

**The Innovation, Volume 2**

## **Supplemental Information**

**From unusual suspect to serial**

**killer: Cyanotoxins boosted by climate**

**change may jeopardize megafauna**

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## **Methods for climatic data processing and analyses**

To investigate the climatic conditions associated with cyanobacterial blooms, temperature and precipitation records are examined. To account for potential observational uncertainty, multiple observational datasets are used. Global monthly temperature datasets used include: (1) GISS (NASA Goddard Institute for Space Studies; 2°X2°) and (2) BEST (Berkeley Earth Surface Temperature); 1°X1°. <sup>1,2</sup> Global monthly precipitation observation is from the GPCP (Global Precipitation Climatology Project); 2.5°X2.5°. <sup>3</sup> Monthly precipitation and evaporation data from the ERA5 reanalysis (0.25°X0.25°) are also used. <sup>4</sup>

Accumulated temperature for cyanobacteria is defined as follows <sup>5,6</sup>:

$$K = N(T - C)$$

where  $K$  is the accumulated temperature,  $C$  is the temperature threshold above which cyanobacteria recruit ( $C=9^{\circ}\text{C}$ ),  $N$  is the length of the cyanobacterial growing period (i.e. when  $T>C$ ), and  $T$  is the mean temperature over the growing period. In this study, annual accumulated temperature is computed from preceding June to May (in  $^{\circ}\text{C}$  days).

Likewise, the annual accumulated net precipitation is calculated from preceding June to May (in mm), which represents the availability of surface water. Here net precipitation (i.e. precipitation minus evaporation) is used so that the enhanced evaporation accompanying hot conditions is taken into account.

The temperature and precipitation records from different datasets consistently reveal the anomalous hot and dry conditions during 2019-2020 in Botswana and Zimbabwe (figure not shown). Thus, only the results from the GISS and ERA5 datasets are provided in the manuscript for brevity.

## **Methods for remote sensing analyses**

NDWI (Normalized Difference Water Index) was calculated according to REF<sup>7</sup>:

$$\text{NDWI} = (\text{GREEN} - \text{NIR}) / (\text{GREEN} + \text{NIR})$$

where GREEN and NIR represent green band and near infrared band of MSI and OLI. And pixels with NDWI greater than 0 are defined as constant water body.

FAI (Floating Algae Index) was calculated according to REF<sup>8</sup>:

$$\text{FAI} = \text{RED} - \text{NIR}'$$

$$\text{NIR}' = \text{RED} + [(\text{SWIR} - \text{RED}) * [(\lambda(\text{NIR}) - \lambda(\text{RED})) / (\lambda(\text{SWIR}) - \lambda(\text{RED}))]]$$

where RED, SWIR and NIR represent red band, near infrared band and shortwave infrared band of MSI and OLI.  $\lambda(\text{RED})$ ,  $\lambda(\text{NIR})$  and  $\lambda(\text{SWIR})$  represent the center wavelength of red band, near infrared band and shortwave infrared band of MSI and OLI.

## **Methods for Figure 1 D**

Response curve of growth rate of cyanobacteria to temperature was modified from REF<sup>9</sup>; Data on elephant density were collected from REF,<sup>10</sup> and data on mammal species richness were compiled based on their distribution range maps from the African Mammal Databank.

## **Text S1 Potential leading causes underlying the mysterious mass mortality.**

### **Ruled-out causes**

- 1 Poaching for ivory – carcasses intact.
- 2 Sabotage by farmers (nine commonly used pesticides) – lab test.
- 3 Starvation or dehydration – found in an area with an abundance of woodlands, 70% near waterholes and ponds.
- 4 Anthrax poisoning – differing from its selective effects on old and young individuals, carcasses of all ages found.

### **Possible causes**

- 1 Cyanotoxins
  - Lab test (posted by Botswana Department of Wildlife and National Parks) (*1*).
  - Carcasses found in/near waterholes or ponds with heavy scum of cyanobacteria.
  - Massive hemorrhage.
  - Walk in circles and appear dizzy before suddenly dropping dead (sometimes face-first), typical neurological symptoms of (*2,3*).
- 2 Encephalomyocarditis virus (hemorrhagic septicemia, a rodent-borne virus infection)
  - Neurological symptoms.
  - Massive hemorrhage.

### **Related media reports:**

1. Weston, P. 2020 Botswana says it has solved the mystery of mass elephant die-off. <https://www.theguardian.com/environment/2020/sep/21/Botswana-says-it-has-solved-mystery-of-mass-elephant-die-off-age-of-extinction-aoe>
2. Boulton, V. 2020. Hundreds of elephants are mysteriously dying in Botswana – a conservationist explains what we know. July 3, 2020. <https://theconversation.com/hundreds-of-elephants-are-mysteriously-dying-in-Botswana-a-conservationist-explains-what-we-know-142004>
3. Maron, D. F. 2020. What's killing Botswana's elephants? Here are the top theories. <https://www.nationalgeographic.com/animals/2020/07/botswana-elephant-death-mystery/>

URL link for photos showing dead elephants in the Okavango Delta.

<https://www.reuters.com/article/us-botswana-elephants-idUSKCN24N2JK>

## **Text S2 Concepts of cyanobacteria, cyanotoxin, and eutrophication.**

### **Cyanobacteria**

Cyanobacteria, also known as blue-green algae, are oxygen-producing bacteria and the Earth's oldest (~ 3.5 bya) organisms, with a major shaping effect on the origin of our modern-day biosphere through oxidization of the Earth's atmosphere.<sup>11</sup>

Cyanobacteria occur throughout the world, especially in calm water rich in nutrients. Cyanobacteria can form dense blooms under nutrient-rich conditions, resulting in increased turbidity, smothering of submersed macrophytes, and oxygen depletion followed by massive deaths of fish and sensitive macroinvertebrates. Cyanobacteria can interfere with the recreational function of waters and their use for drinking water purposes by their generation of obnoxious taste and odor compounds. Cyanobacteria can also produce a variety of toxins, called cyanotoxins, which are detrimental and even lethal to birds, mammals and humans.<sup>12</sup> Cyanobacteria are particularly abundant in nutrient-rich and hot environments such as southeastern Africa.<sup>13,14</sup>

### **Cyanotoxins**

Cyanotoxins can be classified into various types according to their modes of action and target cells and organs: hepatotoxins (the most frequently encountered), neurotoxins, dermatotoxins, and cytotoxins.<sup>12,15</sup> The symptoms of cyanotoxin poisoning include skin irritation, stomach aches, vomiting, nausea, diarrhea, fever, sore throat, and headache. The largest group of cyanotoxins is the cyclic heptapeptide hepatotoxins called microcystins (MCs), in which MC-LR is the best known and most toxic structural variant.<sup>16</sup> Microcystins predominantly cause liver injury.<sup>17,18</sup> An acute, lethal dose exposure can result in almost total breakdown of the tissue architecture and blood accumulation in the liver, where blood loss from the circulation causes death to the animal. Besides hepatotoxicity and tumor promotion, MCs may also induce neurotoxicity, reproductive toxicity, genotoxicity, and potential carcinogenicity.<sup>19-22</sup> Despite their aquatic origin, most cyanotoxins tend to be more hazardous to terrestrial mammals than to aquatic biota.<sup>23</sup> Furthermore, the proportion of toxic species or strain and release of toxins tend to increase with global warming.<sup>24,25</sup>

### **Eutrophication**

Eutrophication is an enrichment process in aquatic ecosystems created by excessive loading of nutrients (e.g. nitrogen and phosphorus), which promotes massive growth of phytoplankton, including cyanobacteria. A shift from a macrophyte-dominated state to a phytoplankton-dominated state will happen when nutrient loading surpasses a critical level.<sup>26,27</sup> Human activities are a common cause of eutrophication, with both point-source and non-point-source discharges accelerating the rate at which nutrients enter ecosystems. In parts of Africa, wild mammals such as hippos contribute significantly to the nutrient loading of freshwater ecosystems by moving nutrients from terrestrial to aquatic ecosystems, and by foraging on land, whereas much non-feeding time is spent in water.<sup>28</sup> Therefore, savanna waters can become highly eutrophic also without the influence of human activities.

## **Text S3 Derivation of the tolerable daily intake and half lethal dose of microcystin for African savanna elephant**

### **Provisional guideline of cyanotoxins by World Health Organization (WHO)**

In order to set safe levels of cyanotoxins, extensive experimental studies on their toxicity have been carried out. Microcystin-LR (MC-LR), the most frequently occurring and most toxic form, is also the most studied. As direct assessment of the risk of cyanotoxin exposure to humans is not feasible, animal studies have been conducted to address the issue. Among these, a 13-week study of mice exposed to pure MC-LR (by gavage) was considered suitable for deriving a guideline value for MC-LR.<sup>29</sup> Based on liver histopathology and serum enzyme level changes, a NOAEL (No Observed Adverse Effect Level) of  $40 \mu\text{g kg}^{-1} \text{ bw day}^{-1}$  was determined. By applying a total uncertainty factor of 1,000, a provisional TDI (Tolerable Daily Intake) of  $0.04 \mu\text{g kg}^{-1} \text{ bw day}^{-1}$  was established as the guideline of safe level.<sup>23</sup> A level of  $1.0 \mu\text{g L}^{-1}$  was then adopted as the provisional guideline based on an average adult body weight of 60 kg and an average water intake for adults of 2 liters per day. For acute exposures, a half lethal dose (LD50) of  $5 \text{ mg kg}^{-1} \text{ bw}$ , administered by gavage, is commonly accepted based on tests on one mouse strain.<sup>29</sup>

### **TDI and LD50 for African savanna elephants**

We derived TDI and LD50 for African savanna elephant (*Loxodonta africana*) as follows, based on an average elephant (5000 kg in body weight, 200 L water ingestion per day)<sup>30</sup>:

$$\text{TDI}_{L.africana}$$

$$= \text{TDI}_{\text{WHO2011}} * \text{bw}_{L.africana} / \text{Water ingestion}$$

$$= 0.04 \mu\text{g kg}^{-1} \text{ bw day}^{-1} * 5000 \text{ kg} / 200 \text{ L}$$

$$= 1.0 \mu\text{g L}^{-1} \text{ d}^{-1}$$

$$\text{LD50}_{L.africana}$$

$$= \text{LD50}_{\text{WHO2011}} * \text{bw}_{L.africana} / \text{Water ingestion}$$

$$= 5 \text{ mg kg}^{-1} * 5000 \text{ kg} / 200 \text{ L}$$

$$= 125 \text{ mg L}$$

**Table S1. Historic events of cyanotoxin-caused animal mortality**

Year	Country	Location	Cyanobacteria species	Toxin	Impacts reported		Reference number
					Death	Illness	
1913-1943	South Africa	Free State and Southeast Transvaal	<i>Microcystis aeruginosa</i>	microcystin	thousands of livestock (horses, sheep, cattle and rabbits)		31
1942	South Africa	Vaal Dam	<i>Microcystis</i> sp.	microcystin	thousands of cattle and sheep		32,33
1973-1974	South Africa	Hartbeespoort Dam	<i>M. aeruginosa</i>	microcystin	cattle		34
1979	South Africa	Klipvoor Dam	<i>M. aeruginosa</i>	microcystin	3 white rhinoceroses		35
1980	South Africa	Vaal Dam	<i>M. aeruginosa</i>	microcystin	cattle		31
1984	South Africa	William Pretorius Game Reserve	<i>M. aeruginosa</i>	microcystin	several black wildebeests		35
1987	South Africa	Eastern Transvaal	<i>M. aeruginosa</i>	microcystin	47 cattle		36
1989	South Africa	Reservoir Bloemhof Dam	<i>M. aeruginosa</i>	microcystin	7 giraffes		37
1989	South Africa	Reservoir Klipdrif Dam	<i>M. aeruginosa</i>	microcystin	livestock	livestock	38
1993	South Africa	Malmesbury	<i>Nodularia spumigena</i>	nodularin	3 cattle	10 cattle	39
1994	South Africa	Lake Zeekoevlei	<i>N. spumigena</i> , <i>M. aeruginosa</i>	nodularin, microcystin	dog (bull terrier bitch)		39,40
1994	South Africa	Malmesbury	<i>N. spumigena</i>	nodularin	34 cattle		39
1994	South Africa	Paarl	<i>M. aeruginosa</i>	microcystin	11 sheep	30 sheep	39
1996	South Africa	Kareedouw	<i>Oscillatoria</i> sp.	microcystin	290 dairy cows	70 stock	41
1998	South Africa	Erfenis Dam	<i>Anabaena</i> spp.	cyanotoxin	livestock		31,42

2000	South Africa	Orange River system downstream of the confluence with the Harts River	<i>Cylindrospermopsis raciborski</i> <i>Anabaena</i> sp., <i>Oscillatoria</i> sp.	not available	fish		41,43
2001	Kenya	Lake Bogoria	<i>Phormidium terebriformis</i> , <i>O. willei</i> , <i>Spirulina subsalsa</i> , <i>Synechococcus bigranulatus</i>	microcystin, anatoxin	mass lesser flamingos		44
2005	South Africa	Nhlanganzwane Dam	<i>M. aeruginosa</i>	microcystin	7 white rhinoceroses, 2 lions, 2 cheetahs, 9 zebras, 23 wildebeest, 1 hippopotamus, 1 giraffe, 5 buffalos, 1 warthog, 1 Kudu		45
2007	South Africa	Nhlanganzwane Dam <sup>a</sup>	<i>M. aeruginosa</i>	microcystin	15 white rhinoceroses, 10 zebras, 10 blue wildebeests	wild animals	43,45,46
2007	South Africa	Sunset Dam	<i>M. aeruginosa</i>	microcystin	1 white rhinoceros, 6 impalas		46
2008	South Africa	Lake Loskop Dam	<i>M. aeruginosa</i> , <i>M. flos-aquae</i>	microcystin	fish		47
2008	South Africa	Shilolweni Dam	<i>Microcystis</i> sp.	microcystin	70 wild animals (zebras, wildebeest, impalas, white rhinoceroses)		45
2008	Botswana	Tuli block	<i>Oscillatoria</i> sp.	microcystin	70 bushbucks and impalas		43
2010	South Africa	Shilolweni Dam	<i>Microcystis</i> sp.	microcystin	17 zebras, 7 wildebeests, 3 white rhinoceroses		45

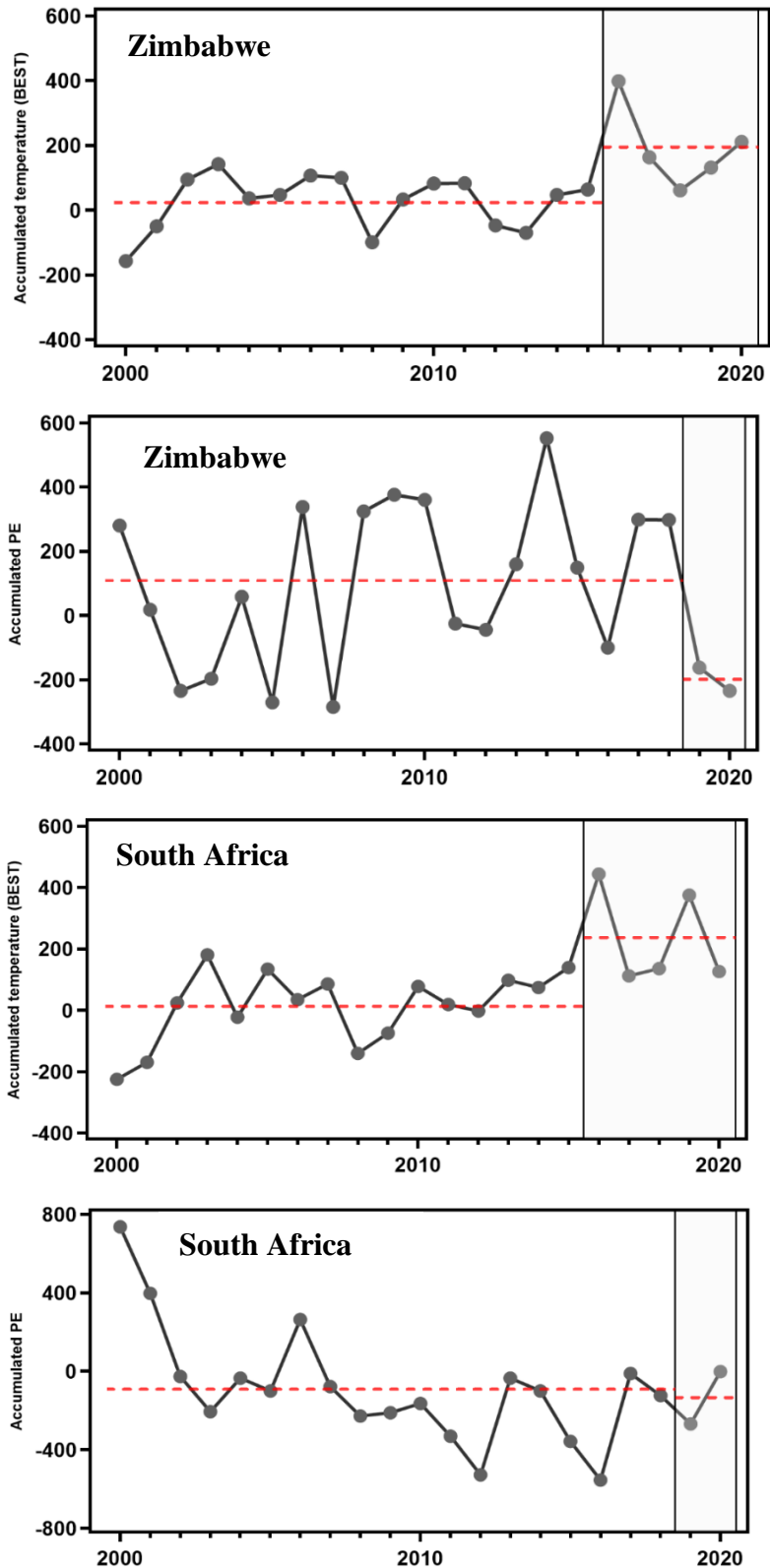
**Table S2. List of published concentrations of microcystins (MCs) /manuscripts on African waters, with calculation of daily intake by African savanna elephant per body weight based on an average individual.**

Code	Zone	Country	Waters	MCs, $\mu\text{g/L}$	Daily intake by African elephant per body weight (MCs*200L/5000 kg), $\mu\text{g kg}^{-1}\text{bw}$	Reference number
1	Northern	Algeria	Ain Zada Dam	69.3	2.8	48
2	Northern	Algeria	Cheffia Dam	28.9	1.2	49
3	Northern	Algeria	Lake Oubcim	46.2	1.8	14
4	Northern	Egypt	Nile River	7.1	0.3	50
5	Northern	Morocco	Lake Lalla Takerkoust	19.9	0.8	14
6	Western	Ghana	Kpong Dam	0.03	0.001	51
7	Western	Ghana	Weija Dam	3.21	0.1	51
8	Western	Nigeria	Zaria aquaculture ponds	6.34	0.3	14
9	Eastern	Ethiopia	Lake Chamo	28.9	1.2	14
10	Eastern	Ethiopia	Lake Chamo	6.1	0.2	52
11	Eastern	Ethiopia	Lake Koka	3.9	0.2	14
12	Eastern	Ethiopia	Lake Koka	51	2.0	52
13	Eastern	Ethiopia	Lake Langano	1.3	0.1	52
14	Eastern	Ethiopia	Lake Ziway	1.3	0.1	52
15	Eastern	Kenya	Lake Simbi	29163	1167	53
16	Eastern	Kenya	Lake Sonachi	64.8	2.6	53
17	Eastern	Kenya	Lake Victoria, Nyanza Gulf	0.4	0.02	54
18	Eastern	Kenya	Nakuru oxidation ponds	1.72	0.1	14
19	Eastern	Kenya	Nyanza Gulf	82.5	3.3	14
20	Eastern	Kenya, Tanzania, Uganda	Lake Victoria	1	0.04	55
21	Eastern	Uganda	Lake Saka	31783	1271	14

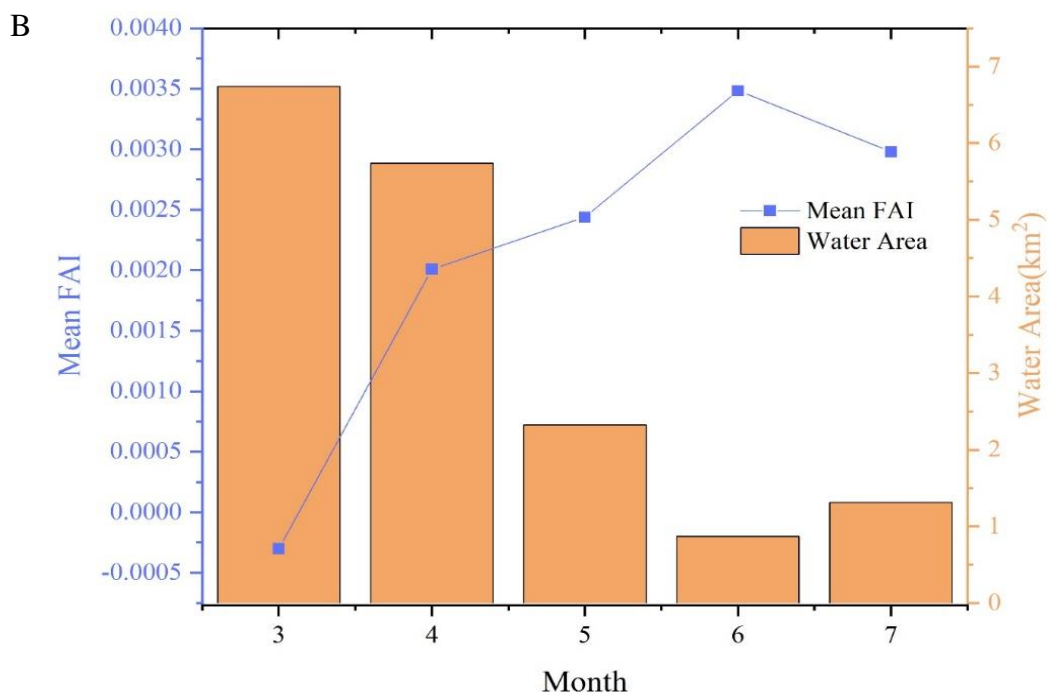
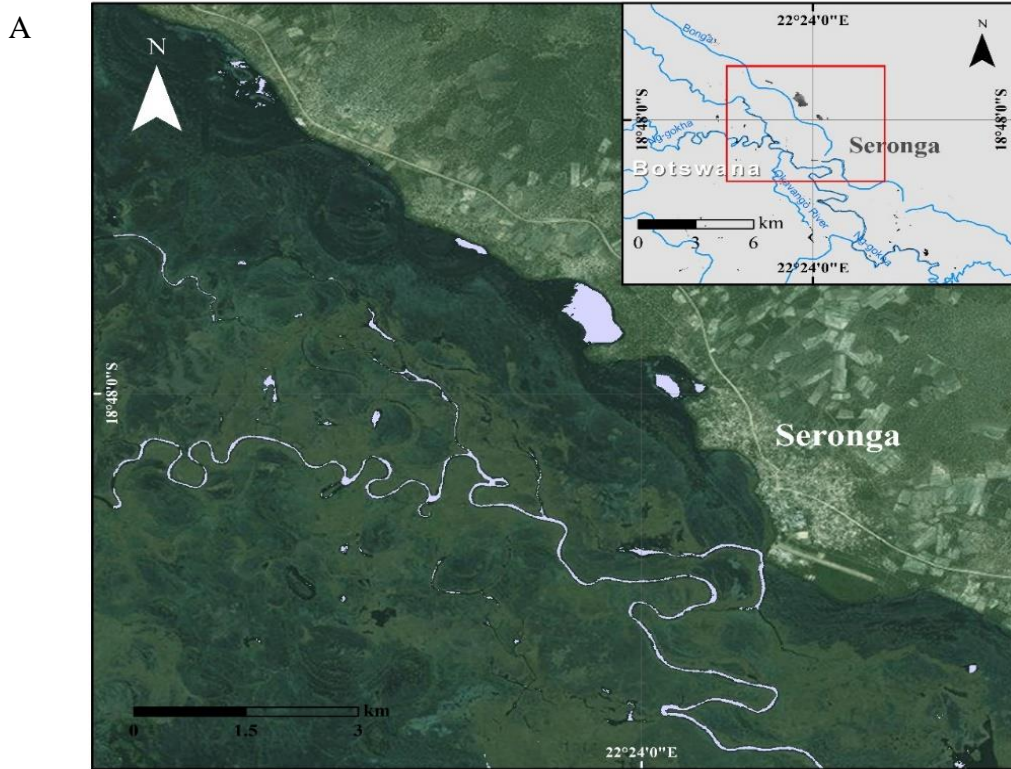


22	Eastern	Uganda	Murchison Bay	3.1	0.1	14
23	Southern	Mozambique	Maputo and Gaza provinces	7.31	0.3	13
24	Southern	South Africa	Hartbeespoort Dam	580	23.2	56
25	Southern	South Africa	Hartbeespoort Dam	1965	78.6	41
26	Southern	South Africa	Hartbeespoort Dam	1861	74.4	41
27	Southern	South Africa	Hartbeespoort Dam	1698	67.9	41
28	Southern	South Africa	Hartbeespoort Dam	414	16.6	41
29	Southern	South Africa	Hartbeespoort Dam	1538	61.5	41
30	Southern	South Africa	Hartbeespoort Dam	1314	52.6	41
31	Southern	South Africa	Hartbeespoort Dam	474	19.0	41
32	Southern	South Africa	Hartbeespoort Dam	4.65	0.2	57
33	Southern	South Africa	Hartbeespoort Dam	12300	492	58
34	Southern	South Africa	Hartbeespoort Dam	44878	1795	59
35	Southern	South Africa	Klipvoor Dam	22330	893	41
36	Southern	South Africa	Klipvoor Dam	21100	844	41
37	Southern	South Africa	Loskop Dam	0.36	0.01	14
38	Southern	South Africa	Loskop Dam	0.09	0.004	14
39	Southern	South Africa	Loskop Dam	3.173	0.1	60
40	Southern	South Africa	Makhohlola Dam	0.2	0.01	46
41	Southern	South Africa	Makhohlolo Dam	3.38	0.1	14
42	Southern	South Africa	Mpanamana Dam	1	0.04	46
43	Southern	South Africa	Nhlanganzwane Dam	24951	998	14
44	Southern	South Africa	Nhlanganzwane Dam	103160	4126	46
45	Southern	South Africa	Nhlanganzwane Dam	23720	949	47
46	Southern	South Africa	Rietvlei Dam	198	7.9	41
47	Southern	South Africa	Rietvlei Dam	184	7.4	41
48	Southern	South Africa	Roodeplaat Dam	981	39.2	41
49	Southern	South Africa	Sunset Dam	124460	4978	46

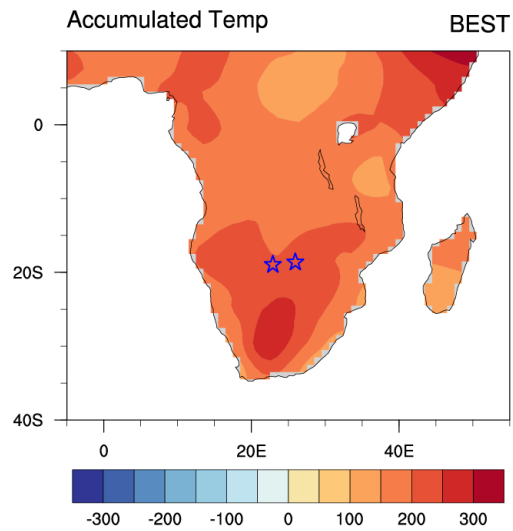
50	Southern	South Africa	Sunset Dam	1110	44.4	46
51	Southern	Zimbabwe	Lake Chivero	19.86	0.8	61
Average	Northern Africa			34.28		
	Western Africa			3.19		
	Eastern Africa			4371		
	Southern Africa			13423		



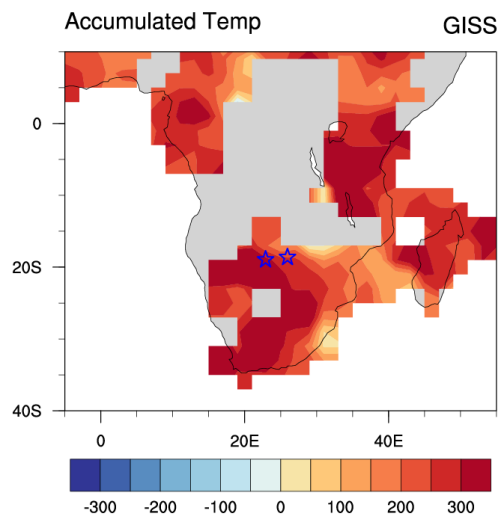
**Fig. S1.** Anomalies (against the 1986-2005 baseline climatology) of accumulated (from preceding June to May) temperature ( $^{\circ}\text{C}$  days) and net precipitation (precipitation minus evaporation, mm) during 2000-2020 in Zimbabwe and South Africa



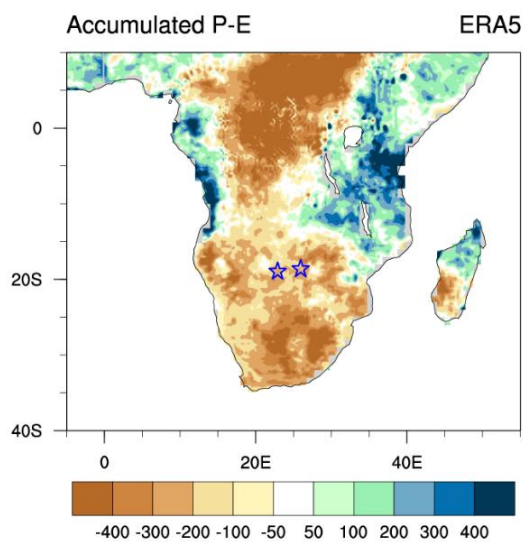
**Fig. S2.** The zone (framed) where most elephant mortality events took place (north-west of Seronga, Okavango Delta, Botswana) and for which the remote sensing analyses were performed (A) and changes in water area and floating algae index (FAI) in the framed area during the period of elephant death (March-July, 2020). The constant water extent area was extracted by NDWI with Sentinel-2/MSI images during the period of elephant death. Changes in water area and floating algae index (FAI) in the framed area were obtained Sentinel-2/MSI and Landsat-8/OLI with at the same time.



**A.** Annual accumulated effective temperature (from preceding June to May; °C days) during 2015-2020 relative to the 1986-2005 baseline climatology from the BEST data. Stars denote Botswana (18.84S, 22.90E) and Zimbabwe (18.51S, 25.95E).

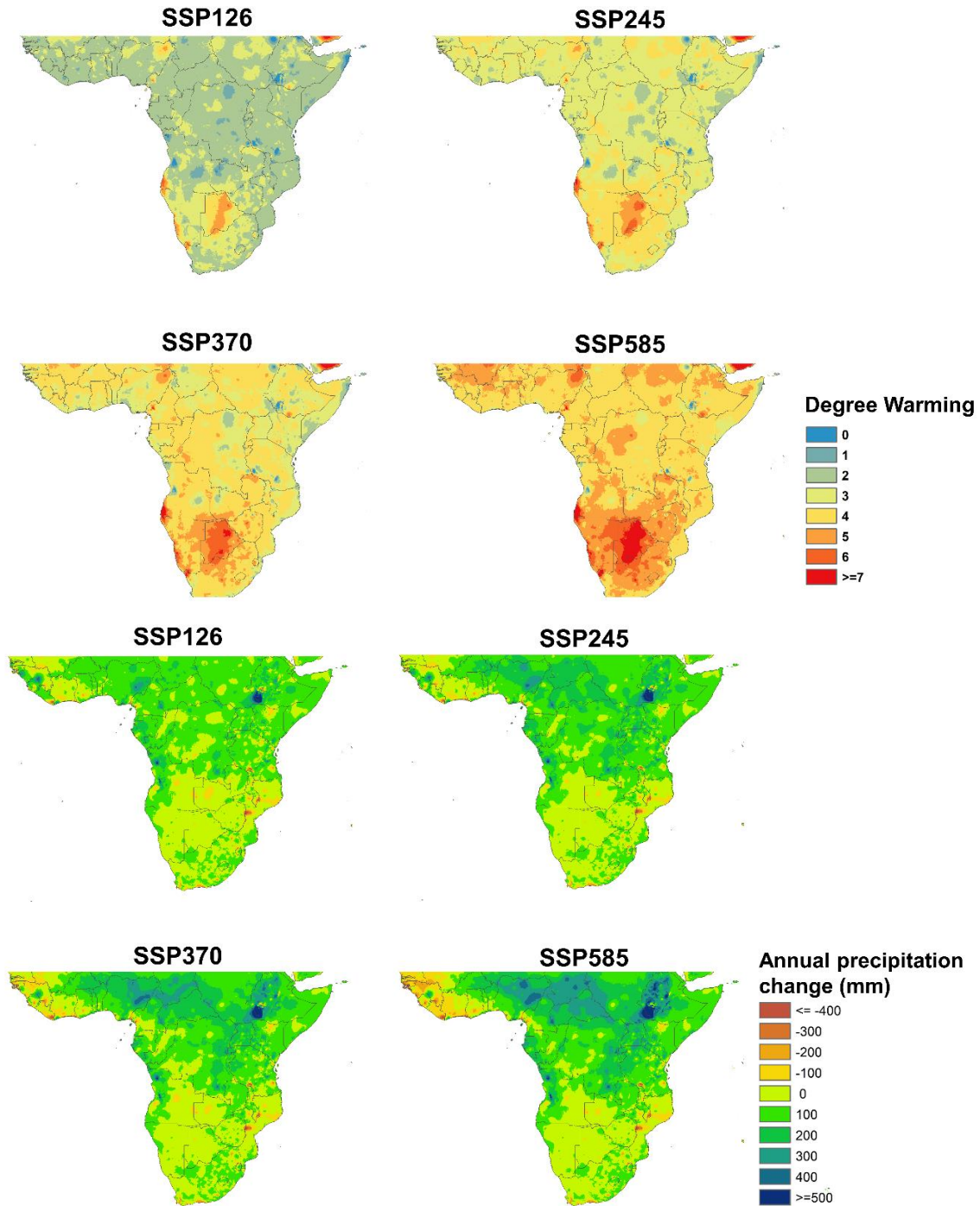


**B.** Same as A but for temperature derived from the GISS data.



**C.** Same as A but for annual accumulated net precipitation (precipitation minus evaporation, mm) during 2019-2020 derived from the ERA5 data.

**Fig. S3.** Spatial distribution of extreme hot and dry climatic conditions associated with the deaths of African savanna elephants in 2020.



**Fig. S4.** Changes in mean annual temperature and precipitation around 2070, with southern Africa identified as a hotspot of climate warming and drying. The mean annual temperature and precipitation data projected for the time period of 2061-2080 are available from 9 CMIP6 global climate models (BCC-CSM2-MR, CNRM-CM6-1, CNRM-ESM2-1, CanESM5, GFDL-ESM4, IPSL-CM6A-LR, MIROC-ES2L, MIROC6, MRI-ESM2-0) for 4 Shared Socio-economic Pathways (SSP126, 245, 370 and 585). WorldClim v2.1 data is used as the current baseline. The climate data with a spatial resolution of ~10 km is downloaded from the WorldClim website.

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