Assessment of the short-term effects of weather conditions on mass mortality of the saiga antelope (*Saiga tatarica tatarica*) in Kazakhstan

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Declaration: The research question and objectives were developed by the author and primary supervisor. The author assisted in post-mortem examinations and sample collection conducted by veterinarians from the Research Institute for Problems of Biological Safety and the Royal Veterinary College. Collection of photographic material and meteorological data during field work, as well as data analysis and write-up were done by the author.

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Abstract

The critically endangered saiga antelope (*Saiga tatarica tatarica*) was subject to a catastrophic mortality event during calving in May 2015, Central-Kazakhstan. More than 140,000 antelopes, representing approximately half of the global population, were estimated to have died, but the causes are unclear. One of the hypotheses is that an environmental factor triggered the precipitation of a polymicrobial disease, primarily haemorrhagic septicaemia caused by Pasteurella multocida serotype B. This study describes gross pathological findings obtained during field investigations and tests if weather conditions preceding the outbreaks acted as a common trigger. For this purpose, weather conditions at different known die-off sites were compared with those from unaffected sites, using meteorological data from regional, ground-based weather stations. To verify if datasets from regional stations adequately reflect weather conditions on calving sites, variation with weather records collected on-site was determined. Subsequently, weather records from before the die-offs in 2015 were assessed for consistencies and compared to datasets from a calving aggregation in a non-outbreak year (2014) and from die-offs in the Ural population in 2010 and 2011. Finally, divergence of weather values in May 2015 from long-term climatic normals was determined. The pathologies observed during post-mortem examinations were consistent with haemorrhagic septicaemia and clostridial enterotoxaemia, with *Clostridium perfringens* being a potential secondary factor in the polymicrobial disease. While weather patterns preceding the 2015 die-offs did show some similarities, there were also substantial variations. Temperature variation was the only parameter consistent across all 2015 die-off sites and significantly different from 2014, however the implication of this is debatable. Overall, no evidence was found for the influence of meteorological variables on the onset of disease in saigas. The agreement between on-site and regional weather records was generally moderate and very low for relative humidity records, which could be useful information for future meteorological research in the region.

Keywords: saiga antelope, *Saiga tatarica,* haemorrhagic septicaemia, *Pasteurella multocida, Clostridium perfringens*, Kazakhstan, mass mortality

Introduction

The saiga antelope (*Saiga tatarica*) is a nomadic, herding ungulate from Central Asia, listed as Critically Endangered on the IUCN Red List of Threatened Species and on Appendix II of the Convention of Migratory Species. The *Saiga tatarica tatarica* consists of one population in Russia (Kalmykia) and three in Kazakhstan (Ural, Ustyurt and Betpak-Dala) while the *Saiga tatarica mongolica* is endemic to western Mongolia (fig.1; Bekenov et al., 1998; Milner-Gulland et al., 2003). The primary threat to saigas throughout history has been hunting. Economic collapse after breakdown of the USSR led to heavy increase in poaching which caused the species to decline by more than 90% by 2002, leaving 50,000 antelopes (Milner-Gulland et al., 2003). Nevertheless, saigas have the ability to rapidly recover their population numbers. They are characterised by very high reproduction rates, with females conceiving annually from the age of one and high twinning rates (25-65%; Kühl, 2008). Annual aerial surveys conducted by the Association for Conservation of Biodiversity of Kazakhstan (ACBK) suggest that the Kazakh population increased by 36% from approximately 137,500 to 187,000 between 2012 and 2013. In this period the Betpak-Dala population increased by 41%, reaching 155,200 animals and representing the largest population globally (Grachev, 2013). The Kazakh population further increased to reach over 256,000 individuals in 2014 (Saiga News, 2014).

Fig. 1: Approximate geographic distribution of extant saiga antelope populations: (1) Kalmykia (2) Ural (3) Ustyurt (4) Betpak-Dala (5) West-Mongolia. (Data from The IUCN Red List of Threatened Species, 2015. Note: summer and spring ranges of the Betpak-Dala population are north from depicted range boundary)

Besides anthropogenic threats population growth is limited by biotic and abiotic factors. The saigas' habitat consists of deserts, semi-arid deserts and steppes, characterised by extreme continental weather, with substantial seasonal and daily variations in temperature and humidity. In Kazakhstan, temperatures can range from 40°C in summer to -40°C in winter, while mean annual precipitation varies from 100-120mm in the south to 250-300mm in the north (Robinson and Milner-Gulland, 2003; Salnikov et al., 2015). In winter, melting and refreezing snow creates a layer of ice on pastures, a phenomenon called *dzhut*. This impedes foraging and may lead to starvation. *Dzhuts* occur periodically, causing population drops of up to 40% every three to four years (Sludskii, 1963; Fadeev and Sludskii, 1982; Bekenov et al., 1998). Susceptibility to starvation is exacerbated when droughts precede *dzhuts*. Droughts are not a direct cause of severe declines of the adult population, however, they may influence nutrition, body condition and subsequently fertility, lactation and calf survival (Fadeev and Sludskii, 1982).

Disease outbreaks have been reported to substantially affect saiga numbers. Foot-and-mouth disease was observed in 1955,1956,1958,1967,1969 and 1974, with the most severe outbreak in 1967 causing death of approximately 50 000 calves. Pasteurellosis was reported to have caused mass mortalities in 1981, 1984 and 1988, with roughly 270 000 adult deaths in 1988 (73% of the Betpak-Dala population; Bekenov et al., 1998). Based solely on the isolation of *Pasteurella haemolytica* from saiga carcasses, the diagnosis of pasteurellosis has been disputed. *Pasteurella sp* are common commensal bacteria in many species and have been identified in nasal and tonsillar swabs from healthy saigas (Lundervold, unpublished data). They may induce disease during stressful situations (Jones et al., 1997), but isolation of the bacterium from diseased animals is insufficient to explain the cause of mass mortalities (Lundervold, 2001). Pasteurellosis was also officially reported as the cause of die-offs in the Ural population after calving in 2010 and 2011, based on isolation of *P.multocida.* Investigation of the clinical signs, meteorological data and pasture conditions suggested Atypical Interstitial Pneumonia due to unusually rich pastures was more likely the cause, but due to the failure to undertake adequate outbreak investigation and pathological examination the officially reported aetiology remains equivocal (Dancer, 2012; Kock, 2012). In general, past mass mortalities in saigas are poorly documented due to inadequate epidemiological and pathological methodologies, which severely hamper our understanding of their drivers (Kock, 2012).

Other diseases such as necrobacteriosis, brucellosis, strains of plague and toxoplasmosis have been observed in saigas, but are not associated with mortalities on a similarly large scale (Bekenov et al., 1998; Lundervold, 2001).

During the calving season in May 2015, the Betpak-Dala population was subject to large-scale mortality events. Unusual deaths were primarily reported in the calving aggregation in the Turgaj region on the 10th of May. Within nine days virtually the entire herd, exceeding 61 000 animals, had died. Similar die-offs with a mortality of nearly 100% were observed in 13 other herds, with a total death count of approximately 134,000 at the start of June (ACBK, 2015).

Following pathological and bacteriological investigations, a current hypothesis is that the die-offs were caused by a polymicrobial disease, triggered by an environmental stressor (Kock et al., 2015). Haemorrhagic septicaemia caused by *P.multocida* serotype B is thought to play a key role, but it is unclear what may have caused its manifestation. It is believed that *Pasteurella sp* cause opportunistic disease following stress-induced suppression of host immunity (Miller, 2001). Possible predisposing factors in livestock include long-distance transportation, starvation, overcrowding, inclement weather and intercurrent disease. *P.multocida* seem to have prolonged survival times in damp conditions and prevalence is thought to increase during the rainy seasons in Africa and Asia (Coetzer and Tustin, 2004). Outbreaks were reported following sudden increases in rainfall and humidity with associated temperature drops or during severe, cold winters, although usually other stress factors are also present (Anosa, 1975; Voigts et al., 1997). Seasonal weather patterns also appear to precipitate epizootics in wild mammals (Miller, 2001). Extreme weather fluctuations, in particular large amounts of precipitation, gusty wind conditions and thawing temperatures resulting in extremely muddy conditions, were believed to contribute to an outbreak of septicaemic pasteurellosis in Elk *(Cervus elaphus*) in the United States (Franson and Smith, 1988). A pneumonia epizootic in muskox (*Ovibos moschatus*) in Norway, involving *Mannheimia haemolytica* and *P.multocida* corresponded with a period of abnormally warm and humid conditions, suggesting an effect of climate change (Ytrehus et al., 2008).

This study tests the hypothesis that adverse weather conditions triggered opportunistic disease outbreaks in saigas. The objectives are to 1) verify whether meteorological data from regional weather stations adequately reflect weather conditions on calving aggregation sites; 2) assess if weather conditions preceding mass mortality events in saigas are consistent between die-off sites and different from unaffected sites; 3) establish whether meteorological values in the period before the outbreaks in 2015 departed significantly from climatic means for that season. Weather conditions associated with the die-offs are discussed in relation to gross pathological findings and laboratory results.

Methods

Mortality events (fig.2)

Field work was conducted during die-offs in May 2015, at two calving aggregations in Betpak-Dala. The first calving aggregation was in the region Turgaj (N49°48’18” E65°27’59”), where mass calving took place between 6-17 May and unusual mortality started on the 10th of May. Highest mortalities were observed on the 15th and 16th, on the 18th of May the die-off had ended. Field work was conducted from the 11th until the 19th of May. At the second site, Tengiz (N51°04’36” E67°23’36”), calving occurred between 16-24 May. The die-off was estimated to have started on the 18th of May and approximately 8500 animals died by the 26th of May. Field work was conducted between 22-26 May. The saiga herds in Tengiz and Turgaj are viewed as two separate subpopulations of the Betpak-Dala population, as they do not come into contact with each other during migration (Zuther, unpublished data). This implies that they were affected independently from each other.

Information on various die-off and unaffected sites from different years and populations was assembled and used for analyses of meteorological data. Three die-off sites were used to represent the mortality events in 2015: Turgaj, Tengiz and Yrgyz. Four outbreaks were identified near Yrgyz (Turgaj subpopulation) with first carcasses observed on the 18th of May (N48°19’30”, E62°22’37”*)*. They were ongoing on the 2nd of June and likely ceased shortly afterwards. The four sites are treated as one in analyses, as there was only one weather station in that region. Moreover, animals are suspected to have moved and died between sites (Zuther, pers. com.). Likewise, in Turgaj and Tengiz a total of six outbreaks were identified. These were clearly distinct, with no carcasses found between sites, but this distinction could not be made in the analyses.

Due to carcass clearing procedures, start dates could not be estimated for two die-off sites at N50°09’14”, E62°22’37” and N50°38’17”, E69°06’25”, which were excluded from analyses.

Weather conditions from the die-off sites in 2015 were compared to those from a calving aggregation in 2014, where no outbreak occurred. Calving occurred between 11-22 May in Turgaj, starting at N49°56’25”, E65°36’08” and ending at N49°52'43", E65°44'35" (Kock, pers. com.).

The Ural die-offs from 2010 and 2011 were included in some analyses to assess if similar weather conditions may have influenced the Ural and Betpak-Dala mortality events. Officially, deaths were reported from the 18th until the 21st of May in 2010 and from the 26th until the 27th of May in 2011, although in 2010 dead saigas were seen by farmers on the 16th (Kock, 2012). For the purpose of this study, the 15th of May will be used as the outbreak start date in 2010. Both die-off locations were overlapping, with a centre point at N50°06’41”, E47°33’21” and the events occurred after calving had ended (5-13 May in 2010 and 13-19 May in 2011).

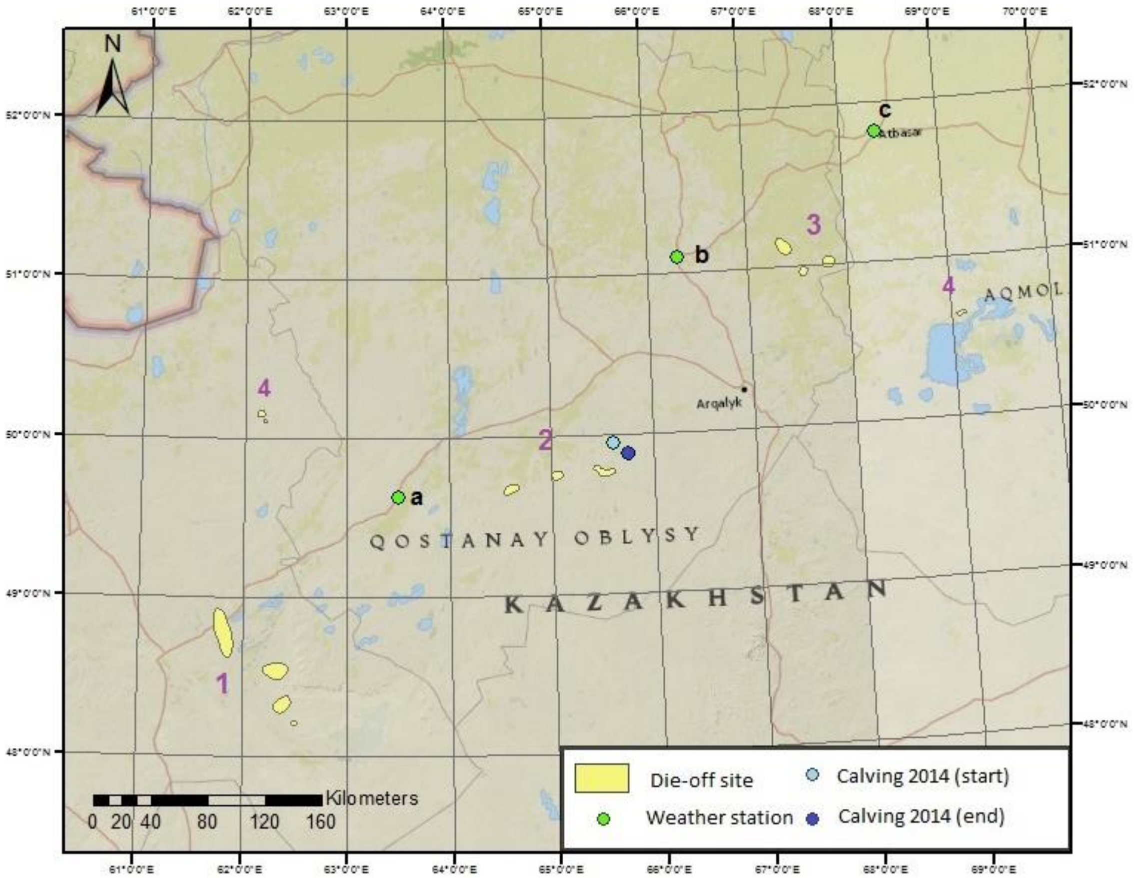


Fig. 2: Saiga die-off locations during calving in 2015, Betpak-Dala. Die-off sites are grouped per region: (1) Yrgyz (Aktyubinsk oblast) (2) Turgaj (Kostanay oblast) (3) Tengiz (Akmola oblast) (4) die-off sites with unknown start dates. Also shown are the start and end point of mass calving in 2014 and the weather stations used for analyses: (a) Turgaj (b) Derzhavinsk (4) Atbasar. (Data from Zuther, pers. com.; Kock, pers. com.)

Clinical observations, pathological investigation and sample collection

Morbid animals were observed, photographed and filmed throughout field work in Turgaj and Tengiz. In Turgaj, post-mortem investigations were conducted by the author from the 11th until the 14th of May. Organ tissue samples were fixed in 10% neutral buffered formalin for histopathology. Between 15-18 May, veterinarians from the Research Institute for Problems of Biological Safety (RIPBS) necropsied dead saigas and collected tissue samples for histopathology, microbiology (50% sterile buffered glycerine, refrigerated) and virology (sterile tube, refrigerated). Blood samples were taken from dying animals and stored in liquid nitrogen. In Tengiz, necropsies were performed by veterinarians from the RIBSP and Royal Veterinary College. Blood samples and tissue samples for histopathology, virology and microbiology were collected. Samples of liver, kidney and rumen content were collected from three cases for toxicology and blood was collected on Flinders Technology Associates filter papers (FTA® cards). Necropsies were carried out following a standardized protocol (Munson, 2000), though there was some variation in the procedures and tissues sampled depending on the person performing the necropsy. All samples were analysed at the RIPBS laboratory (Almaty).

Meteorological data

In Turgaj, a portable weather station (WS3083, Aercus Instruments) was used to record ambient temperature, relative humidity (RH), wind speed, wind direction and precipitation at 30 minute intervals. Records were downloaded on a computer using the software Cumulus (version 1.9.4, Sandaysoft, 2014) and exported to Excel (Microsoft Excel 2013). A temperature and humidity data logger (CEM DT-172) recorded temperature and RH in the shade, every five minutes. Both devices collected data between 11-19 May, however, the first and last day of data were excluded from analyses, as records did not cover all 24 hours of those days. Due to technical problems (loss of signal between data transmitter and receiver for unknown reasons) with the WS3083 weather station, records were missing for multiple time periods. As such, data logger measures were used for analyses involving temperature and humidity. No signal loss occurred during periods of rainfall, thus precipitation records from the weather station are considered reliable. Wind speed was only measured by the WS3083 weather station, therefore these records were used for analyses. Since the anemometer was placed below the recommended of 10 m, measurements were adjusted with a multiplier (Burt, 2012).

Meteorological data from land-based weather stations was obtained from the online Global Historical Climatology Network data set (National Climatic Data Center, 2015). Precipitation records were obtained from Weather Underground (The Weather Channel, 2015) and Raspisaniye Pogodi Ltd. (Raspisaniye Pogodi Ltd., 2015). However, these data had to be excluded from statistical analyses, as there were too many days with zero precipitation.

Datasets from regional weather stations in the towns Turgaj, Derzhavinsk and Atbasar were used to represent the weather conditions in Betpak-Dala (fig.2). Data from all stations was compared to records collected at the Turgaj die-off site during field work, to verify if the former adequately reflect field conditions. The die-off site was at a distance of approximately 144 km from the weather station in Turgaj and 156 km from Derzhavinsk. In further analyses records from Derzhavinsk were used for the Tengiz die-off (75 km), while records from the weather station in Turgaj were used for the Yrgyz die-off (167 km), the Turgaj die-off (referred to as Turgaj ’15), and the 2014 calving period in Turgaj (referred to as Turgaj ’14 or ‘unaffected site’). For the Ural die-offs records from Aleksandrov-Gay (71 km), Russia, were used.

Data from Atbasar were used to construct climatic parameter normals for comparison with the weather during the outbreaks in 2015. It is recommended that climatic normals are constructed with consecutive records from minimum 30 years to account for interannual variation (WMO, 2007), but such long-term data were not available for the weather stations in Turgaj and Derzhavinsk. Atbasar is the second closest station to the die-off site in Tengiz (107 km).

Data analysis

Weather parameters included in statistical analyses were daily mean, maximum and minimum temperature, temperature variation (the difference between maximum and minimum temperature) mean and maximum wind speed, RH and mean and minimum wind chill. Wind chill was calculated using Siple’s formula (Rilling, 1996). All analyses were performed in R (R version 3.1.0), with a significance level of $p<0.05$.

Variation between meteorological datasets from the regional weather stations and records collected in the field was assessed through graphical inspection, two-sample t-tests and evaluation of agreement by Bland-Altman plotting (Bland and Altman, 1986). Two-sample t-test were used to evaluate whether parameter values at the local weather stations were consistently higher or lower than field records. After testing for normality (Shapiro-Wilk test) and equal variance (Levene’s test) of the data, a non-parametric Mann Whitney U-test was used if the two-sample t-test assumptions were violated. Bland-Altman plots allowed for more detailed inspection of the differences between datasets and evaluation of their relevance to the study.

Weather station data from the die-off sites in Betpak-Dala (2015) and Ural (2010 and 2011), as well as from calving in Turgaj (2014) were first visually inspected to recognize any specific weather patterns that may be associated with the die-offs. Selected for further statistical analyses were parameters that appeared to follow consistent trends across the die-off sites, in the period immediately preceding the outbreaks. Tests were conducted on daily records for a selected nine-day period, representing the week immediately preceding the outbreak, followed by the estimated date of the index-case and one day after the estimated start date (to account for uncertainties in start day estimates). For the unaffected site, a nine-day period during calving was selected that showed the most comparable weather pattern to those preceding the 2015 die-offs, based on visual inspection of the data. Similarity of the patterns across sites was evaluated by cross correlation. To assess whether parameters had consistently higher or lower values at the die-off sites than at the unaffected site, a general linear model with parameter records as the response variable and the sites as the categorical explanatory variable was used. User defined contrasts were constructed to make specific comparisons between sites. Firstly, all die-offs in 2015 (Turgaj ’15, Tengiz, Yrgyz) were contrasted against the unaffected site (Turgaj ’14). For parameters that differed significantly, the variation between the 2015 sites was evaluated. Parameters that were similar between the 2015 die-offs were contrasted against the Ural die-offs (Ural ’10, Ural ’11). A log transformation was performed when the residuals followed a skewed distribution.

Mann-Whitney U tests were used to establish if weather parameters preceding and during the die-offs in May 2015 differed from climatic normals. Normals were constructed using data from May from every year during 1985-2014, from the weather station in Atbasar.

Results

Clinical signs

Adult females, calves and the few adult males in the calving aggregations were all affected by disease. Clinical signs of diseased adults observed throughout the study include lethargy, depression, anorexia, weakness, salivation, diarrhoea. In the final stage of disease animals became recumbent and unable to stand up when approached. They suffered from dyspnoea and grunting, with occasional frothy salivation. Pelleted faeces, diarrhoea (occasionally blood-stained) and sometimes urine were found in direct vicinity of dead and morbid animals. Ataxia and little struggling were typically observed before death. Morbid animals died within few hours of becoming recumbent. Calves died usually after their mother, showing the same signs. Case fatality was 100%, while morbidity and mortality in affected herds was close to 100%.

Gross pathology

A total of 33 saiga necropsies were witnessed by the author: six calves and 12 adults in Turgaj and three calves and 13 adults in Tengiz, all adults being females. Most carcasses were in very good condition (76% of 33 animals), with ten animals necropsied immediately after death. All had reasonable or good body condition and rumens were full. The same pathologies were observed in varying degrees in different animals and were consistent with haemorrhagic septicaemia (table1; Coetzer and Tustin, 2004). All cases were characterized by generalized congestion. Widespread ecchymoses and petechiae on the subcutis (fig.3), serosal and subserosal surfaces were common. Lungs were severely congested and oedematous, with occasional haemorrhages and mild emphysema (fig.4). Endocardial haemorrhages were prominent. Severe changes of liver and kidneys suggest rapid toxic change and most likely septicaemia (Kock et al., 2015). There was gastroenteritis, occasionally severe and haemorrhagic. Large accumulation of fluid in the bowel and tissue turgidity suggested dehydration. Lymph nodes were oedematous and haemorrhagic with typically the mesenteric and omental lymph nodes being more affected than the thoracic and peripheral lymph nodes.

Table 1: Pathologies observed during post-mortem examinations of saigas that died during mortality events in Turgaj and Tengiz in May 2015. Total amount of cases examined differs between lesions, as some carcasses were examined less thoroughly due to time constraints or depending on the person performing the necropsy.

Pathology		**number of affected cases affected**	**total number of examined cases**	**percentage (%)**
severe congestion major organs		33	33	100.0
subcutaneous ecchymoses or petechiae		17	29	58.62
congested larynx		15	15	100.0
congested trachea		27	27	100.0
lungs	congested	32	32	100.0
	haemorrhages	16	27	59.26
	mild emphysema	11	22	50.0
heart	congested	20	23	86.96
	endocardial haemorrhage	14	16	87.50
	epicardial haemorrhage	6	13	46.15
liver	congested	20	21	95.24
	mottled	23	24	95.24
	friable	16	17	94.12
enlarged gall bladder		19	25	76.0
congested kidneys		32	32	100.0
spleen	empty	29	32	90.63
	moderately swollen	1	32	3.125
gastroenteritis		17	17	100.0
accumulation of fluid in bowel		12	12	100.0
lymph nodes	oedema	19	19	100.0
	haemorrhagic	15	15	100.0

Fig. 3: Subcutaneous haemorrhages in adult saiga, Turgaj.

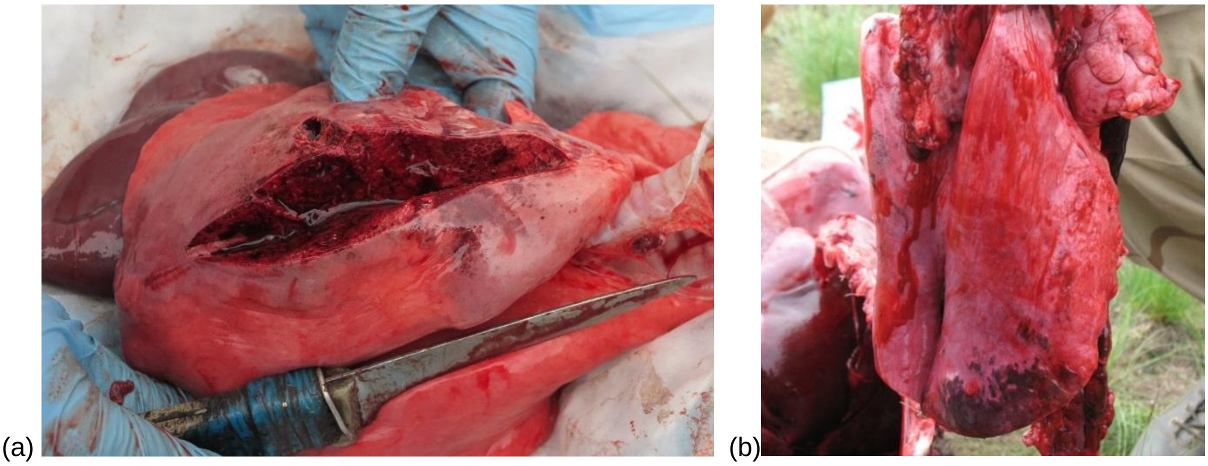


Fig. 4: Lungs of diseased adult saigas, Turgaj (a) oedematous and haemorrhagic lungs (b) severe haemorrhage on the thoracic caudal lobes.

Fig. 5: Linear pattern of congestion on caecum in adult saiga, Tengiz.

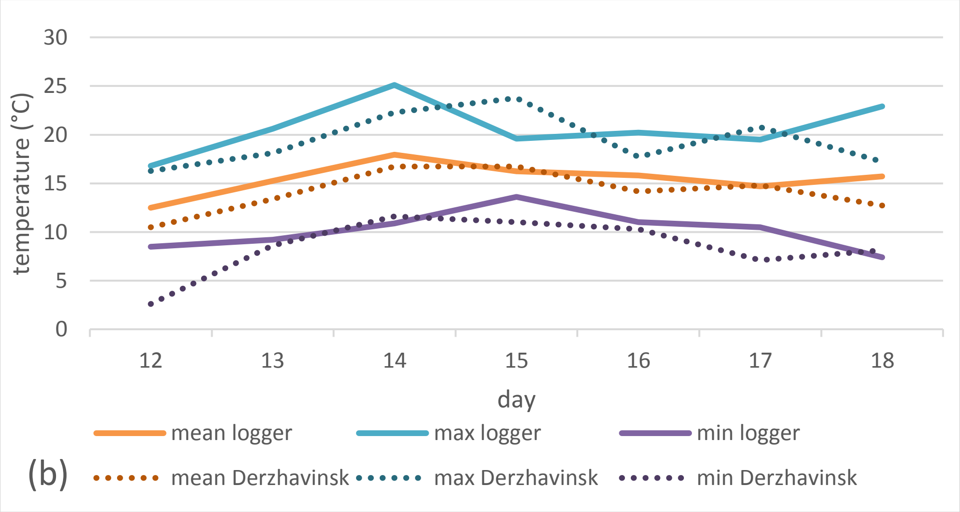
Laboratory findings and preliminary diagnosis (as reported by Kock et al., 2015)

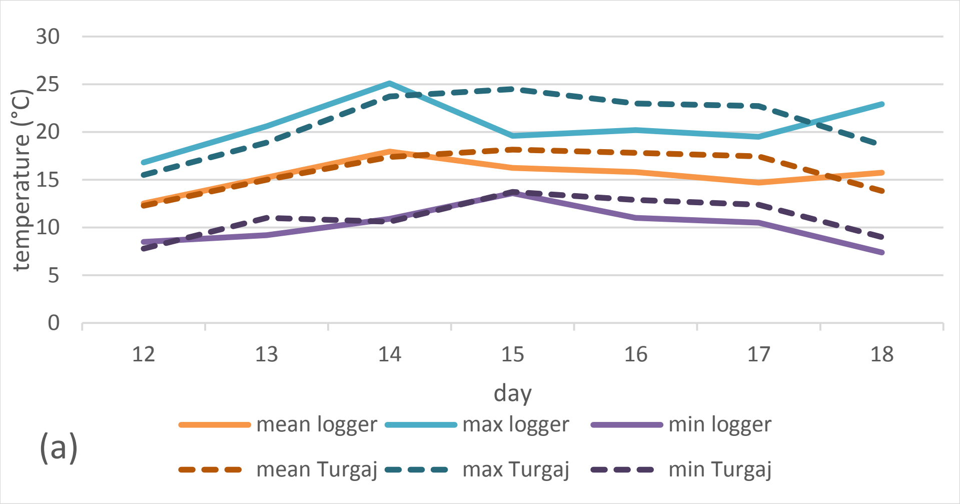
A detailed report of the laboratory results has not yet been released and findings are largely preliminary. *Pasteurella multocida* serotype B with the same genotype was isolated and cultured from all sampled saigas. Additionally, clostridial alpha toxins were found and *Theileria annulata* was identified by PCR in blood samples. Samples tested negative for foot-and-mouth disease, peste des petit ruminants, epizootic haemorrhagic disease, Q-fever, malignant catarrhal fever, mycoplasmosis, Akabanae disease, maedi-visna virus, sheep pox, anthrax, campylobacteriosis, paratuberculosis, brucellosis, listeriosis and bluetongue. RNA of a Flaviviridae virus was detected, but the virus has yet to be identified. Histopathology showed toxic degeneration of kidneys and liver, as well as acute inflammation of the lungs and intestines, consistent with septicaemia. The research team reported a polymicrobial disease*,* primarily haemorrhagic septicaemia caused by *P. multocida* with clostridial enterotoxaemia as a potential secondary factor, as preliminary diagnosis.

Meteorological data

Verification of the use of regional weather station data

Weather records collected on the Turgaj die-off site and data obtained from regional weather stations in Turgaj, Derzhavinsk and Atbasar are presented in figures 6 and 7. Graphs show that on-site temperature readings are similar to those all regional stations (fig.6a-b), while wind speed and wind chill appear to show more variation (fig. 7b-c). RH records from the humidity logger are substantially higher than those from Turgaj over the entire study period (fig.7a). At Derzhavinsk and Atbasar RH shows higher variability, generally increasing throughout the period. There were few days with precipitation at all sites except Atbasar (fig. 7d), which had five rainy days and the highest total precipitation.





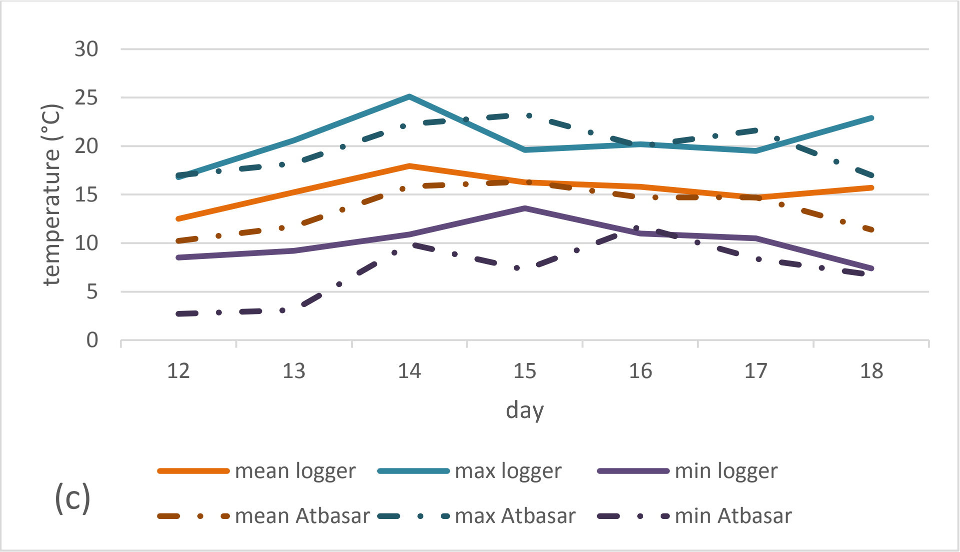
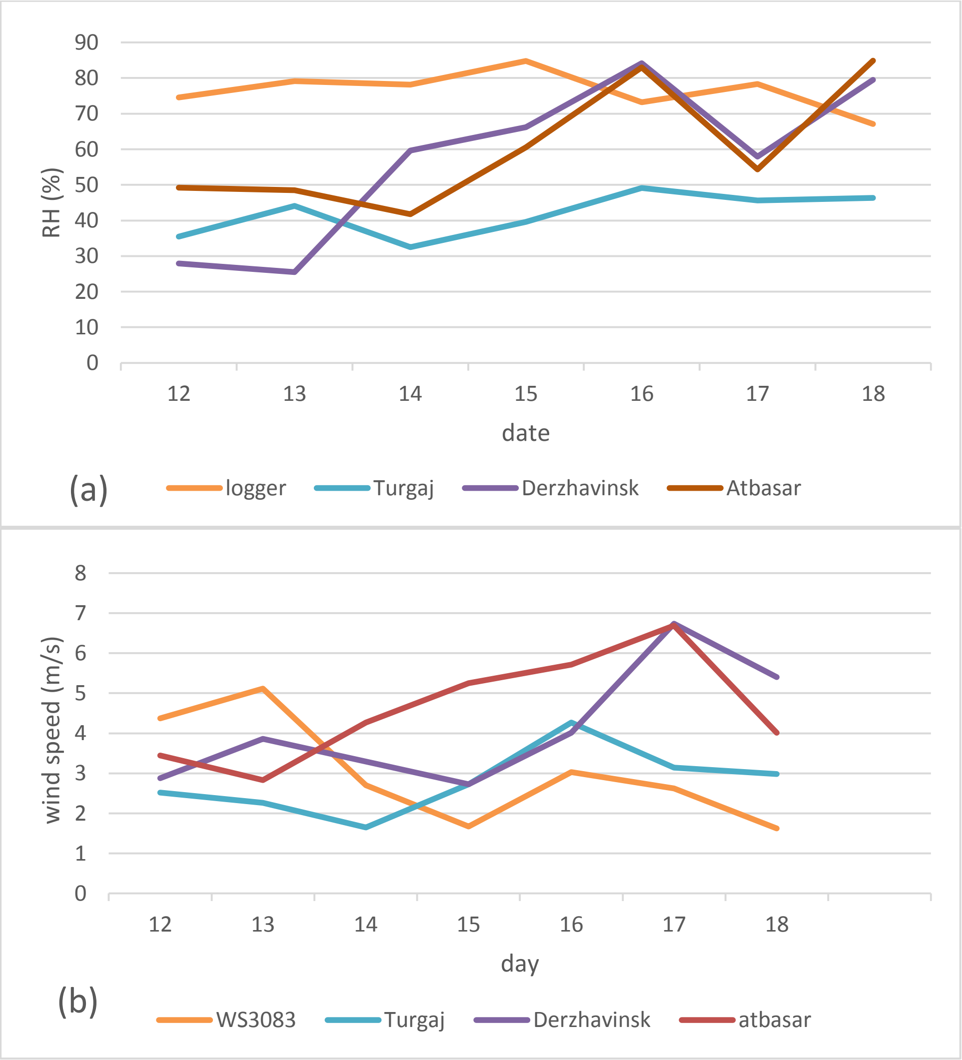
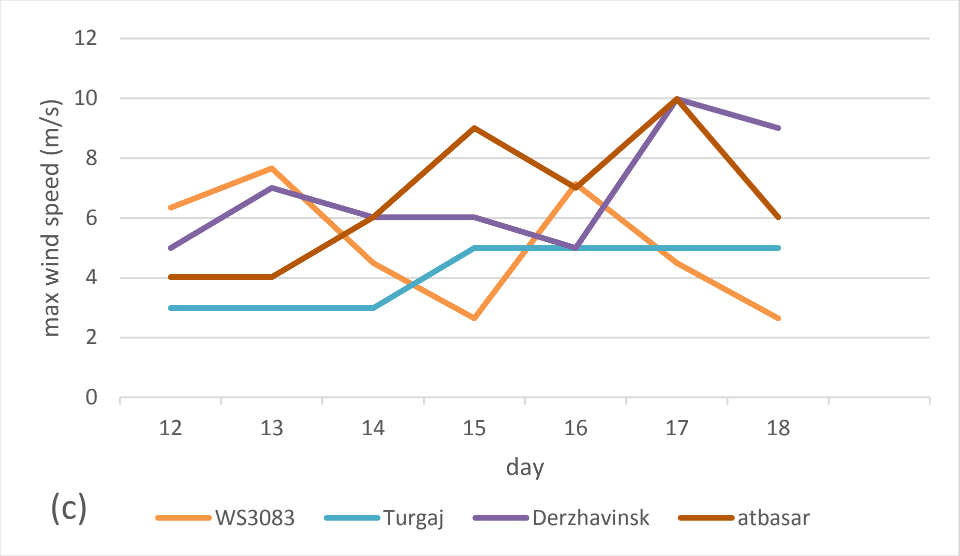


Fig. 6: Daily mean, maximum (max) and minimum (min) temperature recorded by a data logger during field work between the 12th-18th of May at the Turgaj die-off site. Records are compared to datasets from regional weather stations in (a) Turgaj (b) Derzhavinsk. (c) Atbasar. Day 12= 12th of May 2015

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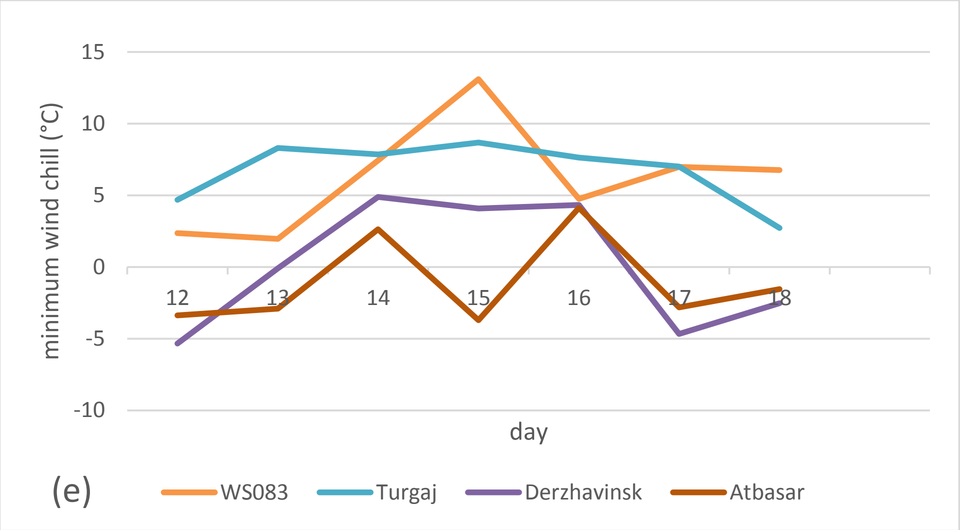
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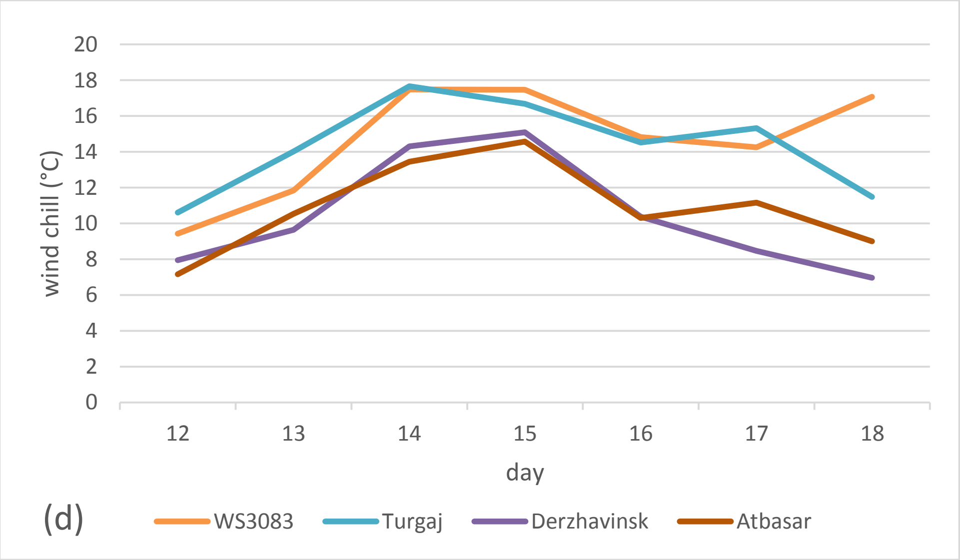
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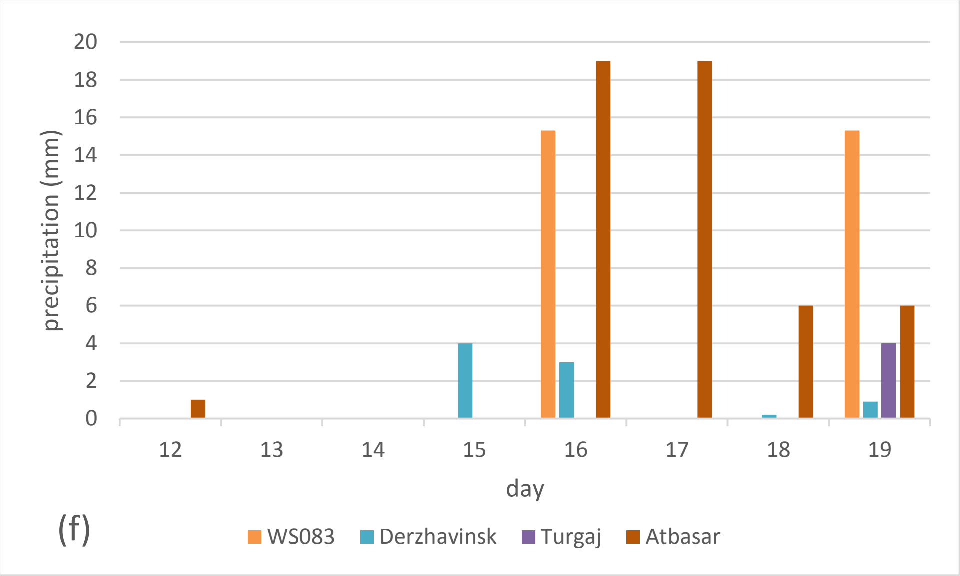


Fig. 7: Comparison of daily weather records from the Turgaj die-off site between 12th-19th May 2015 with datasets from weather stations at Turgaj, Derzhavinsk and Atbasar. Day 12 = 12th of May (a) relative humidity (RH), recorded on-site using a data logger (b) mean wind speed measured on-site by the WS3083 weather station (c) maximum wind speed (WS3083) (d) mean wind chill, calculated from minimum temperature records (logger) and maximum wind speed (WS3083) (e) minimum wind chill (f) total daily precipitation (WS3083). Note: total precipitation on-site on the 19th of May is likely an underestimate, as data collection finished in the afternoon and rainfall was observed afterwards.

According to two-sample t-tests, only RH differed significantly between the die-off site and the station in Turgaj (table2). There were more differences between parameter records with increasing distance from the die-off site. Mean wind speed, maximum wind speed and mean wind chill on-site were significantly different at both Derzhavinsk and Atbasar. Additionally, minimum wind chill (W=49, p=0.000583) was significantly different in Atbasar.

Bland-Altman plotting showed that differences between temperature variables (daily mean, maximum and minimum) from the die-off site and the regional weather stations were overall relatively small (table 3). Bias in temperature variables was smallest for Turgaj, though the limits of agreement were of comparable width across all stations. Most notable is the variation in RH, with the highest bias at Turgaj and very wide limits of agreement for all stations. Wind speed parameters generally had a low bias and moderate to wide limits of agreement. The agreement between wind chill records is rather ambiguous.

Table 2: Two-sample t-test results showing differences between the records of weather parameters from the Turgaj die-of site (devices: logger and WS3083) and weather stations at nearby towns (Turgaj, Derzhavinsk, Atbasar). Only significant results are displayed. Df = degrees of freedom, max=maximum, RH= relative humidity.

weather parameter	**comparison (device & town)**	**mean device**	**mean town**	**95% confidence interval**		**t-statistic**	**df**	**p-value**
				lower	**upper**			
RH	Logger & Turgaj	76.46 ±5.55	41.83 ±6.13	27.82	41.43	11.08	12	1.17E-07
mean wind speed	WS3083 & Derzhavinsk	2.29±0.99	4.13± 1.46	-3.29	-0.39	-2.77	12	0.01708
	WS3083 & Atbasar	2.29±0.099	4.60±1.35	-3.69	-0.93	-3.66	12	0.003294
max wind speed	WS3083 & Derzhavinsk	3.83±1.55	6.86±1.95	-5.08	-0.98	-3.22	12	0.00733
	WS3083 & Atbasar	3.83±1.55	6.58±2.29	-5.02	-0.47	-2.63	12	0.02184
mean wind chill	WS3084 & Derzhavinsk	14.62±3.09	10.40±3.15	0.59	7.85	2.53	12	0.02624
	WS3083 & Atbasar	14.62±3.09	10.89±2.52	0.45	7.02	2.48	12	0.02897

Table 3: Summary of the Bland-Altman statistics for the agreement between daily weather records collected on the die-off site in Turgaj and meteorological data from three regional weather stations (Turgaj, Derzhavinsk, Atbasar), for the period 12-18 May 2015. Data are paired and the bias is the mean difference between the on-site records and the regional station records. It is negative when the regional station values are lower than the on-site records. SD=standard deviation, max=maximum, min=minimum, RH=relative humidity.

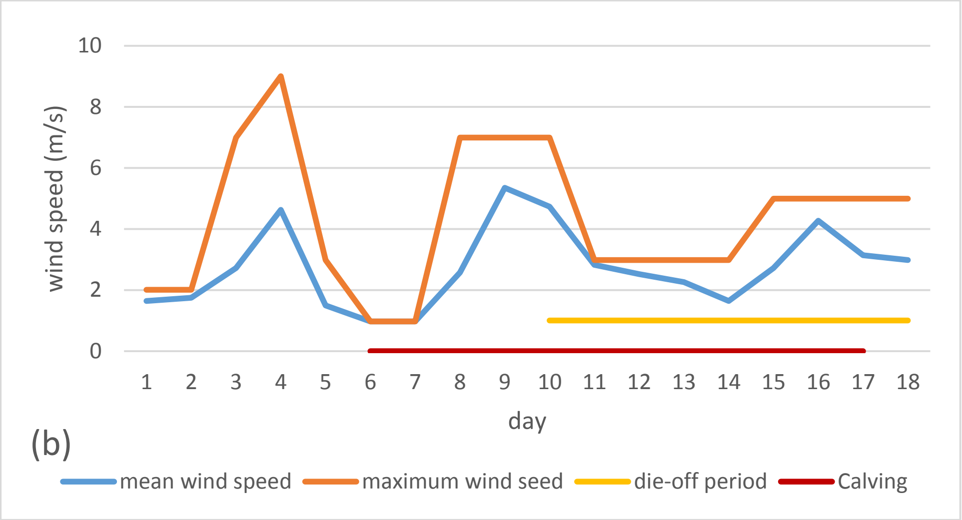
weather station	**weather parameter**	**bias**	**SD**	**limits of agreement**	
				lower	**upper**
Turgaj	mean temperature	-0.54	1.70	-3.87	2.79
	max temperature	-0.32	3.33	6.20	-6.84
	min temperature	-0.9	2.42	1.35	-3.15
	RH	34.63	9.66	15.71	53.55
	mean wind speed	-0.5	1.41	-3.28	2.27
	max wind speed	-0.3	2.29	-4.781	4.178
	mean wind chill	0.289	2.54	-4.689	5.268
	min wind chill	-0.19	3.94	-7.92	7.54
Derzhavinsk	mean temperature	1.31	1.21	-1.07	3.69
	max temperature	1.22	3.2	-5.05	7.49
	min temperature	1.68	1.14	-3.06	6.42
	RH	19.19	25.33	-30.46	68.84
	mean wind speed	-1.84	1.96	-5.68	2
	max wind speed	-3.03	2.966	-8.84	2.79
	mean wind chill	4.22	2.98	-1.62	10.06
	min wind chill	3.63	4.34	-4.87	12.13
Atbasar	mean temperature	1.89	1.69	-1.42	5.2
	max temperature	0.76	3.23	-5.57	7.08
	min temperature	3.04	2.95	-2.75	8.82
	RH	16.14	21.04	-25.1	57.19
	mean wind speed	-2.31	2.08	-6.39	1.76
	max wind speed	-2.75	3.39	-9.39	3.89
	mean wind chill	3.74	2.18	-0.54	8.02
	min wind chill	19.56	4.06	11.6	27.51

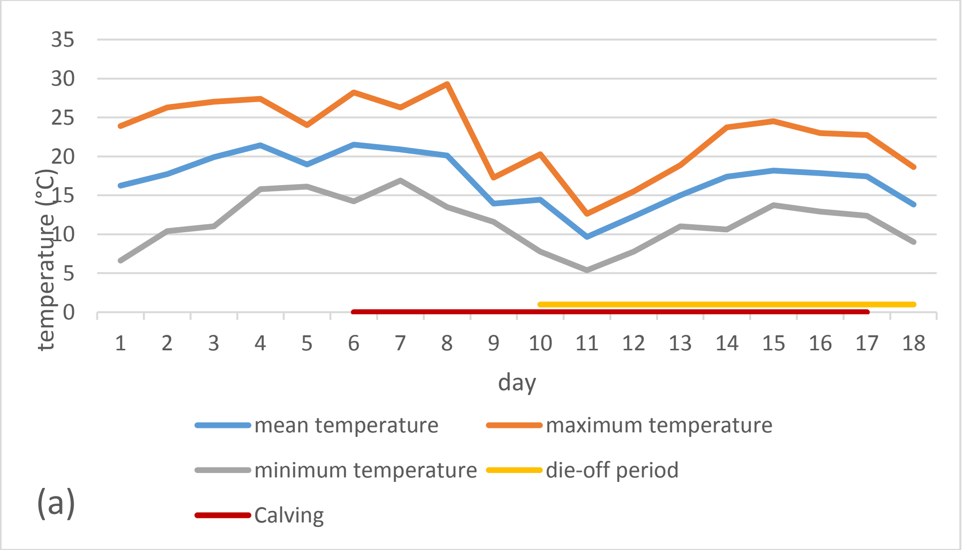
Detection of consistencies in weather conditions preceding die-offs

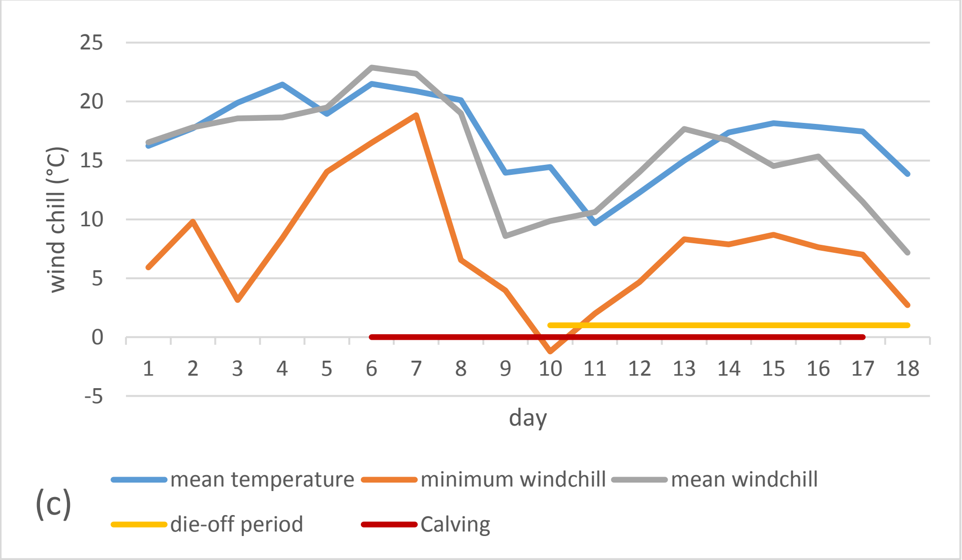
Visual inspection of weather data

Weather data from all sites are included in appendix A (fig.1-5). To illustrate the weather patterns occurring before and during die-offs, data from Turgaj ’15 are displayed in figure 8. The most prominent pattern preceding the 2015 die-offs was a rise and subsequent drop in temperature and wind chill (fig.9a,c). Wind speed generally increased and subsequently decreased slightly (fig.9b), but this is not pronounced, nor different from Turgaj ’14. Temperature variation and RH fluctuated strongly, with no apparent trends in 2015. Precipitation was generally low at all sites (fig.9). There appear to be no similarities between the Betpak-Dala and Ural die-offs, or between the die-offs in Ural in 2010 and 2011.

An increase and subsequent decrease in temperature and wind chill also occurred during calving in Turgaj ’14, between 12th-20th of May (fig11a,b). This period was therefore regarded as the most similar to the nine-day periods preceding the die-offs in 2015 and was used in statistical analyses.







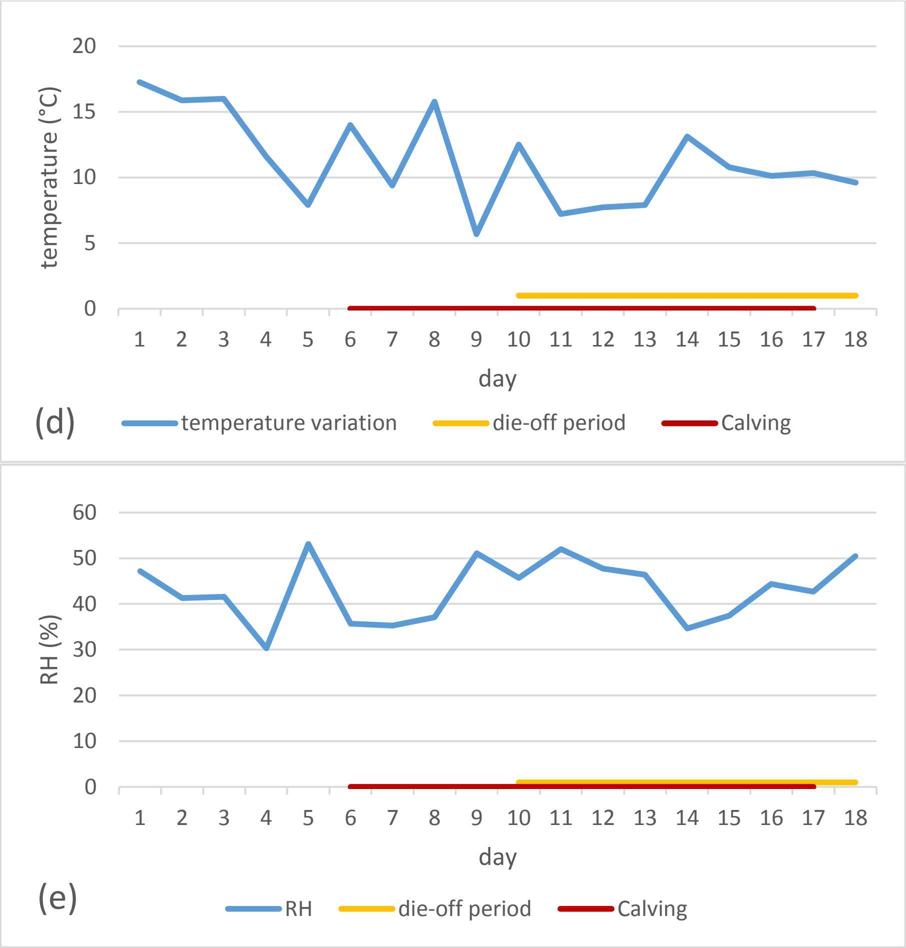


Fig. 8: Weather records from the weather station in Turgaj from before and during the die-off in May 2015. Day 1 = 1st of May (a) mean, maximum (max) and minimum (min) temperature (b) mean and maximum wind speed (c) mean and minimum wind chill (d) temperature variation (e) relative humidity (RH).

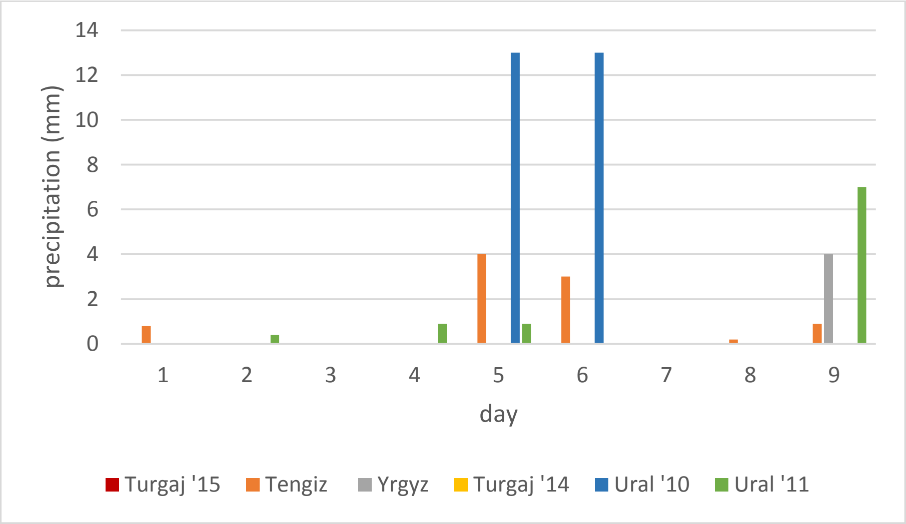


Fig. 9: Precipitation records for the selected nine-day periods for the die-offs in Turgaj ’15 (3-11 May), Tengiz (11-19 May), Yrgyz (11-19 May), Ural ’10 (9-17 May) and Ural ’11 (19-27 May), as well as for calving in Turgaj, 2014 (12-20 May). Day 1 = first day of the respective nine-day period.

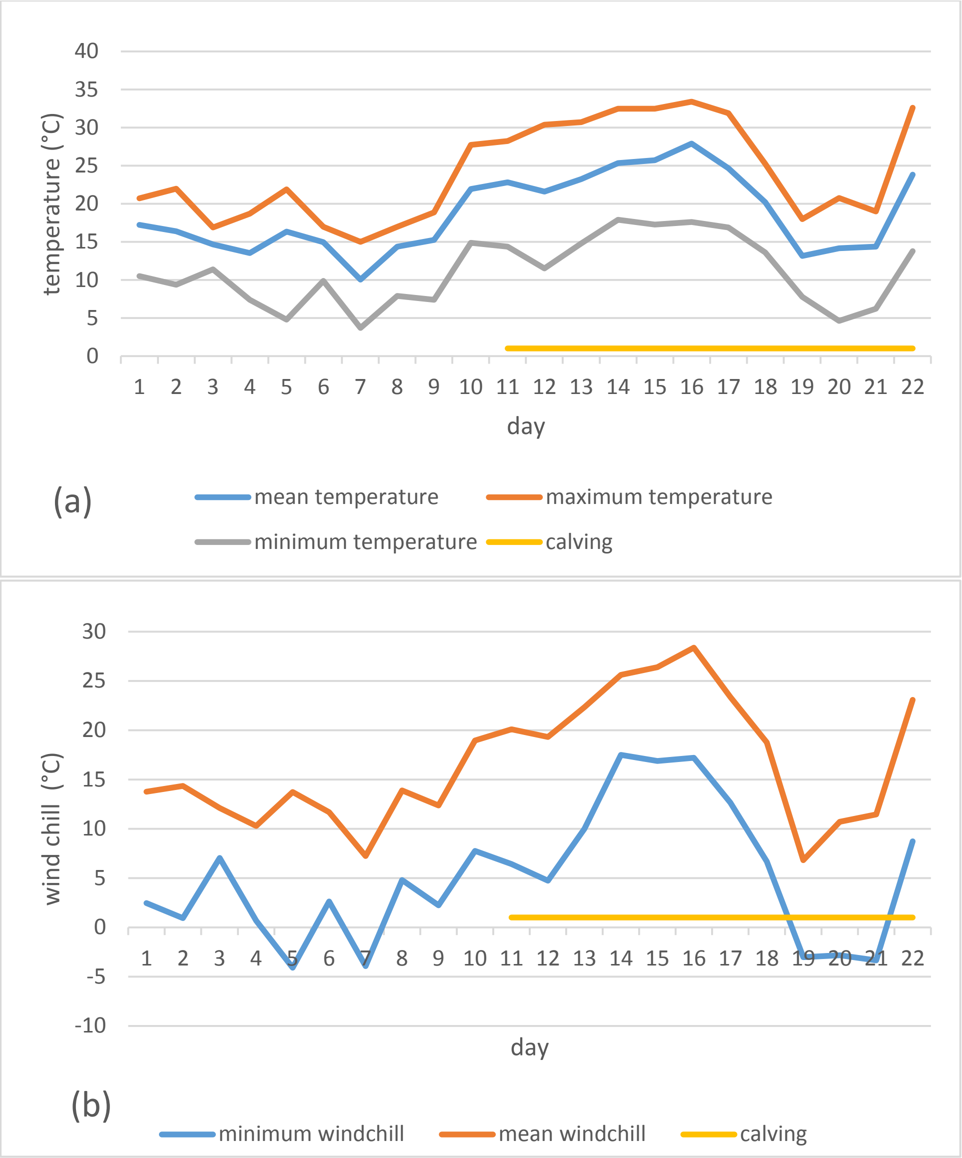


Fig. 10: Weather records from the weather station in Turgaj, May 2014, from before and during mass calving (11-22 May) (a) mean, maximum (max) and minimum (min) temperature (b) mean and minimum wind chill.

Correlation of weather patterns between sites

Cross correlation analysis of temperature and wind chill patterns showed many similarities in the patterns preceding the 2015 die-offs, as well as with those from calving in Turgaj, 2014 (table 4). Minimum temperature is the only variable cross correlated between all three 2015 die-off sites, however, it is also cross correlated between Turgaj 2015 and Turgaj 2014, demonstrating that this pattern is not restricted to unusual mortality events. There was one cross correlation between the Betpak-Dala and Ural die-offs (mean wind chill at Turgaj ’15 and Ural ’11), therefore, it is unlikely that these mortality events were associated with similar weather changes.

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Table 4: Summary of the cross correlations at zero lag between temperature (mean, maximum and minimum) and wind chill (mean and minimum) records from all sites, during the selected nine-day periods. Marked red are cross correlations between the unaffected site (Turgaj '14) and a die-off site. Df = degrees of freedom.

weather parameter	sites	cross correlation	95% confidence interval		t-statistic	df	p-value
			lower	upper			
Mean temperature	Yrgyz & Tengiz	0.89	0.53	0.97	5.04	7	0.00149
	Turgaj '14 & Turgaj '15	0.91	0.64	0.98	5.98	7	0.000555
Maximum temperature	Yrgyz & Tengiz	0.91	0.63	0.98	5.91	7	0.000593
	Turgaj '14 & Turgaj '15	0.86	0.46	0.97	4.45	7	0.00295
Minimum temperature	Turgaj '15 & Tengiz	0.76	0.19	0.94	3.07	7	0.0180
	Turgaj '15 & Yrgyz	0.73	0.13	0.94	2.83	7	0.0254
	Yrgyz & Tengiz	0.76	0.19	0.95	3.07	7	0.0180
	Turgaj '14 & Turgaj '15	0.86	0.46	0.97	4.46	7	0.00295
Mean wind chill	Turgaj '15 & Yrgyz	0.70	0.07	0.93	2.60	7	0.04
	Turgaj '15 & Ural '11	0.71	0.09	0.93	2.70	7	0.0308
	Turgaj '14 & Turgaj '15	0.72	0.10	0.94	2.72	7	0.02973
	Turgaj '14 & Yrgyz	0.90	0.58	0.98	5.41	7	0.000995
Minimum wind chill	Turgaj '15 –Yrgyz	0.81	0.31	0.96	3.62	7	0.00847
	Turgaj '14 & Turgaj '15	0.87	0.49	0.97	4.69	7	0.00224
	Turgaj '14 & Yrgyz	0.88	0.52	0.97	4.92	7	0.00171

Detection of unusually high or low parameter values

There was a significant difference between records from Turgaj ’14 and the die-off sites from 2015 (Turgaj ’15, Tengiz and Yrgyz) for all parameters except mean and maximum wind speed (table 5). Further contrasting showed that parameters also vary significantly between the 2015 die-offs (table 6). Only temperature variation was consistent across the 2015 die-off sites whilst being significantly different from the unaffected site. Temperature variation was subsequently contrasted between the 2015 Betpak-Dala die-offs and the Ural die-offs. The contrast was significant (contrast estimate=-2.16±0.86, t=-2.51, p=0.0151, 95%CI[-3.88, -0.44]) and there was also a significant difference between the two die-offs in Ural (contrast estimate=-3.81±1.33, t=-2.87, p=0.00608, 95%CI[-6.48, -1.14]).

Table 5: Differences in the weather variables between the unaffected site (Turgaj ’14) and the 2015 die-off sites (Turgaj ’15, Tengiz and Yrgyz), generated by general linear models with user defined contrasts. Negative estimates indicate that parameter values were significantly lower in Turgaj ’14 than in 2015 and vice versa.SE=standard error, CI=confidence interval.

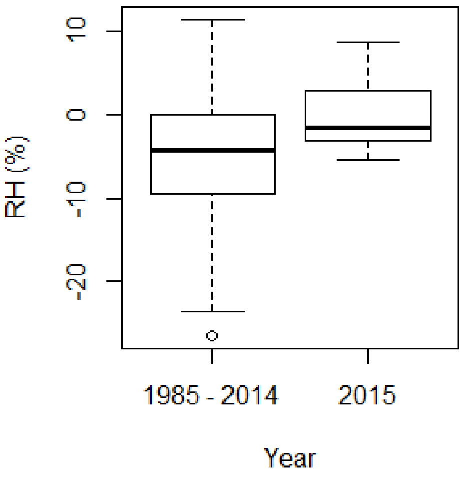
parameter	**contrast estimate**	**SE**	**95% CI**		**t-statistic**	**p-value**
			lower	**upper**		
mean temperature	6.04	1.22	3.59	8.49	4.96	9.15e-06
Maximum temperature	7.19	1.56	4.06	10.33	4.61	2.99e-05
Minimum temperature	3.22	1.16	0.89	5.55	2.78	0.00777
Temperature variation	3.97	1.08	1.79	6.15	3.67	0.000617
RH	-19.30	4.88	-29.11	-9.49	-3.96	0.000252
Mean wind chill	7.17	1.77	3.61	10.73	4.05	0.000189
Minimum wind chill	4.46	1.96	0.52	8.40	2.28	0.0273

Table 6: Differences in weather parameters between the 2015 die-off sites, generated by general linear models with user defined contrasts. Negative estimates indicate that parameter values were significantly lower at first listed site. SE=standard error, CI=confidence interval.

parameter	**contrast**	**contrast estimate**	**SE**	**95% CI**		**t-statistic**	**p-value**
				lower	**upper**		
mean temperature	Turgaj ’15 - Tengiz	2.63	0.75	1.13	4.13	3.53	0.000935
	Turgaj ’15 - Yrgyz	-1.91	0.75	-3.41	-0.41	-2.57	0.0134
	Turgaj ’14 -Yrgyz	6.84	1.49	3.84	9.84	4.59	3.23e-05
Maximum temperature	Turgaj ’15 - Tengiz	3.36	0.96	1.44	5.28	3.52	0.000959
	Turgaj ’15 - Yrgyz	-2.51	0.96	-4.4	-0.60	-2.64	0.0112
Minimum temperature	Turgaj ’15 - Tengiz	2.60	0.71	1.17	4.02	3.66	0.00063
	Turgaj ’15 - Yrgyz	-1.46	0.71	-2.88	-0.03	-2.05	0.0456
RH	Turgaj ’15 - Yrgyz	-7.87	2.99	-13.88	-1.86	-2.63	0.0113
	Tengiz - Yrgyz	-5.99	2.99	-12.00	0.02	-2.01	0.0506
Mean wind chill	Turgaj ’15 - Tengiz	4.09	1.09	1.91	6.27	3.77	0.000449
	Turgaj ’15 - Yrgyz	-2.58	1.09	-4.76	-0.40	-2.38	0.0214
Minimum wind chill	Turgaj ’15 - Tengiz	4.86	1.20	2.45	7.27	4.05	0.000186
	Tengiz - Yrgyz	3.00	1.20	0.59	5.41	2.50	0.0158

Divergence from climatic normals

Comparison of the weather from May 2015 to climatic normals for May from the period 1985-2014 (Atbasar) showed that RH (W=1495; p= 2.612e-05; fig.11a), minimum temperature (W=8951.5, p=0.000534), temperature variation (W=17241, p=0.0351) and minimum wind chill (W=8856.5, p=0.000421) were significantly higher in 2015. However, graphical presentation of the RH records from May for every year separately shows that the records from 2015 are not higher than those from 1987, 1997 and 2001, which are non-outbreak years (fig.11b). There is also no trend visible. This was also the case for the other parameters.



(a)

(b)

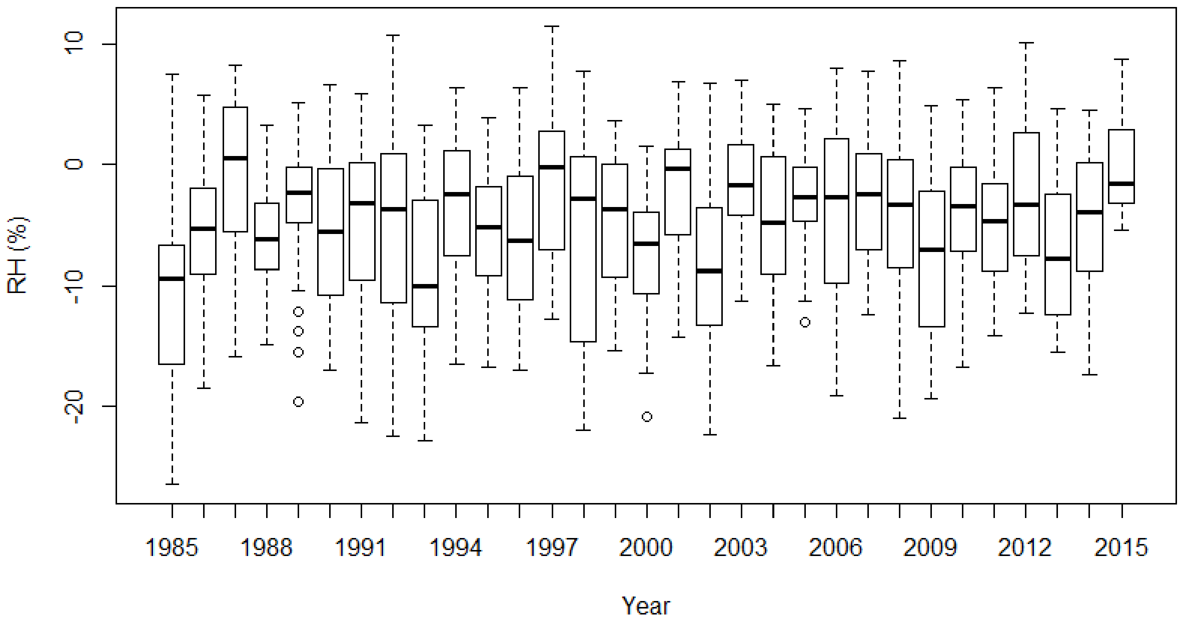


Fig.11: Boxplots for daily mean relative humidity (RH) records in May (Atbasar) (a) compares May 2015 to all records for the month May in the period 2002-2014 (b) same data shown for every year separately.

Discussion

Pathological findings from the 2015 die-offs and isolation of *P.multocida* serotype B are both consistent with haemorrhagic septicaemia. Typical pathologies observed include generalized congestion, petechiae and ecchymoses of serosa, subcutis and in various organs, haemorrhagic and enlarged lymph nodes and gastroenteritis (Jubb and Kennedy, 1963; Coetzer and Tustin, 2004). The condition is potentially complicated by secondary pathogens such as *C.perfringens*. Further toxicological investigations should reveal which type of *C.perfringens* is involved, although generally, some signs and lesions of clostridial enterotoxaemia are mutual with haemorrhagic septicaemia (Coetzer and Tustin, 2004). *T.annulata* is less likely to have played an important role in the mortality events. Some of the observed pathologies are mutual between haemorrhagic septicaemia and theileriosis (haemorrhages, congestion and haemorrhagic, swollen lymph nodes), but carcasses were not characterized by hyperplasia and spleen enlargement, features typical for theileriosis (Coetzer and Tustin, 2004; Kock et al., 2015).

Similarly to *P.multocida, C.perfringens* are commensal bacteria of many species and certain types can become pathogenic after the carrier is exposed to one or multiple stressors. *C.perfringens* type A enterotoxaemia has been reported in livestock after transportation, deworming and sudden dietary changes. Predisposing factors for type D enterotoxaemia include stasis of the intestinal tract, sudden dietary changes, protein-rich diets, wilting of pasture grasses, deworming, coccidiosis and sudden weather changes (Coetzer and Tustin, 2004). Typically, outbreaks are reported after a change from hot and dry to hot and humid weather conditions, associated with pasture overgrowth (Khan et al., 2008; Javed et al., 2009; Ndeereh et al., 2012).

So far, there is no evidence for the effect of weather conditions on occurrence of the outbreaks in 2015. Conditions preceding the outbreaks were generally mild and colder than during calving in 2014 (table 5), although most weather parameters differed significantly between the die-off sites (table 6). Only temperature variation was similar between all investigated die-off sites, while being significantly lower from the previous year. Conversely, high daily temperature variation is more likely to be a stress factor than low variation, as is the case for humans (Kan et al., 2007; Chu et al., 2011). There was a pattern of increase and subsequent decrease in minimum temperature immediately before the die-offs, but this also occurred in 2014. A limitation of these analyses is that datasets for various die-offs were selected based on their start date. These were estimated through field observations at first arrival on a die-off site and may be imprecise. Due to the small sample sizes per site, incorrect estimates would have substantial effects on the results. The die-offs in Yrgyz and Tengiz were both estimated to have started on the 18th of May and as such, there is likely to be confounding.

Comparison of meteorological data from May 2015 to long-term climatic normals showed significantly higher RH, temperature variation, minimum wind chill and minimum temperature, but the implication of this is debatable. Increased RH seems to favour both *P.multocida* and *C.perfringens* infections (Coetzer and Tustin, 2004), but records were similarly high in some non-outbreak years. Moreover, there is a high variation between the meteorological data from Atbasar and weather records from the Turgaj die-off site (table 3), therefore this analysis is only representative for Tengiz.

Agreement between on-site weather records and data from regional stations was lowest for RH and suggests that suitable RH substitutes cannot be obtained from any station. In general, temperature variables appear most homogeneous over large distances, though caution is needed with extreme records (maximum and minimum) for all weather parameters. Data from Atbasar showed considerable variation with all on-site records, likely due to the large distance from the die-off site (304 km). The agreement was moderate (Turgaj) to low (Atbasar) for wind speed records and rather ambiguous for wind chill (all sites), though overall wind chill trends were similar (fig.6d,e).

Bland-Altman plots provide the user with information on the variation or agreement between two sets of measurements, allowing users to choose threshold values for acceptable variation, relevant to their research. However, weather variables act synergistically on host-pathogen systems, therefore, estimating specific cut-off values for agreement is impractical in this study. For example, the temperature-humidity index for livestock shows that at moderate temperatures substantial increases in RH have negligible effects on thermal comfort, while at higher temperatures even small differences in RH can cause dangerous heat stress (Hahn et al., 2009). As such, using substitute RH data that show moderate agreement with field records may be satisfactory for periods with mild temperatures, whilst providing misrepresentative results at high temperatures. Moreover, thermal safety ranges are not known for saigas. In this study, reduced agreement is likely to be partially due to the relatively low quality and sub-optimal positioning of field equipment in comparison with professional meteorological stations. Moreover, due to technical problems, daily wind speed data collected on-site was incomplete. As such, meteorological data from Turgaj was viewed as sufficiently adequate for reflecting conditions on the die-off and calving sites in the same region, for all parameters except RH and minimum wind chill.

The relationship between weather parameters and the host-pathogen system may be subtle complicated by other factors. Detecting associations usually involves long-term meteorological datasets and information on disease epidemiology. Typically, the pathogen is endemic and there are recurring outbreaks or seasonal variation in incidence, such as in human malaria or amphibian chytridiomycosis (Bouma and Kaay, 1996; Murray et al., 2013). Due to poor investigation and documentation of past outbreaks in saiga antelopes, it is unknown whether they are affected by a recurring disease. The die-offs in Ural were characterized by location-specific mortality, associated with lush pastures and bloat (Kock, 2012). This was not the case in 2015, therefore, it is likely that saigas are affected by various syndromes. Consequently, modelling long-term meteorological data to detect associations between outbreak-years could provide deceptive results.

Another factor that could not be included in the study and requires further research is precipitation. The lack of rainy days before the die-offs is not consistent with the typical increase in rainfall preceding *P.multocida* outbreaks (Coetzer and Tustin, 2004), but the effect of precipitation may also be indirect. Heavy snowfall during the former winter resulted in unusual amounts of meltwater and flooding in May (Zuther, pers com). This could affect the vegetation and subsequently saigas. Since climate change models predict an increase in winter precipitation and extreme precipitation events in Central Asia (Christensen et al., 2013), it is probable that floods similar to those observed in 2015 will become more frequent.

Climate change appears to be already affecting saigas. In Kazakhstan, herds follow north-south oriented migration routes, associated with a gradient in vegetation productivity and precipitation, with northern summer ranges being most productive. Calving coincides with maximum vegetation productivity in the spring ranges, but in recent years calving sites have been shifting northwards, possibly due to climate change (Singh and Milner-Gulland, 2011). Increasing temperatures are promoting earlier spring growth (Kariyeva et al., 2012), which could result in a trophic mismatch between peak resource demand and availability, as observed in caribou (*Rangifer tarandus*) in West Greenland (Post and Forchhammer, 2008). Moreover, foraging on pastures of suboptimal quality during calving could affect immunity. Trends and abnormalities in precipitation and vegetation productivity that may have contributed to disease precipitation are currently being investigated through satellite imagery (Singh pers. com.)

While investigation methodologies during past outbreaks in saigas were substandard, in 2015 an early warning scheme and cooperation between Kazakh and international experts made it possible to gather substantially more information and samples than previously. Natural die-offs and recurring epizootics are not uncommon in wildlife, though usually long-term studies are required to develop a comprehensive view of the agents, drivers and co-factors involved (Young, 1994). Although this study did not show a relationship between short-term weather conditions and the occurrence of disease outbreaks in saiga calving aggregations, it provides information on some of the environmental conditions surrounding the events and describes the variation between meteorological records collected in the field and data from regional stations, which can prove useful in future meteorological studies in this region.

It is essential to any investigation of disease occurrence to set the events in time and space to determine the relationship with contributing factors (Wobeser, 2006). Environmental factors are not independent and it is more likely that disease was induced by a complex of interactions, rather than a single factor. Next to weather and climate change, vegetation richness and productivity, quality and pollution of water and soil, human disturbance, density-dependent effects, stress associated with crowding and interspecific interactions should be considered. Understanding the mechanisms behind these events will allow for improvement of surveillance and early warning schemes, as well as development of potential interventions to prevent future mass mortalities. Moreover, it is part of understanding the population dynamics of the species, essential for viability analyses and conservation management.

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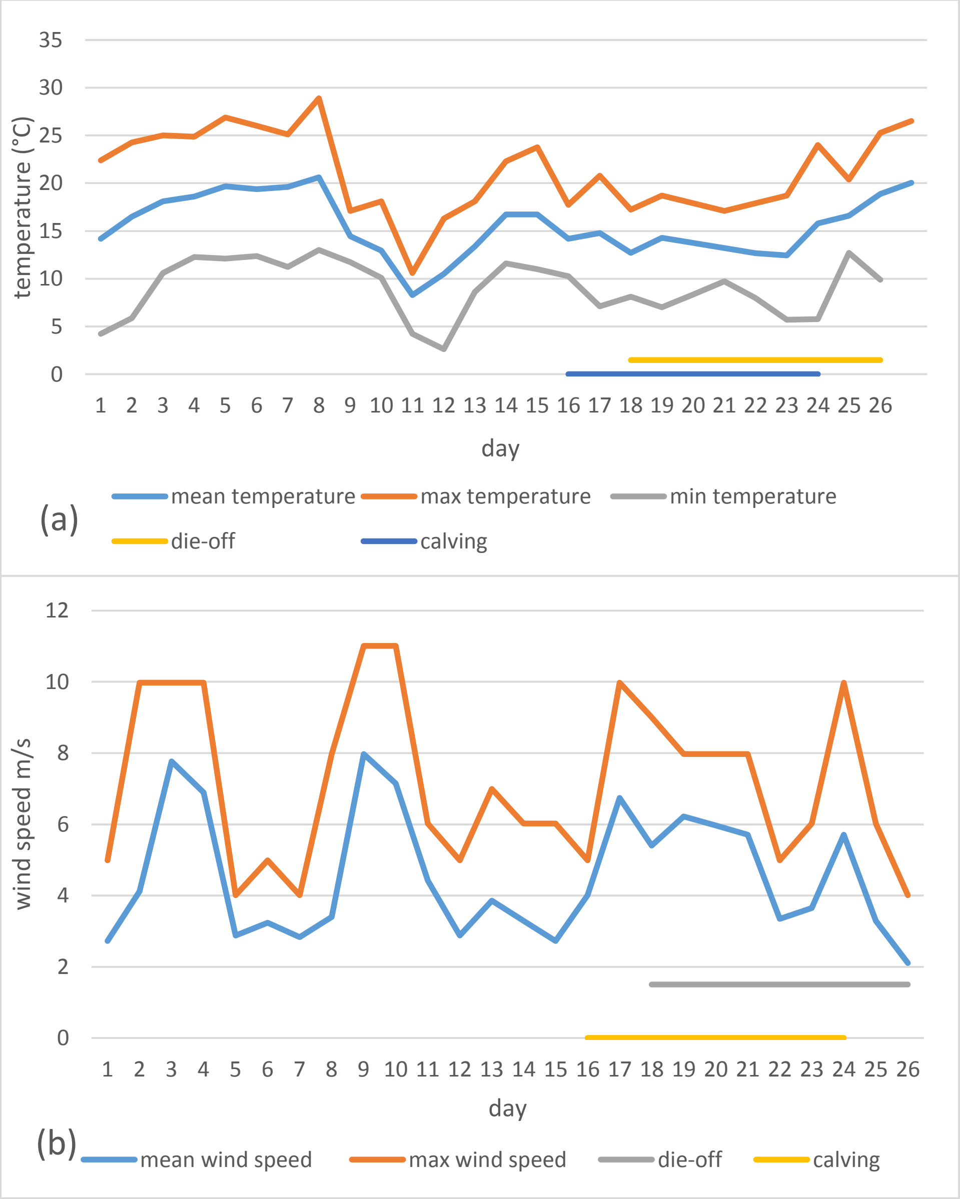
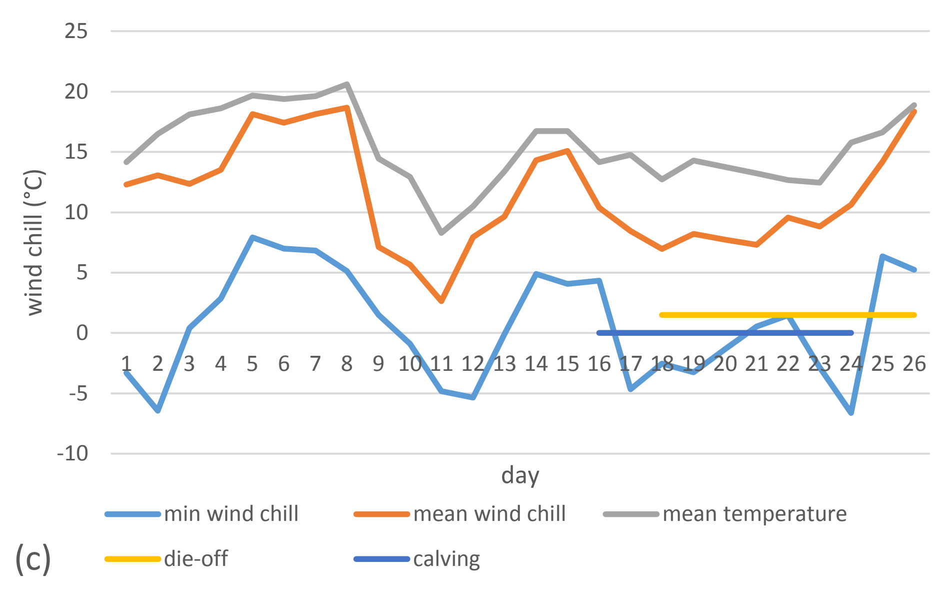
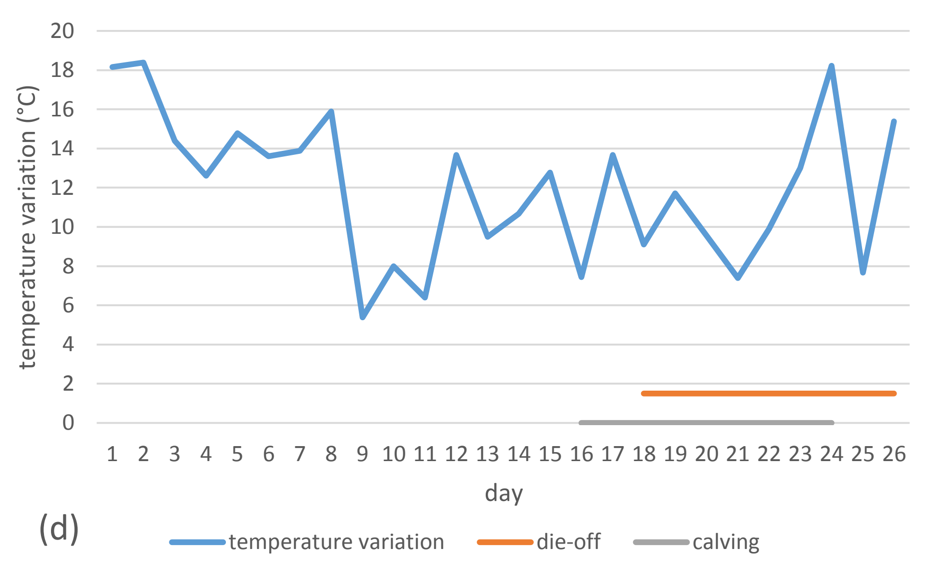
Appendix A

Figure 1: Weather records from the weather station in Derzhavinsk, from before and during the Tengiz die-off in May 2015. (a) mean, maximum (max) and minimum (min) temperature (b) mean and maximum wind speed (c) mean and minimum wind chill (d) temperature variation (e) relative humidity (RH).

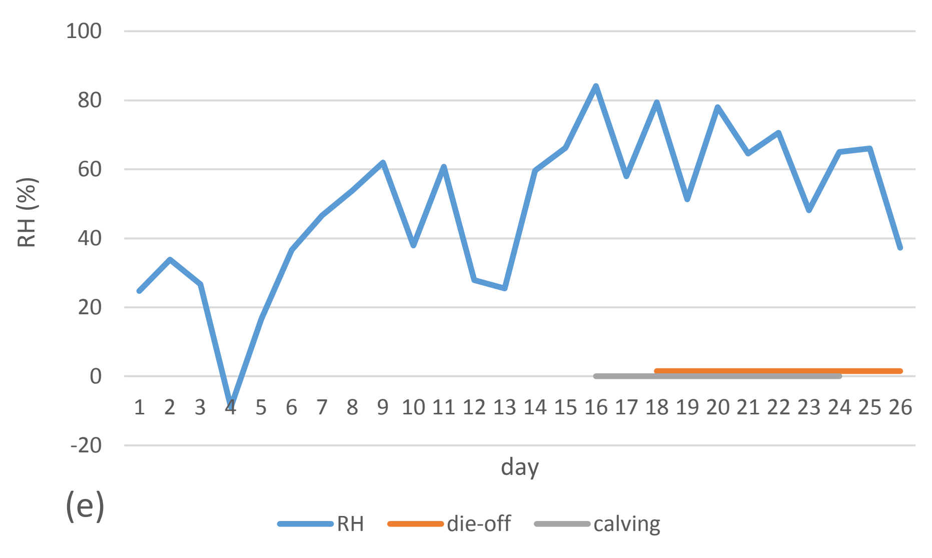



25
20
15
10
5
0
-5
-10
wind chill (°C)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
day
min wind chill
mean wind chill
mean temperature
die-off
calving

(c)

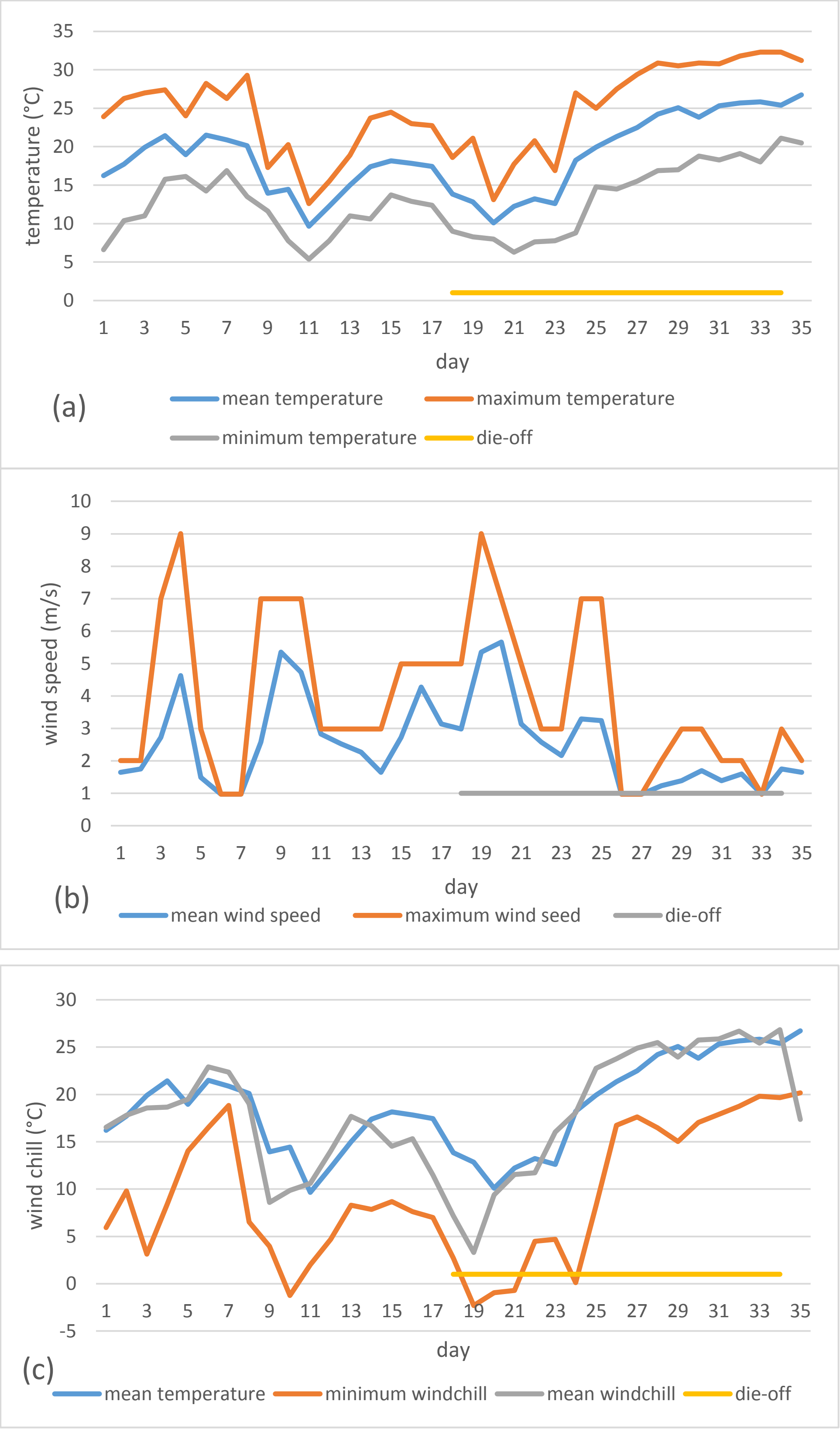

20
18
16
14
12
10
8
6
4
2
0
temperature variation (°C)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
day
temperature variation
die-off
calving

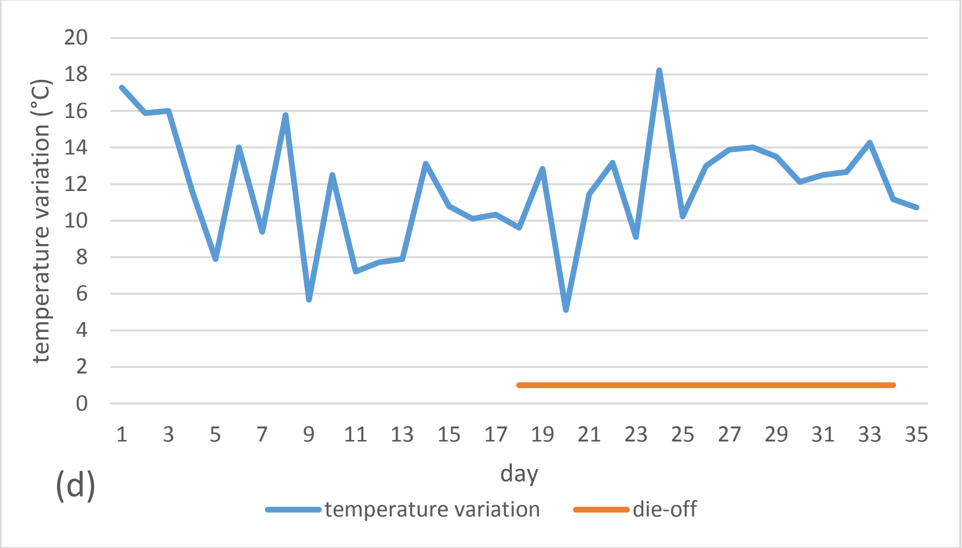
(d)


100
80
60
40
20
0
-20
RH (%)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
day
RH
die-off
calving

(e)

Figure 2: Weather records from the weather station in Turgaj, from before and during the Yrgyz die-off in May 2015. (a) mean, maximum (max) and minimum (min) temperature (b) mean and maximum wind speed (c) mean and minimum wind chill (d) temperature variation (e) relative humidity (RH).




temperature variation (°C)
20
18
16
14
12
10
8
6
4
2
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35
day
(d)
temperature variation
die-off

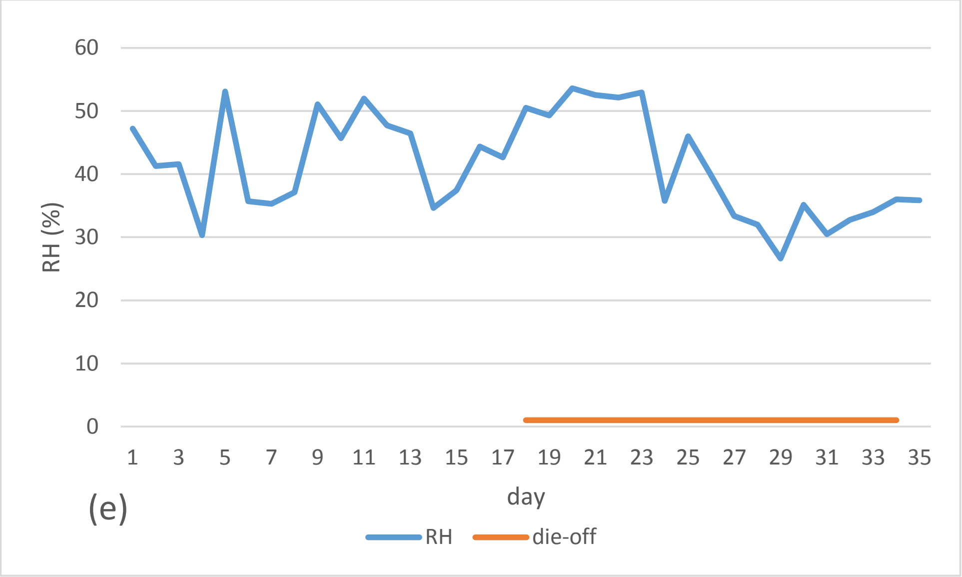

RH (%)
60
50
40
30
20
10
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35
day
(e)
RH
die-off

Figure 3: Weather records from the weather station in Turgaj, from before and during mass calving in May 2014. (a) mean and maximum wind speed (b) temperature variation (c) relative humidity (RH).

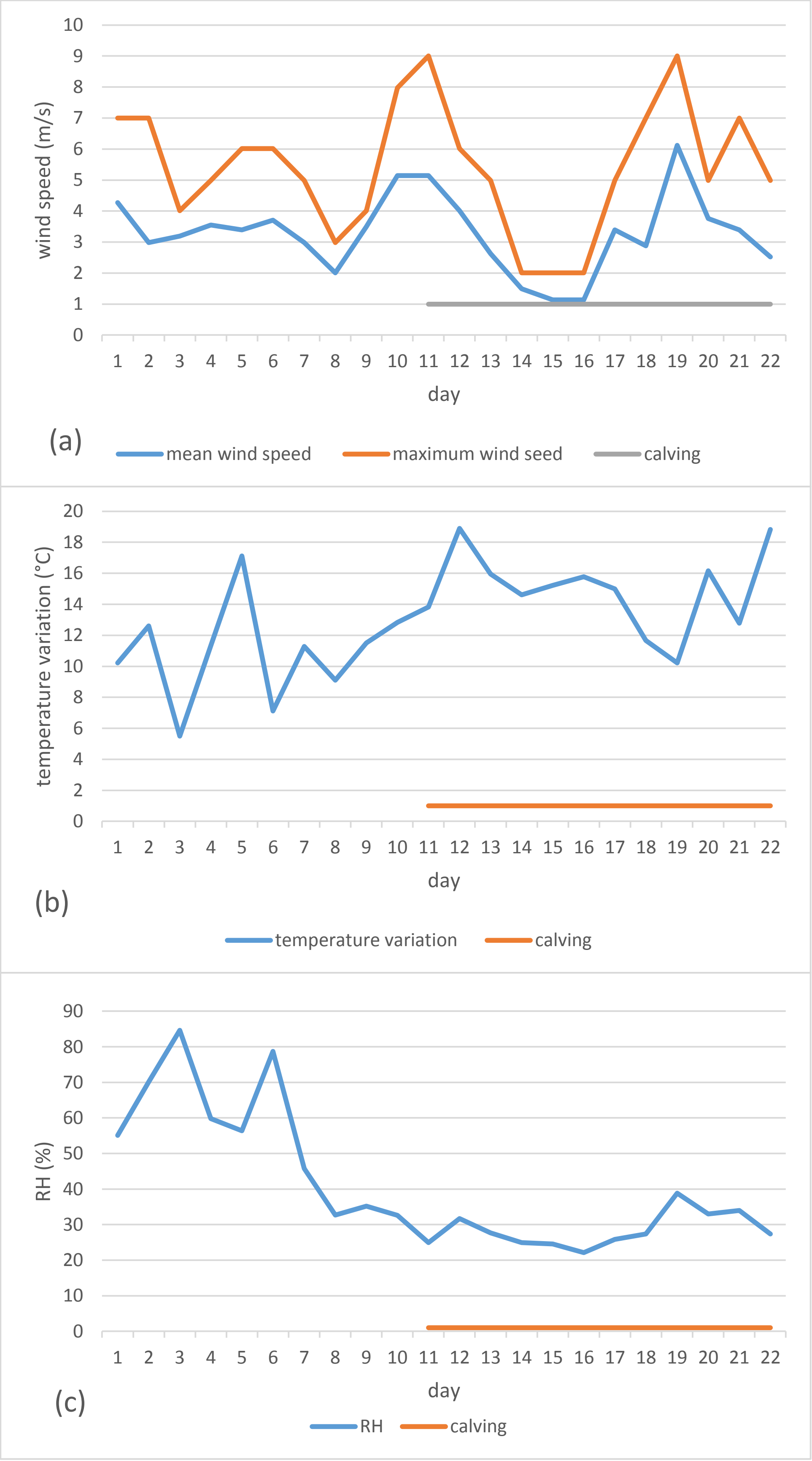
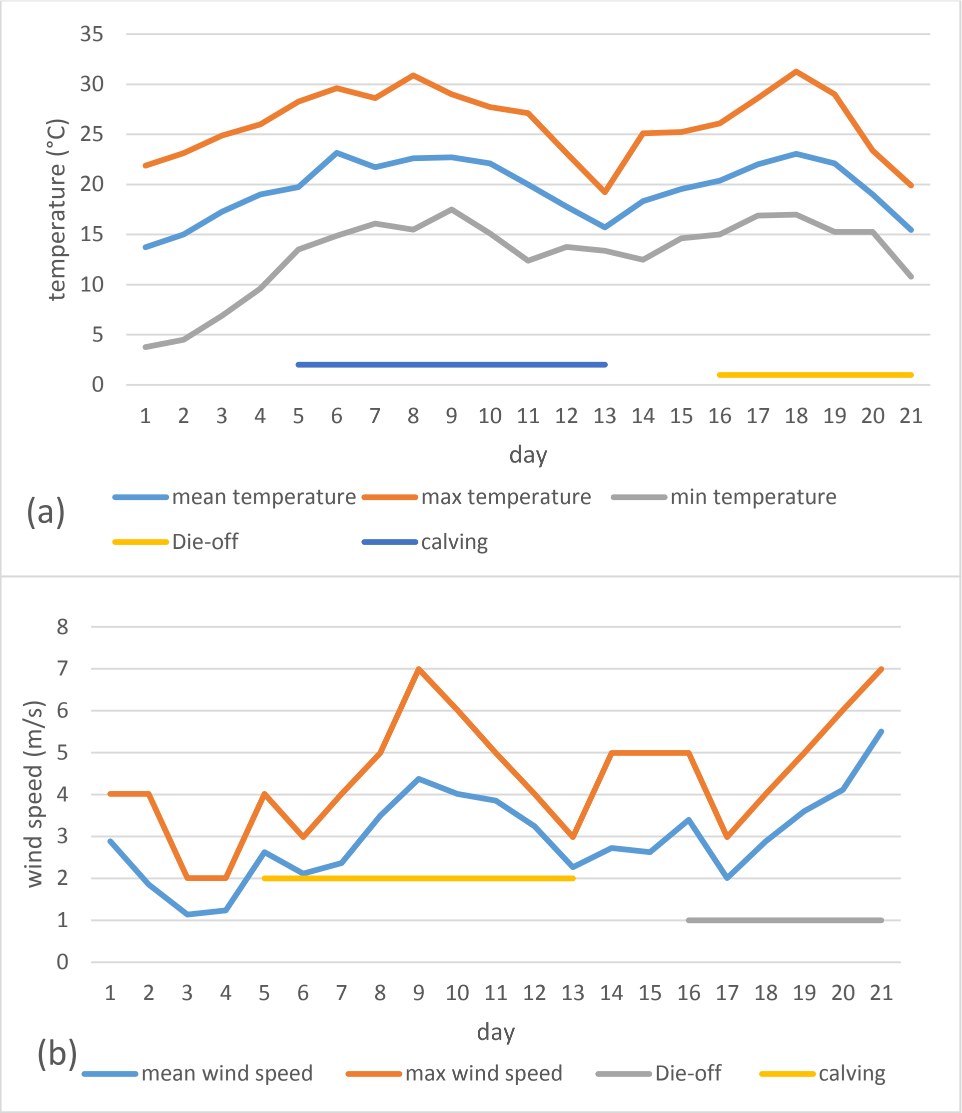


Figure 4: Weather records from the weather station in Aleksandrov-Gay, from before and during the Ural die-off in May 2010. (a) mean, maximum (max) and minimum (min) temperature (b) mean and maximum wind speed (c) mean and minimum wind chill (d) temperature variation (e) relative humidity (RH).



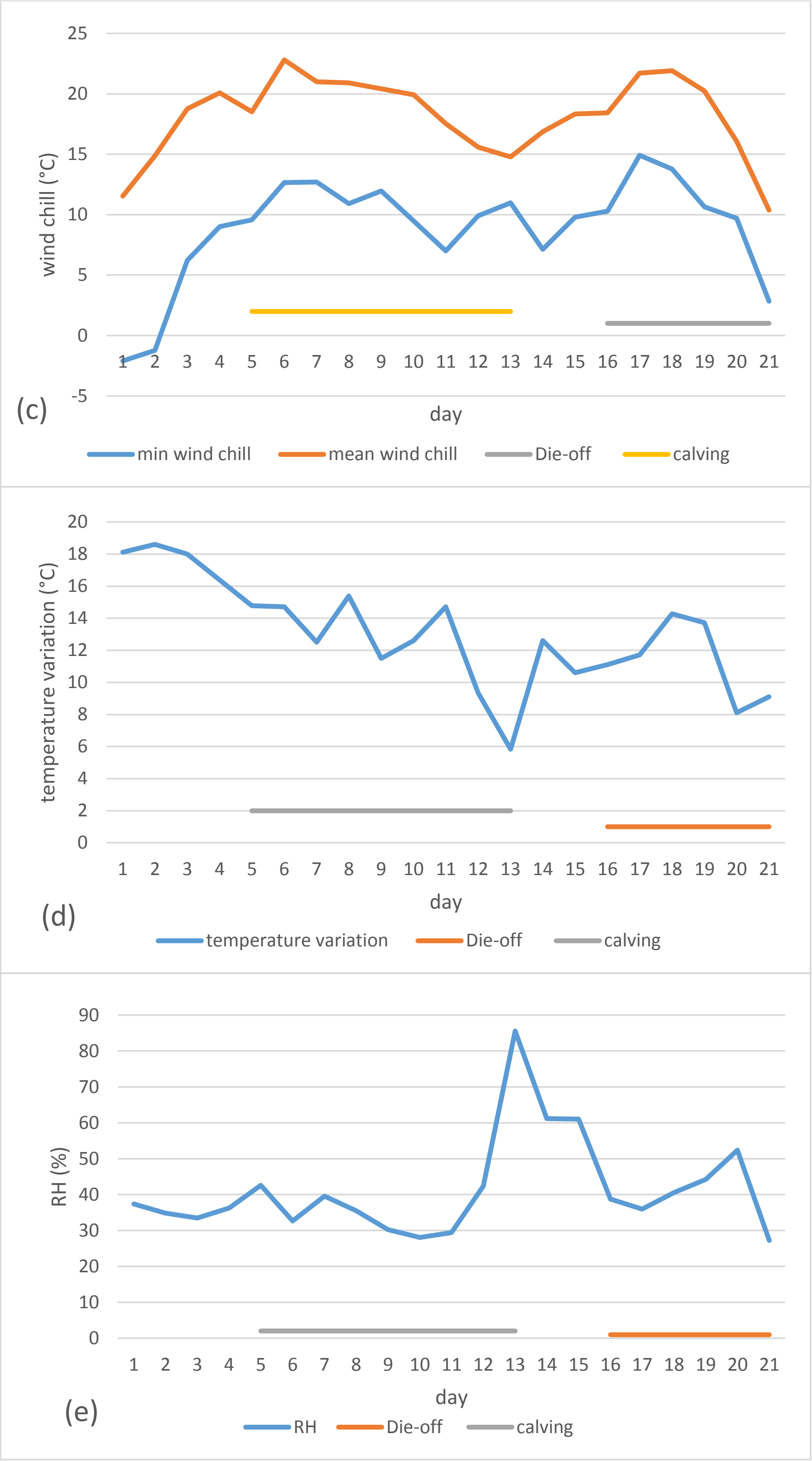
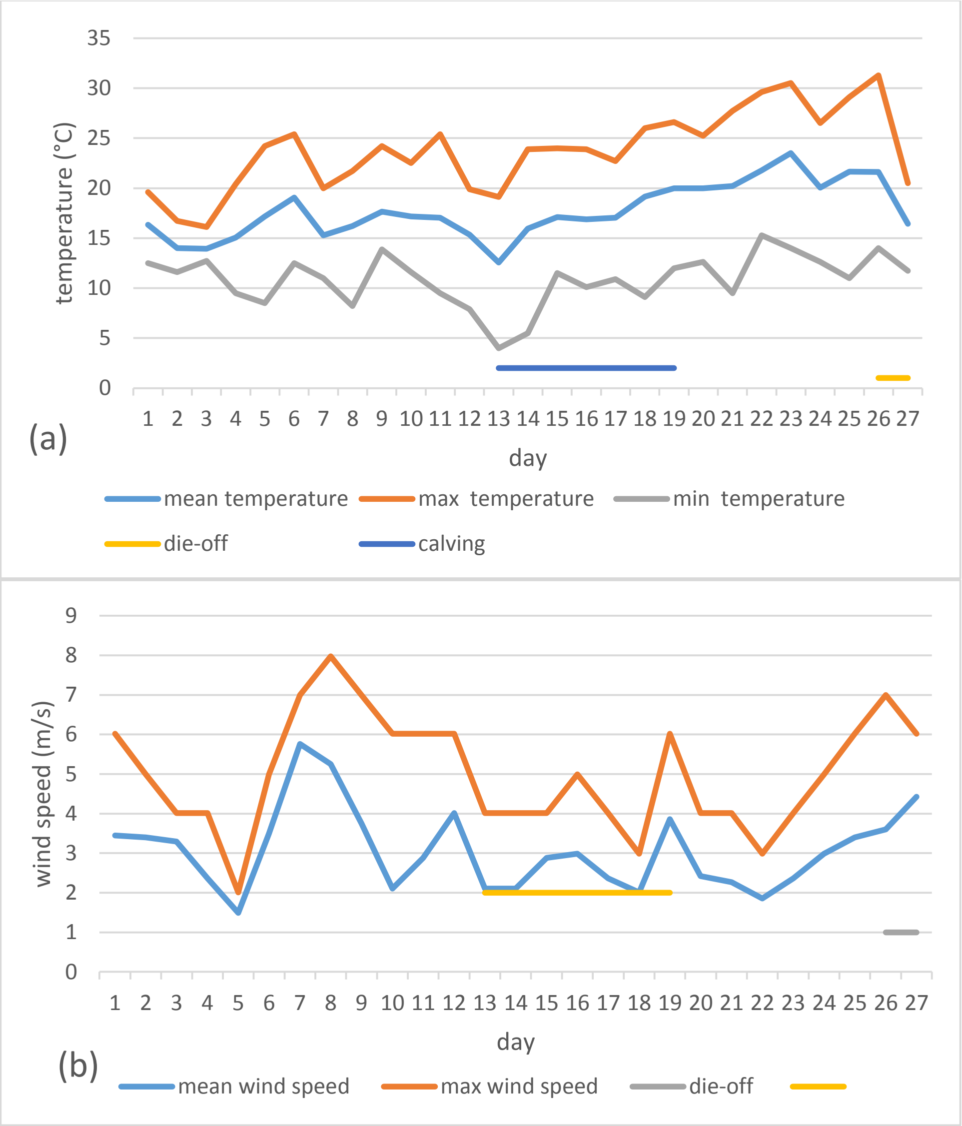
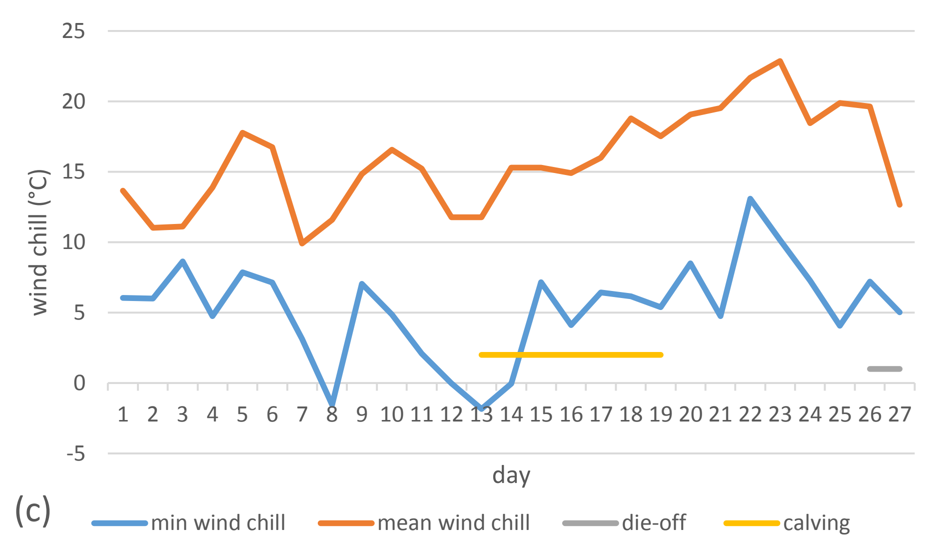
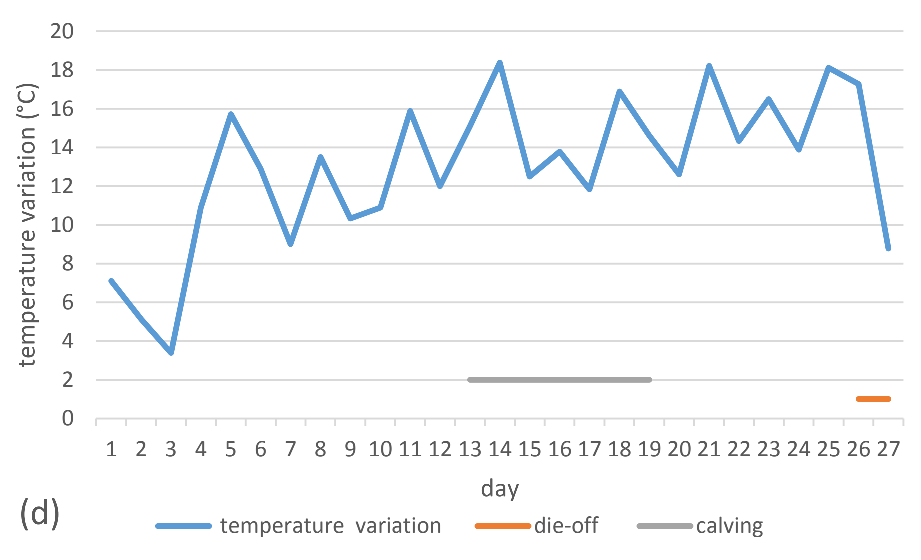
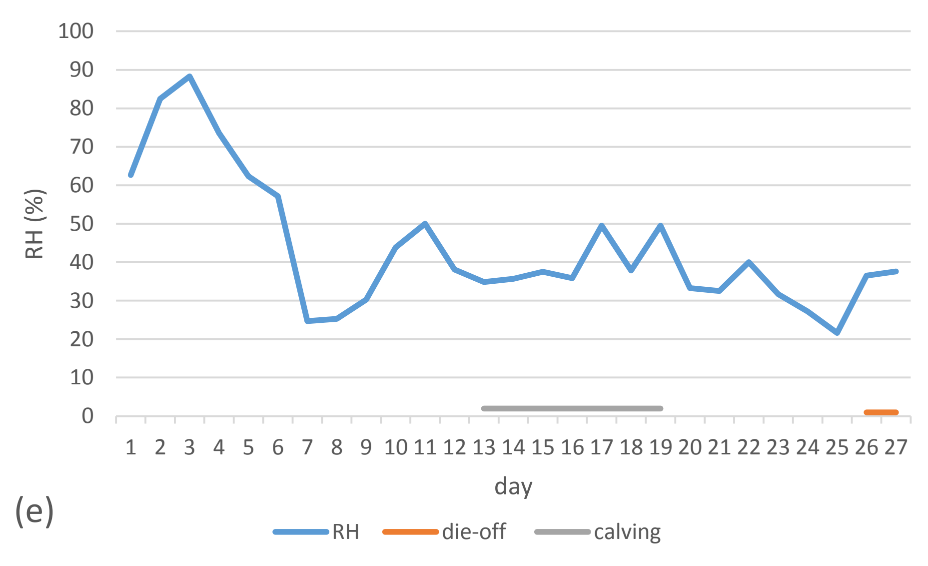

25
20
15
10
5
0
-5
wind chill (°C)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
day
(c)
min wind chill
mean wind chill
Die-off
calving
20
18
16
14
12
10
8
6
4
2
0
temperature variation (°C)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
day
(d)
temperature variation
Die-off
calving
90
80
70
60
50
40
30
20
10
0
RH (%)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
day
(e)
RH
Die-off
calving

Figure 5: Weather records from the weather station in Aleksandrov-Gay, from before and during the Ural die-off in May 2011. (a) mean, maximum (max) and minimum (min) temperature (b) mean and maximum wind speed (c) mean and minimum wind chill (d) temperature variation (e) relative humidity (RH).




(c)
wind chill (°C)
25
20
15
10
5
0
-5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
day
min wind chill
mean wind chill
die-off
calving


(d)
temperature variation (°C)
20
18
16
14
12
10
8
6
4
2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
day
temperature variation
die-off
calving


(e)
RH (%)
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
day
RH
die-off
calving