariate. In agreement with previous studies (Sapir et al. 2004), we assumed that water consumption limits body mass gain through its effect on food intake. Therefore, using ANCOVAs, we examined the effects of consumed water mass (independent covariate) and treatment (independent categorical variable) on DI, consumed mealworms mass and consumed fruit mass (dependent variables). In cases where the treatment was found to be significant in the ANCOVAs, we also report the results of the regressions for each of the treatment groups. If water and fuel load were found to be significant in the ANCOVAs, we tested the effects of both independent covariates and the treatment (independent categorical variable) using ANCOVA. All analyses were made using SPSS ver. 20 (IBM, New York) and were two-tailed tested with a critical α of 0.05. Where relevant, results are reported by their mean \pm SE.

Results

Daily FDR and the mass of consumed water, mealworms and fruits are summarized in Table 1.

Daily and accumulated FDR

During the experiment, accumulated FDR was not affected by the treatment (one-way ANOVA, $F_{2,18} = 1.37$, $p = 0.281$) despite large differences in the mean of the three treatments (4.1, 5.2 and 11.2% for the control, 4.5 and 9 NaCl groups, respectively). In addition, no correlation was found between accumulated FDR and bird fuel load before the start of the experiment (Pearson's correlation, $p = 0.685$). Daily FDR was positively affected by the mass of consumed water, fruit and mealworms in all treatment groups, but not by the treatment and the birds' fuel load (ANCOVA; Table 2). A one-way ANOVA testing daily FDR in relation to treatment was similarly not significant ($F_{1,57} = 1.95$, $p = 0.15$). We then analyzed the data using multiple linear regressions (Table 3) and found that indeed daily FDR was affected by the mass of consumed mealworms (with the standardized model coefficient, $\beta = 0.212$), the mass of consumed water ($\beta = 0.321$), and the mass of consumed fruits ($\beta = 0.458$). The sum of all model coefficients ($0.212 + 0.321 + 0.458 = 0.991$) explains nearly all the variation in bird daily FDR, suggesting that fruit consumption contributes more than twice to bird FDR than mealworm consumption.

Consumed water, mealworms and fruits and the DI

The daily mass of consumed water was not affected by the treatment (one-way ANOVA, $F_{2,53} = 1.96$, $p = 0.15$) and

Table 1. Daily measurements of blackcaps during the experiment.

	Control group		4.5‰ NaCl group		9‰ NaCl group	
	Mean \pm SE	n	Mean \pm SE	n	Mean \pm SE	n
FDR (%)	1.36 \pm 1.03	18	1.47 \pm 0.89	19	3.73 \pm 0.98	21
Consumed water (g)	4.27 \pm 0.75	14	4.83 \pm 0.4	19	6.12 \pm 0.8	21
Consumed fruits (g)	4.83 \pm 0.63	18	4.23 \pm 0.32	19	5.92 \pm 0.37	21
Consumed mealworms (g)	0.66 \pm 0.082	18	0.67 \pm 0.069	19	0.75 \pm 0.066	21

Table 2. The results of an ANCOVA testing the effects of the treatment, bird fuel load and consumed water, mealworm and fruit (g d^{-1}) on bird daily FDR.

ANCOVA source	Sum of squares	DF	Mean square	F	p
Corrected model	553.831	6	92.305	8.955	<0.001
Intercept	3.986	1	3.986	0.387	0.537
Fuel load	3.274	1	3.274	0.318	0.576
Mealworm	47.607	1	47.607	4.619	0.037
Fruit	113.892	1	113.892	11.049	0.002
Water	46.961	1	46.961	4.556	0.038
Treatment	3.456	2	1.728	0.168	0.846
Error	484.449	47	10.307		
Total	1354.409	54			
Corrected total	1038.280	53			

Table 3. Results of multiple regression analysis of independent factors affecting bird daily FDR (%), dependent variable).

Model	Coefficients				
	Unstandardized coefficients		Standardized coefficients		
	B	Std. Error	β	t	p
(Constant)	-1.124	0.250		-4.501	<0.001
Water	0.073	0.032	0.311	2.290	0.026
Fruits	0.155	0.046	0.468	3.408	0.001
Mealworms	0.466	0.218	0.212	2.134	0.038

the same was found for the mass of consumed mealworm (one-way ANOVA, $F_{2,57} = 0.55$, $p = 0.58$). Yet, the daily mass of consumed fruits differed significantly (one-way ANOVA, $F_{2,57} = 3.80$, $p = 0.029$) between the treatments. Bonferroni post-hoc comparisons suggest that only the 4.5 and 9 NaCl groups differed significantly from each other in their overall daily fruit mass consumption ($p = 0.027$) while other groups did not ($p > 0.26$). We found neither an effect of the treatment ($p = 0.771$) nor of the interaction between the treatment and bird fuel load ($p = 0.836$) in ANCOVA, but did find a significant effect of bird fuel load ($p = 0.044$) on the mass of consumed fruits. In addition, no significant difference (one-way ANOVA, $F_{2,57} = 0.78$, $p = 0.46$) in the DI was found between the treatment groups (control group: 0.83 ± 0.032 , $n = 18$; 4.5 NaCl group: 0.86 ± 0.017 , $n = 19$; 9 NaCl group: 0.87 ± 0.017 , $n = 21$). We found no significant effects of fuel load or treatment on the DI, water or mealworm consumption ($p > 0.34$ in all cases, tested using ANCOVAs).

Relations between consumed water, consumed fruits and the treatment

We found a significant effect of consumed water mass ($p < 0.001$) and treatment ($p = 0.009$) on consumed fruit

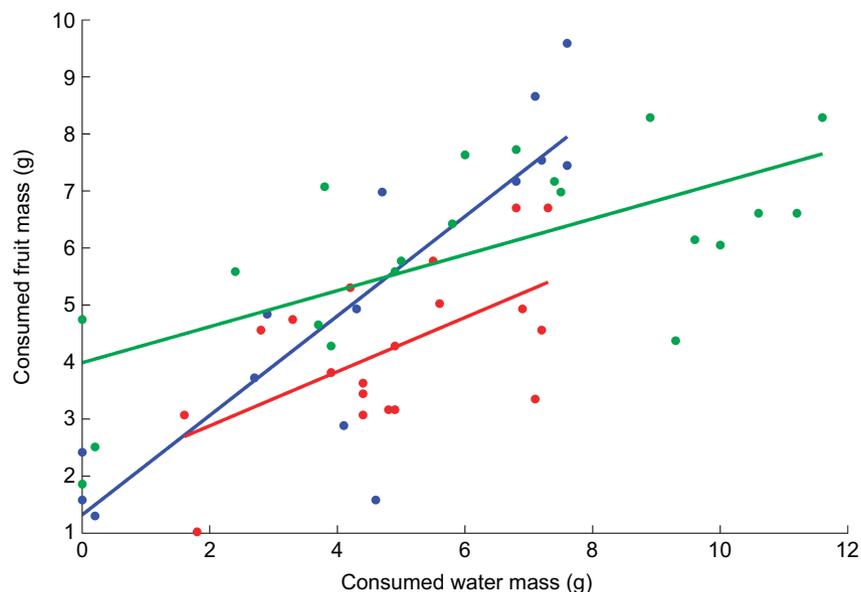


Figure 2. Daily consumption of *Pistacia atlantica* fruits in relation to water consumption in migrating blackcaps *Sylvia atricapilla*. Data are presented in relation to the three water salinity concentration treatment groups. Data and regression lines for the control group, 4.5‰ NaCl group and 9‰ NaCl group are depicted in blue, red and green respectively.

mass in an ANCOVA. Post-hoc comparisons indicated that only the control group differed from the 9 NaCl group ($p = 0.001$), while all other comparisons between the groups were not significant ($p > 0.077$). In addition, the interaction between consumed water mass and treatment was also significant ($p = 0.003$), suggesting that the relationship between water and fruit consumption differed between the treatment groups. Linear regressions performed separately for each treatment group showed a decrease in the regression slope with increasing salinity, with coefficients of 0.87, 0.48 and 0.32 for the control, 4.5 NaCl and 9 NaCl groups respectively. The coefficient of the 9 NaCl group was 2.7 times smaller than that of the control group (Fig. 2, Table 4). Using ANCOVA, we then found that the effects of consumed water mass, bird fuel load and the interaction between treatment and consumed water mass on fruit consumption were significant, but the treatment was not

(Table 4). Using an ANCOVA, we also found that consumed water, the treatment and their interaction had no significant effect on the mass of consumed mealworms (water: $F_{1,53} = 0.822$, $p = 0.369$; treatment: $F_{2,53} = 2.234$, $p = 0.118$; water and treatment interaction: $F_{2,53} = 1.941$, $p = 0.155$). We did find a positive effect (using ANCOVA) of consumed water mass on DI ($F_{1,53} = 10.045$, $p = 0.003$), but no significant effect of the treatment ($F_{2,53} = 2.336$, $p = 0.108$) or the interaction between the treatment and consumed water mass ($F_{2,53} = 2.766$, $p = 0.073$), likely due to the positive relationship between water and fruit consumption (see above). Consequently, the relationship between consumed water mass (WM, independent factor) and DI was significant (regression: $p < 0.001$, $R^2 = 0.58$, $DI = 0.1WM + 0.67$), suggesting that the relative proportion of fruits versus mealworms in the diet of blackcaps increased with increasing consumption of water.

Table 4. The results of an ANCOVA testing the effects of the treatment, bird fuel load and consumed water on bird daily fruit consumption ($g\ d^{-1}$) and regression analyses of the effects of water on fruit consumption in the three treatment groups.

ANCOVA source	Sum of squares	DF	Mean square	F	p
Corrected model	138.917	8	17.365	10.24	< 0.001
Intercept	3.733	1	3.733	2.201	0.145
Treatment	3.538	2	1.769	1.043	0.361
Fuel load	11.913	1	11.913	7.025	0.011
Water	46.797	1	46.797	27.597	< 0.001
Treatment \times Fuel load	2.971	2	1.486	0.876	0.423
Treatment \times Water	13.517	2	6.759	3.986	0.025
Error	76.307	45	1.696		
Total	1651.171	54			
Corrected total	215.224	53			

Treatment group	Intercept (95% CI)	Slope (95% CI)	R ²	p
Control	1.324 (-0.31–2.958)	0.872 (0.549–1.195)	0.742	< 0.001
4.5‰ NaCl	1.935 (0.249–3.621)	0.475 (0.145–0.804)	0.352	0.007
9‰ NaCl	3.991 (2.807–5.176)	0.315 (0.148–0.482)	0.451	0.001

Discussion

Birds experience exceedingly high mortality rates during migration, six times higher than during over-wintering and breeding periods in raptors (Klaassen et al. 2014) and fifteen times higher in passerines (Sillert and Holmes 2002). Large fat stores deposited at stopover sites may enable the birds a buffer against negative environmental conditions and reduce bird mortality, for example by allowing birds to reach their destination even under inclement weather and by extending the duration of fasting when food is unavailable. Habitat conditions may substantially modulate bird fat deposition during stopover (Newton 2006); specifically, water deprivation is known to have a strong and immediate effect on the diet and body condition of migrating passerines (Tsurim et al. 2008). Consequently, it may have a lasting effect on different properties of the migratory journey, such as migration timing and speed, the allocation of time and energy during migration and overall bird mortality (Klaassen 1996). Yet, the question whether migrating birds are affected not only by water availability but also by the properties of the consumed water during stopover has, until now remained unanswered.

In the present study we did not find negative effects of drinking water salinity on blackcap fuel load, but did find other, non-trivial consequences of saline water consumption. The present study is the first to show that in addition to water availability, its salinity may influence bird diet. We found that, in general agreement with results from previous field studies (Sapir et al. 2004), and given a diet of *P. atlantica* fruits and mealworms similar to that recorded in the field, fruits comprised most of the birds' diet. Our results indicate that fruit consumption was affected by water salinity level, but only when disregarding the initial body condition of the birds, represented by their fuel load. These results suggest that body condition is a key factor explaining fruit consumption in blackcaps, with leaner individuals consuming less fruits. In addition, our results indicate that consumed fruit mass during stopover has a positive effect on bird FDR and moreover suggest that water salinity modifies the relationships between fruit and water consumption in migrating blackcaps. The birds ingested more water per fruit when water salinity was high, and this could lead to excessive salt intake. High salt intake is known to induce dehydration that can be lethal, and the consequences of a non-lethal dehydration may still be severe and include reduced bird feeding and lipid deposition, ultimately lowering bird survival (Gutiérrez et al. 2011). Yet, our results suggest that FDR was not affected by the treatment, which means that either these modest salinity levels do not negatively affect the birds, or the birds were able to overcome elevated salt intake, possibly through elevated salt excretion.

Migratory passerines that stop-over in the Middle East and other arid areas found along major migratory flyways might be prone to saline water consumption due to salt concentration in sewage water resources (Fig. 1) and the scarcity of fresh water sources in semi-arid and arid environments (Sapir et al. 2004). The salinity levels of open water sources in Israel that are available to migrating birds (Fig. 1) are similar to those used in our experiment, suggesting a likely similar effect on the migrating blackcaps. Although previous

studies have shown that migrating blackcaps rely on water sources in their journeys (Sapir et al. 2004, Tsurim et al. 2008, Mizrahy et al. 2011), and that excessive salt intake may induce behavioral changes and metabolic costs in birds (Gutiérrez et al. 2011, Peña-Villalobos et al. 2013, Troup and Dutka 2014), no integration between these two lines of research has been made in the context of bird migration thus far. Our findings suggest that such integration is vital for enhanced understanding of key environmental factors affecting bird eco-physiology during migratory stopover. We nevertheless limit our inferences in accordance with the timescale over which the experiment was performed. A longer duration of future experiments may help decipher if high salt intake causes harmful dehydration, or alternatively whether blackcaps are capable of handling even higher salt intake through increased kidney performance. The latter process is nonetheless likely to induce high metabolic costs (Peña-Villalobos et al. 2013), which may have a negative impact on bird fitness.

Our conclusions may not necessarily apply to other species and may be limited to the specific ecological circumstances, specifically water scarcity, prevailing in the study area in autumn. In addition, higher temperatures than the rather moderate temperatures recorded during the course of the experiment may induce higher water loss, thus possibly modulating bird sensitivity to changing water salinity (Gutiérrez et al. 2015). Knowledge from previous studies (Sapir et al. 2004) suggests that not all bird species and not even all *Sylvia* warbler species are influenced by water availability to the same extent as the blackcap. Inter-specific differences in bird response to key environmental factors are worth exploring, in an attempt to understand what has led to these diverging evolutionary pathways in animal response to water availability during migration. It would also be valuable to explore bird response to water salinity over a wider range of salt concentrations, to test if certain threshold levels of water salinity induce specific and identifiable consequences such as reduction of feeding and elevated metabolism. This information may help in management of sewage water treatment practices where reduction of reservoir salinity would have direct conservation implications (Wikelski and Cooke 2006).

Our work dealt with several aspects of passerine stopover ecology under controlled conditions using manipulation of water salinity concentrations. As the first study to deal with this topic in migrating passerines, we were able to demonstrate that blackcaps apparently do not respond strongly to excessive salt intake under rather modest salinity conditions. In almost all cases, the birds were able to maintain an increasing FDR, when ingesting 9‰ saline water, although the short duration of the experiment limited inferences regarding longer term consequences. Interestingly, our results suggest a difference in the overall mass of consumed fruits only between the high and medium salinity levels (9 and 4.5 NaCl), possibly suggesting non-linear effects of saline water consumption on fruit consumption. Yet, since the majority of open water reservoirs in Israel are characterized by salinity levels that are lower than 9‰ (Fig. 1), these water sources do not seem to be a risk to passerines that drink water from them. Instead, they apparently provide an essential resource for birds that are about to cross a wide ecological barrier and

that stage in a semi-arid environment, likely enhancing bird fitness through their positive effect on migrant survival.

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