1	Mass Mortality of Marine Mammals Associated to Highly Pathogenic Influenza Virus
2	(H5N1) in South America
3	Víctor Gamarra-Toledo <sup>1*</sup> , Pablo I. Plaza <sup>2</sup> , Roberto Gutiérrez <sup>1,3</sup> , Giancarlo Inga-Diaz <sup>3,4</sup> ,
4	Patricia Saravia-Guevara <sup>3</sup> , Oliver Pereyra-Meza <sup>3</sup> , Elver Coronado-Flores <sup>3</sup> , Antonio
5	Calderón-Cerrón <sup>3</sup> , Gonzalo Quiroz-Jiménez <sup>3</sup> , Paola Martinez <sup>3</sup> , Deyvis Huamán-
6	Mendoza <sup>3</sup> , José C. Nieto-Navarrete <sup>3</sup> , Sandra Ventura <sup>3</sup> , Sergio A. Lambertucci <sup>2</sup>
7	
8	<sup>1</sup> Museo de Historia Natural (MUSA), Universidad Nacional de San Agustín de
9	Arequipa, Av. Alcides Carrión s/n, Arequipa,
10	Perú.
11	
12	<sup>2</sup> Grupo de Investigaciones en Biología de la Conservación, Laboratorio Ecotono,
13	INIBIOMA, Universidad Nacional del Comahue - CONICET, Quintral 1250
14	(R8400FRF), San Carlos de Bariloche, Argentina.
15	
16	<sup>3</sup> Servicio Nacional de Áreas Naturales Protegidas por el Estado (SERNANP). Calle
17	Diecisiete 355, Urb. El Palomar, San Isidro, Lima, Perú.
18	
19	<sup>4</sup> Asociación Convive Perú, Puerto Maldonado, Madre de Dios, Perú.
20	
21	* Corresponding author: vgamarrat@unsa.edu.pe
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## 37 Biography

38 Víctor Gamarra-Toledo, MSc(c). Biologist, research associate at the Museum of Natural

39 History (MUSA), Universidad Nacional de San Agustín de Arequipa, Peru. With

40 experience in wildlife conservation in coastal ecosystems of Peru.

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## 43 ABSTRACT

- 44 We report a massive mortality of more than 3,000 sea lions (*Otaria flavescens*) of Peru
- 45 associated with a Highly Pathogenic Influenza Virus (H5N1). The transmission pathway
- 46 of H5N1 may have been through the close contact of sea lions with infected wild birds.
- 47 We cannot rule out direct transmission among sea lions.

48	The recent panzootic event (2020-2022) caused by the highly pathogenic avian
49	influenza (HPAI) A (H5N1) is the largest observed so far, several global outbreaks
50	having been caused (1). At the end of 2022, the H5N1 virus reached South America
51	(Peru, Ecuador, Colombia, Venezuela and Chile), with alarming bird mortalities in Peru
52	(2). This pathogen was detected for the first time in wild birds in Peru on 13 November
53	(2). Reports suggest the virus killed more than 50,000 wild birds by the end of 2022,
54	particularly Peruvian pelicans (Pelecanus thagus) and Peruvian boobies (Sula
55	variegata) (2,3). The large biomass of infected wild birds may have led to a spillover
56	event affecting predators and scavengers, including marine mammals cohabiting with
57	them, as reported in other parts of the world (4). Here, we report the death of 3,108 sea
58	lions (Otaria flavescens) on Peruvian coasts over 5 weeks and necropsies of some
59	individuals that suggest they were affected by the Highly Pathogenic Avian Influenza
60	Virus (H5N1), this was lately confirmed by government reports.
61	During January and February 2023, we performed detailed survey of died mammals in
62	marine protected areas of Peru with SERNANP personal (Servicio Nacional de Áreas
63	Naturales Protegidas por el Estado), throughout a surveillance system for avian
64	influenza in high biodiversity areas. More than three thousand sea lions were found

65 dead or dying on Peruvian beaches (Fig. 1.1, Table 1). The high mortality observed was

66 worrisome; for instance, up to 100 dead individuals were found floating together in the

67 sea, or 1,112 individuals died just in one island (Isla San Gallan); one of the places with

the highest populations of sea lions in Peru. Those are unprecedented observations for

69 the Southern Hemisphere (Fig. 1.1, Fig. 1.2 A and B, Table 1). The clinical signs of

70 dying individuals were mainly neurological, such as tremors, convulsions and paralysis.

71 They also showed respiratory signs such as dyspnea, tachypnea, nasal and buccal

72 secretions and pulmonary edema (Fig. 1.2 C). At the time of the deaths many sea lions

73 were pregnant or recently calved females; several abortions were observed. There are no 74 other records of such high mortality of aggregated sea lions, and this is the highest 75 mammal mortality recorded in the world that can be associated to HPAV until now. 76 Individuals were examined by veterinarians and some dead animals were necropsied. 77 The body condition of the sea lions necropsied ranged from good to very good, 78 suggesting they died due to an acute health problem. Substantial quantities of whitish 79 secretions filling the upper respiratory tracts (trachea and pharynx) were observed in the 80 necropsies and in dying animals (Fig. 1.2 C), which explains the severe dyspnea and 81 tachypnea clinically observed by veterinarians. Lungs were congestive, with 82 hemorrhagic focus compatible with interstitial pneumonia. Brains were also congestive 83 with hemorrhagic focus compatible with encephalitis, which explains the neurological 84 signs observed in dying individuals (Fig. 1.2 D); this agrees with the result of other 85 study on mammals infected with H5N1 (5). The small intestine showed necrotic focus 86 compatible with duodenitis. At the time of writing this letter (February 2023) sea lions 87 mortalities continue and have reached up to 3,108 died individuals. Given the 88 epidemiologic situation produced by H5N1 in wild birds that cohabit with these sea lions (2,3), our most plausible diagnosis was acute disease caused by HPAV. Official 89 90 information from Peruvian government confirmed that some of those sea lions tested 91 positive to HPAI (H5N1) (3,6,7).

92 In conclusion, sea lions of Peru developed a deadly associated disease that have 93 produced massive mortality in several regions of the Peruvian coastline (Fig. 1.1). The 94 sea lion mass mortality described is compatible with systemic HPAI that resulted in 95 acute encephalitis and pneumonia. Some specific cases of infection and mortality of 96 marine mammals due to HPAV with similar clinical and anatomopathological 97 characteristics have been reported in previous studies around the world (4,8,9).

98 The source of the HPAI affecting these sea lions was most probably the large number of 99 infected alive birds or their carcasses on the Peruvian coastline (2,3). Sea lions may be 100 infected by close contact with these carcasses and even through their consumption (see 101 an example of a sea lion feeding on a sick bird in Fig. 1.2 E). However, based on recent 102 research suggesting the first mammal-to-mammal infection in *Neovison vison* (10) and 103 the large number of sea lions currently affected at the same time, we cannot rule out that 104 the virus has adapted to mammals and that sea lion-sea lion transmission has begun in 105 Peru, and this needs to be urgently studied. 106 Worryingly, at the time of this submission Bolivia, Uruguay and Argentina add to list of 107 countries that have reported HPAV in their territories for the first time (11). Moreover, 108 authorities from Chile have reported the first sea lion dead due to HPAV in the north of 109 the country with similar clinical signs that we reported in Peru (11). 110 Further research is required to address the transmission pathway in this social species. 111 We would like to call attention to the fact that in this geographical region of the world, 112 human-infected animal interaction is common (e.g., people stain in beaches with sea 113 lions carcasses, (12) (Fig. 1.2 F), so infections might begin to rise and this must be 114 addressed if we are to avoid the risk of a pandemic. 115 References 116 1. Wille M, Barr IG. Resurgence of avian influenza virus. Science. 2022 Apr 117 29;376(6592):459-60.

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- 152 **Table 1.** Sea lion mortality associated with Highly Pathogenic Avian Influenza Virus
- 153 (H5N1) in protected areas of Peru between January and February 2023. Abbreviations
- 154 for the Natural Protected Areas are: RNSF = Reserva Nacional San Fernando,
- 155 RNILLESCAS = Reserva Nacional Illescas, RNPARACAS = Reserva Nacional
- 156 Paracas, ZRANCÓN = Zona Reservada Ancón, RNSIIPG = Reserva Nacional Sistema
- 157 de Islas, Islotes y Puntas Guaneras.

Species	Date	Natural Protected Area	Island & Guano island	Symptoms	Total deaths
South An	nerican S	sea Lion ( <i>Otaria fle</i>	ivescens)		
	6-Jan	RNILLESCAS			38
	14-Jan	RNPARACAS	Lagunillas, Supay, Atenas	Eyes closed and watering, nosebleeds and difficulty breathing	3
	16-Jan	ZRANCÓN		and announcy broathing	4
	18-Jan	RNPARACAS	La Mina		1
	19-Jan	RNPARACAS	La Aguada		1
	i) tuii	RNSIIPG	Isla Cavinzas		2
	20-Jan	RNPARACAS	Lagunillas, La Roja, Yumaque, Atenas		32
	21-Jan	RNPARACAS	Santo Domingo		1
		RNSIIPG	Punta Lomitas	Foaming at the mouth, convulsions, paralysis of the forelimbs	1
	22-Jan	RNSIIPG	Isla Asia		80
	23-Jan	RNPARACAS	Barlovento		6
			Isla San Gallan		33
		RNSIIPG	Isla Asia		17
			Isla Cavinzas		6
			Isla Pachacamac		7
	24-Jan	RNSIIPG	Isla Asia		20
			Isla Cavinzas		4
	25-Jan	RNPARACAS	Lagunillas		36
		RNSIIPG	Isla Asia		21
			Isla Cavinzas		6
			Punta Lomitas		8
	26-Jan	RNPARACAS	Los viejos		132
		RNSF		Carcasses floating in the sea	5
		RNSIIPG	Isla Asia		12
			Punta Lomitas		1
			Punta San Juan	Convulsions (dying individuals)	2

27-Jan	RNSF			20
	RNSIIPG	Isla Asia	Carcasses floating in the sea	100
		Isla Pescadores		2
		Punta Lomitas Punta San Juan		3
	ZRANCÓN	i untu Sun Suni		8 3
28-Jan	RNPARACAS RNSIIPG	Arquillo Punta Lomitas		1 2
29-Jan	RNSIIPG	Punta Lomitas		10
		Punta San Juan	December for the second	2
30-Jan	RNPARACAS	Ancla	Dyspnea, tremors (dying individuals)	17
		Caclio		4
		Chucho La Raya		4 12
		Punta Prieto		3
	RNSIIPG	Rancherio Chincha		5 46
31-Jan	RNPARACAS	Santo Domingo		2
		Sequion	Ataxia, tremors (dying individuals)	2
1-Feb	RNPARACAS	Yumaque	,	6
2-Feb	RNPARACAS	Karwas, La Raya	Dyspnea, tremors (dying individuals)	9
3-Feb	RNSF RNSIIPG	Chincha		188 10
4-Feb	RNPARACAS	Yumaque,		13
4-1.00	KINI AKACAS	Punta Ballena		15
5-Feb	RNPARACAS	Yuyos, Caracolito, Lagunilla, La Roja		13
	RNSIIPG	Chincha		3
6-Feb 7-Feb	RNSIIPG RNSF	Chincha Morro		2 432
0 7 1		Tunga		31
8-Feb	RNPARACAS	Arquillo Caracolito		8
		La Raya		42
	RNSF	Yumaque La Ensenada		27
	RNSIIPG	Punta San Juan		1
9-Feb	RNPARACAS	Antana Arguille		2 20
		Arquillo Barlovento		3
		Caclio		22
		El Negro La cruz		5 6
		Playa Roja		6
		Santa María Supay		1 13
		Supuy		15

	RNSIIPG	Chincha		5
10-Feb	RNPARACAS	La Aguada		4
		Lagunillas		4
		Supay		5
	RNSIIPG	Isla Asia		13
11-Feb	RNPARACAS	Supay		2
12-Feb	RNPARACAS	Playa Roja		2
14-Feb	RNPARACAS	El Negro		1
		Santo Domingo		1
17-Feb	RNPARACAS	Arquillo		3
		Camino hacia Lagunilla		1
		Caracolito		1
		Lagunillas		1
		Los yuyos		11
		Morro Quemado		332
		Playa Roja		3
		Sequion		1
		Talpo		2
19-Feb	RNPARACAS	Carhuaz		1
		Lagunillas		1
		Yumaque		1
	SNLM	Playa		7
20-Feb	RNPARACAS	Isla San Gallan		1112
		Playa Roja		3
		Sacasemita		2
21-Feb	RNPARACAS	Playa Roja		1
			Fotal	3108

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160 **Figure 1.1.** Map of geographical distribution of high mortalities in sea lions in January

- 161 and February 2023 on the Peruvian coastline. Figure 1.2. Images showing the field
- 162 work in the Paracas National Reserve on the Peruvian coastline, February 2023. A) Sea
- 163 lion carcasses on the beach; B) Dying sea lion with ataxia; C) Dead sea lion with avian
- 164 flu symptoms (whitish secretions); D) Sea lion necropsy showing a congestive brain; E)
- 165 Sea lion trapping and eating an infected Guanay cormorant on January 23th of 2023 in
- 166 the Reserva Nacional Paracas; F) Field work sampling on a beach with a large number
- 167 of bathers in the surroundings of infected carcasses (the red arrow indicates SERNANP
- 168 personal with health protection equipment conducting field survey). Photo credits: A, B
- and D, Daniel Ampuero; C and F, Giancarlo Inga; E, Sandra Lizarme.





Figure 1.2