CADAVERIC ENTOMOFAUNA IN STRANDED MARINE VERTEBRATES **ON THE CENTRAL COAST OF PERU**

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ABSTRACT

The aim of the present study was to assess the cadaveric entomofauna in stranded marine vertebrates on the Central Coast of Peru. The diversity of the entomofauna in the decomposition states of 291 carcasses of 18 species of marine vertebrates was determined. Each vertebrate was assigned to one of five stages of carcass decomposition: (1) fresh, (2) bloated, (3) active decomposition, (4) advanced decomposition and (5) skeletal remains. In relation to the total number of carcasses and species richness, birds were predominant. The four vertebrate species with the highest number of corpses corresponded to Otaria flavescens (Shaw, 1800), Sula variegata (Tschudi, 1843), Pelecanus thagus (Molina, 1782) and Phalacrocorax brasilianus (Gmelin, 1789). Advanced decomposition and skeletal remains were the most frequent corpses. The four orders of the cadaveric entomofauna present were Dermaptera, Hymenoptera, Coleoptera, and Diptera, with Tenebrionidae and Muscidae being the two most abundant families. There were four main species of coleopterans: Phaleria gayi, Dermestes maculatus, Phaleria maculata, and Dermestes frischii, and the five most abundant species of Diptera were Musca domestica, Lucilia sericata, Sarcophaga sp., Piophila casei and Calliphora nigribasis. In relation to ecosystem services, the following sequence was observed based on the richness and abundance of species: necrophagous > necrophilous > omnivores. The alpha and beta diversity indices showed different patterns according to the state of development of the entomofauna, the state of decomposition of the vertebrate corpses, year of evaluation and, finally, according to the four most frequent corpses of vertebrates.

Keywords: Cadaveric entomofauna, Coleoptera, diversity, ecosystem services, states of decomposition, Diptera.

RESUMEN

Entomofauna cadavérica en vertebrados marinos varados en la costa central del Perú

La presente investigación evalúa la entomofauna cadavérica en vertebrados marinos varados en la costa central del Perú. Se determinó la diversidad de la entomofauna presente en diferentes estados de descomposición de 291 carcasas de 18 especies de vertebrados marinos. Cada vertebrado fue asignado a uno de los cinco estados de descomposición del cadáver: (1) fresco, (2) hinchado, (3) descomposición activa, (4) descomposición avanzada y (5) restos esqueléticos. Con relación al número total de cadáveres y de rigueza de especies, las aves fueron las dominantes. Las cuatro especies de vertebrados con mayor número de cadáveres correspondieron a Otaria flavescens (Shaw, 1800), Sula variegata (Tschudi, 1843), Pelecanus thagus (Molina, 1782) y Phalacrocorax brasilianus (Gmelin, 1789). La descomposición avanzada y los restos esqueléticos fueron

los cadáveres más frecuentes. Los cuatro Órdenes de la entomofauna cadavérica presentes fueron Dermaptera, Hymenoptera, Coleoptera y Diptera, siendo Tenebrionidae y Muscidae las dos familias más abundantes. Para los coleópteros las cuatro especies principales fueron *Phaleria gayi*, *Dermestes maculatus*, *Phaleria maculata* y *Dermestes frischii*, y para los dípteros fueron *Musca domestica*, *Lucilia sericata*, *Sarcophaga* sp., *Piophila casei* y *Calliphora nigribasis*. Con relación a los servicios ecosistémicos, se observó la siguiente secuencia con base en la riqueza y abundancia de especies: necrófagos > necrófilos > omnívoros. Los índices de diversidad alfa y beta mostraron diferentes patrones según el estado de desarrollo de la entomofauna, el estado de descomposición de los cadáveres, los años de evaluación y según los cuatro cadáveres de vertebrados más frecuentes.

Palabras clave: Coleoptera, diversidad, entomofauna cadavérica, estados de descomposición, Diptera, servicios ecosistémicos.

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Introduction

Arthropods comprise the largest taxon of fauna associated with decomposing animal remains, also known as cadaveric entomofauna (Capo et al., 2004; Alboshabaa & Al Musawy, 2016; Griffiths et al., 2020). For these organisms, a carcass constitutes a temporary microhabitat that offers a rich food source (Dawson et al., 2021). In fact, within applied entomology, forensic entomology involves the use of insects in a judicial investigation, and is based on estimating the time since the first spawning by necrophagous insects in a corpse and, therefore, in the estimation of the postmortem interval (Griffiths et al., 2020; Byrd & Sutton, 2021; Meeds & Parrott, 2021), representing the main application of forensic entomology after the discovery of a cadaver (Magaña, 2001; Pasquerault et al., 2006; Salazar & Donoso, 2015; Hall, 2021; Moreau, 2021; Tembe & Mukaratirwa, 2021).

In relation to cadaveric entomofauna, works that study some aspect of the natural history of these species are mainly related to their forensic importance (Charabidze & Martín-Vega, 2021). The succession process of cadaveric entomofauna occurs by the addition or substitution of species according to the stages of decomposition of the organism (Carvalho *et al.*, 2000; Griffiths *et al.*, 2020; Dawson *et al.*, 2021; Tembe & Mukaratirwa, 2021).

On the other hand, it is also known that insects provide ecosystem services (ES) directly and indirectly, and are often evaluated based on their functional guilds (Noriega *et al.*, 2018). ES may include habitat conservation for biological diversity, maintenance of soil productive capacity and nutrient recycling, biological control, and pollination (Moreno & Sánchez-Rojas, 2007; Genung *et al.*, 2017). In cadaveric entomofauna, four ES are known based on their food guilds, which are associated with the decomposition of a corpse (Goff, 2009). These ES are: (1) necrophagous; species that feed on decomposing tissue and are represented by Diptera (Calliphoridae Sarcophagidae families) and Coleoptera and (Dermestidae and Silphidae families), and generally appear during putrefaction, depending on the time of year and the location of the carcass (Engasser et al., 2021), and their activity accelerates putrefaction; (2) necrophiles; these include parasitoids (Hymenoptera that parasitize Diptera larvae and pupae) and predators such as Coleoptera (families Staphylinidae, Histeridae and Cleridae); (3) omnivores; species that feed on the carcass and other arthropods; among these, there are ants (Formicidae), wasps (Vespidae) and some beetles; and finally (4) opportunists, species that use the carcass as an extension of their natural habitat or that are present by chance, such as some species of mites, spiders and scorpions, among others (Goff, 2009; Griffiths et al., 2020; Tembe & Mukaratirwa, 2021).

Many insect species have acquired the ability to tolerate saline environments (Ruiz-Delgado *et al.*, 2014; Richards *et al.*, 2015; Saavedra-Alburqueque *et al.*, 2019). Sandy beaches are intertidal marine habitats that provide contact between the terrestrial and marine environments. These conditions provide favorable conditions for the development of varied entomofauna, which are usually excluded from biodiversity studies (Mouna *et al.*, 2011; Fattorini *et al.*, 2017).

In marine intertidal habitats, cadaveric decomposition of stranded vertebrates can be affected by various biotic and abiotic factors, and depends mainly on the texture of the sediment, the waves and the drying time during low tide (Mouna *et al.*, 2011; Corrales & Sibaja, 2015; Chauca *et al.*, 2021). There are currently few entomological studies in the marine intertidal of the South Pacific (Ruiz-Delgado *et al.*, 2014; Saavedra-Alburqueque *et al.*, 2019), but the

Orders Coleoptera and Diptera have been reported to make up the greatest diversity of species in intertidal habitats in the sandy marine littoral of this region (Camus & Barahona, 2002; González *et al.*, 2014).

In this context, taxonomic inventories related to the biodiversity of the cadaveric entomofauna in carcasses of marine vertebrates stranded on the central coast of Peru are of great importance, because they are the basis for ecological, biogeographical and conservation studies of the coastal marine ecosystem (Mourglia *et al.*, 2015; Giraldo-Mendoza, 2019). The goal of this study is to provide updated information on the diversity of the cadaveric entomofauna in marine vertebrate carcasses stranded on the central coast of Peru.

Material and methods

STUDY AREA

Seven sandy intertidal areas were studied on the central coast of Peru (South Pacific), between the department of Lima and the Callao region from May 2015 to October 2019 (Fig. 1). These beaches were: Playa Arica (Lurín) (12°18'6"S; 76°51'14"W), Playa Costa Azul (Ventanilla, Callao) (11°52'55"S; 77°9'14" W), Playa Grande (Santa Rosa) (11°47'30"S; 77°09'30"W), Playa Mamacona (Lurín) (12°15'48"S; 76°55'0"W), Playa San Pedro (Lurín) (12°17'21"S; 76°52'40"W), Playa Venecia (Villa El Salvador) (12°13'52"S; 76°58'30"W) and Playa Villa (Chorrillos) (12°12'42"S; 77°1'8.4"W). Each beach was surveyed twice a year, from May to June (autumn) and from September to October (spring). The seven beaches are characterized by being located less than 1 km from an urban center. The beaches are not "cleaned" by mechanical means, and there is no sand removal. In a walk of a few meters, some solid waste can be seen on the sand, such as paper, plastic containers and cigarette butts. All seven beaches have moderate to high human use during the summer (González et al., 2014). For climatic factors, the values of annual average, minimum and maximum temperature, annual relative humidity and annual precipitation were taken from the National Institute of Statistics and Informatics (INEI in Spanish) (2020) (Table 1).

STRANDED MARINE VERTEBRATES

A panoramic inspection of each beach was carried out twice a year. The areas where the vertebrate carcasses were found stranded (between 1 and 7 m from the high tide line, Portflitt-Toro *et al.*, 2018) were located and marked. Photographic records of each stranded marine vertebrate carcass were taken with a digital camera, and the date of assessment was noted. Subsequently, each vertebrate was assigned one of the five stages of carcass decomposition: (1) fresh, (2) bloated, (3) active decomposition, (4) advanced decomposition, or (5) skeletal remains (Castillo, 2002; Griffiths *et al.*, 2020).

Each of the carcasses of birds and mammals evaluated were identified at the species level using specialized scientific literature for their taxonomic identification (Bello, 2005; Barrio & Guillén, 2014; Senner & Angulo, 2014; Chauca *et al.*, 2021), which was corroborated by experts.

CADAVERIC ENTOMOFAUNA

A combination of several methods was used for the collection of colonizing insects from the carcasses of stranded birds and mammals (Richards et al., 2015). The entomofauna was collected twice a day; in the morning from 8:00 a.m. to 10:00 a.m. and in the afternoon from 4:00 p.m. to 6:00 p.m., including a total of 4 hours a day. Entomological nets were beaten over the vertebrate carcasses for 10 min and used to capture adult flying insects, and tweezers and brushes were used to capture immature and non-flying adults (Richards et al., 2015; Whitman et al., 2019). Adult individuals were sacrificed using a lethal chamber with ethyl acetate vapors and preserved in vials with 70% ethyl alcohol (C_2H_6OH) (Whitman *et al.*, 2019). The immature stages were sacrificed using the Adams & Hall (2003) scalding technique and preserved in 70% ethyl alcohol (McCravy, 2018). Each sample was packaged, labeled and transported to the laboratory for its correct identification. Direct mounting with entomological pins was the most widely used preservation technique. Finally, the insects were placed in entomological boxes and these inside cabinets (Ferro & Summerlin, 2019).

Table 1.– Average climatic factors of temperature (°C), relative humidity (RH) (%) and precipitation (mm) for 2015 to 2019 in the department of Lima, on the central coast of Peru. From INEI (2020).

Tabla 1.– Factores climáticos promedios de temperatura (°C), humedad relativa (HR) y precipitación (mm) desde 2015 al 2019 para el departamento de Lima, en la costa central del Perú. From INEI (2020).

Climatic factors	2015	2016	2017	2018	2019
Temperature (°C)	20.8	20.3	20.1	19.5	19.6
Minimum temperature (°C)	19.0	18.3	18.1	17.4	17.6
Maximum temperature (°C)	23.4	23.3	23.1	22.4	22.5
Relative humidity (%)	86	85	86	87	88
Annual precipitation (mm)		7.5	11.3	21.4	26.4



Fig. 1.- Location of seven beaches on the central coast of Peru where the cadaveric entomofauna of stranded marine vertebrates was investigated.

Fig. 1.- Localización de siete playas en la costa central del Perú donde la entomofauna cadavérica de vertebrados marinos varados fue estudiada.

The taxonomic keys used for the order Diptera were those of Dale (1985); Dale & Prudot (1986), Carvalho & Mello-Patiu (2008), Amat et al. (2008), Williams & Villet (2014) and Grisales et al. (2016), while for the order Coleoptera, the taxonomic keys of Díaz et al. (2008), Almeida & Mise (2009), and Aballay et al. (2013) were used. Likewise, various specialized keys were used (Schuster, 1989; Florez & Wolff, 2009; Rafael, 2012; Vidal & Guerrero, 2017) for the different stages of development of the Coleoptera and Diptera collected. Insect species and morphospecies were assigned to three ES based on their functional groups: necrophagous, necrophilous, and omnivore (Castillo, 2002; Noriega et al., 2018). The specimens mounted dry and in 70% ethyl alcohol were deposited in the Entomological Collection of the Natural History Museum, Animal Ecology and Biodiversity Laboratory (LEBA), Federico Villarreal National University, Lima, Peru.

DATA ANALYSIS

Softwares Microsoft Excel and Past 3.0 were used to calculate the alpha biological diversity of the cadaveric entomofauna based on global abundance, by stages of development of the entomofauna (larvae, pupae and adult), by stages of decomposition of vertebrate carcasses (fresh-bloated, active decomposition, advanced decomposition and skeletal remains), between years of evaluation (from 2015 to 2019) and for the four most frequent vertebrate corpses. The following eight indices were used: species richness, abundance, Menhinick diversity index (D_m), Margalef diversity index (Da), Shannon diversity index (H'), Pielou evenness index (J'), Berger-Parker dominance index (BP) and Simpson dominance index (D). Two types of analysis were carried out to calculate the relationships between the cadaveric entomofauna based on the states of decomposition of the vertebrate corpses, between years of evaluation and for the four most frequent vertebrate corpses. The first was the construction of a dendrogram based on the Jaccard qualitative similarity index and the second was based on the Bray-Curtis quantitative index using the UPGMA method (agglomerative hierarchical clustering method).

Pearson's (r) correlation was calculated between species richness according to the developmental stage of the cadaveric entomofauna and the number of stranded marine vertebrate carcasses; between the species richness of the cadaveric entomofauna according to the state of decomposition of the stranded marine vertebrate carcasses and the number of stranded marine vertebrate carcasses evaluated; between the species richness of the cadaveric entomofauna according to the year of evaluation and the number of stranded marine vertebrate carcasses evaluated; and finally, between the species richness of the cadaveric entomofauna according to the vertebrate species and the number of stranded marine vertebrate carcasses evaluated. For the calculation of inferential statistics based on Pearson's correlation, the statistical package SPSS version 25.0 was used. For all cases, Levene's homogeneity of variance test and Shapiro Wilk's normality test were previously performed to ensure assumptions for the proper use of the Pearson correlation test were met. All tests were conducted with an alpha value of 0.05.

ETHICAL ASPECTS

Authorization was obtained to carry out this research for scientific research purposes with General Directorate Resolution No. 534-2019-MINAGRI-SERFOR-DGSPFFS with scientific authorization code AUT-IFS-2019-075.

Results

STRANDED MARINE VERTEBRATES

A total of 291 carcasses of 18 species of marine vertebrates were found stranded on the beaches of Lima and Ica on the central coast of Peru during the study period (Table 1). In relation to the total number of carcasses and species richness, birds made up 75.94% (n = 221) and 72.22% (n = 13), respectively, while mammals constituted 24.05% (n = 70) and 27.78% (n= 5), respectively (Table 2). The most frequently found Orders based on the number of taxa were Charadriiformes, Carnivora and Suliformes. The Laridae family presented the highest number of species among the vertebrates evaluated. The four vertebrate species with the highest number of carcasses corresponded to Otaria flavescens (Shaw, 1800), Sula variegata (Tschudi, 1843), Pelecanus thagus (Molina, 1782) and *Phalacrocorax brasilianus* (Gmelin, 1789) (Table 2).

In relation to the stages of decomposition, in descending percentage order the following was observed in relation to the number of vertebrate corpses analyzed: advanced putrefaction (n= 137; 47.08%)> skeletal remains (n= 117; 40.21%)> active decomposition (n= 26; 8.93%)> fresh- bloated (n= 11; 3.78%) (Table 2). The species richness of stranded marine vertebrate carcasses presented the following pattern in descending order in relation to the decomposition levels evaluated: advanced decomposition (n= 16) > skeletal remains (n= 13) > active decomposition (n= 9) > fresh-bloated (n= 5).

In relation to the five years evaluated, the following was observed in descending percentage order in relation to the number of vertebrate carcasses examined: 2015 (n= 90; 30.93%) > 2017 (n= 57; 19.59%) > 2018 (n= 53; 18.21%) > 2019 (n= 51; 17.52%) > 2016 (n= 40; 13.75%) (Table 2). *Sula variegata* and *O. flavescens* were the only two carcasses found along the entire period 2015 to 2019. The species richness

Table 2.– Family, species and number of corpses by stages of decomposition and by year of marine vertebrates stranded on beaches of the central coast of Peru. *Not typically a marine vertebrate. 1 and 2 =fresh-bloated. 3 =active decomposition. 4 =advanced decomposition. 5 =skeletal remains.

Tabla 2.– Familia, especie y número de cadáveres por estados de descomposición y por año de vertebrados marinos varados en playas de la costa central del Perú. *No es un vertebrado marino típico. 1 y 2 = fresco-hinchado. 3 = descomposición activa. 4 = descomposición avanzada. 5 = restos óseos.

Family	Species	Common name		name Number Stage of of decomposit			of sitio	Year					
			corpses	1	2	3	4	5	2015	2016	2017	2018	2019
Haematopodidae	<i>Haematopus ater</i> (Vieillot & Oudart, 1825)	Blackish Oystercatcher	3	0	0	1	0	2	0	2	1	0	0
Haematopodidae	<i>Haematopus palliatus</i> (Temminck, 1820)	American Oystercatcher	2	0	0	0	2	0	0	0	0	2	0
Sternidae	<i>Larosterna inca</i> (Lesson, 1827)	Inca Tern	19	3	0	0	8	8	15	0	3	1	0
Laridae	<i>Larus belcheri</i> (Vigors, 1829)	Belcher's Gull	7	0	0	0	3	4	6	0	0	1	0
Laridae	<i>Leucophaeus modestus</i> (Tschudi, 1843)	Gray Gull	3	0	0	1	2	0	0	3	0	0	0
Laridae	<i>Leucophaeus pipixcan</i> (Wagler, 1831)	Franklin's Gull	3	0	0	1	1	1	0	0	0	0	3
Pelecanidae	<i>Pelecanus thagus</i> (Molina, 1782)	Peruvian Pelican	54	1	0	6	20	27	30	3	7	0	14
Phalacrocoracidae	<i>Phalacrocorax bougainvillii</i> (Lesson, 1837)	Guanay Cormorant	12	0	0	1	5	6	0	6	5	0	1
Phalacrocoracidae	<i>Phalacrocorax brasilianus</i> (Gmelin, 1789)	Neotropic Cormorant	44	0	0	4	19	21	7	0	6	17	14
Spheniscidae	Spheniscus humboldti (Meyen, 1834)	Pingüino de Humboldt	10	0	0	0	6	4	3	0	1	5	1
Columbidae	<i>Streptopelia decaocto</i> (Frivaldszky, 1838)	Eurasian Collared-Dove	1	0	0	0	0	1	0	0	0	1	0
Sulidae	<i>Sula nebouxii</i> (Milne- Edwards, 1882)	Blue-footed Booby	8	0	1	1	5	1	1	0	0	7	0
Sulidae	<i>Sula variegata</i> (Tschudi, 1843)	Peruvian Booby	55	0	3	5	30	17	7	14	17	5	12
Canidae	Canis lupus familiaris (Linnaeus, 1758)*	Domestic Dog	1	0	0	0	1	0	0	0	0	1	0
Mustelidae	<i>Lontra felina</i> (Molina, 1782)	Marine Otter	1	0	0	0	1	0	0	0	0	1	0
Otariidae	<i>Otaria flavescens</i> (Shaw, 1800)	South American Sealion	62	0	3	6	29	24	20	12	17	8	5
Phocoenidae	<i>Phocoena sinus</i> (Norris & McFarland, 1958)	Gulf of California Harbor Porpoise	1	0	0	0	1	0	0	0	0	0	1
Delphinidae	<i>Tursiops truncatus</i> (Montagu, 1821)	Bottlenose Dolphin	5	0	0	0	4	1	1	0	0	4	0

of vertebrate carcasses presented the following pattern in descending order in relation to the years evaluated: 2018 (n= 12) > 2015 (n= 9) > 2017 (n= 8) = 2019(n= 8) > 2016 (n= 6) (Table 3).

CADAVERIC ENTOMOFAUNA IN STRANDED MARINE VERRTEBRATES

A total of 5981 specimens of cadaveric entomofauna were collected. These were divided into four Orders in relation to the total number of specimens: Dermaptera (0.05%), Hymenoptera (0.02%), Coleoptera (65.45%) and Diptera (34.48%) and into a total of 17 families, Tenebrionidae and Muscidae being the most abundant. The sequence based on the abundance of the stages of development of the entomofauna showed a higher percentage of adults > larvae > pupae (Table 3).

There were four main species of beetles: *Phaleria* gayi (Laporte, 1840), *Dermestes maculatus* (De Geer, 1774), *P. maculata* (Kulzer, 1959) and *Dermestes* frischii (Laporte, 1840). Among Diptera, the five most abundant species were *Musca domestica* (Linnaeus, 1758), *Lucilia sericata* (Meigen, 1826), *Sarcophaga* sp., *Piophila casei* (Linnaeus, 1758) and *Calliphora* nigribasis Macquart, 1851 (Table 3).

Table 3.– Cadaveric entomofauna in marine vertebrates stranded on beaches of the central coast of Peru. ES = EcosystemService. 1 = necrophagous. 2 = necrophiles. 3 = omnivorous. * = nymph.

Tabla 3.- Entomofauna cadavérica en vertebrados marinos varados en playas de la costa central del Perú. ES = Servicios ecosistémicos. 1 = necrófago. 2 = necrófilos. 3 = omnívoro. * = ninfa.

Order	Family	Species	Larvae	Pupae	Adult	Abundance	ES
Dermaptera	Labiduridae	Labidura riparia (Pallas, 1773)	NA	NA	2	3(1)*	2
Coleoptera	Tenebrionidae	Phaleria maculata (Kulzer, 1959)	0	1	209	210	1
		<i>Phaleria gayi</i> Laporte, 1840	551	10	2550	3111	1
		Tenebrio molitor Linnaeus, 1758	1	0	57	58	1
	Dermestidae	Dermestes maculatus De Geer, 1774	218	0	158	376	1
		Dermestes frischii Kugelann, 1792	0	0	51	51	1
		Dermestes peruvianus Castelnau, 1840	7	3	22	32	1
	Cleridae	Necrobia rufipes (De Geer, 1755)	0	0	0	35	2
		Necrobia ruficollis (Fabricius, 1775)	0	0	2	2	2
	Histeridae	Saprinus caerulescens (Hoffmann, 1803)	0	0	4	4	2
		<i>Euspilotus decoratus</i> (Erichson, 1834)	0	0	3	3	2
		<i>Hister</i> sp.	0	0	3	3	2
	Silphidae	Silpha obscura Linnaeus, 1758	0	0	2	2	2
	Staphylinidae	morfotipo gen. sp.	0	0	14	14	2
	Scarabeidae	morfotipo gen. sp.	0	0	14	14	1
Hymenoptera	Formicidae	Pheidole aff. chilensis Mayr 1862	0	0	1	1	3
Diptera	Muscidae	Musca domestica Linnaeus, 1758	685	3	19	707	1
		Synthesiomyia nudiseta Wulp, 1883	0	0	40	40	1
		Ophyra aenescens Wiedemann, 1830	0	0	2	2	1
	Calliphoridae	Calliphora nigribasis Macquart, 1851	45	61	0	106	1
		<i>Lucilia sericata</i> (Meigen, 1826)	397	28	24	449	1
		Cochliomyia macellaria (Fabricius, 1775)	55	8	3	66	1
		Chrysomya albiceps (Wiedemann, 1819)	67	4	14	85	1
	Fannidae	Fannia scalaris (Fabricius, 1794)	43	0	43	86	1
	Sarcophagidae	Sarcophaga sp.	198	0	14	212	1
	Psychodidae	Clogmia albipunctata (Williston, 1893)	0	0	1	1	1
	Piophilidae	Piophila casei Linnaeus, 1758	85	1	119	205	1
	Phoridae	morfotipo gen. sp.	0	8	0	81	1
	Tachinidae	morfotipo gen. sp.	9	0	13	22	2

Regarding ES, the following species richness and abundance were observed for necrophagous (65.52% and 98.51%), necrophilous (31.03% and 1.47%), and omnivores (3.45% and 0.02%), respectively (Table 3).

ALPHA AND BETA DIVERSITY INDICES

Stages of development of the cadaveric fauna. In the case of the larvae and pupae of the cadaveric entomofauna, low values of D and BP and high values of J' were observed. For adults, an opposite pattern was found, with relatively high values of D and BP and low values of J'. The larvae showed higher values of H', D_{Mn} pupae and D α than adults. The number of carcasses of stranded marine vertebrates with the presence of adults was greater than that of larvae, and this in turn was greater than that of pupae (Table 4). No correlation was observed between species richness according to the stage of development of the cadaveric entomofauna and the number of carcasses of stranded marine vertebrates (r= 0.77; p= 0.43).

The cluster analysis of beta diversity showed that the cadaveric entomofauna present in the stages of fresh-bloated decomposition and active decomposition were more associated with each other, while advanced decomposition and skeletal remains presented greater similarity based on the qualitative index of Jaccard (Fig. 2A). In the case of the Bray-Curtis quantitative index, the cadaveric entomofauna present in active decomposition, advanced decomposition and skeletal remains were more associated with each other. In contrast, the entomofauna collected in the fresh-swollen decomposition stage was less similar to the rest (Fig. 2B).

Decomposition states of stranded marine vertebrate carcasses. Active decomposition showed the highest D_{Mn} , $D\alpha$ and J' values in relation to the other decomposition states. Advanced decomposition presented higher values for H' and, finally, the skeletal remains were higher for D (Table 4). A correlation was observed between the species richness of the cadaveric entomofauna according to the state of decomposition

Table 4.– Indices of alpha diversity for stages of development of the cadaveric entomofauna and for the stages of decomposition of the carcasses of marine vertebrates stranded on beaches of the central coast of Peru. 1 and 2 = fresh-bloated; 3 =active decomposition; 4 =advanced decomposition; 5 =skeletal remains.

Tabla 4.- Índices de diversidad alfa para estados de desarrollo de la entomofauna cadavérica y para los estados de descomposición de los cadáveres de vertebrados marinos varados en playas de la costa central del Perú. 1 y 2 = frescohinchado; 3 = descomposición activa; 4 = descomposición avanzada; 5 = restos esqueléticos.

Indices of alpha diversity	Larvae	Pupae	Adult	Global abundance	1 - 2	3	4	5
Species richness	13	9	26	29	11	12	23	22
Abundance	2361	127	3384	5981	152	75	2765	2085
Simpson dominance (D)	0.19	0.30	0.58	0.30	0.18	0.16	0.23	0.41
Diversity of Shannon (H')	1.93	1.60	1.14	1.87	2.04	2.14	2.22	1.51
Menhinick (D _{Mn})	0.27	0.89	0.45	0.38	1.14	1.50	0.78	0.81
Margalef (Dα)	1.55	1.86	3.08	3.22	2.59	2.78	5.05	4.71
Equitability (J')	0.75	0.69	0.35	0.55	0.77	0.83	0.60	0.42
Berger-Parker (BP)	0.29	0.48	0.75	0.52	0.34	0.32	0.44	0.61
Number of corpses	176	23	202	291	11	26	137	117



Fig. 2.– Cluster analysis of beta diversity. **A**. Qualitative similarity using the Jaccard index (Correlation coefficient = 0.99). **B**. Quantitative similarity using the Bray-Curtis index (Correlation coefficient = 0.95), based on the UPGMA method (simple agglomerative hierarchical grouping method) for cadaveric entomofauna by stages of decomposition of marine vertebrates stranded on beaches of the central coast of Peru. 1 and 2= fresh-bloated. 3 = active decomposition. 4 = advanced decomposition. 5 = skeletal remains.

Fig. 2.– Análisis Cluster de diversidad beta. **A**. Cualitativo de similaridad empleando el índice de Jaccard (Coeficiente de correlación = 0,99). **B**. Cuantitativo de similaridad empleando el índice de Bray-Curtis (Coeficiente de correlación = 0,95), en base al método UPGMA (método de agrupamiento jerárquico aglomerativo simple) para la entomofauna cadavérica por estados de descomposición de vertebrados marinos varados en playas de la costa central del Perú. 1 y 2= fresco-hinchado. 3 = descomposición activa. 4 = descomposición avanzada. 5 = restos esqueléticos.

and the number of stranded marine vertebrate carcasses evaluated (r= 0.99; p= 0.002).

Between evaluation years. Low values of D and BP were observed along the five years of evaluation between 2015 and 2019. The year 2017 presented the highest species richness, H', D_{Mn} , D α and J' compared to the other four years of evaluation (Table 5). No correlation was observed between the species richness

of the cadaveric entomofauna according to the year of evaluation and the number of stranded marine vertebrate carcasses evaluated (r=0.40; p=0.49).

Cluster analysis of beta diversity showed that the cadaveric entomofauna presented two groups, the years 2015-2018-2019 that were more associated with each other, and the second group 2016-2017 based on the Jaccard qualitative index (Fig. 3A). In the case



Fig. 3.– Cluster analysis of beta diversity. **A**. Qualitative similarity using the Jaccard index (Correlation coefficient = 0.73). **B**. Quantitative similarity using the Bray-Curtis index (Correlation coefficient = 0.82), based on the UPGMA method (simple agglomerative hierarchical grouping method) for cadaveric entomofauna of marine vertebrates stranded on beaches of the central coast of Peru between the years 2015-2019.

Fig. 3.– Análisis Cluster de diversidad beta. **A**. Cualitativo de similaridad empleando el índice de Jaccard (Coeficiente de correlación = 0,73). **B**. Cuantitativo de similaridad empleando el índice de Bray-Curtis (Coeficiente de correlación = 0,82), en base al método UPGMA (método de agrupamiento jerárquico aglomerativo simple) para la entomofauna cadavérica entre los años 2015-2018 de vertebrados marinos varados en playas de la costa central del Perú.

of the Bray-Curtis quantitative index, the cadaveric entomofauna presented greater similarity between the years 2015-2017-2018-2019. In contrast, the entomofauna collected in 2016 was less similar to the rest (Fig. 3B).

Cadaveric entomofauna in the four most frequent stranded marine vertebrates. The cadaveric entomofauna was low for D, low-medium for BP and medium-high for J' for the four marine vertebrates (Table 5). The highest values for the H' index were for *P. thagus* and *O. flavescens*. *Phalacrocorax brasilianus* presented the highest values for the D_{Mn} and Da species richness indices (Table 5). No correlation was observed between the species richness of the cadaveric entomofauna according to the vertebrate species and the number of stranded marine vertebrate carcasses evaluated (r= 0.08; p= 0.81).

The cluster analysis of beta diversity showed that the cadaveric entomofauna presented two groups, that of the cadavers of the vertebrates *P. brasilianus - P.*

Table 5.– Indices of alpha diversity of cadaveric entomofauna among the years evaluated and in the four most frequent carcasses of marine vertebrates found stranded on beaches on the central coast of Peru. Pt = Pelecanus thagus. Pb = Phalacrocorax brasilianus. Sv = Sula variegata. Of = Otaria flavescens.

Tabla 5.– Índices de diversidad alfa de la entomofauna cadavérica entre años y en cuatro cadáveres más frecuentes de vertebrados marinos varados en playas en la costa central del Perú. Pt = *Pelecanus thagus*. Pb = *Phalacrocorax brasilianus*. Sv = *Sula variegata*. Of = *Otaria flavescens*.

Indices of alpha diversity	2015	2016	2017	2018	2019	Pt	Pb	Sv	Of
Species richness	17	13	20	15	17	15	18	15	19
Abundance	1411	401	1117	1048	1932	784	863	930	1980
Simpson dominance (D)	0.18	0.31	0.16	0.27	0.46	0.15	0.32	0.32	0.19
Diversity of Shannon (H')	2.15	1.68	2.36	1.80	1.41	2.31	1.77	1.48	2.29
Menhinick (DMn)	0.67	0.90	0.93	0.68	0.71	0.96	1.06	0.62	0.72
Margalef (Dα)	3.31	2.84	4.27	3.02	3.97	3.90	4.44	2.63	4.08
Equitability (J')	0.67	0.58	0.69	0.58	0.41	0.70	0.52	0.50	0.66
Berger-Parker (BP)	0.34	0.50	0.31	0.46	0.66	0.29	0.53	0.50	0.39
Number of corpses	90	40	57	53	51	54	44	55	62



Fig. 4.– Cluster analysis of beta diversity. **A**. Qualitative similarity using the Jaccard index (Correlation coefficient = 0.90). **B**. Quantitative similarity using the Bray-Curtis index (Correlation coefficient = 0.88), based on the UPGMA method (simple agglomerative hierarchical grouping method) for cadaveric entomofauna in four marine vertebrates stranded on beaches of the central coast of Peru. Pt = *Pelecanus thagus*. Pb = *Phalacrocorax brasilianus*. Sv = *Sula variegata*. Of= Otaria flavescens.

Fig. 4.– Análisis Cluster de diversidad beta. **A**. Cualitativo de similaridad empleando el índice de Jaccard (Coeficiente de correlación = 0,90). **B**. Cuantitativo de similaridad empleando el índice de Bray-Curtis (Coeficiente de correlación = 0,88), en base al método UPGMA (método de agrupamiento jerárquico aglomerativo simple) para la entomofauna cadavérica en cuatro vertebrados marinos varados en playas de la costa central del Perú. Pt = *Pelecanus thagus*. Pb = *Phalacrocorax brasilianus*. Sv = *Sula variegata*. Of = *Otaria flavescens*.

thagus - O. flavescens more associated with each other, and the entomofauna of S. *variegata* determined with the index of presence/absence of Jaccard (Fig. 4A). The cadaveric entomofauna showed greater similarity for the Bray-Curtis quantitative index among the cadavers of the vertebrates *P. brasilianus - P. thagus -S. variegata*. In contrast, the entomofauna collected in *O. flavescens* was less similar to the rest of carcasses (Fig. 4B).

Discussion

The results of this study show that based on the total number of corpses and the richness of the stranded species, ornithological fauna was more frequently found in comparison to mammals. In regard to corpse number, in their study Chauca *et al.* (2021) reported that the total number of corpses for the northern zone of Peru, specifically for the Regions of Lambayeque $(06^{\circ}22'S-07^{\circ}10'S)$, Piura $(4^{\circ}50'S-06^{\circ}22'S)$ and Tumbes $(3^{\circ}23'S-4^{\circ}50'S)$, was higher for mammals (60.6%) than for birds (39.4%). On the other hand, when evaluated based on species richness, a greater number of birds (n= 19; 67.85%) was observed compared to mammals (n= 9; 32.15%). Recently, in Ite Bay, Tacna on the marine coasts of southern Peru, Ortiz-Álvarez *et al.* (2022) described a dominance

of seabirds (92.4%) compared to marine mammals (7.6%).

In this investigation, the corpse Orders most frequently found were Charadriiformes, Carnivora and Suliformes based on abundance. The Laridae family had the highest number of carcass species (n=3). When comparing our results with previously published studies, it is evident that based on the number of species the Orders most frequently found were Cetartiodactyla (n=7), Suliformes (n=6) and Procellariformes (n=6), being Carnivora, Suliformes and Pelecaniformes based on abundance. Finally, the Delphinidae (n=6) and Laridae (n=4) families had the highest number of Species of vertebrates stranded in the northern zone of Peru, (Chauca *et al.*, 2021).

The chordates with the highest number of stranded carcasses in the present study were a mammal (*O. flavescens*), and three species of birds (*S. variegata*, *P. thagus* and *P. brasilianus*). Our results are consistent with Chauca *et al.* (2021) who recorded data on the stranding of marine mega vertebrates on the north coast of Peru (2017-2018), the most abundant species being *O. flavescens* (50%), *S. variegata* (14%) and *P. thagus* (13 %). Among the causes of stranding of carcasses, these authors described a higher percentage of anthropic origin, mainly associated with interaction with fishing activities, demonstrating a conflict between marine vertebrates and fishing boats (Portflitt-

Toro *et al.*, 2018; Chauca *et al.*, 2021). Portflitt-Toro *et al.* (2018) reported that the birds most frequently stranded were *S. variegata* (30%), *P. bougainvillii* (22%), *Larus dominicanus* (17%), *P. thagus* (9%) and *S. humboldti* (8%), in the Coastal System of Coquimbo, Chile. It has been suggested that various factors influence the presence of vertebrates stranded on marine beaches, such as oceanographic conditions, especially the direction and intensity of the wind, the body size of the mammal or bird, the action of predators and scavengers, and finally the drifting time at sea (Portflitt-Toro *et al.*, 2018).

We found four main species of beetles: *P. gayi* (Tenebrionidae), and the dermestids *D. maculatus*, *P. maculata* and *D. frischii*. On the southern coast of Chile, it has been recorded that the most abundant order was Coleoptera followed by Diptera (Camus & Barahona, 2002; Salazar & Donoso, 2015).

The Tenebrionidae family was the most abundant in the present study. This family registers more than 20,000 species and 2,300 genera worldwide, being the seventh family with the greatest diversity and well represented in the tropical region (Bouchard et al., 2017). Giraldo-Mendoza (2021) described a preliminary list of Coleoptera of forensic importance in Peru, and cited the family Tenebrionidae as being among the 18 families of the Order Coleoptera of importance in cadaveric decomposition. Tenebrionid abundance was the highest during the entire evaluation (Ruiz-Delgado et al., 2014). More than 60 species of the genus Phaleria Latreille have been found on sandy marine beaches (Giraldo-Mendoza, 2019). These insects are found buried in the sand, frequently associated with stranded remains of algae or marine animals (Giraldo-Mendoza, 2019). Phaleria gayi was the most abundant necrophagous species throughout the evaluation, with a lower percentage of the congeneric species P. maculata being found. Gonzalez et al. (2014) reported an inverse relationship between the level of urbanization on the beaches of northern Chile and the abundance of larvae and adults of the dark beetle P. maculata, which according to these authors confirms the hypothesis that human intervention affects the occurrence of these organisms. Consequently, these researchers reported that the sectors with high values of urbanization showed the absence or a very low abundance of beetles, while sectors with low levels of urbanization showed a greater abundance of beetles.

Dermestes (Linnaeus, 1758) is a genus of forensic importance in Peru, and it is the most frequent genus in 35 studies of cadaveric succession in South America (Giraldo-Mendoza, 2021). The dermestids *D. maculatus*, *P. maculata* and *D. frischii* were found in the present study mainly in adult and larval form in vertebrate carcasses stranded on beaches of the central coast of Peru. These three species have been previously recorded in Peru and other South American countries, and are considered as "known" species in entomological studies of cadaveric succession, and with a high proportion and indices of species of forensic importance (Medina-Achín *et al.*, 2018; Giraldo-Mendoza, 2021). Among the dermestid species collected, the presence of *D. maculatus* was of note, being registered as a necrophagous species according to its ES. It should be noted that Medina-Achín *et al.* (2018) reported that *D. maculatus* is a species that consumes the remains of skin and dry stroma of the tissues that remain in the stage of advanced decomposition of a corpse.

According to their ES, the necrophilous beetles found were the hysterids *Hister* sp., *E. decoratus*, and *S. caerulescens*. This group of beetles is associated with stages of advanced decomposition and dry remains. *Hister* sp., *E. decoratus*, and *S. caerulescens* have previously been recorded by Giraldo-Mendoza (2021) as cadaveric fauna in various studies on pig carcasses, and they are considered to have high forensic importance.

Various Diptera species from at least 11 families are known to colonize carcasses at different stages of decomposition (Cruz *et al.*, 2021). In the present work eight different families of Diptera were registered: Muscidae, Calliphoridae, Fannidae, Sarcophagidae, Psychodidae, Piophilidae, Phoridae and Tachinidae. Diptera has previously been described as a predominant Order in this type of vertebrate cadavers (Wangko *et al.*, 2015; Alboshabaa & Al Musawy, 2016; Cruz *et al.*, 2021), and among Diptera, the five most abundant species were *M. domestica* (Muscidae), *L. sericata* (Calliphoridae), *Sarcophaga* sp. (Sarcophagidae), *P. casei* (Piophilidae), and *C. nigribasis* (Calliphoridae).

Musca domestica is a cosmopolitan species that is quite common in the Neotropics (Battan-Horenstein and Gleiser, 2018). The abundance of M. domestica (with a total of 707 individuals) was higher compared to any other species of the Order Diptera, similar to what has been reported in previous studies (Battan-Horenstein & Gleiser, 2018). Other studies described other species of Muscidae, such as Ophyra albuquerquei Lopes, 1985 as the predominant in vertebrate carcasses (da Silva et al., 2014). In the present study, M. domestica was found mostly in larval form in the fresh-swollen decomposing state. Other authors such as Medina-Achín et al. (2018) recorded this species in a covered, dressed pig from the first to the twenty-first day of exposure, and in an uncovered pig from the fourth to the nineteenth day. In both carcasses this species was present in the swollen state until advanced decomposition.

Within the species of the Calliphoridae family, the most abundant are *L. sericata* (63.60%), followed by *C. nigribasis* (15.01%), *C. albiceps* (12.04%) and *C. macellaria* (9.35%). These species are more associated with fresh-swollen states and active decomposition. *L. sericata* is a Holarctic necrophagous

species with a wide distribution worldwide, and is most frequently found in slightly urbanized environments (Battan-Horenstein & Gleiser, 2018). In relation to C. albiceps, its larval stages prefer decomposing food substrates of animal origin and are predators of other Diptera larvae. It is considered a species of primary colonization and is found from the fresh state to the state of advanced decomposition (Medina-Achín et al., 2018). The calliphorid C. macellaria occurs in warm and humid areas because it is sensitive to low temperatures and is considered a species of secondary colonization (Medina-Achín et al., 2018). In pig (Sus scrofa) carcasses in preserved forest fragments in Recife, Pernambuco, Brazil, Cruz et al. (2021) found that among calliphorids C. albiceps was the most abundant in adult form in the dry season. Chrysomya species can cause deleterious effects on the population of native Calliphoridae, such as C. macellaria (da Silva et al., 2014; Cruz et al., 2021). In the present study, the calliphorids C. albiceps and C. macellaria were abundant mainly in the larval stage in vertebrates stranded on the beaches of the central coast of Peru, similar to previous reports (Medina-Achín et al., 2018).

In the present study, a high abundance of Sarcophaga sp. (Sarcophagidae) was observed in the larval stage (n= 198). Medina-Achín et al. (2018) reported that for an indeterminate genus of Sarcophagidae, the females have viviparity (depositing the larvae directly on the carcass, without going through the egg stage). and that they are present mostly in the pupal state from the fresh state to the skeletonized state, and are considered a species of primary colonization. While other authors did not achieve a taxonomic identification for all the Sarcophagidae species, they recorded at least 10 different species, which reinforces the significant diversity of this family in Neotropical environments, especially of the genera Oxysarcodexia and Peckia, and with a potential use as bioindicators in vertebrate corpses (Cruz et al., 2021). Sarcophagidae species are apparently opportunists on a wide variety of decomposing carcasses. This behavior is more frequently observed in other generalist necrophagous species (da Silva *et al.*, 2014).

Piophila casei was the fourth most abundant dipteran species (205 individuals) in the present study. This species has been recorded as a necrophagous species in other studies using *S. scrofa* as a model, for example, in Brazil (Cruz *et al.*, 2021), Colombia (Ramos-Pastrana *et al.*, 2018), Uruguay (Castro *et al.*, 2019; Remedios-De León *et al.*, 2019), among other South American countries. The fifth most abundant dipteran species in this study was the necrophagous *C. nigribasis* (106 individuals), which has been recorded in pig carcasses in urban environments in Colombia (Ramos-Pastrana *et al.*, 2018).

In relation to the ES, the following sequence was observed in descending order for species richness and abundance: necrophagous > necrophilous > omnivores. The community of necrophagous insects that is dominant in stranded vertebrates may be determined by various processes such as intraspecific and interspecific competition, predation and even cannibalism, which can produce a marked dominance of a few species of insects, especially invasive taxa (Cruz *et al.*, 2021). The higher richness and abundance of scavengers compared to the other ES are in agreement with the results of other studies (Benbow *et al.*, 2013).

The cluster analysis showed that the cadaveric entomofauna present in active decomposition, advanced decomposition and that of skeletal remains of stranded marine vertebrates were more associated with each other in relation to the fresh-bloated state according to decomposition states. These results suggest the fulfillment of the so-called "discrete process" hypothesis, which would imply a greater similarity in the composition of insect species within a given stage of decomposition than between stages, taking into account the ephemeral period of vertebrate carcasses stranded (Cruz *et al.*, 2021).

Benbow et al. (2013) described a low richness of taxa at the beginning of the decomposition; later, when the carcasses enter the stage of bloated and active decomposition, the richness and variation of entomofauna increases considerably. In addition, taxon richness is higher during active decomposition in a decomposing carcass. Finally, it has been reported that the richness of taxa decreases as the carcasses progress towards the advanced stages of decomposition and skeletal remains. The results of the present study suggest that active decomposition showed the highest values in D_{Mn} (1.50) and J' (0.83) for the cadaveric entomofauna in relation to the other decomposition states. However, the highest species richness (n= 23), H(2.22) and D α (5.05) were higher in advanced decomposition. These differences in the alpha diversity indices of the cadaveric entomofauna in the different stages of decomposition of a corpse could be due to the volatile by-products produced by the microbial communities that could act as signaling agents to attract or repel certain insects during the decomposition process. In addition, necrophilous insects with a predatory role can be attracted by necrophagous insects that colonized carcasses in previous stages of decomposition (Benbow et al., 2013; von Hoermann et al., 2021).

The highest species richness for cadaveric entomofauna was found in 2017 (n= 20) in H' (2.36), D_{Mn} (0.93), $D\alpha$ (4.27) and J' (0.69) compared to the other four years of evaluation. Among climatic factors, the year 2017 presented more central values of annual temperature, minimum and maximum annual temperature, and annual precipitation compared to 2015, 2016, 2018 and 2019 (INEI, 2020). It has been suggested that the differences in the structure of

these insect communities in carcasses are probably attributable to abiotic factors such as variations in temperature and relative humidity, rather than to the type of vertebrate carcass (Da Silva *et al.*, 2014). This could explain the variations in the structure and composition of the entomofauna along the five years evaluated (2015 to 2019). Thus, this type of interannual studies should be replicated in order to provide a broader basis for inferential statistics (Cruz *et al.*, 2021).

It has been reported that near the cities of Pelotas and Capão do Leão in Brazil the carcasses of four road-stranded wild terrestrial vertebrates, Didelphis albiventris Lund, 1840 (Mammalia, Didelphidae), Tupinambis merianae Linnaeus, 1758 (Reptilia, Teiidae), Nothura maculosa Temminck, 1815 (Aves, Tinamidae), and Cerdocyon thous Linnaeus, 1766 (Mammalia, Canidae) with a greater biomass developed a higher abundance of cadaveric dipterans, possibly due to the histological constitution of the different species of carcass types. The authors of this study did not find a relationship between carcass size and necrophagous insect community structure (Da Silva et al., 2014). Other authors showed that increasing the abundance or quantity of a carcass (size of the resource) should increase the number of trophic levels within local food webs and result in a greater diversity in species associated with carcasses with greater or biomass. Thus, large dipteran larval masses on large vertebrate carcasses (=high carcass biomass) are preferred feeding sites for necrophilous beetles (von Hoermann et al., 2021). The present study showed a pattern similar to that suggested by von Hoermann et al. (2021), with higher values being found for the H' index for *P. thagus* (2.31) with an average weight of 5 to 7 kg and for O. flavescens (2.29) with an average weight of 190 kg. These two species presented higher weights and higher values of H' compared to two other bird species such as *P. brasilianus* with 1.2 to 1.4 kg and *S. variegata* with an average weight of 1.2 to 1.67 kg. The higher biomass of O. flavescens could explain that according to the Bray-Curtis quantitative index the entomofauna collected from this marine mammal was less similar to the other three more frequent carcasses with a lower biomass (P. thagus, P. brasilianus and S. variegata).

The present evaluation carried out on cadaveric entomofauna found in marine vertebrates stranded on beaches of the central coast of Peru during the period 2015-2019 provides a conservative estimate of the real diversity of Diptera, Coleoptera, Dermaptera and Hymenoptera, which are mostly necrophagous and necrophilous. Further studies on the evaluation of diversity, biological cycles of bioindicator species and how these species that make up the cadaveric entomofauna in marine vertebrates stranded on the coasts respond to biotic and abiotic factors.

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