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HAL Id: hal-01076080

https://hal.science/hal-01076080

Submitted on 1 Jun 2021

THE COMPANY OF
Biologists

RESEARCH ARTICLE

Impacts of extreme climatic events on the energetics of long-lived vertebrates: the case of the greater flamingo facing cold spells in the Camargue

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ABSTRACT

Most studies analyzing the effects of global warming on wild populations focus on gradual temperature changes, yet it is also important to understand the impact of extreme climatic events. Here we studied the effect of two cold spells (January 1985 and February 2012) on the energetics of greater flamingos (*Phoenicopterus roseus*) in the Camargue (southern France). To understand the cause of observed flamingo mass mortalities, we first assessed the energy stores of flamingos found dead in February 2012, and compared them with those found in other bird species exposed to cold spells and/or fasting. Second, we evaluated the monthly energy requirements of flamingos across 1980–2012 using the mechanistic model Niche Mapper™. Our results show that the body lipids of flamingos found dead in 2012 corresponded to 2.6±0.3% of total body mass, which is close to results found in woodcocks (*Scolopax rusticola*) that died from starvation during a cold spell (1.7±0.1%), and much lower than in woodcocks which were fed throughout this same cold spell (13.0±2%). Further, Niche Mapper™ predicted that flamingo energy requirements were highest (+6–7%) during the 1985 and 2012 cold spells compared with 'normal' winters. This increase was primarily driven by cold air temperatures. Overall, our findings strongly suggest that flamingos starved to death during both cold spells. This study demonstrates the relevance of using mechanistic energetics modelling and body condition analyses to understand and predict the impact of extreme climatic events on animal energy balance and winter survival probabilities.

KEY WORDS: Bioenergetics, Energy requirements, Energy stores, Fasting, Body condition, Niche Mapper™, Mechanistic modelling, Winter stress

INTRODUCTION

Climate change is the second most important driver of biodiversity change after human land use (Sala et al., 2000). Strong effects of climate on species dynamics have been highlighted, such as shifts in geographic range (McCarty, 2001), changes in food web structure (Petchey et al., 1999), changes in population life-history features (Forchhammer et al., 2001) and fluctuations in population patterns (Birkhofer et al., 2012; Duriez et al., 2012). Studies on the effects of climate change on species dynamics primarily focus on consequences of gradual increase in temperature (Britton et al., 2010; Moses et al., 2012). However, climatologists also predict an increase in the frequency, intensity and duration of extreme climatic events (IPCC, 2011; Rahmstorf and Coumou, 2011). Extreme climatic events are often ignored as potential drivers of population dynamics, although they can have dramatic impacts on wild populations. For instance, population crashes due to severe heat waves have been reported in terrestrial and aquatic ecosystems (Bailey, 1955; Welbergen et al., 2008; McKechnie and Wolf, 2010). Moreover, although many regions undergo warming during winter (e.g. northwestern North America and Europe), the number of cold spell days has been high for several decades (Vavrus et al., 2006), substantially impacting animal populations (Chan et al., 2005; Péron et al., 2011).

Most studies forecasting consequences of climate change on species dynamics are correlative and ignore underlying physiological mechanisms (Pearson and Dawson, 2003; Dormann, 2007). Yet, studying the energetic constraints of species facing extreme or gradual climatic changes can help predict impacts on population dynamics (Chown et al., 2010; Bozinovic et al., 2011). Moreover, forecasting animal energetics as a function of climate also helps predict species range shifts (Kearney and Porter, 2009; Stillman and Goss-Custard, 2010).

During a cold spell, non-hibernating and non-torpid endotherms are confronted with increasing energy requirements to enhance thermogenesis and maintain homeostasis while food is scarce or even unavailable (Boos et al., 2007). Animals may therefore undergo a fasting period leading to death (Davidson and Evans, 1982). Fasting is characterized by three phases. During phase I, the organism uses energy from its glycogen reserves (glycogenesis). Phase II corresponds to a prolonged period of protein sparing and preferential mobilization of fat stores (lipolysis). Finally, the fasting animal enters phase III, which is characterized by an increase in net protein catabolism and an increase in body mass loss (proteolysis) (Robin et al., 1988). Phase III is usually lethal (Cherel et al., 1988).

The physiological impacts of cold spells on organisms may be studied through a joint evaluation of animal body condition (energy reserves) and energy requirements. Body condition can be inferred from lipid and protein contents in different tissues to determine in which fasting phase the animal died (Owen and Cook, 1977). For instance, an animal dead in phase II had remaining energy reserves and probably died from hypothermia. Conversely, an animal that died in phase III likely died from starvation (Robin et al., 1999).

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The Journal of Experimental Biology

Daily energy expenditure can be determined either by direct empirical measures (i.e. doubly labelled water technique and/or heart rate recordings) or by indirect methods such as mechanistic modelling (Porter et al., 2000a; Fort et al., 2009; Fort et al., 2011). Mechanistic modelling assesses the theoretical energy requirements of animals through calculations based on the fundamental principles of thermodynamics (Kearney and Porter, 2004; Kearney et al., 2009). Such thermodynamic equations allow the assessment of energy expenditure with no or limited need for time-consuming observations or manipulations (Fort et al., 2011). Porter and colleagues developed a thermodynamic model, Niche Mapper[TM] (Porter and Mitchell, 2006), which has been validated for a large range of species (Porter et al., 2000b; Porter et al., 2002; Kearney and Porter, 2004; Kearney et al., 2009). Niche Mapper[TM] computes heat and water mass balances of a model individual with user-defined morphological, physiological and behavioural properties to estimate energetic requirements and thereby self-maintenance and survival in a given environment (see Porter et al., 2000b; Porter et al., 2002). Here, we focus on the greater flamingo (*Phoenicopterus roseus* Pallas 1811; hereafter 'flamingo'), a long-lived bird emblematic of Mediterranean wetlands. Flamingos are filter feeders (Jenkin, 1957) foraging on a diversified diet including aquatic invertebrates and seeds (Deville et al., 2013; Yohannes et al., 2013). The commercial saltpans of Salin-de-Giraud in the Camargue (southern France) are one of the most important flamingo breeding sites in the world, with an average of 10,500 pairs (Johnson and Cézilly, 2007). In autumn, approximately half of all individuals present in summer migrate to distant wintering areas across the western Mediterranean and North Africa, whereas others spend the winter near the breeding colony (Barbraud et al., 2003; Johnson and Cézilly, 2007). Survival rates for both strategies depend on experience and environmental factors. Young birds wintering close to or at medium distances from their birth site survive better than those wintering further away, whereas adults survive better in the southernmost wintering areas (Sanz-Aguilar et al., 2012). However, these survival trade-offs were strongly modified during a cold spell in the Camargue in January 1985 (Sanz-Aguilar et al., 2012) that caused the death of more than 3000 flamingos (Johnson et al., 1991). In February 2012, another cold spell caused the death of more than 1500 flamingos (A. Béchet, unpublished data). In 1985, the cold spell lasted three more days and reached lower temperatures compared with February 2012. During both cold spells, most of the ponds were nearly completely frozen over, preventing the birds from accessing food.

Our objective was to determine the causes of flamingo mass mortality from the Camargue during these two cold spells. To this end, we examined the morphometrics of birds that died during these two events, determined the body condition of birds that died during the 2012 cold spell, and used the mechanistic model Niche Mapper[TM] (Porter and Mitchell, 2006) to estimate the monthly energy requirements of flamingos across the 1980–2012 period.

RESULTS

Two cold spells were identified during the 1980–2012 study (January 1985 and February 2012). The lowest temperatures were −10.6°C in 1985 and −6.6°C in 2012 (Fig. 1).

In 1985, the sex ratio of dead birds was biased towards males (number of dead males/overall number of dead flamingos=0.63; χ^2=113.13, d.f.=1, $P<0.005$, collected dead birds were 670 females and 1120 males). In 2012 the sex ratio was equal to 0.43, so significantly biased towards females (χ^2=13.6, d.f.=1, P=0.0002, collected dead birds were 133 females and 79 males).
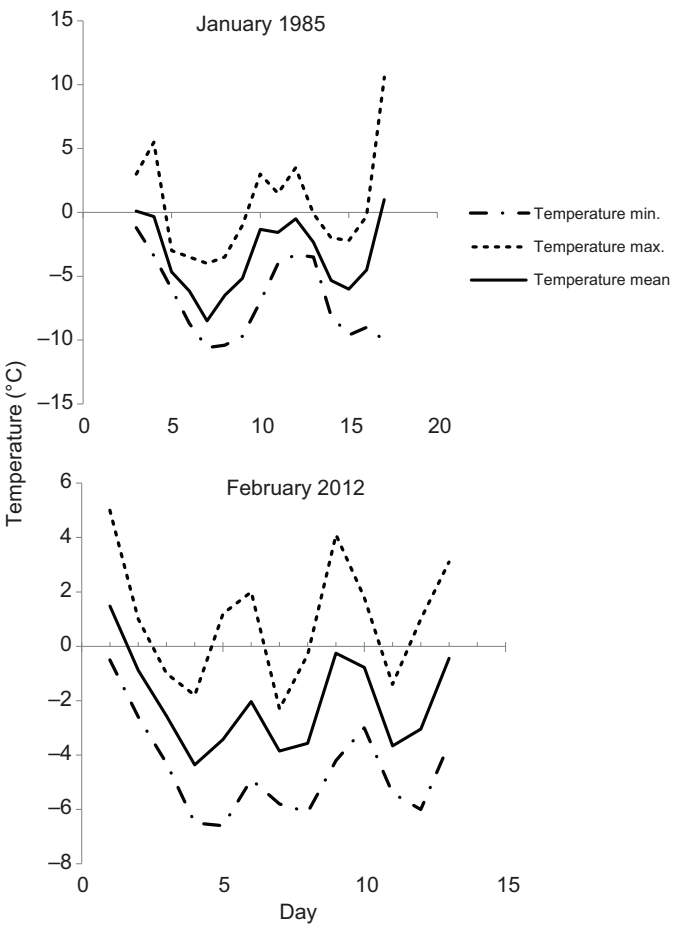

Fig. 1. Air temperatures during the January 1985 and February 2012 cold spells. Source: Tour du Valat/Meteo France station, based on hourly recordings.

Flamingo body condition

The body composition of six birds collected during the 2012 cold spell is summarized in Table 1. Average lipid proportion was 2.6±0.3% of total body mass (with 1.7±0.2% lipid for the carcass, 0.7±0.1% for the skin, 0.2±0.01% for legs and pectoral muscles, 0.003±0.0004% for abdominal fat pads and 0.003±0.001% for

Table 1. Lipid and protein stores for six flamingos found dead in the cold spell of February 2012

	Mean (g)
Intact body mass	2056±197
Plucked body mass	1812±177
Body mass	1766±188
Protein	
Total protein mass	314.0±39.1
Pectoral	26.0±2.5
Leg	30.1±5.4
Skin	32.0±4.0
Carcass	225.9±29.2
Lipid	
Total lipid mass	45.9±8.7
Pectoral + legs	3.8±0.4
Skin	11.8±3.2
Carcass	30.2±5.4
Digestive tract fat pads	0.06±0.03
Abdominal fat pads	0.05±0.009

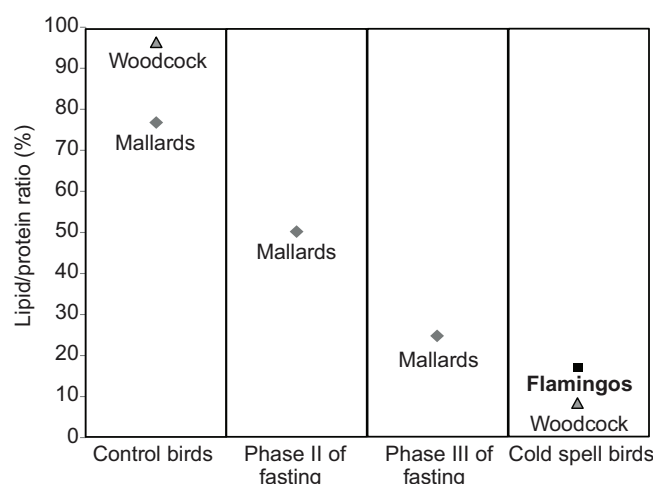Body mass, intact body mass minus feathers and digestive tract content.

Fig. 2. Comparison of lipid/protein ratios for mallards in different phases of fasting [fed, in phase II or in phase III (Robin et al., 1999)], woodcocks [fed or dead during a cold spell (Péron et al., 2011)] and flamingos that died during the February 2012 cold spell.

digestive tract fat pads). Protein accounted for 17±0.2% of the total body mass. The lipid/protein ratio averaged 0.11±0.74. When compared with those found for mallards (*Anas platyrhynchos*) and woodcocks in different phases of fasting, this ratio suggests that flamingos were beyond phase II of fasting (Fig. 2). Leg muscle proteins and pectoral muscles accounted for 1.7±0.3% and 1.5±1.1%, respectively, of the total protein mass.

Further, the residuals of an RMA regression calculated with body parameters of flamingos in good condition [log body mass=1.85×log tarsus length−5.23 (R^2=0.85, P<0.001)] allowed us to compare the body condition of the birds. Residuals were significantly smaller for a sample of 152 adult individuals randomly picked among the 2700 flamingos collected during the 1985 cold spell compared with the 142 adult flamingos collected during the 2012 cold spell (Wilcoxon, W=15170.5, P<0.001). Indeed, the difference from the weight reference was 1.6 times higher for birds that died in 1985 than for birds that died in 2012. Also, flamingos that died in 1985 (Wilcoxon, W=4786, P<0.001) and in 2012 (Wilcoxon, W=3909.5,
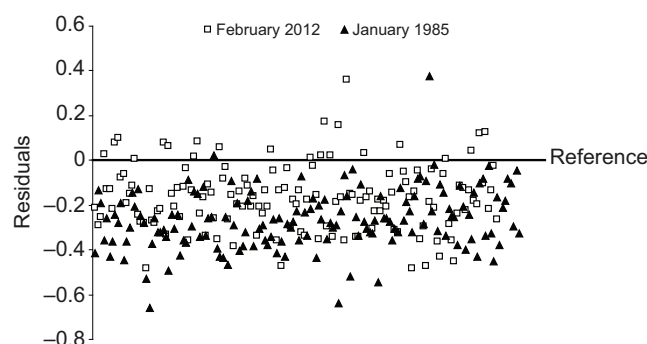

Fig. 3. Comparison of Camargue flamingo body conditions for birds that died during/outside cold spells. The average of the residuals of the RMA regression between log body mass and log tarsus length (see text for details) for 34 flamingos in good condition that died outside cold spells is represented by the horizontal line (reference birds). Triangles are residuals for flamingos that died during the January 2015 cold spell along the RMA regression for reference birds; squares are residuals for flamingos that died during the February 2012 cold spell along the RMA regression for reference birds.

P<0.001) had a significantly lower body condition than flamingos in good condition used as reference flamingos (Fig. 3).

Niche Mapper[TM] modelling

Flamingo monthly energy requirements showed substantial within-year, between-year and intersexual variability (Fig. 4). Overall, energy requirements were lowest in July and greatest in January for both sexes. Over the whole study period, excluding cold spells, males had a mean energy requirement of 2170 kJ day^{-1}, s.d.=235. Outside cold spells, their highest energy expenditure was 2575 kJ day^{-1} in December 1980 and their lowest was 1720 kJ day^{-1} in July 2006. Females had significantly lower energy requirements than males (mean excluding cold spells=1785 kJ day^{-1}, s.d.=208; t=3.18, P<0.001). Outside cold spells, their highest energy expenditure was 2143 kJ day^{-1} in December 1980 and their lowest was 1387 kJ day^{-1} in July 2006. Allometric equations estimated average flamingo energy requirements at 2763 kJ day^{-1} for adult males and 2178 kJ day^{-1} for adult females. These values are higher than those provided by Niche Mapper[TM] (+23.2% for males and +22.0% for females).

Both cold spells caused a peak in energy requirements for both sexes, with values higher than at any time during 1980–2012 (Fig. 4). For males, energy requirements reached a maximum in January 1985 (2639 kJ day^{-1}) and in February 2012 (2582 kJ day^{-1}). This corresponded to an increase of 6.5% and 5.7%, respectively, compared with the mean values for the same months outside cold spells. Female energy requirements also reached a maximum in January 1985 (2201 kJ day^{-1}) and in February 2012 (2151 kJ day^{-1}). This corresponded to an increase of 7% and 6.1%, respectively, compared with the mean values for the same months without considering cold spells. Our sensitivity analysis showed that plumage depth, body dimensions, body temperature and air temperatures were key factors influencing flamingo energy requirements (supplementary material Tables S3, S4). A 10% variation of these parameters led to a variation of energy requirements greater than 1% compared with the energy requirements of an 'average' adult. These parameters had the same effect across sexes (supplementary material Table S3).

DISCUSSION

Our study provides, to the best of our knowledge, the first estimations of monthly energy requirements in a vertebrate species across a >30 year period, and the first body condition measurements of flamingos exposed to cold spells. Our results strongly suggest that flamingo mass-mortality events occurring during cold spells are caused by starvation. Specifically, body composition of flamingos that died in February 2012 revealed that birds were extremely lean, with energy stores lower than those of mallards in phase III of fasting and close to those of woodcocks that died from starvation during a cold spell (Fig. 2). Further, our results showed maximum energy requirements during both cold spells for male and female flamingos (Fig. 4). Therefore, slightly higher levels of energy requirements and harsher climatic conditions observed in January 1985 compared with February 2012 (lower temperatures and a cold spell lasting three more days; Fig. 1) probably explain the higher mortality in January 1985. Finally, our results indicate that, on average, flamingos are close to their energetic limit, suggesting that relatively small decreases in temperature are causing disproportionately large increases in mortality.

Methodological aspects

Allometric equations predicted 24.5% higher energy requirements than those calculated with Niche Mapper[TM]. Similarly, Fort and
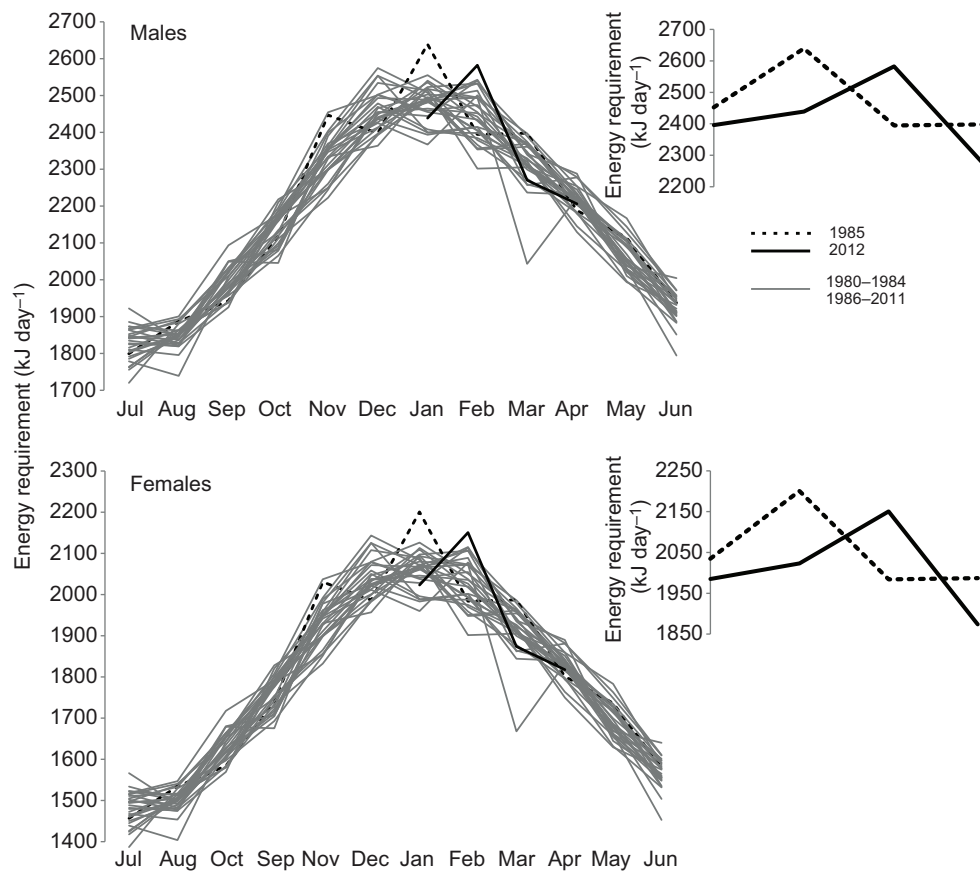
Fig. 4. Monthly energy requirements predicted by the model Niche Mapper™ for female and male flamingos living in the Camargue from January 1980 to April 2012. The insets show the energy requirements in January 1985 and February 2012.

colleagues (Fort et al., 2011) showed that allometric equations overestimated energy requirements by almost 21% in great cormorants (*Phalacrocorax carbo*). They found that allometric equations did not perform well because they were not species specific, and only used body mass as an input value (Fort et al., 2011). In contrast, they showed that mechanistic, thermodynamic modelling provides accurate and reliable estimates of energy requirements, which were validated using the doubly labelled water method (Fort et al., 2011). Therefore, although Niche Mapper™ outputs have not yet been validated using the doubly labelled water method in our case, they provide the first credible estimates of the temporal variations of flamingo energy requirements over extended time periods.

Because of our limited sample size we could not explore inter-sexual differences in terms of body composition, which may be linked to the fact that male flamingos are 20% larger than females. Although all six birds were found to be extremely lean, including the one male, we therefore cannot completely exclude that males generally may have had slightly more body reserves than females. However, our results show that cold spells did not systematically kill more females than males, with the sex ratio of dead birds being skewed towards males in 1985 and towards females in 2012.

What kills flamingos during cold spells?
Our results suggest that the primary cause of flamingo death during cold spells was prolonged fasting. Indeed, their body masses were significantly lower than those of flamingos in good body condition. Further, their body condition was poorer than that of mallards in phase III of fasting. Finally, their body condition and their ratio of pectoral protein to leg protein were close to those of woodcocks found dead during a cold spell where fasting was the primary cause of death (Robin et al., 1999). Specifically, the ratio of leg protein to pectoral

protein (1.2) is close to that found for woodcocks that died during a cold spell (1.8), showing that the pectorals were the most depleted muscles and substantially contributed to total protein depletion. However, we cannot exclude the possibility that birds died from causes other than starvation, including disease. Unfortunately, during the 1985 cold spell, none of the bird carcasses were kept and subjected to detailed autopsies and screening for parasites and pathogens. During the 2012 mass-mortality event, 30 birds were dissected. They did not display abnormal parasite loads or any internal damage. Sampled tissues are currently being analyzed.

Overall, cold temperatures probably accelerated the process leading to death by increasing flamingo energy requirements. When confronted with very low temperatures, homeotherms must use more energy to maintain a constant body temperature. During both cold spells, the water surface of most ponds of the Camargue froze, preventing flamingos from accessing their invertebrate prey, whose own activity and availability might also have been reduced by low temperatures. As flamingos were unable to maintain body reserves, they probably also could not maintain their homeothermic status. Energy stores and then body temperature thus decreased until death.

Birds died during the cold spells, and not afterwards. Hence, cold temperatures likely accelerated energy store usage, especially during the cold spell of January 1985, when lower temperatures were reached for a longer period. Consequently, one can speculate that the factor ultimately controlling winter survival was not ambient temperature, but rather food availability. This situation mirrors polar studies analysing the survival strategies of homeothermic animals confronted with harsh environmental conditions, which also stressed the importance of food availability (Grémillet et al., 1999; Grémillet et al., 2001; Mathewson and Porter 2013). Further, the timing of the cold spell might also be important, with cold spells occurring later in

winter predicted to kill more birds than earlier in this season because of depleted energy stores. Along these lines, one might speculate that the February 2012 cold spell was severe because of its timing (Fig. 4). Also, one might conclude that the 1985 cold spell might have killed even more flamingos if it had occurred in February.

Further studies are required to understand why flamingos are particularly susceptible to cold spells compared with other waterbird species such as ducks. Cold spells also kill ducks (Newton, 2007), but duck carcasses are less conspicuous, which may lead to an underestimation of duck mortality (Pain, 1991). Nevertheless, ducks also show more plasticity in their management of body reserves (Lovvorn, 1994) and in their migratory behaviour (Ridgill and Fox, 1990), which may enhance winter survival probabilities.

Population and migratory implications

Cold spells kill adult individuals within a population, and therefore have profound impacts on population dynamics. This is the case for flamingos in the Camargue (Johnson et al., 1991) and for numerous other vertebrate species. For instance, a 1996 cold spell killed thousands of cliff swallows (*Petrochelidon pyrrhonota*), reducing their population by approximately 53% in southwestern Nebraska (Brown and Brown, 1998). Furthermore, severe winters significantly reduced the survival probabilities of woodcocks wintering in France (Péron et al., 2011), and a Svalbard reindeer population (*Rangifer tarandus*) underwent an 80% reduction in population size during an extreme icing event in 1993–1994 (Chan et al., 2005).

Beyond acute mortality, adverse environmental conditions such as cold spells have carryover effects on wild populations. Those effects are defined as non-fatal impacts on individuals during one period of the annual cycle, which influence breeding success and survival later in life (Webster et al., 2002; Runge and Marra, 2005). In flamingos, the 2012 cold spell may explain delayed laying during the following breeding season (which started ca. 3 weeks later; A. Béchet, unpublished data). Also, as a long-lived species, flamingos must face numerous unpredictable winters. As mentioned in the Introduction, Camargue flamingos are partial migrants with a varying proportion of migratory and resident individuals. The forecasted increase in extreme climatic events (Walsh et al., 2001; Tank et al., 2002; Cellitti et al., 2006; Vavrus et al., 2006) may have consequences for this trade-off, as it may enhance the probability of winter mass-mortality for 'resident' individuals. Forthcoming migratory studies will show whether flamingos have the capacity to migrate in order to escape cold spells. The fattest individuals, who have sufficient energy stores to migrate over thousands of kilometres, may leave immediately at the onset of the cold spell.

Other, leaner individuals may, on the contrary, adopt a wait-and-see strategy, expecting a short cold spell that may not cause mortality, after which they could refuel body stores sufficiently to either stand the entire winter or initiate migration. In this context, flamingos may have a predetermined fuel-depletion threshold, as do many migratory waders. For instance, in knots (*Calidris canutus*) undergoing a fasting period, a refeeding signal triggers active food searching at the onset of the last phase of energy catabolism [i.e. when lipid reserves are almost exhausted and when protein catabolism increases (Piersma and Poot, 1993)]. A similar mechanism also exists in King penguins (*Aptenodytes patagonicus*) (Groscolas et al., 2000).

To conclude, our case study has shown that accurate determination of both the amount of body energy reserves and energy requirements is essential to improve our understanding of animals energy balance facing climatic changes, including extreme climatic events. Such a complementary approach is key to future studies predicting the effects of harsh weather conditions on the mortality and population dynamics of wild populations.

MATERIALS AND METHODS
Study period and data collection

We defined a cold spell as a period of at least six consecutive days with daily mean temperatures below the 10th percentile of the temperature distribution in the Camargue for each calendar day across the 1980–2012 period (Tank et al., 2002). Only two cold spells were identified (Fig. 1): the first lasted 15 days in 1985 (between 3 and 17 January) and the second 13 days in 2012 (between 1 and 13 February). Approximately 3000 flamingos died in January 1985, of which 2700 were collected. In February 2012, approximately 1500 flamingos died, of which 233 were collected. A set of basic morphological measurements was performed on each collected individual (Appendix 1). Birds were sexed by cloacae analysis in 1985 (Johnson, 1985), and by wing length measurement in 2012. Additional measurements were performed in February 2012 to parameterize Niche Mapper™ (see Tables 2, 3; Fig. A1).

Flamingo body condition

Six dead flamingos (one male and five females) collected during the 2012 cold spell were weighed (±1 g). The digestive tract was cleared of its contents. Pectoral muscles, legs muscles and skin were dissected and weighed (±0.1 g). These latter tissues and the entire carcass were freeze-dried to constant mass and ground under liquid nitrogen to obtain a homogeneous powder for analysis. For each tissue, nitrogen content was determined in triplicate using 100–150 mg aliquots following the Kjeldahl method (Campbell and Leatherland, 1980). Protein content was calculated as nitrogen×6.25 (Campbell and Leatherland, 1980). Lipids were determined in duplicate using 1 g aliquots following a gravimetric method derived from Folch and colleagues (Folch et al., 1957). Total lipids were extracted from a 1 g aliquot using chloroform/methanol (2/1, v/v). Adiposity was the total fat mass percentage of body mass. Ash content was determined in duplicate by a gravimetric method on 1 g dry samples placed in a muffle furnace for 24 h. Samples were freeze-dried once again prior to analysis in order to eliminate any remaining traces of water. Reproducibility between duplicate measurements was 1.1±0.2% (proteins) and 0.7±0.1% (lipids).

Despite our efforts at screening through the literature and consulting with experts, we could not find any data on energy stores for flamingos in good condition. We therefore compared the ratio of their total body lipid/protein content with those of wintering woodcocks that died during a cold spell (Robin et al., 1999) and with mallards in different phases of fasting (Robin et al., 1991) (J.-P. Robin, unpublished data). Moreover, because the first proteins catabolized during phase II of fasting are those from pectoral muscles before proteins from legs (Robin et al., 1999), we also compared the ratio of proteins in pectoral muscles/proteins in legs with those found in the woodcocks that died in advanced phase III during a cold spell.

We used residuals (*y*-axis deviations) from a Reduced major axis regression type II model (RMA; also called standardized major axis regression) as empirical indices of body condition for flamingos that died during both cold spells. An RMA was used instead of an ordinary least squares linear regression, which has been criticized (Green, 2001). The RMA of log body mass and log tarsus length was performed on a sample of 34 flamingos used as references. Nine of these birds died with good body condition (mostly due to powerline collision) and were collected at the Tour du Valat (A. Béchet, unpublished data). The remaining 25 birds were a combination of birds that died with good body condition outside cold spells and birds living in captivity (Gallet, 1949). Regression residuals along this allometric function were then calculated for all flamingos and compared with Wilcoxon tests between 'flamingos dead in 1985', 'flamingos dead in 2012' and 'flamingos in good condition'.

Niche Mapper™ modelling

Niche Mapper™ (US Patent 7,155,377B2) is divided into two sub-models: an endotherm model with morphological, physiological and behavioural characteristics of the animal, and a microclimatic model that computes local environmental parameters experienced by the animal. We used the last

Table 2. Morphological parameters used in the endotherm model for flamingos

Parameter	Units	Males (n=9) Dorsal	Ventral	Females (n=10) Dorsal	Ventral
Head					
Plumage density	feathers cm^{-2}	66.0±16.1	74.7±26.9		
Rachis diameter	μm	108.33±28.23	110.00±31.62		
Feather length	mm	13.50±3.22	16.77±22.53		
Plumage thickness	Cm	1.19±0.33	1.14±0.17		
Reflectivity	%	64.80±4.27	67.40±4.40		
Neck					
Plumage density	feathers cm^{-2}	21.3±7.3	21.3±6.6		
Rachis diameter	μm	122.73±42.89	110.00±31.62		
Feathers length	mm	23.04±5.38	19.42±5.04	22.44±6.25	19.38±4.91
Plumage depth	cm	1.64±0.51	1.48±0.42	1.63±0.52	1.58±0.44
Reflectivity	%	76.5±3.3	75.8±3.7	79.3±3.9	80.9±2.4
Head + neck (see Appendix 1 for more details)					
Plumage density	feathers cm^{-2}	34.7±9.9	37.2±12.7		
Rachis diameter	μm	118.41±38.50	110.00±31.62		
Feathers length	mm	20.18±4.73	17.64±4.50	20.74±11.14	18.60±10.20
Plumage depth	cm	1.51±0.45	1.40±0.40	1.49±0.42	1.45±0.36
Reflectivity	%	73.00±3.60	72.50±3.86	75.70±4.08	76.90±3.01
Torso					
Plumage density	feathers cm^{-2}	2.1±0.8	1±0.4	3.3±3.2	1.3±0.4
Rachis diameter	μm	573.33±314.35	431.11±159.29	557.78±358.34	413.33±91.95
Feathers length	mm	129.07±51.00	82.47±14.30	124.82±51.75	83.1±16.75
Plumage depth	cm	2.25±0.85	2.04±0.80	2.45±1.03	2.30±0.86
Reflectivity	%	66.5±2.8	71.7±2.3	69.8±2.0	74.3±2.3
Legs					
Reflectivity	%	25.0±2.6	22.6±2.0	25.6±1.6	23.6 ±1.7

Data are means ± s.d. All values are from the present study.

version of the climatic model (Micr2010a) and a recently updated version of the endotherm model (Endo2013a) developed by Fitzpatrick, Mathewson and Porter (Mathewson and Porter, 2013). This updated version is adapted for species such as flamingos as it includes variations in core temperature along the length of the leg. Briefly, the endotherm model receives the outputs from the microclimate model and estimates the flamingo's daily energy requirements for each month by calculating the metabolic rate required to maintain the animal's core temperature given its heat exchange with the surrounding microclimate (i.e. radiant, convective, evaporative and solar heat flux with the environment). The model used the Julian day at the center of each month as a representative day for the entire month.

The endotherm model was parameterized using flamingo-specific information summarized in Tables 2 and 3 (see also Appendices 1, 2, and supplementary material Table S1). As the shape of the animal directly influences heat exchange with its environment, the endotherm model also requires parameters related to the shape of the modelled animal. The flamingo shape was represented by two ellipses for the torso and the head

and three cylinders for the neck and legs. These body parts were measured on 32 adults found dead in February 2012 (13 males and 19 females).

Meteorological hourly data of air temperature and standard meteorological variables (rainfall, humidity, solar radiation and wind speed at 2 m height) used in this study (see supplementary material Table S2) were collected at the Tour du Valat/Meteo France weather station (43°30.6′N, 4°41.63′E).

All measurement resolutions and accuracies complied with international standards. Analyses for males and females were conducted separately. Flamingo body characteristics were kept constant and energy requirements varied because of observed climatic fluctuations. Energy requirements were calculated for non-reproductive and non-migrant birds. Average monthly flamingo energy requirements were estimated from January 1980 to April 2012, except between January 1987 and March 1988 and between January 1993 and December 1994 as climatic data were unavailable during these periods.

Niche Mapper^{TM} outputs were validated for aquatic birds in a previous study using the doubly labelled water technique, time–energy budgets and

Table 3. Physiological and flight parameters used in the endotherm model for flamingos

Parameter	Units	Mean value (min–max) Male	Female	Reference
Physiology and body condition				
Body mass	kg	3.579	2.525	Gallet, 1949
Body temperature	°C	39.5 (37.5–45.0)		Bech et al., 1979
O$_2$ extraction efficiency	%	24.5		Bech et al., 1979
Animal density	kg m^{-3}	932.9		Porter et al., 2006
Digestive time	days	0.125		MacDonald, 1980
Thermal conductivity of flesh	W m^{-1} °C^{-1}	0.5		Cheng and Plewes, 2002
Animal height	cm	130		Cramp and Simmons, 1977
Flight				
Flight speed	km h^{-1}	60		Amat et al., 2005; Bruderer and Boldt, 2001
Flight altitude	m	100		Bruderer and Boldt, 2001
Time spent flying	%	2		Galicia and Baldassarre, 1997
Flight metabolism	W	219	129	Present study; Software Flight 1.24; Pennycuick, 1989; Pennycuick, 2008

allometric equations (Fort et al., 2011). We could not use the first two techniques in flamingos because adults are difficult to capture and manipulate, and little is known about their year-round time budgets. We thus used generic allometric equations for non-passerine birds to estimate flamingo field metabolic rates (kJ day^{-1}) as $10.5 M_b^{0.681}$, where M_b is body mass (g) (Nagy, 2005).

We then performed two sensitivity analyses (supplementary material Tables S3, S4). First, we used average climatic data across the last 10 years and mean bird parameters (males and females confounded) to identify key input parameters affecting bird energy requirements. Input values were either modified according to their observed variation (minimum and maximum), or they were set to a standard variability of 10% of the average when minimum and maximum values were not available (Grémillet et al., 2003; Fort et al., 2009). The sensitivity analysis was also performed for males and females separately. Additionally, to highlight the most critical parameters affecting energy requirements during cold spells, we ran a second sensitivity analysis considering climatic data during these two particular periods. Mean bird parameters (males and females combined) were used. All statistical analyses were performed in R (R Development Core Team, 2012).

APPENDIX 1

Morphological measurements of greater flamingos and their feathers used to run the endotherm sub-model of Niche Mapper™

Feather measurements were conducted for four different body parts: head, neck, torso and legs. 'Head' was considered as one homogeneous area, whereas for the others we distinguished the dorsal and ventral sides. Feather lengths were determined without plucking them using callipers for five feathers of each area. The same number of feathers was measured for each area under a binocular microscope to determine the diameter of the rachis in their central part. Plumage depth was also measured five times for each area, with a ruler placed perpendicular to the skin. Finally, plumage density was determined for each area by counting feathers plucked per unit area. Plumage reflectivity was measured on bird carcasses that were selected as the cleanest, and were perfectly dry at the time of measurement. Measurements were performed using a portable ASD spectrometer with a wavelength ranging between 350 and 2500 nm, covering 97% of the solar spectrum at the earth's surface. The probe was held perpendicular to the bird plumage surface, and 10 replicates were performed for each bird area, except for the head, where four replicates were taken. The spectrometer was calibrated

Fig. A1. Morphological measurements of greater flamingos. The following measurements were taken: (1) head length; (2) head width; (3) beak width; (4) beak length; (5) neck diameter; (6) neck length; (7) torso length; (8) torso width; (9) wingspan; (10) wing length; (11) wing width; (12) rectangle length; (13, 14) sides of the triangle; (15) tibia length; (16) tibia diameter; (17) tarsus length; and (18) tarsus diameter. Division of the wing into a triangle and a rectangle (11–14) allowed the calculation of wing area.

using a white reference surface each 10 min. Data were analyzed using Indico™ Pro software.

APPENDIX 2

Calculations of the areas of head and neck for NicheMapper™ parameterization

Some parameters in Niche Mapper™ (especially for feathers) consider the head and neck together. However, in flamingos, the neck area is much more important than the head area. Hence, we calculated the approximate area of each part to consider the head and neck in real proportions. The head was considered as a cylinder. Its area was calculated as follows: $\pi \times$mean diameter$\times$mean length$=152$ cm^2, with mean diameter$=0.99$ cm and mean length$=48.8$ cm.

Two formulae were used for the head area calculation. First, we considered the head as a cylinder and then as an ellipsoid: cylinder: $\pi \times 3.4 \times 6.7 = 72$ cm^2, and ellipsoid: $4\pi(a^p b^p + a^p c^p + b^p c^p)^{1/p} = 60$ cm^2, where $p=1.6$ and a, b and c are the semi-axes of the ellipsoid (1.685, 1.685 and 3.31 cm, respectively). We then calculated a mean of both formulae: mean$=(72+60)/2=66$ cm^2.

Hence, the head represented 30% of the total head+neck surface and the neck 70%. For each parameter, the mean value (head+neck) was thus calculated as follows: mean value$=0.3\times$head value$+0.7\times$neck value.

Supplementary material
Supplementary material available online at
http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.106344/-/DC1

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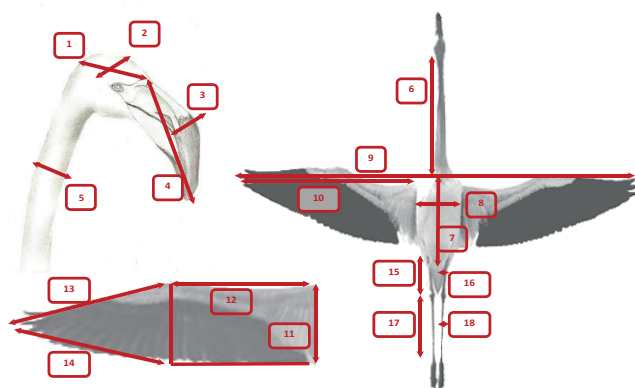
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Table S1. Protein, lipid and carbohydrate content, and dry matter proportion for the two main prey of flamingos in the Camargue (*Artemias* and chironomid larvae).

	Artemias	Chironomids
Protein	0.56	0.50
Lipid	0.12	0.14
Carbohydrates	0.12	0.15
Water	0.87	0.87

Table S2. Example of climate data used for modelling flamingo daily energy requirements. Here we show data for the two years during which cold spells occurred, and for two 'normal' years (for 2012, only data for four months were available).

Year	Month	Air Temperature (°C) Min	Air Temperature (°C) Max	Relative Humidity (%) Min	Relative Humidity (%) Max	Wind speed (m s⁻¹) Min	Wind speed (m s⁻¹) Max	Cloud cover (%) Min	Cloud cover (%) Max
1985	Jan.	-1.89	6.45	54.51	78.87	3.03	14.8	0	1
	Feb.	4.35	13.28	60	88.21	1.5	10.75	0	1
	March	4.34	12.43	58.87	82.58	3.16	15.12	0	1
	April	8.48	18.72	47.5	81.16	2.76	15.33	0	1
	May	11.04	19.78	58.54	87.58	1.29	10.54	0	1
	June	14.45	24.86	50	81.16	1.93	11.66	0	1
	July	17.9	29.44	53.38	82.58	1.16	10.83	0	1
	August	16.18	27.38	52.9	83.38	1.45	11.38	0	1
	Sept.	14.57	27.31	50	86.33	1.6	10.33	0	1
	Oct.	11.58	21.35	60.32	89.35	2.5	12.25	0	1
	Nov.	3.55	11.18	58	82.66	3	13.92	0	1
	Dec.	4.73	12.29	70.8	87.9	3.12	13.16	0	1
2012	Jan.	3.65	12.39	51.65	84.26	2.2	13.58	0	1
	Feb.	-0.32	8.1	34.24	66.48	5.5	21.36	0	1
	March	6.53	17.87	40.19	84.13	1.74	13.7	0	1
	April	9.24	17.47	47.7	84.9	2.15	16.67	0	1
1984	Jan.	2.15	10.57	65.32	87.9	1.48	12.58	0	1
	Feb.	1.74	10.33	58.27	84.13	2.62	13.86	0	1
	March	3.95	13.01	53.87	82.9	4.06	16.19	0	1
	April	5.93	17.92	42.66	81	2.43	14.5	0	1
	May	9.36	18.55	55.8	85.8	2.64	13.35	0	1
	June	14.09	25.41	48.33	83.83	1.86	12.76	0	1
	July	16.55	28.16	56.29	83.38	2.7	13.35	0	1
	August	16.17	27.38	50.64	85.48	2	11.64	0	1
	Sept.	13.41	23.3	51.33	85.83	1.8	12.36	0	1
	Oct.	9.96	20.06	59.67	91.29	2.32	12.22	0	1
	Nov.	8.19	16.96	63.5	87.66	1.86	11.73	0	1
	Dec.	3.14	11.59	65.96	87.58	2.22	12.35	0	1
2011	Jan.	2.84	9.95	62.81	86.26	2.91	13.68	0	1
	Feb.	4.55	12.62	56.39	86.68	1.84	13.46	0	1
	March	6.3	15.39	53.68	88.06	1.54	12.89	0	1
	April	10.16	21.5	38.37	87.9	1.22	12.9	0	1

May	13.15	25.03	34.55	87.03	1	13.1	0	1
June	15.81	25.27	44.17	86.03	1.26	12.64	0	1
July	17.08	27.24	39.26	82.71	1.64	13.25	0	1
August	16.88	28.68	42.61	90.52	0.74	10.78	0	1
Sept.	15.48	27.07	43.4	90.57	0.86	10.94	0	1
Oct.	11.4	21.99	45.06	86.61	1.88	13.08	0	1
Nov.	10.32	16.65	65.17	90.27	1.64	11.83	0	1
Dec.	5.04	13.16	56.26	87.68	1.3	13.45	0	1

Table S3. Sensitivity analysis for estimated flamingo energy requirements, expressed as the % increase (positive value), or decrease (negative value) of the average daily energy expenditure (kJ d⁻¹). +10% and −10% mean climatic parameters and mean adult input parameters are used to simulate variable input. Significant variations in energy requirements (>1%) are in bold. For climatic parameters, the minimum and maximum considered do not include values from cold spells.

Parameter	Average for males and females		Males		Females	
	Min/-10%	Max/+10%	Min/-10%	Max/+10%	Min/-10%	Max/+10%
Morphology						
Feathers length	0.48	-0.41	0.53	-0.40	0.55	-0.42
Feathers diameter	0.081	0.14	0.053	0.18	0.10	0.11
Plumage depth	**3.97**	**-3.54**	**3.99**	**-3.54**	**3.97**	**-3.55**
Plumage density	0.37	-0.23	0.35	-0.22	0.38	-0.25
Plumage reflectivity	-0.058	0.069	-0.054	0.00	-0.064	0.073
Body dimensions	**-10.29**	**10.99**	**-10.16**	**10.79**	**-10.64**	**11.18**
Physiology						
Body temperature	**-4.44**	**12.10**	**-4.33**	**11.80**	**-4.58**	**12.49**
O₂ extraction efficiency	0.94	-0.74	0.93	-0.73	0.96	-0.75
Digestive time	0.00	0.00	0.00	0.00	0.00	0.00
Flight parameters						
Fly altitude	0.011	-0.011	0.01	-0.01	0.012	-0.12
Flight velocity	0.00	0.51	-0.52	0.46	-0.64	0.57
Time spent flying	-0.32	0.32	-0.37	0.37	-0.26	0.26
Flight metabolism	-0.34	0.34	-0.39	0.39	0.00	-0.26

Climatic parameters					
Air temperature	**6.15**	**-9.15**	**5.99**	**-8.95**	**6.35**	**-9.41**
Relative humidity	-0.16	0.22	-0.15	0.21	-0.16	0.23
Wind speed	-0.0084	-0.001	-0.012	-0.00091	-0.0032	-0.0015

Table S4. Sensitivity analysis for estimated flamingo energy requirements for birds wintering in the Camargue in January 1985 and February 2012. Variability is expressed as the % increase (positive value), or decrease (negative value) of the average daily energy expenditure (kJ d^{-1}). +10% and −10% mean climatic parameters and mean adult input parameters are used to simulate variable input. Mean parameters values for males and females confounded are used. Significant variations in energy requirements (superior to 1%) are in bold.

Parameters	January 1985		February 2012	
	Min/-10%	Max/+10%	Min/-10%	Max/10%
Morphology				
Feathers length	0.47	-0.40	0.48	-0.40
Feathers diameter	0.019	0.210	0.024	0.200
Plumage depth	**4.33**	**-3.85**	**4.30**	**-3.83**
Plumage density	0.34	-0.20	0.34	-0.21
Plumage reflectivity	-0.023	0.017	-0.031	0.023
Body dimensions	**-11.63**	**12.42**	**-11.50**	**12.28**
Physiology				
Body temperature	**-3.32**	**9.04**	**-3.41**	**9.30**
O$_2$ extraction efficiency	0.49	-0.40	0.52	-0.42
Digestive time	0.00	0.00	0.00	0.00
Flight parameters				
Fly altitude	0.013	-0.013	0.013	-0.013
Flight velocity	-0.81	0.73	-0.78	0.70
Time spent flying	-0.24	0.24	-0.25	0.25

| Flight metabolism | -0.26 | 0.26 | -0.26 | 0.26 |