Proceedings of the Royal Society B

The supplementary material for

## Male density, a signal for population self-regulation in

## Alligator sinensis

DOI: 10.1098/rspb. 2019.0191

Lan ZHAO<sup>1</sup>, Li-Ming FANG<sup>2</sup>, Qiu-Hong WAN<sup>1</sup>, & Sheng-Guo Fang<sup>1, \*</sup>

<sup>1</sup> MOE Key Laboratory of Biosystems Homeostasis & Protection, State Conservation

Center for Gene Resources of Endangered Wildlife, College of Life Sciences,

Zhejiang University, Hangzhou 310058, China

<sup>2</sup> Changxing Chinese Alligator Nature Reserve, Changxing 313100, China

Correspondence and requests for materials should be addressed to

sgfanglab@zju.edu.cn

#### Supplementary methods S1

Number count. Initially, the population size was small, and the staff could count the alligators during their daily feeding. The feeding season lasts from May to October every year. Once the population reached 200–300 individuals, counting in this way became difficult. The Chinese alligator has become a national-level protected animal; therefore, the nature reserve staff captured the alligators to confirm the number and sex every spring so that they can report the data to State Forestry Bureau of China.

Incubation. Eggs were collected every year from the alligator nests after 50 days of incubation, i.e. ~1 week before hatching. Eggs were incubated in artificial hatchery rooms. During the egg collection, the number of nests and the total number of eggs were recorded, and the number of hatchlings were record after hatching. The fertilisation status was examined in the nests in which all egg remained unhatched. Hatchlings were raised to survive the first winter and sexed.

*Identification of fertilised eggs.* Egg candlers were used to determine the fertilisation of eggs, whereby light was shone on the eggs in a dark environment. The opaque banding on the shell would be observed if the egg was fertilised [1], and infertile eggs would be translucent [2].

**Sexual identification.** The sex of adults and sub-adults was determined by examining the cloaca for the presence of a penis. In male adults, the penis was determined by touch by moving the finger from side to side along the ventral floor of the cloaca [3,

4]. The sex identification of hatchlings was conducted following the methods initially used for Crocodylus johnstoni and C. porosus. This involved inserting fine curved forceps into the cloaca and spreading them to view the clitero-penis. In females this was defined as "sharp" and "small", while in males it was defined as "tubular" and "large" [5]. The sexing of hatchlings was usually conducted when they reached three months old. Before 1996, the hatchlings were released into a small part of a pond in the original habitat and protected, and the juveniles and the founders all coexisted in the same pond. It was difficult to distinguish the size of the clitero-penis in hatchlings raised to 4–5 years old in the original habitat (> 30 cm snout-vent length), i.e. to reconfirm their sex and then release back into the pool. After 1996, the sex of hatchlings was identified at 2–3 months old with reference to the identification methods used for A. mississippiensis. This method involved determining the colour of the clitero-penis, an important character for sexing alligators. The male clitero-penis is typically red, round, and large in comparison to the female organ, which is white, sharper, and smaller [6]. After sex identification, the alligators were released into the same pool as their mothers.

Age identification. Male and female Chinese alligators mature at 10 and 11 years old, respectively. Juveniles were mainly categorised by age. If the age was unclear, the body length (i.e. total length) was assessed and used to identify the age. The distinguishing standards used were as follows:

Hatchlings-yearlings:  $\leq 2$  years-old, body length  $\leq 30$  cm;

Juveniles: males = 3–6 years old or females = 3–7 years old, body length = 31–120 cm;

Sub-adult: males = 7–9 years old or females = 8–10 years old, body length = 121–150 cm;

Adult: males  $\geq 10$  years old; females  $\geq 11$  years old, body length  $\geq 151$  cm. Originally, the age structure data were not recorded each year for each area. However, the selection criteria of individuals removed to the new areas was explicit, and the number and age of dead individuals were also recorded. The selective order of founders in the C1–C6 area was: large offspring > small offspring > founders of the original area. In each age group, half of the offspring were left behind in the original habitat and the other half were moved to the new area in age order, i.e. from old to young. If more than half the individuals were removed, then the older offspring were selected first and then the younger offspring. The founders in the original area were considered last. The age structure of areas C and C1 were calculated for every year and the data are presented in Table S2. However, this age structure is not precise. It was difficult to determine the age structure in areas C2, C3, C4, C5, and C6 to a high degree of accuracy because they should be based on the age structure of areas C1 and C2. Therefore, the age structure data of areas C2–C6 are absent.

**Data selection.** A destructive snow and ice disaster occurred in 2008 which led to the death of 1184 alligators. To avoid another such disaster, the reserve changed its management protocol after 2011. In 2011, areas C–C6 were divided into 18 new

habitats in succession and all the hatchlings were moved to a greenhouse to overwinter, and then released into separate pools where they were protected during the following spring. These juveniles were moved to a reintroduction area when they were 6 years of age. Owing to this artificial control, we mainly analysed the data before 2011.

#### **Supplementary references**

- 1. Grahame J.W.W., Manolis S.C., Whitehead P.J., Dempsey K. 1987 The Possible Relationship between Embryo Orientation Opaque Banding and the Dehydration of Albumen in Crocodile Eggs. *Copeia* **1987**(1), 252-257. (doi:10.2307/1446070).
- Chen B., Hua T., Wu X., Wang C. 2003 Research on the Chinese alligator (In Chinese). Shanghai Scientific and Technological Education, Shanghai, pp.312-313.
- 3. Bradt G.W. 1938 A study of beaver colonies in Michigan. *Journal of Mammalogy* **19**(2), 139-162. (doi:10.2307%2F1374609)
- 4. Chabreck R.H. 1963 Methods of capturing, marking and sexing alligators. In *Proceedings of the Annual Conference of the Southeastern Association of Game and Fish Commissioners*, pp. 47-50. (doi:10.1007/978-1-349-03711-7\_2)
- 5. Webb G., Manolis S.C., Sack G.C. 1984 Technical Note: Cloacal Sexual of Hatchling Crocodiles. *Wildlife Research* **11**(1), 201-202. (doi:10.1071/WR9840201).
- 6. Allsteadt J., Lang J.W. 1995 Sexual Dimorphism in the Genital Morphology of Young American Alligators, Alligator mississippiensis. *Herpetologica* **51**(3), 314-325. (doi:10.1016/0162-3095(95)90001-2).

# Supplementary table S1

The population events and breeding situation in each of the evaluated years.

"NC" means Not Clear for the data scarcity;  $\mathcal{D}$ : female;  $\mathcal{D}$ : male. The data in the table represent the number of individuals.

		Manual intervention events before breeding season			Population situation in breeding season			reeding situ	ation		Notes					
Area name	Year	Emigration	Imigration	Total individual	Male number	Female number	Nest number	Egg number	Hatchlings		Survive to 3 months-old		Deaths events (after 3 months-old)	Surv numb mat	er to	
				number						Survival number	Male hatchings	Female hatchings		M F		
	1979		1♂2♀are original inhabitants; 1♂2♀captured from Er-jie-ling; 2♀ captured from Guan-yin-qiao;	11	3	8	NC	NC	0	0	0	0	0	0	0	The egg laid on
			2♀captured from Xian-shan; 1♂captured from Si-an													the island, but the island was
	1980			11	3	8	NC	NC	0	0	0	0	0	0	0	submerged by
	1981			11	3	8	NC	NC	0	0	0	0	0	0	0	floods
	1982			11	3	8	NC	NC	0	0	0	0	0	0	0	
	1983			11	3	8	NC	NC	0	0	0	0	0	0	0	
	1984			11	3	8	2	27	10	10	2	8	0	2	7	
C	1985			21	5	16	6	83	64	52	9	43	1♀ born in 1984; $4 \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ $	2	19	
	1986			41	7	34	6	138	134	101	17	84	0	6	29	
	1987			142	24	118	4	79	31	29	7	22	90 (11 $355$ 9 born in 1986, $6318$ 9 born in 1987)	1	4	
	1988			81	14	67	5	98	51	48	8	40	0	8	40	
	1989			129	22	107	6	123	74	68	12	56	0	12	56	
	1990			197	34	163	5	94	43	38	7	31	0	6	9	
	1991			235	41	194	6	111	62	42	7	35	36 (3♂22♀ born in 1990; 5♂6♀born in 1991)	0	3	
	1992	10 ♀ were denoted to Hangzhou zoo	2 ♂ (Captured from wild on April 23 1992)	233	42	191	8	159	47	26	4	22	17 (1♂10♀ born in 1991; 6♀ born in 1992)	3	14	
	1993			244	45	199	6	117	0	0	0	0	0	0	0	

	1	ı				ı			I					
1994			242	45	197	3	57	0	0	0	0	$2^{\bigcirc}_{+}$ (Fight for nest site and died)	0	0
1995			241	45	196	1	14	0	0	0	0	1 (Fight for nest site and died)	0	0
1996			241	45	196	0	0	0	0	0	0	0	0	0
1997	81 individuals (14♂67♀) were remove to C1		160	31	129	11	151	104	83	15	68	0	15	68
1998			243	46	197	11	131	0	0	0	0	0	0	0
1999			243	46	197	2	27	0	0	0	0	0	0	0
2000			243	46	197	1	7	0	0	0	0	0	0	0
2001	80 individuals (15♂65♀) were remove to C2		163	31	132	14	291	163	74	13	61	0	13	61
2002			237	44	193	0	0	0	0	0	0	0	0	0
2003	162 individuals (30♂132♀) were remove to C3	13 mature individuals (6♂7♀) were introduce from Anhui nature reserve	75	14	61	13	258	209	172	31	141	0	31	141
2004			247	45	202	2	12	0	0	0	0	0	0	0
2005			247	45	202	4	81	0	0	0	0	0	0	0
2006	83 individuals (15♂68♀) were remove to C6		164	30	134	14	272	168	97	16	81	0	NC	NC
2007			261	46	215	5	47	0	0	0	0	3♀ born in 2006	0	0
2008			258	46	212	1	8	0	0	0	0	0	0	0
2009			258	46	212	0	0	0	0	0	0	0	0	0
2010	80 individuals ( 20♂60♀) were remove to C6		178	26	152	10	194	127	111	19	92	0	19	92
2011			289	45	244	3	29	0	0	0	0	0	0	0
1997		14♂67♀were remove from area C	81	14	67	5	107	69	54	33	21	0	33	21
1998			135	47	88	4	81	64	42	23	19	0	23	19
1999			177	70	107	7	128	0	0	0	0	0	0	0
2000			177	70	107	0	0	0	0	0	0	0	0	0
2001	80 individuals (15♂65♀) were remove to C2		97	55	42	5	44	36	23	14	9	0	14	9
2002			120	69	51	0	0	0	0	0	0	0	0	0
2003	64 individuals (39♂25♀) were remove to C4	13 mature individuals (6♂7♀) were introduce from Anhui nature reserve	69	36	33	10	174	111	75	33	42	0	33	42
2004			144	69	75	4	52	0	0	0	0	0	0	0
2005			144	69	75	3	52	0	0	0	0	0	0	0

	2006	29 individuals (29♂) were remove to C6		115	40	75	13	255	193	170	28	142	0	NC	NC	
	2007	remove to Co		285	68	217	6	48	0	0	0	0	0	0	0	
	2008			285	68	217	2	5	0	0	0	0	0	0	0	
	2009			285	68	217	0	0	0	0	0	0	0	0	0	
	2009			263	08	217		U	U		U	U	U	U	0	
	2010	120 individuals ( 30♂90♀) were remove to C6		165	38	127	5	90	58	45	31	14	0	31	14	
	2011			210	69	141	1	23	0	0	0	0	0	0	0	
	2001		15♂65♀were introduced from area C; 15♂65♀were introduced from area C1	160	30	130	17	238	199	114	34	80	0	34	80	
	2002			274	64	210	2	7	0	0	0	0	0	0	0	
	2003	174 individuals ( 44♂130♀) were remove to C5	13 mature individuals (6♂7♀) were introduced from Anhui nature reserve	113	26	87	16	334	203	137	39	98	0	39	98	
	2004			250	65	185	1	21	0	0	0	0	0	0	0	
C2	2005			250	65	185	1	9	0	0	0	0	0	0	0	
	2006	65 individuals ( 25♂40♀) were remove to C6		185	40	145	18	141	58	46	25	21	0	NC	NC	
	2007			231	65	166	2	14	0	0	0	0	$1 \stackrel{?}{\circlearrowleft} 1 \stackrel{?}{\hookrightarrow} $ born in 2006	0	0	
	2008			229	64	165	2	14	0	0	0	0	0	0	0	
	2009	80 individuals ( 20♂60♀) were remove to C6		229	64	165	1	4	0	0	0	0	0	0	0	
	2010			149	44	105	6	103	51	44	22	22	0	22	22	
	2011			193	66	127	1	16	0	0	0	0	0	0	0	
	2003		162 individuals (30♂132♀) were introcued from C	175	36	139	5	107	61	45	34	11	0	34	11	
	2004			220	70	150	5	64	0	0	0	0	0	0	0	
	2005			220	70	150	5	33	0	0	0	0	0	0	0	
	2006	120 individuals ( 30♂90♀) were remove to C6		100	40	60	11	207	101	89	29	60	0	NC	NC	
C3	2007			189	69	120	5	33	0	0	0	0	1♂ born in 2006	0	0	
	2008			188	68	120	1	17	0	0	0	0	0	0	0	
	2009			188	68	120	3	24	0	0	0	0	0	0	0	
	2010	90 individuals ( 30♂60♀) were remove to C6		98	38	60	4	71	40	37	15	22	0	15	22	
	2011			135	53	82	5	102	46	41	6	35	0	6	35	

	<u> </u>		40							1					
			64 individuals $(39 \stackrel{?}{\bigcirc} 25 \stackrel{?}{\bigcirc})$ were												
	2003		introduced from C1;13 mature	77	45	32	15	308	222	189	27	162	0	27	162
			individuals ( $6 \circlearrowleft 7 \circlearrowleft$ ) were introduce from												
			Anhui nature reserve												
	2004			266	72	194	8	144	82	76	23	53	0	NC	NC
	2005			342	95	247	3	17	0	0	0	0	0	0	0
		210 individuals ( 35♂175♀)													
C4	2006	were remove to C6		132	60	72	16	289	154	137	36	101	0	NC	NC
	2007			269	96	173	8	75	0	0	0	0	0	0	0
	2007			269	96	173	5	55	0	0	0	0	0	0	0
	2009			269	96	173	0	0	0	0	0	0	0	0	0
	2009			209	90	173	U	U	U		U	U	U	U	0
	2010	90 individuals ( $30 \stackrel{\wedge}{\circ} 60 \stackrel{\circ}{\circ}$ )		179	66	113	5	95	42	37	30	7	0	30	7
		were remove to C6											·		
	2011			216	96	120	2	32	0	0	0	0	0	0	0
			174 individuals ( $44 \stackrel{?}{\circlearrowleft} 130 \stackrel{?}{\hookrightarrow}$ ) were												
	2003		introduced from C2; 15 mature	189	51	138	15	308	222	108	37	71	0	37	71
	2003		individuals ( $7 \stackrel{\wedge}{\circ} 8 \stackrel{\cap}{\circ}$ ) were introduce from	189	31	138	13	308	222	108	3/	/1	0	3/	71
			Anhui nature reserve												
	2004			297	88	209	10	204	138	119	29	90	0	NC	NC
	2005			416	117	299	0	0	0	0	0	0	0	0	0
		259 individuals ( 37♂222♀)													
C5	2006	were remove to C6		157	80	77	20	377	187	163	38	125	0	NC	NC
	2005			220	440	202		<b>7</b> 0	0		0	0		0	
	2007			320	118	202	11	53	0	0	0	0	0	0	0
	2008			320	118	202	3	20	0	0	0	0	0	0	0
	2009			320	118	202	6	118	0	0	0	0	0	0	0
	2010	120 individuals (40♂80♀)		200	78	122	6	112	64	52	38	14	0	38	14
	2010	were remove to C6		200	70	122	O	112	04	32	36	14	U	30	14
	2011			252	116	136	6	127	0	0	0	0	0	0	0
	-		766 individuals ( $171 \circlearrowleft 595 $ ) were					-			-	-			
	2006		introduced from area C, C1, C2, C3, C4,	766	171	595	35	692	559	486	97	389	0	NC	NC
	2000		C5	700	1,1		35	0,2				207	O .	1,0	
	2007			1252	268	984	31	568	443	401	101	300	1184 (301♂883♀)	NC	NC
C6	2007			469	68	401	15	276	144	99	43	56	0	NC NC	NC NC
	2009			568	111	457	6	144	47	38	24	14	0	NC NC	NC NC
	2010			1186	305	881	4	51	23	19	4	15	0	NC NC	NC NC
							•								
	2011			1205	309	896	13	251	84	69	31	38	0	NC	NC

### **Supplementary table S2**

The calculated sex-age structure of the Chinese alligator (Alligator sinensis) subpopulation in areas C and C1

The data in the table represent the number of individuals.

Amaa	Voor	Total			Mal	le		Female						
Area	Year	Total	<=2	3~6	7~9	>=10	Total	<=2	3~7	8~10	>=11	Tota		
	1984	11	0	0	0	3	3	0	0	0	8	8		
	1985	21	2	0	0	3	5	8	0	0	8	16		
	1986	41	4	0	0	3	7	26	0	0	8	34		
	1987	142	19	2	0	3	24	103	7	0	8	118		
	1988	81	7	4	0	3	14	33	26	0	8	67		
	1989	129	9	10	0	3	22	44	55	0	8	107		
	1990	197	20	11	0	3	34	96	59	0	8	163		
	1991	235	19	17	2	3	41	87	99	0	8	194		
	1992	233	6	27	4	5	42	38	145	0	8	191		
	1993	244	5	25	10	5	45	37	138	16	8	199		
	1994	242	4	25	9	7	45	18	128	45	6	197		
	1995	241	0	21	15	9	45	0	142	49	5	196		
	1996	241	0	9	21	15	45	0	102	73	21	196		
C	1997	160	0	5	18	8	31	0	46	58	25	129		
C	1998	243	15	4	15	12	46	68	37	65	27	197		
	1999	243	15	0	9	22	46	68	18	64	47	197		
	2000	243	0	15	5	26	46	0	68	46	83	197		
	2001	163	0	15	4	12	31	0	68	37	27	132		
	2002	237	13	15	0	16	44	61	68	18	46	193		
	2003	75	3	3	0	8	14	11	18	0	32	61		
	2004	247	31	3	3	8	45	141	29	0	32	202		
	2005	247	31	3	3	8	45	141	11	18	32	202		
	2006	164	0	19	3	8	30	0	106	8	20	134		
	2007	261	16	19	0	11	46	81	106	8	20	215		
	2008	258	16	16	3	11	46	78	106	0	28	212		
	2009	258	0	32	3	11	46	0	173	11	28	212		
	2010	178	0	6	9	8	26	0	113	11	28	152		
	2011	289	19	6	6	11	45	92	48	76	23	244		

	1997	81	0	0	6	8	14	0	0	42	25	67
	1998	135	33	0	2	12	47	21	0	40	27	88
	1999	177	56	0	0	14	70	40	0	20	47	107
	2000	177	23	33	0	14	70	19	21	0	67	107
	2001	97	0	41	0	14	55	0	0	0	42	42
	2002	120	14	41	0	14	69	9	0	0	42	51
	2003	69	14	9	0	13	36	9	0	0	24	33
C1	2004	144	33	23	0	13	69	42	9	0	24	75
	2005	144	33	14	9	13	69	42	9	0	24	75
	2006	115	0	27	0	13	40	0	51	0	24	75
	2007	285	28	27	0	13	68	142	51	0	24	217
	2008	285	28	27	0	13	68	142	51	0	24	217
	2009	285	0	55	0	13	68	0	184	9	24	217
	2010	165	0	25	0	13	38	0	103	0	24	127
	2011	210	31	25	0	13	69	14	103	0	24	141